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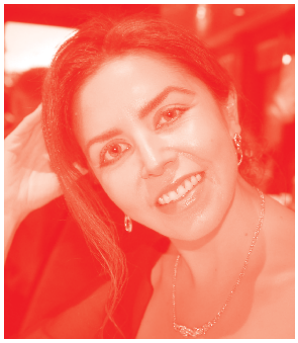

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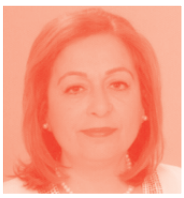
Meet the editors



Prof. Dr. Ayzin B. Küden is an academic staff member at the Faculty of Agriculture, Department of Horticulture, Çukurova University, Turkey, where she served as vice dean from 1997 to 1999 and dean from 2005 to 2012. She took part in the establishment of the ZIDEK Association with the assignment of the Agriculture, Forestry and Fisheries Deans Council in 2008, and still serves as the association's vice president. She chaired the TYYÇ Working Group on Agriculture, Forestry, Livestock and Fisheries, which was published in 2011. Her research interests include conservation of genetic resources in fruit growing, physiological studies, stone fruit breeding, almond and fig selection and cultivation, the use of molecular markers in breeding, the physiology of dormancy in temperate climate fruits, and the effects of climate change on fruit growing. She has made short- and long-term visits and training studies in many countries on these issues. Since 1996, she has been the chairperson of the Temperate Fruits in the Tropics and Subtropics (TFTS) Working Group of the International Society for Horticultural Science (ISHS). She became a country member of ISHS in 2018. Dr. Küden has edited and written books and book chapters and published around 200 research articles. She has served as a referee for journals. She has participated in six international symposiums, several workshops, and national meetings. She retired in 2021 but continues to be active in horticultural research and as an executive in two EU Prima projects. Dr. Küden is married and has two children.

Prof. Dr. Ali Küden is an academic staff member at the Çukurova University, Faculty of Agriculture, Department of Horticulture, and served as the Director of the Çukurova University Horticultural Research and Application Center between 1997-2017. Ali Küden is a full professor, and pomologist, studying on propagation and training of temperate fruit trees, especially pome and stone fruits, and nursery plants, at the University of Cukurova. He has been also working for 37 years on the selection and collection of fig and almond germplasm at the University of Cukurova. He has been extending his theoretical and practical knowledge to the growers, nurseries, and private sector in the whole country. He has published more than 170 scientific papers. He is one of the members of the "Temperate Fruits in the Tropics and Subtropics" (TFTS) Working Group of the "International Society for Horticultural Science" (ISHS). In addition, he took on the role in the organization of some scientific meetings of several organizations in and out of the country. He has been involved in several national and EU Prima projects, Prof. Dr. Ali Küden is married and has two children.

Contents

Preface

This book discusses breeding, germplasm, fruit tree physiology, pruning, production, and nutritional studies of the *Prunus* species. *Prunus* is one of the most important genera of fruit. It includes peaches, plums, cherries, apricots, and other stone fruits. The main species include *Prunus persica* L., *Prunus domestica* L., *Prunus armeniaca* L., and *Prunus avium* L., all of which are discussed in this book.

The wide adaptability of *Prunus* allows it to be grown in many parts of the world. The peach (*Prunus persica* L. Batsch) is native to China. However, it is widely produced in different parts of the world in various ecologies for fresh and fruit juice consumption. It is grown at 25-45 north and south latitudes.

Plum (*Prunus domestica* L.) is another stone fruit species that has spread and adapted to different climatic regions of the world with a high number of species and cultivars.

Sweet cherry (*Prunus avium* L.) originated in South Caucasus, the Caspian Sea, and Northeast Anatolia. These gene centers spread to the east and west covering a wide area of the world. Sweet cherry is abundantly found in the wild areas of North Anatolia and the Taurus Mountains in Turkey.

Apricot (*Prunus armeniaca* L.) is a stone fruit originating from Central Asia, Western China, and Iran-Caucasus. It is economically cultivated in many countries in the world, especially in the Mediterranean countries. Adaptation of apricot to different climatic conditions is weaker than that of the peach.

This book includes two sections: "Molecular and Breeding Studies and Germplasm Diversity in Prunus Species" and "Physiological and Nutritional Studies on Prunus Species."

The first section includes the following chapters: "Genetic Diversity in Almond (*Prunus dulcis*) "; "Varietal Wealth of *Prunus* Species"; "Expansion in Cultivating Almond Trees in Egypt"; "Advances in Breeding of Peach, Plum and Apricot"; and "Gene Editing in *Prunus* Spp.: The Challenge of Adapting Regular Gene Transfer Procedures for Precision Breeding."

The second section includes the following chapters: "Stock Influence on Growth, Morphological and Biochemical Leaf Parameters *Prunus domestica* L."; "Recent Techniques and Developments on Cherry Growing in Turkey"; "Logistics Chain and Cost Assessment of Pruning-to-Energy Value Chains: Application of Life Cycle

Cost Analysis Approach"; "Nutritional and Antioxidant Values of the Black Plum (*Vitex doniana*)"; and "Behavior of *Prunus persica* as Green and Friendly Corrosion Inhibitor for Corrosion Protection."

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Section 1

Molecular and Breeding Studies and Germplasm Diversity in Prunus Species

Chapter 1

Genetic Diversity in Almond (*Prunus dulcis*)

Sadia Sana, Naheed Akhter, Fozia Amjum, Samreen Gul Khan and Muhammad Akram

Abstract

Almond (*Prunus dulcis*), a stone fruit belonging to a family *Rosaceae* (rose) is broadly cultivated for ornament and fruit. Within this genus, *the* almond is very much associated with the peach, and these two fruits share the same subgenus the *Amygdalus*. About 430 species are spread all through the northern temperate regions of the world. The Mediterranean climate region of the Middle East like Turkey and Pakistan eastward to Syria is native to the almond and its related species. Almond is one of the ancient fruit trees known to the Asian as well as European regions with the most primitive proof of cultivation dating about 2000 B.C. *Prunus dulcis* (Almond) is a nutrient-loaded nut crop. Almond possesses a great genetic diversity due to the genetically controlled self-incompatibility system which can be estimated by a morphological characteristic including molecular markers and isoenzymes with a wide range of marker techniques. Simple sequence repeats (SSR) involving RFLP or SNP are the most commonly used molecular techniques among the DNA-based molecular symbols. Particular agronomic characters, e.g. kernel bitterness or self-compatibility can also be traced by these molecular markers. The direct association between the level of diversity and the basis of the germplasm cannot be understood by the studies of genetic diversity. Genetic diversity cannot be seriously lost by self-compatibility in almonds. The breeding, conservation, and cultivation of wild-growing almonds may similarly advantageous after the genetic diversity research studies (especially those applying molecular markers).

Keywords: *Prunus dulcis*, Genetics diversity, Simple sequence repeats, Molecular mechanism

1. Introduction

Nuts generally are part of functional foods and their consumption was reported, to protect against cardiovascular disease, certain types of cancer, diabetes, and other disease states, including neurodegenerative conditions. African Black Walnut and Almonds are important but underutilized nuts with inherent medicinal and therapeutic potentials for promoting human health reported that Almonds (*Prunus dulcis*) and Black walnuts (Tetracarpidium conorphorum) help to lower cholesterol levels in the blood, the risk of diseases of heart, control of body weight and control of diabetes. These nuts (Walnut and Almond) are also believed to naturally comprise and polyunsaturated and monounsaturated fatty acids, dietary fiber, and

protein, as well as various necessary nutrients including several trace elements and vitamins which contribute significantly to healthy living.

Almonds are prunes, which are small to medium-sized trees of fruit that have their place in the family of rose, which is Rosaceae. They were usually positioned in a Prunoideae (or Amygdaloideae), that is a sub-family, but sometimes, they are positioned in their individual Prunaceae (or Amygdalaceae) family [1]. In recent times, it has become seems that almonds evolved from the Spiraeoideae that is a sub-family [2, 3]. The group Prunus consists of several economically significant fruit trees classes such as apricot, cherry, plum, peach, and almond [4, 5]. In Southcentral Asia, the peach and almonds almost developed from the identical inherited species [6, 7]. 26 classes of Prunus form a distinct taxonomic group on the earth. 21 almond classes and 6 natural hybrids present in Iran [8, 9].

Almonds were cultivated at least by 3000 BC [8, 10]. The almond was spread beside the seashore of southern Europe by Greeks, Egyptians, and Romans, and the Mediterranean in northern Africa [11]. Thus the almond and its associated kinds are native to the Mediterranean environment area of the Middle East indicating Pakistan eastward to Turkey and Syria [12, 13]. In the 1700s, Spanish Padres established the Task at Santa Barbara and carried almonds to California [14, 15].

In the late 1800s, the industry started in California due to the growth of great cultivars on almonds, and importers were forced to defend the industry. From then till about 1960, the industry technologically advanced at a moderate speed [14]. Furthermore, approximately 8% of the total world's almonds are cultivated in California.

Under farming, many fruits trees classes have converted from sexual reproduction into vegetative propagation [1]. Outdated systems of production have continued, where cultivar propagation is established on a diverse reproductive system [16]. For millennia, *Prunus dulcis* has been cultivated by seeds. *Prunus dulcis* grafting continued with little significance until recently [15]. Both clonal and sexual reproductions are used for *Prunus dulcis* propagation [16–18].

2. Botanical description

The *Prunus dulcis* is botanically categorized as a drupe with skin like pubescent exocarp, hell is like a fleshy mesocarp and shell is like a hard endocarp [19]. The embryo is enclosed by a pellicle in the seed, composed of a nucleus, endosperm remnants, and seed coat. *Prunus dulcis* is distinguished from other *Prunus* classes by its leathery and dry mesocarp, at maturity which is dehisced [20–22].

2.1 Plant

Small to the medium that is average-sized tree exist with open canopy with linear or ovate with notched margins leaves sized between 3 to 5 inches, about 3-4 times longer than wide having finely notch margins and sharp tips [21, 23].

2.2 Flowers

Almond tree flowers are sweet-scented with white or light pink and almost identical to peachtree flowers. Almond flowers have a perigynous ovary and many elongate stamens with 5 petals and sepals [22, 23]. Flowers are borne laterally on short lateral branches and spurs, or occasionally on elongated shoots in lateral position [24, 25].

2.3 Pollination

Almonds involve cross-pollination because they are self-incompatible [26]. *Prunus dulcis* is a crop that is a large consumer of fertilizer and water, and it is extremely pollinator-dependent, its production may be dependent on variations in these resources [27]. All pollinators like honey bees are entirely crucial for pollination, particularly since wet and cool weather can arise at the comparatively early blooming period [28, 29]. Moreover in California, almost 8% of the total world's almond fruits are cultivated, where the temperature change is predicted to decrease water accessibility [30].

2.4 Fruit

Almond is a nut fruit. The whole nut fruit includes the hull is a drupe, though the hull dries and splits to reveal the pit of the fruit. Its fruiting starts in 3 to 4 years old trees, and 6-10 years old tree leads with maximal production [26, 31]. On average, an almond tree can produce for more than 50 years. For best fruit cropping high ratio of flowers should be maintained. Almond fruit trees produce flowers in February. Fruits development is considered by an increased cotyledon size and diminishing endosperm and nucleus [23, 32]. The growth of the different fruit tissues in the 4 genotypes presented sequential deviations as has formerly reported in other *Prunus dulcis* cultivars [33]. Therefore, ripening and growth of the different fruit tissues continued in a somewhat shifted mode from one genotype to another. Till April, the tenderly derived fruit tissues as endocarp, mesocarp, exocarp, and tegument are surrounded and protected by cotyledon. The endocarp is soft and green in color that is easy to open [34]. The endosperm and growing embryo with its typical white cotyledons are noticeable in all genotypes and nucleus size has decreased in May [32]. The endocarp has become appear a woody texture and hard to open and is turnoff into brown color. To end, the maturing season ends and the white cotyledons fill the full space inside the tegument. Mesocarp with the exocarp is starting to dry ultimately exposing the endocarp [34].

3. Harvest, postharvest handling techniques

In agronomical processes, irrigation is the most significant aspect affecting almond seed weight, quality, and yield, however, there is no significant impact was observed on the lipid concentration and composition of fatty acid [35]. Practically crop of almonds does not disturb the lipid composition but induces discrepancy to the physical characters of almond grain due to the greater seed moisture concentration. A late harvest of drupes fruits prompts a higher concentration of dry material in the grain. Genetic features, weather and soil conditions, fertilizers usage, and the condition of the plant's maturity can affect at harvest level, and also the concentration of minerals in a plant [36].

3.1 Maturity

At maturity, the hull of the almond splits and physically nuts separate from the tree at this spot. Harvesting of the almond tree started when hulls of almond nut fruit in the inside of the canopy are open [37]. During maturation, the drying of the seed coat proceeds, and the seed coat turns brown. If harvesting is delayed it increases the threat of navel orange worm invasion [38].

3.2 Harvest method

Almond trees are harvested by mechanical or automatic tree shakers. While shaking the young trees may be damaged, therefore in the first few years, the young trees are harvested by hand knocking. Almond nuts are spread on the ground for drying for 1-2 weeks [39].

3.3 Postharvest handling

Immediately after crop harvesting the fruits can be dried and hulled instantly or stocked for fumigation against Navel Orange worm [37]. Fruit nuts are dried under hot air till the moisture content reaches 5 to 7 percent [40]. Then the nuts are dehulled and shelled. If final processing is pending the nuts in the shell can be stored in a container for many weeks or months [41]. Nuts are then shelled and sorted for size and appearance [42]. In the last the nuts are bleached for color development, then salted, roasted, and/or flavored before wrapping. Furthermore, former studies described that storing at low oxygen and low-temperature atmosphere caused less off-flavors development [43].

3.4 Storage

Either in-shell or shelled if dry, almonds may be stored for many months, or frozen for very long periods in years [41]. Commercially, for long-term storage, the nuts are fumigated for navel orange worm and kept at a temperature below 40°F [39, 43].

4. Genetic engineering for the improvement of production yield

The objective of the study was to investigate the genetic diversity of almonds. Genetic engineering contains direct handling of an organism's genome through biotechnology to adapt the genetic makeup of cells, containing the transmission of genes within and across different kind's limits to yield advanced crops [44]. The process can be used to remove, (knock out) or target a specific part of the genome. Genetic engineering techniques have been applied in various fields including medicine, research, industrial biotechnology, and agriculture [45].

There are four main targets in making genetically improved crops. The first aim is to provide defense against environmental pressures, such as pathogens or cold or resistance to herbicides. The second aim is to alter the quality of the crop by raising the nutritious value and providing additional industrially valued qualities and quantities. Thirdly, to construct materials that it does not normally make or to provide the novel model. Forth is to honestly improve yield by accelerating growth, or making a tolerant organism, for example improving salt, cold, or drought tolerance in plants [46, 47]. The basic chromosome number of wild-type almonds is eight and it's DNA substances are small. Almond fruit occupies a very anomalous place between other fruit trees. Almond is considered the main crop and is cultured in diverse climatic areas after tolerance to drought, salinity, and cold [47].

The genotypes of almonds are clustered into 2 main groups, one is wild and the other one is cultivated almonds. The group of cultivated almonds is distributed into four subgroups which are comprised of 2, 3, 44, and 42 genotypes, respectively. The wild group of Prunus almonds has genotypes that had the less average for a maximum of the studied characters, but an average of this group for characters such as kernel color, ease of hulling, shell color, leaf basal shape, sensitivity to *Anarsia Lineatella*, marking of the outer shell, and leaves arrangement was greater than

the other cultivated group of almonds. In cultivated almonds average genotypes in the first subgroup for certain main characters such as thickness, kernel weight, and width, double flower in buds, growth tree habit, bearing habit, ease of hulling, flower density, sensitivity to *Anarsia Lineatella,* petiole length, sensitivity to *Myzus persicae* and *Pterochloroides persica* was greater than the other subgroups. The second subgroup of the cultivated almonds had the maximum average for suture opening of the shell, kernel length, leaf length, shriveling of the kernel, leaf width, leaf shape and leaf area, duration of flowering, and sensitivity to *Pseudomonas syringe* [48].

Numerous study of the literature shows that almond tree size and seedling juvenility is the key hurdle to developing the genetic potential of almond fruit and nut breeding stocks. Dwarf trees with usual cultivars revealed that fruit size was not destructively affected by dwarfing whereas the yields considered being high [47]. As in the dwarf tree, the fruit value was reduced it was observed that heritability for fruit firmness, skin color, size, and percentage of soluble solids was in high concentration. This suggested that these characteristics can be improved to meet up the commercial standards within one or two selected cycles. Thus it is recommended that mass selection will be helpful in genetically reducing the juvenile stage [49].

To increase the yield of almond trees different studies were carried to facilitate genetic manipulation, and to increase its production efficiency. The genetically engineered almond tree can be dwarfed (compressed), by manipulating the dwarf (DW) gene of peach [50]. Dwarf almond tree revealed that the heritability of these dwarf traits is high as well as spur density also differs widely. The flower production of dwarf hybrids is copious. While the yield potential of dw/dw dwarf almonds will stay unidentified until fertility is restored. As the international reputation increases, demands are motivating almonds yield to continue to rise across worldwide. Different research studies on varietal chilling necessities involving the particular microclimates in a defined state will provide better assistance in reducing the risks of wasted bloom. Chilling prototypes must also be considered for regional application and accuracy to increase consideration of the causes affecting the timing and the length of the almond bloom and also the association among characteristics of bloom. Continuous changes in climate intensely affect the area where Prunus almonds are grown up. Cultivators have to need to take care to develop different varieties in different climates with sufficient chilling, and also to care for young buds and shoots from chill damage. And also more researches are required on precise climate thresholds and their association to physiological variations during Prunus almond pollination and bloom. Though, the simple training of heat and chill monitoring will permit cultivators to anticipate flowering like to prepare the optimum bee activity in bloom and idea for crop reduction in very warm bloom times [47].

In recent years, molecular markers have been used to study genetic diversity and cultivar identification of almonds. Methods based on knowledge provided by advances in molecular genetics, notably molecular markers, promise faster and more efficient approaches to cultivar improvement. In fact, important tools such as molecular markers, maps, DNA sequences, and quantitative trait loci (QTLs) have been developed and made available to researchers, and applications at the breeding program level have already started. In genetics, a molecular marker is a fragment of DNA that is associated with a certain position within the whole genome [51]. Molecular markers are used to identify a particular sequence of DNA in an unknown DNA pool [52].

For this purpose different types of molecular markers are used for the assessment of the genetic diversity like microsatellite markers, simple sequence repeat (SSR) markers, Informative Markers for morphological traits of almond (*Prunus dulcis*) [53]. Edifying markers are the most suitable and reliable genetic statistics for

breeding purposes and are considered as a first fact to examine the genome for the associated characters. In almond Prunus, the practice of breeding faces a distinctive task due to the limited genetic experience of commercial cultivars.

Morphological traits such as tree altitude in cm, leaf length in cm, leaf shape, flowering duration, leaf width in cm, blooming time, petiole length in cm, kernel length in cm, kernel yield in gram, kernel width in cm, kernel thickness in cm, nut weight in g, kernel nut weight in g and kernel percentage are frequently used for cultivar identification in almond. Though, morphological characters are restricted because of their environmental oscillation [54].

The basic worries of present agriculture are the utilization and conservation of priceless genetic resources of different plant crops. The requirement for precise recognition applies to cultivars and sequences, in parallel to their type of maintenance, whether they are preserved in an ex-situ and in-situ gene bank or another in the Vitro gene bank [55]. The tools developed for biodiversity classification may permit explanations of synonyms and improvement in the origin of cultivars and species. At times for cultivars, the description and determination of trees of fruits are hard by using conservative approaches. Although the morphological symbols are prone to uncertain explanations, molecular methods should be applied in the identification and programs of breeding for the cultivars [56]. Molecular markers facilitate distinguishing labeling mistakes and repetitive documentation of cultivars in nurseries etc. Moreover, it reduces the work programs of breeding by speed up the process of breeding by permitting an assortment before the first crop of fruit, by following certain genes or genotypes among offspring of crosses [57]. The use of molecular symbols based on PCR has been the option of plant genetic research studies and in making to impression for many types of fruits. These symbols can be used to regulate the varieties by agreeing a plant to be recognized at any step and vegetative cycle and may undo cases involving plants with undefined sources and names [58].

5. Isozymes detection

Isozymes are various forms of enzymes that catalyzed the conjoint substrate but are mixed based on their physical appearances for example shape, electrical charge, molecular mass, and protein structures [57]. Isozymes can be separated and analyzed due to the difference in their electrophoretic mobility [54].

In-plant genetic and breeding isoenzymes have been used due to their individuality like simple inheritance, lack of gene interactions, co-dominant expression, and polymorphism present in various plant species and lack of environmental effect [59].

Isozymes can be identified in different tissues by different processes. Iso enzyme's variability is the key source of genetic markers which can be used for recognition of hybrids and cultivars, initial selection, recognition of genetic diversity, quantification of genetic associations among populations [55].

In Prunus almond fruit following Isozymes are present these include glutamate dehydrogenase, alcohol dehydrogenase, malate dehydrogenase, formate dehydrogenase, and shikimate dehydrogenase [60, 61]. Isozymes can be separated by using the polyacrylamide gel electrophoresis method and these isoenzymes can be used to recognize genetic variability in Prunus almonds [56].

6. Therapeutic applications

Almond numerous active components as dietary fiber [62], proteins like albumin, globulins & amandine, amino acids, certain important essential minerals as

magnesium and calcium, vitamins especially B vitamin, and monounsaturated fats [63, 64]. Furthermore, almonds contain phenolic and phytates that constrain the amylase enzyme activity and are supposed to perform synergistically to reduce starch digestibility [65]. The reduced rate of digestion of carbohydrates may describe reported growths in blunted blood glucose response and satiety with consumption of almonds nuts, which describes them as a low glycemic food [66]. Almond flour mixed with honey or sometimes with sugar is often used as a gluten-free food substitute for wheat flour in baking and cooking [67].

These components showed the therapeutic activities. Almond oils also comprise of fatty acids like stearic acid, palmitic acid, palmitoleic acid, oleic acid, eicosanoid acid, linoleic acid, arachidic acid, behenic acid, alpha-linolenic acid, and erucic acids due to these fatty acids almond oil has outstanding emollient properties [68]. The oil can be used for massage therapy to relieve sprains [13].

7. Hypoglycemic action

Almonds nuts, flowers, and seeds lowered the blood glucose level oxidative stress in diabetic patients and also decrease post-prandial glycemia as the almond nuts ingestion is related to a reduction in oxidative damage and blood glucose level [31].

8. Cholesterol-lowering action

Prunus dulcis have a reliable effect of LDL-cholesterol lowering in healthy people and persons with diabetes and high cholesterol [69]. Prunus Almonds are rich in unsaturated fatty acids and low in saturated fatty acids and plant protein, contain fiber, α-tocopherol, phytosterols, magnesium, arginine, manganese, copper, potassium, and calcium [70]. The responsible mechanism for the LDL-cholesterol decline is probably to be linked with the presence of nutrients Prunus almonds, like reduced bile acid and cholesterol absorption, increased excretion of cholesterol and bile acid, and LDL-cholesterol receptor activity is also increased. Prunus Almonds also comprise phytosterols which are accompanying properties of lowering cholesterol [66]. The nutrients present in Prunus almonds control the enzymes involved in the production of bile acid and cholesterol. Almonds also reduced the biomarkers of lipid peroxidation in hyper-lipid emic patients. Regular ingesting of Prunus almonds can be supportive in the regulation of blood pressure as they are low in Sodium and high in potassium [71].

9. Immunostimulant action

Almonds enhanced the immune surveillance of blood mononuclear cells against the infectious virus because the *Prunus dulcis* nuts are associated with high levels of cytokine production including interleukins, interferon-A, (TNF-α) tumor necrosis factor, and INF-gamma. Almonds also induce a considerable decrease and control in the Herpes simplex virus replication [65].

10. Pre-biotic potential

Almond seeds possess prebiotic activity. Prebiotics are non-digestible nutrition elements that stimulate bacterial activity and growth in the system of digestion [13].

In this way, prebiotics is stated to be beneficial to health. Characteristically the prebiotics is carbohydrates (such as oligosaccharides) in nature [71]. As nutritionally soluble fibers are the most common classification of pre-biotic. To a certain level, many forms of dietary fibers reveal some level of prebiotic effects. It has also been shown that Prunes almonds altered the composition of bacteria in the gut by stimulating the Eubacterium rectal and bifid bacteria's growth [68].

11. In amnesia

Almonds have a memory-enhancing activity as they are found to raise the Ach level in the brain and finally improve the brain memory [72]. It may be useful to examine the potential of the almond plant in the management of Alzheimer's. Regular consumption provides power to the brain as they comprise vital nutrients which can essentially help to increase intellectual capabilities [73].

12. Anti-oxidant action

Almonds possess anti-radical and anti-oxidant activities and their phenolic extract may be useful in inhibiting and reducing the process of different oxidative stress linked to disease [72]. The scavenging capacity and reducing the power of the phenolic extracts for hydrogen peroxide, superoxide, and radical nitrite were calculated [74].

13. Hepato protective action

Almond Prunus showed hepatoprotective activity against hepatitis and also improves the biochemical markers to see the hepatic damage like ALP, SGOT, SOD, SGPT, GSH, total bilirubin, catalase, direct bilirubin, and LPO [62].

One study in the almond was tried for its hepatic protecting effect against Paracetamol-induced and CCl_4 hepatitis in rats. The management with the almond fruit extracts carried out the changed levels of the biochemical markers to close to normal levels [72].

14. Conclusion

As the international reputation increases, demands are motivating almonds yield to continue to rise across worldwide. In the end, we concluded that microsatellite indicators can be effectively used to examine the genetic diversity of almonds and to classify useful markers for important traits breeding.

The tools developed for biodiversity classification may permit explanations of synonyms and improvement in the origin of cultivars and species. At times for cultivars, the description and determination of trees of fruits are hard by using conservative approaches. In almond Prunus, the practice of breeding faces a distinctive task due to the limited genetic experience of commercial cultivars. To increase the yield of almond trees different studies were carried to facilitate genetic manipulation, and to increase its production efficiency. And also continuous changes in climate intensely affect the area where Prunus almonds are grownup. Cultivators have to need to take care to develop different varieties in different climates with sufficient chilling, and also to care for young buds and shoots from chill damage.

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Chapter 2

Varietal Wealth of *Prunus* Species

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Abstract

Genus *Prunus* includes all the stone fruits (peach, nectarine, plum, apricot, almond and cherry) comprise around 98 species and classified under three subgenera namely: *Amygdalus* (peaches, nectraine and almonds), *Prunophora* (plums and apricots) and *Cerasus* (cherries). Genus *Prunus* have attained a prime position among all the temperate fruit crops as delicious edible drupe, and many species have ornamental values as well. Major species of importance are *Prunus persica* (peach), *Prunus armeniaca* (apricot), *Prunus salicina* (Japanese plum), *Prunus domestica* (European plum), *Prunus americana* (American plum), *Prunus avium* (Sweet cherry), *Prunus cerasus* (Sour cherry), *Prunus dulcis* (almond), *Prunus ceracifera* (Cherry plum), *Prunus mira* (Behmi), *Prunus cerasoides* (Wild Himalayan cherry), *Prunus mahaleb* (Mahaleb cherry) etc. Interspecific hybrids namely: plumcots, pluots and apriums also produce very delicious edible fruits. Commercial cultivars of different stone fruits are J H Hale, Cresthaven, Flordasun, Florda Prince, Elberta, Glohaven, July Elberta, Redhaven, Kanto 5, Sun Haven etc. of peaches, Fantasia, Mayfire, Red Gold, Snow Queen etc. belongs to nectarine, Turkey, Charmagz, Perfection, St. Ambroise, Royal, New Castle etc. are apricots, Santa Rosa, Black Beauty, Kelsey, Green Gage, Methley, Satsuma, Frontier, Burbank etc. are plums, Regina, Burlat, Lapins, Kordia, Stella, Bing, Van, Black Heart, Compact Lambert, Compact Stella etc. are cherries, and California Paper Shell, IXL, Mission, Nonpareil, Drake, Ne Plus Ultra, Pranyaj, Merced etc. are almonds.

Keywords: *Prunus*, varieties, stone fruits, peach, cherry, almond

1. Introduction

Stone fruit is a generic term used to define fruits which includes peach, nectarine, plum, apricot, almond and cherry which are generally grown in temperate climatic conditions. The main feature of stone fruit is having fleshy layer, mesocarp, as edible pulp surrounding a relatively large, hard pit commonly known as 'stone' that shields and protects a seed. The commercial production of stone fruits is confined between the latitude of 30 and 40°N and S, although it is now grown almost all over the world. The major stone fruit-producing country is China accounting about 50 per cent share of the total world production. In India, stone fruits are grown on a commercial scale in mid-hill Himalayan states, viz. Himachal Pradesh, Jammu and Kashmir, Uttarakhand, as well as in a limited scale in north-eastern states. These fruits are generally grown on soils having bulk density, parasitic nematodes, root rot problems, fungal pathogens or other soil and replant problems.

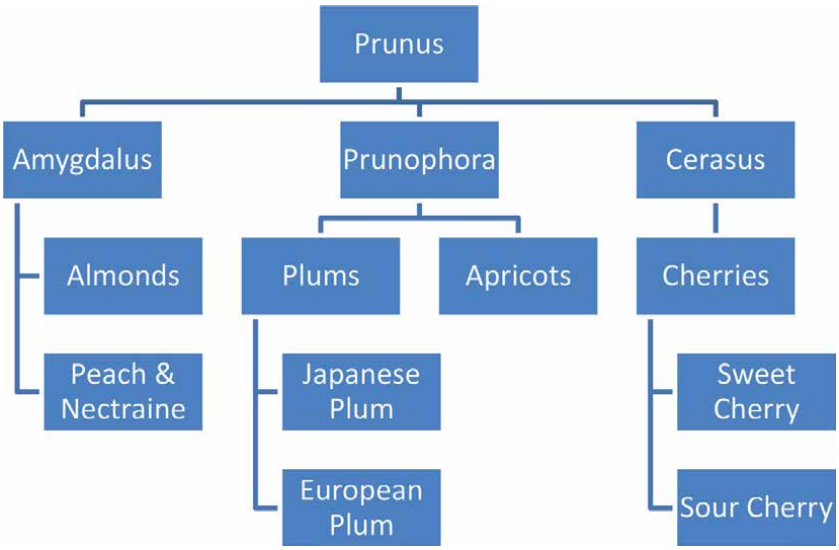

Figure 1.
Botanical classification of Prunus genera showing various stone fruit crops.

There are over 400 to 430 species in the genus *Prunus*, (includes edible as well as ornamentals fruit crops) but only 89 are listed in the Genetic Resource Information System [1, 2]. In India, about 36 *Prunus* species have been reported so far and 18 species are useful for cultivation for different purposes [2–4] (**Figure 1**).

2. Almond

Almond is classified in the subgenus *Amygdalus* within the genus *Prunus*, distinguished from the other subgenera (*Prunophora* and *Cerasus* sections) by the corrugated seed shell [5]. Twenty two almond species were classified into five taxonomic sections including: *Euamygdalus*, *Spartioides*, *Lycioides*, *Chameamygdalus* and *Leptopus* [6]. Being a temperate crop, almost all the varieties of almond can be grown successfully in the temperate regions of India. However, most promising cultivars which have been tried or being cultivated in various parts of the world are described here under.

2.1 Blanquerna

It is derived from self-pollinating 'Genco', self-compatible almond cultivars developed at Zaragoza, Spain [7]. The cultivar do not requires any foreign intervention for their proper pollination and consequently for the production of a commercial crop. In addition, kernels from these new cultivars show no doubles.

2.2 Butte

California and Mission type cultivar, harvested 25–30 days after Nonpareil. Shell is semi-hard without suture opening, excellent crop potential. Nut and kernel size is small, short, wide in shape with wrinkled surface. The plants are spreading in nature, a late bloomer variety and suitable pollinizers for Mission.

2.3 California Paper Shell

Tree is erect which is suitable for high density plantations. It bears flower and nuts on both spurs as well as on long shoots. Regular bearer, blooms in mid March and ready to harvest after 152 days from the date of full bloom. Nut and kernel are longer in shape with extra light color. Thin shelled, yielding 50 shelling percentage.

2.4 Cambra

This variety is derived from a cross between 'Tuono' and 'Ferragnes', self-compatible almond cultivars developed at Zaragoza, Spain [7]. The cultivar do not requires any foreign intervention for their proper pollination and consequently for the production of a commercial crop. In addition, kernels from these new cultivars show no doubles.

2.5 Carmel

California type cultivar, harvested 25–30 days after Nonpareil. Shell is soft, good shell integrity and fair suture opening. Nut medium in size, narrow in shape with slightly wrinkled surface and long attractive kernel. The plants are medium upright in habit, late in harvesting and a good pollinizer for Nonpareil variety.

2.6 Drake

A soft-shelled cultivar which yields medium-sized nuts of good shape and color. Nut shape is oblong with the tip rounded or slightly tapering and the shell is whitish or light brown in color. Cultivar shows irregular bearing and is susceptible to brown rot disease.

2.7 Felisi

This variety is originated from a cross between 'Titan' and 'Tuono', self-compatible almond cultivars developed at Zaragoza, Spain [7]. The cultivars do not requires any foreign intervention for their proper pollination and consequently for the production of commercial crop. In addition, kernels from these new cultivars show no doubles.

2.8 Fritz

California and Mission type, harvested 40–60 days after Nonpareil. Semi-hard shell, light in color. Nuts are small, medium plump in shape, dark brown in color with wrinkled surface. The tree has upright vigorous tree, good cropper, a good pollinizer for Nonpareil variety, but susceptibility to bacterial spot.

2.9 Himachal selection no. 10

This cultivar has been selected from Himachal Pradesh, India. The tree is medium vigorous and spreading growth habit. The nut is medium in size, elongated in shape and brown in color. Kernel weight is about 78 per cent of the nut weight.

2.10 Hybrid no. 15

This hybrid has been released from Punjab Agricultural University, Ludhiana. The tree is spreading type. The nuts are uniform in size and are elongated. The

kernel is dark brown in color. The taste and flavor of this variety is poor. The kernel weight is 51 per cent of the nut weight.

2.11 IXL

A regular bearing cultivar, blooms towards end March and ready to harvest after 151 days from the date of full bloom. The tree is spreading type and of intermediate vigor. It bears on both spurs as well as on long shoots with good ability to renew fruiting wood. The nut and kernels are medium and shell color intensity is intermediate. Shells are soft that gives a high shelling percentage of about 55 per cent. It gives good yield in the foot-hills and valley areas but is incompatible with Nonpareil and should, therefore, not be planted with it. It is susceptible to gummosis disease.

2.12 Makhdoom

A regular bearer cultivar, bloom during 1st week of March and ready to harvest after 141 days from the date of full bloom. The tree is spreading/drooping in growth habit. It bears flowers on long shoots and spurs with good ability to renew fruiting wood. The shell color is medium, soft type plump which yields about 42 shelling percentage. The average productivity is more than 2.0 t/ha.

2.13 Marcona

A Spanish variety with a small, precocious habit. The shells are hard, making the nuts more difficult to shell, resulting in a weaker meat to shell ratio. However, Marcona meats are generally worth 20 per cent more per pound than Nonpareil. Good compatibility with Sonora.

2.14 Merced

A regular cultivar, blooms during 3rd week of March and ready to harvest after 152 days from the date of full bloom. The tree is upright which is suitable for high density plantations. It bears on both spurs as well as on long shoots with good ability to renew fruiting wood. Shell color is intermediate with papery shell giving shelling percentage of about 56 per cent. The variety is also suitable for export of kernels. The average productivity is 2.0 t/ha.

2.15 Mission

This cultivar is harvested 40–60 days after Nonpareil. It is a hard shelled without suture, opening. Nut are small, short wide in shape and dark brown in color with deep wrinkled surface. Medium small but plump kernel but unsuited to blanching. It is a late bloomer variety so resistant to frost.

2.16 Monterey

It is a California type cultivar, harvested 40–60 days after Nonpareil. It is soft shell variety, exhibits high percentage of double kernels, which are large in size, kernel brown in color with smooth surface. Nut is large in size, long narrow in shape and surface is deep wrinkled. Very good crop potential and late harvester.

2.17 Ne Plus Ultra

A popular cultivar for big sized nuts and have attractive shell. Plants are somewhat small with spreading branches, good cropper, a good pollinizer for Nonpareil. The kernel are large with many doubles, becomes defective when the moisture is lacking and the nuts are frequently gummy when the soil becomes slightly water-logged. It is soft shell, earlier bloomer. The plants are susceptible to some diseases.

2.18 Nonpareil

An early and one of the most important and best commercial cultivar of almond with soft shell which is brown in color. Nuts are medium in size, flat in shape, light in color and have smooth surface. Shell is papery with high kernel to the nut ratio fetching high price. Nuts are elongated in shape, flattened and pointed at the apical end. The shell is white and the kernel is flat, light brown, sweet with good flavor. This variety grows well in Punjab and Himachal Pradesh. It bears on both spurs as well as on long shoots having good ability to renew fruiting wood and is relatively resistant to frost. Nuts have an extra light color, papery shell which yields high shelling percentage upto 60 per cent.

2.19 Padre

Hard shelled, good consistent cropper, California and Mission type cultivar, harvested 25–30 days after Nonpareil and similar to Butte. Nut and kernel is small to medium in size, short and wide in shape, dark brown in color with wrinkled surface.

2.20 Parbat

The tree is regular bearer which blooms in the 3rd week of March. Fruits are born on one year shoots a some on spurs. Nut size small, shell color whitish yellow, matures at 140 days after bloom. Kernels are smooth having good color and very good appearances and taste, shelling percentages on an average 49 per cent. The average yield (in shell) is around 0.5–1.0 t/ha under normal conditions.

2.21 Peerless

Hard shell type cultivar, blooms early in the season and susceptible to frost, harvested 7–10 days after Nonpareil. Shell is hard, light in color and surface is smooth. Nuts are medium in size, wide in shape with fairly wrinkled surface. It is a best pollinizer variety for Nonpareil.

2.22 Pranyaj

This is a regular bearer cultivar which blooms during mid-March and ready to harvest after 144 days from the date of full bloom. The tree growth habit is upright and is suitable to grow under high density orcharding. Bears on long shoots and spurs. Shell color is light, shell is soft. Nuts are medium in size, kernels are plump and yields shelling percentage of about 44 per cent. The average productivity is more than 2.0 t/ha.

2.23 Price

It is good cropper cultivar but tends to be biennial, California type cultivar, harvested 7–10 days after Nonpareil. High percentages of double kernels are produced. Shell is papery, dark brown in color, surface is rough. Nuts are small to medium in size, short narrow in shape with fairly wrinkled surface, tends to form in clusters. It is a best pollinizer variety for Nonpareil.

2.24 Primorskij

Trees are spreading and moderately vigorous, mid to late blooming, nuts are medium to large, bold, slightly flattened and brown in color, kernel medium to large. Soft paper shelled and late season maturity.

2.25 Shalimar

A regular bearer cultivar, blooms in mid March and ready to harvest after 143 days from the date of full bloom. The tree growth habit is spreading/drooping type. It bears flowers on both long shoot and spurs with good ability to renew fruiting wood. The shell is light in color, papery type and yields shelling percentage of about 50 per cent. This variety is suitable for export. The average productivity is 2.0 t/ha.

2.26 Sonora

California type cultivar, harvested 7–10 days after Nonpareil. Shell is papery, dark brown in color and surface is rough. Nuts and kernels are medium to large in size, long narrow in shape, light in color with smooth surface. It is a heavy bearer variety which tends to biennial bearing.

2.27 Texas

It is a late ripening variety and yields regularly. The kernel tastes slightly bitter. The nut is small and poor in appearance.

2.28 Thin shelled

An early and regular bearing cultivar. Tree is very vigorous, upright and with a fairly dense head. The nut is light brown in color, attractive and smooth. The shell is very thin and papery. The kernel is brown, well developed, sweet and delicious.

2.29 Waris

The variety is a regular bearer, blooms during mid-March and ready to harvest after 145 days from the date of full bloom. The tree growth habit is upright and is suitable for high density orcharding. It bears fruit on long shoots and spurs. The shell color is medium, soft shelled, nut are medium in size, soft shelled with plump kernels with upto 48 shelling percentage. The average productivity is more than 2.0 t/ha.

2.30 Belona and Soleta

These are two new almond (*P. amygdalus* [*P. dulcis*]) cultivars from Zaragoza, Spain. Both cultivars were obtained from artificial pollinations following the steps

of a traditional breeding programme. Both came from the cross made in 1988 of Selection E-5-7 (seedling of open-pollinated 'Genco', a self-compatible Italian cultivar) by the French cultivar 'Belle d' Aurons', characterized by its kernels of excellent quality. This cross was made with the aim of utilizing a self-compatibility source other than 'Tuono', so far the mostly utilized parent in almond crosses [8].

3. Apricot

According to different apricot classifications, reported by Lingdi and Bartholomew [9], there are 11 accepted apricot species within the section *Armeniaca* including *P. brigantina* Vill. (alpine apricot), *P. mandshurica* Maxim. (Manchurian apricot), *P. sibirica* L. (Siberian apricot), *P. armeniaca* L. (common apricot), *P. mume* Sieb & Zucc. (Japanese apricot), *P. dasycarpa* Ehrh. (black apricot, a natural plum-apricot hybrid), *P. holosericea* Batal. (Tibetan apricot), *P. hongpingensis* Li., *P. zhengheensis* Zhang & Lu., and *P. hypotrichodes* Cardot. Also, the desert apricot (*P. fremontii* S. Wats.), originated from southern California deserts, is worth mentioning among the listed species, can be freely hybridized with them, and has close morphological traits to other species of apricot [10]. All the Apricot varieties in the Mediterranean region and USA are of European group and have very little genetic diversity [11]. The European group of Apricots is the youngest in origin and Dzhugar Zailij (Soviet Central Asia such as Kazakhstan) is the oldest. The varieties belonging to European group are self-compatible. Majority of the varieties of Central Asian, North African, Near East and Caucasian origin are self-incompatible [12]. Around one hundred thirty one cultivars and thirteen rootstocks have been reported in USA [13]. The description of major apricot varieties is described here.

3.1 High chilling apricot varieties

3.1.1 Australian

It is an introduction and grown in cold desert area in India. Fruit round, very large, medium sweet, freestone, sweet kernel and suitable for processing and not like for table purpose. Matures in end July to end August. Shelling and kernel percentage are 72 per cent and 28 per cent, respectively.

3.1.2 Bergeron

It is a late apricot, found from the beginning of August in the Northern Hemisphere, original from the valley of Rhone in France. It is suitable both for fresh consumption as for processing. The cultivar has flowering duration of 7 days. The tree at the age of 4 years 15–16 kg fruits on an average. The fruit weight 51.0 g almost the size of Pinkcot but with little bigger stone (3.43 g). Fruits are large with orange-yellow skin, with some red traces. Flesh is juicy and delicious. The fruit is sweet with a TSS/acid ratio of 10.7 [14].

3.1.3 Charmagz

A self-incompatible cultivar which requires pollination with varieties like Turkey. Skin straw yellow, light yellow flesh which is very sweet. Fruit is medium in size; roundish flat in shape; kernel is sweet. Suitable for dessert and drying purposes. It is widely grown in high hills of Himachal Pradesh and also in cold deserts.

3.1.4 CITH Apricot-1

It is self-sterile and mid-season blooming type variety. Fruits are very large (79.0 g), round, symmetrical with smooth distal end, yellowish-orange colored with reddish coloration on one side (25–30%), low acidity, high TSS (14°Brix), early maturing and good quality, consume as table purpose and suitable for processing. Tolerant to major pests and diseases. The plant yields around 15–20 tons/ha. This variety can be grown under entire temperate region of North Western Himalayan agro-ecological zones [15].

3.1.5 CITH Apricot-2

This variety is self-fertile and early to mid-season blooming type. Fruits are very large (80.0 g) oblate, asymmetrical with slightly pointed beak, yellowish orange with reddish on exposed surface, low in acid, high TSS (14°Brix) and high yielding (12–15 tons/ha) early maturing, superior quality having and widely acceptability by growers and consumers. The plants are tolerant to leaf curl and stigmina blight. Used for table and processing purpose. This variety can be grown under entire temperate region of North Western Himalayan agro-ecological zones [15].

3.1.6 CITH Apricot-3

This variety is self-fertile and early to mid-season blooming type. Fruits are very attractive in color, 30 to 40 per cent area of fruit with orange back ground, fruit medium (74.0 g), obtale, symmertrical with slightly pointed beak, yellowish orange with very little reddish tinge, low in acid, high TSS (16°Brix), early-mid season maturing and good quality. Fruit yields upto 10–12 tons/ha. Tolerant to major pests and diseases. This variety can be grown under entire temperate region of North Western Himalayan agro-ecological zones [15].

3.1.7 Dora

The plants are upright in nature and self-compatible. Blooming in the first fortnight of March and harvests in the last week of May. Fruits are 45.58 g in weight, peel color was intense yellow. In ripe fruits 11.0°B TSS was observed [16].

3.1.8 Early Shipley

Fruits are creamy white and medium in size. Flesh sweet and juicy, kernel is bitter in taste. Fruit matures in mid-May.

3.1.9 Ema

It is suitable for mid hills of Himachal Pradesh. The fruit is round, medium in size, yellow orange in color, sweet and mature in end May to first week of June (10–15 days before New Castle.

3.1.10 Halman

An important dessert cultivar of cold arid zone of India especially, Ladakh region of Jammu and Kashmir. The name 'Halman' means grafted in Tibetan language. Spreading and vigorous tree habit, fruits are orange red in color; round, small in size (around 13.0 g) with compressed pedicle end, free stone. Fruit not so juicy and

matures in early August. The fruit is primarily used for drying as fruit has less juice with high TSS (15.8°Brix) and 4190 mg per kg potassium [17]. It has the highest TSS in all the cultivated varieties of apricot and the sweet kernel is also eaten. Shelling and kernel percentage is 72 per cent and 28 per cent, respectively [18].

3.1.11 Hamidi

This variety comes from Tunisia but it is now-a-days cultivated in Greece. It is available in the markets from the end of May, beginning of June. It is pale yellow, it has a very pleasant aroma, although the pulp is not very juicy.

3.1.12 Harcot

It is suitable for mid hills of Himachal Pradesh. Trees are upright to spreading and vigorous. Fruits are medium to large with roundish heart shape. Fruits with yellow orange skin color, pink to red blush, sweet kernel. It is resistant to leaf spot, fruit spot and fire blight. Fruit matures in mid-June.

3.1.13 Imola Royal

It is one of the most important Italian varieties. Because of the firmness of its flesh, it is very appropriate for the industry. It is available in June and July, coming not only from Italy, but also from Spain and Israel. It is asymmetrical, large, elongate, orange-yellow with some pink traces where it has been touched by the sun. The pulp is also yellow orange and its juice is sweet with a remarkable aroma.

3.1.14 Kaisha

The trees are vigorous and spreading, medium sized, Fruit yellow in color with a red blush. Fruits are roundish flattened shape with prominent suture and medium in size. Flesh orange yellow in color, sweet and delicious. Matures in early June, high yielding variety with bitter kernel.

3.1.15 Moongold

The fruit is soft golden colored and of medium size. The fruit is attractive with orange yellow flesh, firm and sweet with excellent flavor and is good for eating fresh or making preserves. Early to mid-season variety.

3.1.16 Moorpark

Fruits are orange in color with a red blush. Fruits are round and large. Flesh juicy, sweet and excellent in flavor. A mid-season variety which matures in first week of June but, shy bearer.

3.1.17 Nari

It is an exotic cultivar and grown in India. Fruits skin pale yellow. Fruits were small, oblong and depressed at ends. Flesh light yellow color and slightly fibrous. Fruits have sweet aroma; free stone and kernel is sweet. It is a late variety and matures in end May to first fortnight of June in high hills and after mid-August in dry temperate areas. Dual purpose variety suitable for table as well as drying purpose.

3.1.18 Nella

The plants are upright in nature and self-compatible. Blooming starts in the end of February and ends in the second half of March month and harvests in the last week of May. Fruits are 42.44 g in weight with 40–50 per cent reddish area. In ripe fruits 11.3°B TSS was observed [16].

3.1.19 New Castle

A popular cultivar commonly grown in the mid hills of Himachal Pradesh. Trees are vigorous and spreading, Fruits are yellow in color, round and medium in size. Used as fresh fruit and for drying; kernel is sweet. It is an early maturing cultivar and fruit matures in May [19].

3.1.20 Nugget

Regular bearing and self-fruitful cultivar with good quality fruit. Fruit skin deep orange with vermillion blush. Fruits are round in shape, medium to large. The flesh is fine textured, deep yellow, firm and juicy. The flesh and kernel is sweet. It ripens in the end of May or first week of June.

3.1.21 Perfection

Traditionally, this variety has been produced both in Washington and California. A mid-to late season variety that is oval, oblong in shape with excellent flavor. The fruit has orange skin and flesh and is somewhat flattened, but usually with equal halves. Skin is pebbly. Fruit is relatively large with 6.4 diameter, medium suture and small pit. Flavor and quality are good for both fresh marketing and processing. The variety is somewhat more acidic than other leading commercial varieties [20].

3.1.22 Pinkcot

The French cultivar yields 11.0 kg fruit per plant at the age of 4 years. The fruit size is little less than Sylred with 5.5 per cent stone share. The fruit is very sweet with total soluble solids of 14.0 per cent acidity 1.12 per cent [14].

3.1.23 Rakchey Karpo

Second most important dessert cultivar after Halman in Ladakh region, Jammu and Kashmir. Indigenous to Ladakh region; 'Rakchey' means stone and 'Karpo' means white hence, the name symbolizes apricots with white stones which is generally uncommon [18]. The fruits are medium to large in size (18.7 g) with high TSS (19.6°B) and 4800 mg per kg potassium [17]. Early maturing matures in end July to early August. Fruits pale yellow with red blush, light pale pulp, juicy, sweet and mild acidic with pleasant flavor, freestone with sweet kernel, shelling is 68 per cent and kernel 32 per cent. Kernels are used for oil extraction. Matures in August month in Ladakh region.

3.1.24 Rogan

An early table purpose variety grown in Leh district which matures in early July. Fruits are glossy skin straw yellow, smallest in cultivated apricot varieties, round in

shape, very soft, juicy and slightly acidic, freestone, with small stone, kernel sweet. Shelling and kernel percentage is 62 per cent and 38 per cent.

3.1.25 Royal

Fruit is yellow in color and firm. Fruits are large in size with juicy flesh. Good for dessert and canning.

3.1.26 Shakarpara

An introduction from Afghanistan and suitable for growing in Himachal Pradesh and Kashmir valley. Fruits are medium in size, round in shape, Fruit skin is creamy yellow with pinkish blush on shoulders. Flesh is pale yellow and very sweet, less acidic with good aroma. It can be used for table purposes. The kernel is bold and sweet. It ripens by the third week of May in high hills and in cold deserts matures in late July to mid-August. It is a shy bearer variety with high chilling requirements.

3.1.27 St. Ambroise

Fruit is orange yellow in color with tiny dots of pink. Fruits are oval in shape and large in size. Flesh is deep yellow, firm and sweet. Matures late in end of June to first week of July.

3.1.28 Suffaida/Safaida

An exotic cultivar and an introduction for cold deserts of India. Fruits are medium to large in size, round in shape, light yellow skin glossy, smooth and flesh very sweet, less acidic with pleasant flavor. Fruit matures in first week of June. Stone is small and kernel sweet. Shelling percentage is 72 per cent whereas kernel 28 per cent.

3.1.29 Sungold

Fruits are of medium size, colored bright colored clear gold with attractive orange blush, nearly round and tender skin. The fruit is good for eating fresh or making preserves.

3.1.30 Sylred

This is an French cultivar with an average yield of 15.6 kg/tree. The cultivar flowers during March–April with a flowering duration of 7–8 days. The fruit weighs 59.0 g with 4.52 per cent stone share. The fruit is sweet with the TSS/acid ratio of 10.0 [14].

3.1.31 Tilton

Regular in bearing, firm and good in quality. An important canning cultivars in the USA and Canada.

3.1.32 Tokpopa

A table and drying type apricot indigenous to Leh district in Ladakh region. Fruit ripe late and matures in the month of August. Fruits are dull yellow in color,

medium sized fruit, round in shape compressed with smooth skin, acidic to sweet in taste, freestone with a shelling percentage (68%) and kernel (32%).

3.1.33 Turkey

The trees are vigorous with spreading habit, fruits are medium in size and almost round shape. Fruits with deep yellow skin, brownish orange in color with dots, free stone and sweet kernel and mid-season maturity.

3.1.34 Wenatchee

Fruits are yellow with green shoulders, oblong shaped, large in size. Regular in bearing, firm and good in quality. A chance seedling and important fresh fruit variety in the USA and Canada.

3.1.35 Wild apricot

It is found in temperate regions of Western Himalayas within Himachal Pradesh and Kashmir and locally known as chulli. It is a prolific bearer in comparison to cultivated apricot and fruits are eaten fresh or sun dried. The kernel mostly tastes sweet is also eaten but, it may be bitter. The kernel contains around 50 per cent oil which is extracted and used for cooking, massage or hair oil. It flowers in second half of March and fruits ripen in May to June in high hills and latter in dry temperate region. It is widely used as a rootstock for apricot and plum in India.

3.2 Low chilling apricot cultivars

3.2.1 Benazir

An introduction from Pakistan at Patiala in Punjab known as Benazir flowers and fruits profusely and ripens in the first fortnight of May.

3.2.2 Chaubattia Alankar

Hybrid between Kaisha x Charmgaz. Regular bearing, low chilling and very early maturity. Good keeping quality.

3.2.3 Chaubattia Madhu

Hybrid between Turkey x Charmagz. Early ripening, highly productive, regular in bearing.

3.2.4 Chaubattia Kesri

Hybrid between St. Ambroise x Charmagz. Regular bearing, mid-season, good quality fruits, mature during end May to first week of June.

4. Cherries

There are over 30 species of cherries, most of which are endemic to Europe and Asia. In the subgenus *Cerasus*, *P. avium*, *P. cerasus*, *P. fruticosa*, and also *P. tomentosa*

and *P. pseudocerasus* in China and domesticates of *P. serotina* in South America are grown for their fruits [21, 22].

Sweet cherries can be divided into subgroups, based on fruit color, shape, and texture [22]. The subgroups include Geans which are heart shaped with tender flesh, black Geans which have dark-colored flesh, amber Geans which have light yellow fruit with translucid flesh and skin, Bigarreaux which has firm and cracking flesh, and Hearts which are dark in color with flesh texture between Geans and Bigarreaux.

Sour cherries have also been further categorized, based on skin and juice color and fruit shape, into either Amarelles (pale red fruits with more or less fattened shape and colorless juice) or Morellos (dark red fruits with globular or cordiform shape and red to dark red in juice color) [23]. Duke cherries (with dark red skin and semiacid juice) are considered to be a hybrid between sweet and sour cherry and now classifed as *P. x gondouinii* Rehd. [24].

Although there are numerous other cherry species in the *Cerasus* and *Padus* subgenera, few of these species have been used to genetically improve sweet and sour cherry scion cultivars. Most of the species and interspecific hybrids developed are used for rootstock improvement. *Prunus avium* is primarily an European species, which ocuurs abundantly in wild form on the forest slopes of Southern, Central and Western Europe. Pomologically, according to fruit firmness, cherry cultivars are divided into the Heart cherry group, with mainly early ripening cultivars that have a soft flesh and the Bigarreau group. Among them few have been described here under.

4.1 Bada

Resistant to fruit doubling. The fruit ripens earlier than Napoleon. Blooms relatively late but overlaps with 'Bing' and 'Napoleon', serves as good pollen source for these varieties.

4.2 Balaton

Originated in Hungary, as a local variety. Compared to 'Montmorency' the fruit is larger, firmer, sweeter and redder and it has a juicy flesh. The pits are slightly larger than other varieties and may cause problems for processors.

4.3 Bing

It is originated in United States from an open-pollinated population of Black Republican which is the most traditional and representative cherry of America. Fruits are firm, sweet, medium to large in size, attractive black color with excellent flavor. Its dark red flesh is firm, not very fibrous, juicy, sweet and very good in quality. Bing produces an excellent canned product but is inferior for brining unless picked before fully ripe. However, its susceptibility to canker, severe fruit splitting, and sensitivity to our cold climate does not make it an ideal variety.

4.4 Blackgold

A late mid-season, self-fertile, sweet cherry selection and primary use is for fresh eating. This is the latest blooming sweet cherry and has remarkable tolerance to spring frost.

4.5 Black Republican

Fruit are medium sized, good quality, dark red turning black when fully ripe. Primarily used as a pollinizer for other sweet cherries.

4.6 Black Tartarian

Fruits are small-medium in size, purplish black in color, heart shaped and good quality. The flesh is dark red, soft and juicy. Early ripening and early bearing. An excellent pollinizer for most varieties but the small, soft fruit of relatively poor quality is not commercially desirable.

4.7 Cavalier

It is a black variety which is early ripening. Fruit is medium to large, dark red and has firm flesh with good flavor. Fruits are of high quality and resistant to fruit cracking.

4.8 Chelan

This is the earliest of the fresh sweet cherries grown in the Pacific Northwest, ripening 10–12 days before 'Bing' or 'Van'. Fruit size is small and similar to Bing or slightly smaller. It has a very mid-flavor but the flavor seems to be acceptable. Bing, Van, Lapins and Sweetheart cultivars are used as pollinizers. Due to its high productivity, most growers prefer Mazzard rootstock and it is incompatible on Mahaleb.

4.9 Chinook

Chinook is a cross between Bing and Gil Peck and was introduced in 1960 by Harold Fogle. Nearly black colored fruits which are about 1 inch in size. The plants are medium in hardiness and productivity. The fruits are susceptible to severe fruit cracking.

4.10 Christiana

It is a Bigarreau type sweet cherry, vigor is medium with wide branched habit. Fruit large in size (9–10 g), skin color dark red, fruit stone small, flesh has good acidulated sweet taste and it is medium firm. This variety is quite resistant to damage of flowers by late spring frosts and fruit cracking. The tree productivity is precocious and very high.

4.11 CITH Cherry-01

Developed through clonal selection from an old variety 'Double Bigarreau Na Yield' is higher in comparison to parent variety. It is suitable for cultivation under Kashmir valley and other Himalayan regions. Consumer acceptability is excellent due to its attractive bright glossy red color, taste and overall appearance. Trees are semi-spreading, suitable for high density planting. Five to six flower/spur and a prolific bearer with spur and bloom density is medium to high. Average yield is 9.35 t/ha at 8 years of age. Fruits medium to large in size, ovoid to heart shaped like 'Double' with long pedicles and firm fleshed. Fruit is red blushed on yellow background having high TSS (15.47° Brix) with good acid/sugar blend. It takes 52–55 days to mature after full bloom and is 10 days earlier to cv. Mishri [25].

4.12 CITH Cherry-02

It is a clonal selection from old variety 'Mishri' (Bigarreau Noir Grossa). It is regular in bearing, precocious and yields higher (3.85 kg/tree) than 'Mishri'. It is early to mid maturing variety having better quality and attractive red skin color. It is suitable to grow under high altitudes of temperate area of North Western Himalaya. Trees are upright, suitable for high density planting, leaves are glossy having drought escape ability. Spurs are bold and prominent having 5–6 flowers/spur. Spur and bloom density is medium and higher than 'Mishri'. Annual average yields of 10.24 t/ha after 7–8 years of age. Fruits are medium to large in size, dark red in color with prominent white dots on fruit surface. Fruits have high TSS (15.43°B) along with good acid/sugar blend. It takes about 51–56 days after full bloom to mature [26].

4.13 Compact Lambert

It is a radiation induced mutant of Lambert. Fruit are the same as Lambert. Trees are of reduced size, the dwarf tree is about 80 to 90 per cent of the size of the standard cherry tree, but there has been a problem with both the stability and the virus status of this cultivar. It bears heavily. Fruit size is good and color is black.

4.14 Corum

Corum is a light colored cherry with a pronounced red blush. It ripens 4 to 5 days before Royal Ann. It is a semi-firm, but productive and hardy cultivar. The flesh is not quite as firm as Ann's. Used as pollen source for cultivar Napoleon. The tree is considerably less susceptible to bacterial canker than Ann. It branches more freely and tend to spread more and to bear at an earlier age.

4.15 Danube

It is a new tart cherry for fresh consumption and ripens a few days earlier than 'Montmorency'. The fruit is dark red, medium to large, and sweeter than most tart cherries. This variety is being planted widely in Europe.

4.16 Early Burlat

Fruits are large and moderately firm. The fruits are harvested approximately two weeks before 'Bing'.

4.17 Emperor Francis

It is a high quality cherry of Napoleon type. It is less susceptible to cracking than Napoleon. It is the major cultivar being used for brining. The fruits are large, yellowish white with a red blush, firm, attractive, good quality, moderately hardy and productive. Used for both brining and fresh consumption.

4.18 Gold

It is easily bleached for brining as it has no red pigment. This is an early maturing variety. Trees are hardy and productive and fairly resistant to cracking and bacterial canker. Much cold tolerant as compared to most of other cultivars. Fruit

are small and is in a unique pollination group as it is able to serve as a pollinizer for many other brining varieties.

4.19 Hardy Giant

Fruits are large in size, dark red in color, good in flavor and resemble 'Bing'. It is a late bearer and a good pollinizer for other sweet cherries, especially 'Lambert'.

4.20 Hedelfinger

It is an old European variety. The trees are early bearing and very productive. The fruit is resistant to cracking than most other varieties. The black fruit is medium to large, firm fleshed, high quality late cherry of Lambert type. Fruits are very soft and if not picked at the proper stage of maturity, fruit quality becomes low. The fruit is good for fresh consumption, freezing and processing. It ripens just ahead of Windsor and Lambert.

4.21 Hudson

This is a hybrid resulted from a cross between 'Oswego' x 'Giant'. Fruits are dark red, medium to large in size, sweet, very firm, very good quality, low field susceptibility to fruit cracking and ripen very late. The plants are medium in hardiness and productivity. Fruit can be harvested over an extended period of time because of its firmness. Good for refrigerated storage of fruit.

4.22 June Bright

This variety was selected from open-pollinated seedlings of Nanyo. The plants are vigorous and cold tolerance. It is early ripening cultivar, mature in late-June. The fruits are medium to large (6–8 g), skin color is bright red blush on a yellow background. The flesh is white coloured, sub-acidic with TSS (14–15%) and acidity (0.52–0.64%).

4.23 Kiona

This cultivar originated from a cross between Glacier and Cashmere cultivars and released in 2007. It blooms mid-late season, generally 4–7 days after cv. Bing and the large red-purple fruit ripens 6–9 days before Bing. The plant produces very flavourfull fruits and taste. The TSS content of Kiona fruit is similar or greater than that of Bing. However the early sugar development and high acidity ensure a good balance of sweetness and acidity that contributes to the unique flavor of this cultivar that is highly sought after by consumers [27].

4.24 Kordia

This is a popular variety of European countries, originated in Germany. It is a self-sterile variety which matures late in the season. The fruits are large in size, dark black in color, firm with good flavor. The fruits are resistant to fruit cracking. 'Regina', 'Stella', 'Sweetheart' and 'Sunbrust' are best pollinizers for 'Kordia'

4.25 Kossara

Originated from the parent combination of 'Rannacherna' x 'Bigarreau Burlat' by the method of embryo culture under *in-vitro* conditions. The tree is medium

in growth; it is very fertile and displays good compatibility with rootstocks viz. Gisela-5, *P. mahaleb* and *P. avium*. Fruit are medium to large in size (7.8 g), cordate in shape, dark red color of fruit skin, red flesh, juicy, having a pleasant sweet sour taste. The cultivars 'Rivan', 'Nalina' and 'Bigarreau Burlat' are good pollinators [28].

4.26 Kristin

It resulted from a cross between 'Emperor Francis' and 'Gil Peck', and was introduced in 1982. This was named due to its outstanding performance, yield and quality in Norway. Trees are vigorous, precocious in bearing and moderately productive. Fruits are large (1.0 inch in size), aromatic, firm, sweet, dark red, attractive good in quality, combining good flavor and high soluble solids. Fruit can be used for fresh consumption and processing. Fruits are moderately resistance to rain cracking.

4.27 Lambert

It is grown primarily as a late maturing black variety for freezing and shipping in Oregon. Fruits forms black flesh has a super flavor when fully mature, medium sized (7/8 inch size) but tends to be quite small with a heavy crop. The fruit is distinctly heart-shaped and pointed. Very susceptible to fruit cracking and plants are productive.

4.28 Lapins

Originated as a result from a cross between 'Van' and 'Stella' and was introduced in 1983. The plants are very productive. A late maturing dark sweet cherry with commercial possibilities. The fruit is resistant to rain induced fruit splitting. Fruits are large in size, high yielding, good flavor and taste.

4.29 Montmorency

It originated in the Montmorency Valley of France before the 17th century. The trees are productive and the fruit are relatively large, bright red, white fleshed, have clear juice, firm flesh, and are of good quality. This is the standard tart cherry variety and about 90% of the tart cherries grown are 'Montmorency'.

4.30 Napoleon

It is also called 'Royal Ann' or 'Napolean Wax'. The fruit is medium to large, oval and yellow in color with a red blush, firm, sweet and juicy fruits which are of good quality. It flowers late and fruit matures by the end of June. It is fairly resistance to cracking and below average hardiness are the major drawbacks.

4.31 Northstar

It is a cross between 'English Morello' and 'Serbian Pie'. The mahogany red fruit has red juice, and is medium size. Trees are small, which makes them easy to cover with bird netting. The trees possess some resistance to leaf spot and brown rot.

4.32 Rainier

Originated from a cross of 'Bing' x 'Van'. The plants are vigorous, extremely hardy and very productive. It bears early and ripens four days before Napoleon.

Fruits are large, firm and are of high quality. The skin is attractive yellow with considerable high red blush. The flesh is light yellow and juice is colorless. It is fairly sweet. The fruit is good for brining or fresh consumption. It is effectively pollinated by Napoleon and Emperor Francis. Susceptible to moderate rain cracking results in large crop losses. Bruise susceptibility may require field packing to minimize loss.

4.33 Regina

This variety was originated in Germany and is self-sterile. The fruit matures late ie. one month after Burlat. The fruits are large in size with good taste and very good resistance to cracking. This variety is compatible with Gisela 5 rootstock. The cultivars 'Kordia', 'Lapins', 'Merchant' and 'Stella' are good pollinizers for 'Regina'.

4.34 Rosita

Selection from an open-pollinated population of 'Bigarreau Burlat' cultivar. The tree is moderate in growth, highly productive and it has a good compatibility with the rootstocks viz. Gisela-5, *P. mahaleb* and *P. avium*. Fruit ripens very early, medium to large size (7.1 g). The fruit is kidney-shaped and the fruit skin is basically pale yellow in color with a light red tint covering upto 50 per cent of the surface. Fruit flesh is soft, gentle, pale yellow in color, very juicy, with a pleasant sour–sweet taste and transparent juice. The cultivars 'Rivan', 'Nalina' and 'Bigarreau Burlat' are good pollinators of 'Rosita' [29].

4.35 Ruby

It is a new promising early sweet cherry cultivar. It is precocious and productive. Fruit are medium in size (7.1–10.2 g) red in color, having a firm juicy flesh, high sugar (14.8%) and low acid (1.2%) content. The acid-sweet flavor is quite popular to consumers. It is resistant to fruit cracking and has a good shipping quality, suitable for both open-field and protected cultivation. Its chilling requirement is low. Under 0-5°C condition it could be stored for more than 40 days without losing flavor.

4.36 Sam

The fruits are tolerant to cracking, lacks in firmness of the fruits. Plants are hardy, but only moderately productive. Late in blooming. Fruits are black in color with medium (3/4–7/8 inch) in size. Often listed as bacterial canker resistant, but it is susceptible to certain strains of this disease.

4.37 Schmidt

Fruits are large, firm, attractive and of good quality. Trees are slow coming into bearing and unreliable in cropping. The effective pollination period is very short so that unless conditions are ideal for pollination and fertilization, light fruit set can be a problem. Susceptible to sever cold.

4.38 Skeena

This variety was derived from 2C-60-07 and 2C-38-32. A dark mahogany cherry that have very large fruits (11.1 g), symmetrical and kidney-shaped, red to ark red flesh color. Fruit flavor is strong and good with a pleasant sweet/acid balance. It

is superior to 'Lapins' and the fruits are produced in looser clusters than 'Lapins'. The fruits are resistant to rain induced cracking [30]. This variety is self-fertile and blooms in mid-season.

4.39 Sonata

Developed in British Columbia and introduced in 1996. It is a self-fertile variety, plants are vigorous with good productivity. The fruit is very large, firm with lustrous mahogany to black skin, a plump kidney shape and a well-balanced, sweet flavor. This variety does not resist cracking. The fruits are harvested before Lapins but after Bing variety.

4.40 Starblush

It is a large-fruited, self-fertile blush cherry that ripens later than Rainier and Lapins. The fruit is heart-shaped with medium to long stems and firm flesh. The trees are vigorous and somewhat upright in growth habit. It had low levels of rain-induced splitting. The crop levels are medium to high. Flavor is medium to good after storage and pitting is only slight [31].

4.41 Starkrimson

Only for the home market in areas where rain cracking is not a problem. It is productive, firm, and of good quality, but it is extremely susceptible to rain induced fruit cracking.

4.42 Stella

Originated from a cross between 'Lambert' and 'John Innes Seeding' and was introduced in 1968. It is the first self-fertile cherry which ripens early i.e. starting June. Fruits are dark red in color, large, one inch in size, heart shaped and flesh is semi-firm. Moderately susceptible to rain induced fruit cracking. Trees are very vigorous and productive but tender to winter cold.

4.43 Summit

Originally from Canada, matures sixteen days after Burlat. Outstanding for its large fruit size, firm, excellent flavor and quality. The fruits are susceptible to cold and rain cracking of the fruit.

4.44 Sunburst

A mid-season dark sweet cherry, reported to be outstanding for large fruit size, high yield, and self-fertility. The plants are productive. It is more resistant to rain splitting than many commercial cultivars. Lacks in fruit firmness, so not recommended for transportation to distant markets where long term storage is required.

4.45 Sweetheart

It is a self-fertile cherry resulting from a 'Van' x 'Newstar' cross in 1975. It is very late maturing sweet cherry cultivar. Trees are productive and fruit is medium to large in size (9.9 g), very firm and has good flavor. The fruit is dark red and moderately resistant to cracking.

4.46 Symphony

This is a self-fertile variety originated in Canada having high productivity. The fruit is bright red in color, medium to large in size with good taste with and matures late in the season. This variety is moderately resistant to rain-cracking.

4.47 Tehranivee

It is a new mahogany colored self-fertile sweet cherry with black red juice. It has excellent flavor as well as size, sweetness and firmness. It is a mid-season cherry developed in Ontario, Canada which ripens in the end of July. The fruits are susceptible to fruit cracking.

4.48 Tieton

It produces a very large cherry that has a beautiful cluster. The flavor is very mild. Susceptibility to rain cracking of fruits is very high. This variety ripens early but just after Chelan, bloom time is just before Bing. Bing, Van and Rainier can serve as pollinizers.

4.49 Ulster

It is a cross between 'Schmidt' and 'Lambert' and was introduced in 1964. Fruits are large, sweet, firm, crisp and nearly black in color, (dark red) 3/4 to 7/8 inch in size. Trees are medium hardy and productive. Resembles 'Schmidt' but more productive. The fruits are moderately resistant to rain cracking. Good for fresh consumption and processing.

4.50 Utah Giant

This variety ripens with 'Bing' and is susceptible to severe fruit cracking. Fruits are large and of good quality.

4.51 Valera

Ripens a few days before 'Bing'. Fruits are medium sized, semi-firm, good quality fruit. Trees are vigorous and early bearing. Fruits are less clustered, and not as susceptible to brown rot as 'Venus'.

4.52 Vandalay

It is originated from a cross between 'Van' x 'Stella'. Large, wine-red colored fruit have a kidney shape and purple juice. It is a self-fertile cherry which is resistant to cracking and canker.

4.53 Van

It is an open-pollinated seedling of Empress Eugenie, introduced at the Summerland in 1944. Trees are very vigorous and hardy. It comes into bearing early and is very productive. Fruits are large, black in color, with bright luster, very firm with short stem, 3/4–7/8 inch in size. It is very susceptible to bruising. Fruit develop in clusters so brown rot control becomes a problem. It is less susceptible to cracking and quality is good.

4.54 Vega

Fruits are very large and attractive. It has a small, easily removable pit. It is larger, firmer, and earlier than most white cultivars, but it remains tart until it is very ripe.

4.55 Venus

Fruits are dark red in color, 3/4–7/8 inch in size and semi-firm in texture. Medium in hardiness and very productive. It has a tendency to overbear in some years, especially under conditions which favor good cross pollination.

4.56 Viscount

The fruits are medium to large in size, kidney shaped, firm, good quality, dark red glossy in color. It ripens with 'Bing'. It is highly resistant to rain-induced fruit cracking. It is also resistant to diseases caused by *Monilinia fructicola* and *Pseudomonas syringae.*

4.57 Vista

Fruits are nearly black in color, 7/8 inch in size, semi– firm in texture. The plants are medium in hardiness and productivity. Cracking is often a serious problem, especially in young plantings with light crops.

4.58 Viva

Fruits are dark red in color, 3/4 inch in size, semi-firm in texture. Good fruit crack resistance but this may be due to its soft texture.

4.59 Vogue

A large, shiny, dark red sweet cherry with a small pit. Ripens with Bing and is productive. In heavy crop years it sets in bunches so that careful spraying is required for brown rot control.

5. Peaches and nectarines

Peaches and nectarines are fruit species which are typically self-fertile and naturally self-pollinating. Although polyploidy is common in the *Prunus* genus, fve species may be referred to as 'Peach'. *P. persica*, *P. davidiana* (Carr.) Franch, *P. mira* Koehne, *P. kansuensis* Rehd., and *P. ferganensis* Kov. & Kost., all of which are diploid (2n = 2x = 16) [32, 33].

According to geographical distribution, the peach cultivars have been divided into three groups namely, Northern, Southern and European or Persian group. Peach cultivars can also be divided into two groups high chilling and low chilling on the basis of their chilling requirements. Low chilling cultivars developed in Florida during last three to four decades have become very popular in the sub-mountainous Himalayan region. Description of different high and low chill varieties of both peach and nectarine grown in various parts of the world is here under.

5.1 High chilling peach varieties

5.1.1 Alexander

It is an excellent early season cultivar, ripening in the last week of May to first week of June and is a mediocre bearer. Fruit is medium to large in size, round with unequal sides, skin is smooth, beetroot purple, with some patches of pod green color, flesh is soft, greenish white juicy, very sweet, aromatic and free stone, keeping quality is not so good.

5.1.2 Allstar

The fruit matures two days prior to Harrow Beauty and is a medium sized, bright red fruit with clear yellow flesh. The fruit is medium firm with fair quality. The fruit and the tree have shown signs of bacterial spot.

5.1.3 Babcock

The plants are vigorous and productive. The fruit is small to medium round to ovoid spherical, having prominent ventral suture [13]. The skin is light pink with a deep red blush. Flesh is white, very sweet and is free stone. It ripens two weeks before Elberta.

5.1.4 Babygold

It is an introduction by Rutgers in 1961 which ripens early and is suitable for processing purpose. Its parentage involves several cultivars including J H Hale and Gold Finch. It is a canning non-melting clingstone with yellow flesh and red at the pit. It is much less rubbery when canned than the other non-melting clingstones, the fruit is of large size, tree is productive and winter hardy, although prone to crotch injury. The fruit is susceptible to brown rot disease and tends to drop at maturity.

5.1.5 Blazingstar

The fruit matures three days after Redhaven. Fruit are highly coloured, round and attractive, firm, yellow fleshed peach. Size and fruit quality are acceptable. The fruit and the tree are susceptible to bacterial spot.

5.1.6 Blushingstar

Fruits are harvested 2–4 days after Loring and are round, medium to large fruit attractively blushed with pinkish red over color, firm flesh white and good quality. Plants are productive but moderately susceptible to bacterial spot disease.

5.1.7 Bounty

The fruit ripens two days before Loring. It has more color compared to Loring, and is round in shape with better flavor. Bounty has had light crops under extreme cold winter conditions in Ontario.

5.1.8 Candor

It is a hybrid originated from a cross (Red Haven x Early Red Free) in Carolina, USA [13]. Tree is small, a prolific bearer, regular, self-fruitful and has

a medium chilling requirement. Fruit is medium in size, round in shape with pointed blossom and with excellent flavor. The skin is bright red on rich yellow ground and is deep red near the blossom end. Flesh is yellow and texture is fine, firm and freestone. Fruit ripens before the onset of rains and is a promising cultivar for hills.

5.1.9 Coralstar

The fruit matures four days prior to harrow Beauty and is medium to large in size, bright red and has clear yellow flesh. The fruit is medium firm with only fair to poor quality. The fruit and the plants are susceptible to bacterial spot.

5.1.10 Cresthaven

The fruit ripens ten days after Harrow Beauty with firm, large fruit. It is moderately susceptible to bacterial spot. The fruit lacks sufficient color to compete against other cultivars.

5.1.11 Dixigem

It resulted from a cross between Admiral Dewey and St John in 1944. Tree is vigorous, productive and needs about 950 hours chilling. The fruit is of medium size, round, with a bright yellow skin having light blush [13]. Flesh is light yellow with good texture and is free of stones. It matures about a month before Elberta.

5.1.12 Early Amber

It is a low chilling cultivar (350 hours), bred in Florida. Tree is vigorous, fruit is medium in size. Skin is yellow with dark-red blush [34]. Flesh is yellow of good quality and is clingstone.

5.1.13 Early Redhaven

This bud sport of Redhaven, ripens few days after Garnet Beauty and 10–12 days before Red haven, but with similar characteristics. The fruit may be more highly colored and smaller, and the firmer flesh tends to be clingy. Early Redhaven has been widely planted during recent years.

5.1.14 Early White Giant

The fruits are medium to large, free stone, flesh sweet and most appealing in flavor, good bearing quality, ripens in the second week of June.

5.1.15 Elberta

It is an open-pollinated seedling of 'Chinese Cling', introduced in 1889. The tree has non-showy blossoms. This cultivar is best known as yellow canning peach. Fruits are large, oblong, very pubescent, fairly attractive, skin smooth, pale yellow with red a splash. The fruits are firm and juicy, sweet in taste, flesh yellow and clingstone. The fruits are best suited for canning. It is a mid to late season variety and the fruits are harvested in the last week of August. The tree is moderately resistant to bacterial spot.

5.1.16 Florda Red

It is an excellent mid-season table peach maturing in the beginning of June. Tree is vigorous in growth. Fruit are large, almost red at maturity, juicy with soft white flesh and free stone.

5.1.17 Flordaqueen

It was evolved by R H Sharpe in Florida and needs 550 chilling hours. Tree is productive and the fruits are round yellow with a light red blush. Flesh is firm and yellow. It is clingstone with a fair quality.

5.1.18 Flordasun

It is a low chilling cultivar developed in Florida. Plants are spreading and vigorous. It is an early maturing cultivar which ripens towards last week of April to first week of May. Fruit is medium sized, freestone, round in shape with red blush on the surface, pulp yellow, acidic-sweet taste with 11.5 per cent total soluble solids.

5.1.19 Garnet Beauty

This bud sport of Redhaven ranks second in number of trees to its parent in Ontario. Garnet Beauty is a good-quality peach, ripening about a week after Harrow Diamond. It is attractive, usually not subject to split-pits, but not fully freestone [35].

5.1.20 Glohaven

It is a cross between an open pollinated seedling of J H Hale and Kalhale. It was introduced by South Heaven in 1964. This is a yellow free stone variety of good size and quality. Its outstanding characteristics are its dark red, tough skin with very slight pubescence. The flesh is firm. Resistant to flesh browning, and has almost no red around the pit. It is satisfactory both for fresh fruit and canning. Large nearly round, attractive yellow freestone fruit. Very tough, mostly red skin is practically fuzzless with a deep yellow ground color. Firm yellow flesh is resistant to browning, superior for canning and freezing qualities. Plants are vigorous and productive, excellent quality for fresh market and commercial processing. Keeps and ships very well.

5.1.21 Glowingstar

The fruit ripens 11 days after Harrow Beauty and has medium to large sized fruit with good crops. The fruit has a bright red color with good blush and quality. It has good tolerance to bacterial spot.

5.1.22 Harbrite

The fruit ripens four days after Redhaven. It is bud hardy and a good peach for its season. The fruit is medium to large, round, with an attractive red color and resistant to bacterial spot and brown rot.

5.1.23 Harcrest

The fruit ripens just before Redskin and promises to be a late season cultivar with good disease resistance. The fruit of Harcrest are medium large, quite firm, good quality and have good winter hardiness and disease resistance but no better blush than other cultivars in this late season.

5.1.24 Harrow Beauty

The fruit ripens with Loring and Canadian Harmony but is more winter hardy. The very firm, highly attractive, medium sized fruit ships well. The rich yellow flesh has a red pigment around the pit cavity. Leaves and fruit have good resistance to bacterial spot and brown rot.

5.1.25 Harrow Dawn

This variety ripens eleven days before Redhaven. Plants are hardier than Redhaven, vigorous, productive and medium-to-high field resistance to bacterial spot, brown rot and canker. Fruit is very attractive, bright red blush on a yellow background, uniform ripening, medium size, firm yellow flesh, usually freestone when ripe, medium-to-good quality, very few split-pits.

5.1.26 Harrow Diamond

The fruit ripens about a week before Garnet Beauty. It is winter hardy, disease resistant and has few split-pits. The fruit have an attractive red blush over a bright yellow background; the deep yellow, low oxidizing flesh is of good quality and is nearly freestone when fully matured [35]. Because the fruit is small tomedium sized, this cultivar must be thinned early and adequately to obtain suitable size.

5.1.27 J H Hale

Around 1900, a peach grower J.H. Hale found an off type plant in his farm which performed well at Georgia due to its superior firmness. After that this variety is widely planted in USA and was often used in breeding programmes. It is thought to be a chance seedling of Elberta. The cultivar is self-unfruitful. Plants grow vigorously with an average plant height of 15–20 feet. The plants have a low flowering intensity (up to 1.50 flowers per inch). Fruits are fuzzy, yellow skinned with slight red blush. Fruit flesh flavored, texture fine, yellow and freestone. The fruit weight is 81.1 g with a TSS (11.1°B), acidity (0.81%) and TSS/acid ratio (14.7).

5.1.28 July Elberta

The plants vigorously growing with a rounded tree top. The peach is the most adaptable of all fruit trees for home gardens. At 3 or 4 years of age they begin to bear large crops and reach peak productivity at 8–12 years. Peaches need clear, hot weather during their growing season and require well-drained soil as well as a regular fertilizing program. They also require heavier pruning than any other fruit trees to maintain size and encourage new growth. The fruits are large, sweet, freestone with a golden yellow flesh. The fruits skin is highly pubescent. The average fruit weight was (73.9 g) with TSS (12.0°B) and acidity (1.03%). The fruit matures a fortnight before Elberta.

5.1.29 Kanto 5

This is a late maturing cultivar, the trees have vigorous and upright growth habit. Fruit skin color is yellow with red blushes. The flesh is yellow, juicy and clingstone. The average yield of five year old plants yields 120.5 kg of fruit. Fruits weigh of 127.8 g with TSS (13.5°B) and acidity (0.73%).

5.1.30 Loring

This late season peach is large, firm, yellow-fleshed, freestone and known for its good quality. Loring lacks winter hardiness and should not be planted on marginal sites. Once an industry standard, it now lacks sufficient red skin color to compete with newer cultivars.

5.1.31 Khurmani

The tree is medium and upright in growth. It blooms in early February and fruit ripens slightly earlier than Suffeda. The fruit is large, weighing about 70 g and is attractive with red colouration. It is slightly pointed at the base. It is a clingstone cultivar with white, soft and juicy flesh.

5.1.32 Maygold

It is a cross of Sunhigh and Southland, selected in Georgia, USA. Tree is vigorous, productive, precocious and self-fruitful. Its chilling requirement is 650 hours. Fruit is medium to large, ovate, skin is yellow with more than half red. Flesh is yellow, firm, melting is of good flavor and is clingstone [34]. It ripens in second week of June and is an early promising cultivar.

5.1.33 Pratap

It matures a week earlier than the Flordasun cultivars take 76 days for maturity. The color of its fruit is yellow with red blush and flesh color is also yellow with red coloration. It yields 70 kg fruit per plant and its average fruit weight is slightly higher than the Flordasun. Due to its significantly better firmness and slightly acidic characteristic it has better keeping quality than Flordasun. Also its tree remains smaller in size as compared with Flordasun.

5.1.34 Redhaven

It is a cross of Halehaven and Kalhaven introduced by Stanley Johnston at Michigan in 1940. It is a widely planted cultivar. It has firm, excellent flesh which enables easy picking and handlings. Fruit is of red color and good size [35]. It is a regular cropper and the tree set heavy crops and must be adequately thinned to attain size. It is a mid-season variety and ripens about 30 days before Elberta. It has a chilling requirement of 950 hours.

5.1.35 Redstar

The fruit ripens with Redhaven and is medium sized with good crops. The fruit has a scarlet orange color with good blush, fair quality and few split-pits and tolerance to bacterial spot.

5.1.36 Redskin

The fruits are of medium sized, good quality, late ripening freestone with fairly good color. Trees tend to be somewhat willowy but are very productive.

5.1.37 Red Globe

It is originated in Maryland from (Admiral Dewey x St. John) x Fireglow cross. Plants are vigorous, productive and its chilling requirement is similar to that of Elberta. Fruit is medium in size, round with a bright red blush and is suitable for freezing, canning and for long shipments. Flesh is yellow fine textured, firm, melting with a small stone.

5.1.38 Rio Oso Gem

It had originated in California. Its fruit is large, round to elongate, with an attractive skin. Flesh is yellow, fine textured, firm with good quality and freestone. Its chilling requirement is 850 hours. It resembles J H Hale and ripens a week before Elberta.

5.1.39 Risingstar

The fruit ripens one day before Garnet Beauty and medium in size with fair to good crops. It has an orange red color with good blush, fair to good quality and few split pits and tolerant to bacterial spot.

5.1.40 Shimizu Hakuto

The fruit matures in the second week of July. Plants are dwarf and spreading. Fruits are medium in size, quite firm roundish, red blushed on creamish background. TSS recorded was 18.0 per cent with excellent flavor, flesh white, cling stone.

5.1.41 Springcrest

Fruit ripens two days prior to Harrow Diamond and is considered an early peach for the local fruit stands and fruit markets [35]. Fruit size is small and has several early split-pit fruit. Skin can be very deep purple as it matures. This cultivar is also winter sensitive.

5.1.42 Starfire

It ripens few days after Redhaven and has medium fruit with good crops. It has scarlet orange red blush with good fruit quality and few split pits. It has good tolerance to bacterial spot.

5.1.43 Suncrest

This variety was originated as a result of a cross between Alamar and Gold Dust. It is late maturing in the first week of July. Fruits are large, round, freestone, firm and hold well during shipping. The fruit is uniform and highly color with excellent quality for both fresh and canned products. Fruits have attractive color to about 80

per cent bright red blush over yellow background. Flesh is yellow and exceptionally firm with good texture and flavor. Vigorous, self-fruitful, productive trees have a good hardiness record where tested in Eastern sites. Good shipper that is proving to be a good commercial market peach.

5.1.44 Sun Haven

Originated as a result of crosses involving Red Haven, J.H. Hale and Halehaven. It is a fair sized yellow melting flesh, clingstone, with firm texture and good flavor, and is very resistant to flesh browning. It matures during mid-June almost ten days before Red Haven. It has fairly good skin color. Fruits are bright red with yellow gold cheeks.

5.1.45 Veteran

It is a cross between Vaughan and Stark's Early Elberta originated in Canada. Tree is very productive. Fruit is medium to large, round, oblate and is harvested ten days before Elberta. Skin lacks red color and flesh is soft. It is suitable for both fresh and canning purpose. The chilling requirement is also high being 1150 hours [20].

5.2 High chilling nectraine varieties

5.2.1 Fantasia

Originated as a hybrid from a cross of Gold King x an open-pollinated seedling of Red King. The fruit ripens late in the season with Cresthaven peach. The fruits are medium to large, attractive, bright red with a yellow ground color, freestone and firm fleshed. The plants are moderately hardy and moderately resistant to bacterial spot [35]. Fantasia is the main commercial nectarine in the Niagara Peninsula.

5.2.2 Flavortop

The fruit ripens just after Loring and are large, ovate and freestone with excellent quality. Skin is highly blushed over an attractive undercolour. Flesh is yellow, firm and smooth textured. Trees are vigorous but produce light crops and are tender to winter cold. Fruits are also susceptible to bacterial spot [35].

5.2.3 Harblaze

This cultivar has promise as a commercial type nectarine that ripens during the late Redhaven season. The vigorous, productive trees bear attractive medium to large sized fruits that are semi freestone. The fruit tends to soften quickly near maturity during final swell. Harblaze is relatively winter hardy and has a good level of resistance to bacterial spot, brown rot and powdery mildew.

5.2.4 Harflame

The fruit ripens few days before Harblaze. The plants are hardy as Redhaven, medium vigor, somewhat upright and moderately productive. It has good field resistance to bacterial spot, brown rot and canker. The fruit is attractive, medium in size with 80 per cent blush on yellow background. It is semi freestone, ripens uniformly with a medium firm yellow flesh, medium quality and a low incidence of split pits.

5.2.5 Independence

It is freestone variety, early ripening. Fruits are golden having a red cherry blush. Fruit firm, sweet with acidic blend, good textured and flavor. Ripening in the first week of July. Fruit covered with red color, ground color yellow. Flesh of the fruit is yellow and firm.

5.2.6 May Fire

Plants are vigorous and require more than 150 chilling hours. Fruits are medium, smooth, skin color green to white with deep red over color. Flesh white, attractive, juicy, clingstone and sweet with good quality. Fruit matures very early to mid-May.

5.2.7 Red Gold

This nectarine variety was developed by crossing 'Halberta Giant' x 'Sunrise'. A late maturing variety with harvesting season is mid-August. The plants are productive and have non-showy blossoms. The plants are vigorous, heavy and regular bearer. Average diameter of this nectarine is about 3.0 inches. The fruit is freestone having 50–70 per cent of the surface covered rich red over yellow background. The flesh is yellow with red around the pit and has the ability to hold firmness making it an excellent storage and shipping nectarine. The flavor is very good to excellent, taste is sweet. This nectarine is the standard in its season. Fruits are also susceptible to bacterial spot and mildew.

5.2.8 Silver King

This is an early maturing variety ripening in the third week of May. Fruits are medium in size with ground color is greenish white, 3/4 fruit covered with dark red blush. Fruits are very attractive, taste sweet and flesh is greenish white. The plants are vigorous and are heavy yielders. Fruit set is very high, require heavy thinning and heavy pruning.

5.2.9 Snow Queen

The plants are heavy bearer having fruits of medium size, skin color white with shinning red over color, smooth without fuzz, Flesh white, good flavor and clingstone.

5.2.10 Spring Bright

This variety was developed as a hybridized seedling from May Diamond and an unnamed seedling. This is a mid-season variety, ripening in third week of June. Fruits are large in size with greenish yellow ground color. Fruit is very attractive, almost whole fruit is covered with maroon red. Skin little rough, flesh is firm, crunchy, crispy and yellow. Red tinge or stripes may appear in the flesh if harvested late. This is a freestone variety having sweet taste with acidic blend. Trees are compact type, semi-dwarf, regular, heavy bearer and require little thinning.

5.2.11 Summer Bright

The is fruit large, clingstone in type, very firm, deep red over a reddish orange background in skin color, and both acidic and very sweet in flavor. The variety is a cross between Red Diamond and an unnamed nectarine.

5.3 Low chilling peach and nectarine varieties

5.3.1 Early Grande

It is a selection from the cross Fla558 [(Southland x Jewel) open pollinated] x Early Amber which performed very well in Punjab. Plants are semi-vigorous, high yielding and fruit maturity occurs in the first week of May (4 days earlier than Shan-i-Punjab). Fruits are large (90.0 g) with red blush on the surface with TSS (10.5%) and acidity (0.7%). Pulp yellow, firm (firmness of 10.9 lbs./inch^2) and some red colouration next to the pit and semifree from stone when fully ripe. Fruits possess excellent shipping quality.

5.3.2 Florda Prince

Plants are vigorous and the fruits mature in the fourth week of April. Fruit size medium (65–70 g); yellow with red blush at maturity, flesh firm and free stone at full ripe stage. Average yield 100 kg per tree with a (TSS 12.0%) and acidity (0.5%).

5.3.3 Pant Peach 1

This variety was released by GBPUA&T, Pantnagar in 1998. This is a chance seedling selection from the population of cv. Sharbati under Pantnagar conditions. It ripens about one week prior to Sharbati but fruit quality is similar to Sharbati.

5.3.4 Saharanpur Prabhat or Prabhat

It is a variety developed at HTTC, Saharanpur by crossing Sharbati and Flordasun. The trees are upright having strong and thin primary limbs. Fruits are medium in size with attractive red blush, round, truncate in shape with pointed apex with good taste having good keeping quality. Flesh is sweet, freestone, white having excellent flavor. This variety ripens in the third week of April, about 4 to 6 days earlier than the Floridasun which is one of its parents. The fruits are very similar to Floridasun in shape. It gives good fruit yield on peach and plum seedling rootstock.

5.3.5 Shan-i–Punjab

This is early cultivar, maturing in first week of May. Plants are vigorous in growth. It produces large fruits of 5–5.5 cm diameter weighing about 90 g each. The color of the fruit is yellow with red blush, juicy and sweet with excellent taste, flesh yellow and completely freestone. The fruit is quite firm in texture and can withstand transportation. In addition to its table use, this cultivar has also been found suitable for canning. The average yield is about 70 kg per tree.

5.3.6 Sharbati

It is chance seedling selected at Saharanpur. It has medium sized fruit, which is clingstone and is round-oblong shape, flesh is white, soft, juicy and aromatic. It is a heavy yielder and ripens late in second week of June. It is very popular in plains of Western Uttar Pradesh. Total soluble solids and acidity observed was 13.0 per cent and 0.33 per cent, respectively. Chilling requirement ranges between 30 and 40 hours.

5.3.7 Tropic Beauty

This variety was released in 1988. Fruit have a high percentage red over color on bright yellow background with very short fuzz, making the fruit highly attractive. The round, firm fruit have melting, deep yellow flesh that frees from the pit at soft ripe. Fruit is medium in size, red skinned semi freestone with soft, yellow flesh, excellent flavor. The fruit ripens in mid-May. It is low chill variety which requires around 150 chill hours.

5.3.8 Tropic Snow

Fruit have a moderate to light yellow with red blush, flesh sweet and white. Fruit taste tart, but sweet very early and firm at harvest. It is a medium sized, delicious white freestone peach that has creamy white, firm, aromatic flesh with balanced acid and sugar and superb flavor. It requires 175–200 hours chilling requirement.

5.3.9 Tropic Sweet

Fruit skin color yellow, flesh color yellow, good quality freestone. Fruit are very large when thinned properly. The fruit ripens just after Tropic Beauty and the chilling requirement is 100–200 hours.

5.3.10 Sunred

It is low chilling nectarine bred by R H Sharpe in Florida. Tree is large heavy bearer and requires 300 chilling hours. Fruit is small, round with bright red skin and has excellent dessert quality. Flesh is yellow, firm and semi free stone. It ripens in first or second week of May.

5.3.11 Punjab Nectarine

The plants are vigorous and spreading. Fruit matures in 2nd week of May. Fruit large, weighing 90 g, round, attractive with 90–100 per cent red blush over yellow ground color at maturity, flesh yellow, firm, melting and freestone at full ripe stage. The fruits are sweet with acidic blend having TSS (11.5%) and acidity (0.8%). Average yield is about 40 kg/plant. It is a low chill nectarine which is suitable for plains of Punjab and lower areas of Himachal Pradesh.

5.3.12 Sunrise

It is a low chilling nectarine. Fruits are globose and medium thick skin having mahogany red color [20]. Flesh is yellow, firm, crisp and mildly sub acidic. It is semi freestone and ships well. The cultivar is medium in vigor, upright and bears a good annual crop.

6. Plums

Most plum cultivars belong to only two species: the hexaploid European plum (*P. domestica*) (2n = 6x = 48) and the diploid Japanese plum (*P. salicina*) (2n = 2x = 16). *P. domestica*, which categorized in European plums, is grown not only in Europe but also in other continents as the most common plum. Based on the fruit characteristics, this species can be divided into several groups including plums, prunes,

gage plums, and mirabelles, as well as the wild plums like cherry plums, bullaces, damsons, and sloes [36].

P. salicina, Japanese plums tend to be oval or heart-shaped and come in yellow, black, or red varieties. These types of plums have firm flesh and are often eaten fresh. Types of European plums are usually very sweet with juicier flesh and are used in baking or for making jams and jellies. There are many varieties of plums ranging in taste from sweet to tart. Some types of plums have a red sour flavored-skin that surrounds sweet juicy yellow flesh. Other varieties of plums are extremely sweet with dark purple skin and amber-colored flesh. Some of the varietal descriptions of plums include:

6.1 High chilling plum varieties

6.1.1 Au-Rosa

A dark red plum, medium to large in size with red flesh. Trees are very vigorous, spreading in nature and moderately productive.

6.1.2 Beauty

It is a Japanese plum which is vigorous, heavy and regular bearing variety. It does not require pollinizers, but cross pollination may increase fruit set and also good pollinizers for other plums. The fruit is heart shaped, clingstone, greenish yellow to bright crimson and the flesh color is amber streaked with scarlet. The fruit ripens in June–July months.

6.1.3 Black Amber

A mid-season plum with black red skin and an amber flesh. The fruit is alrge and very firm. This variety is susceptible to bacterial diseases caused by extreme humid climates and is not recommended under these conditions.

6.1.4 Black Beauty

It is a Japanese plum that has bright yellow flesh and dark, deep purple-red skin. This drupe fruit is extremely juicy when biting into its firm flesh. This variety is dark red in color with oval shape, medium to large in and preferred for eating fresh. It has an excellent balance of sweetness with only hints of tartness.

6.1.5 Black Ruby

It is one of the most popular types of black Japanese plums. It was originated from a cross of Queen Ann x Santa Rosa which was released in mid-90s by the USDA. The fruit is firm, juicy with reddish-black skin that surrounds yellow flesh. This round plum is one of the few sweet plum varieties that which ripens in late August. This variety is recommended for fresh eating. This variety is tolerant to humid climates, making it a great choice for southern districts.

6.1.6 Bluebyrd

Released by USDA in 1998, Bluebyrd is an excellent European type plums for the commercial orchard and home garden use. The fruit is blue with amber flesh,

medium to large in size with excellent flavor and high sugar content. The tree is vigorous and productive, showing great resistance to black knot. This variety blooms before Stanley and requires cross pollination.

6.1.7 Bradley's King (Prunus insititia)

An extremely hardy variety which blooms late and is a heavy bearer. It makes a vigorous tree and unlike other damsons, the wood is not brittle. Fruit is large for a damson. Purple skin with a blue bloom. Greenish yellow, dry flesh. Quite sweet with little of the bitter flavor characterizing damsons.

6.1.8 Bradshaw

A Lombard plum, the fruit is medium large, oblong, purplish red, attractive and medium soft. The quality of the fruit is fair to good. The stone is semi-cling to clingstone. It is a late bloomer cultivar and are better adapted to areas with late frosts or cool, rainy spring weather.

6.1.9 Bruce

One of the toughest plum trees, a Chickasaw Japanese plum hybrid. Flavor is tops; A frost hardy tree, ideal for low areas. Semi dwarf, weeping habit. Sunset orange fruit with a sweet mellow flavor. Needs a pollinator, ripens late May–June. It requires 500 chill hours.

6.1.10 Burbank

The Burbank is a well-known old variety. The fruit is medium-sized and has attractive orange-red color that covers most of the surface with a base color that is amber-yellow. The flesh is yellow, fine-grained, firm and juicy, sweet and very good tasting. The peak harvest is in the second part of August-beginning of September.

6.1.11 Czar

The fruits are on the large size with dark purple plums. The flesh is yellow. Traditionally use for cooking. Czar is also a good eater if the fruit are allowed to fully ripen. It has distinct advantage over some other plum trees of being self-fertile. It resists frost damage well. Fruit is produced in August.

6.1.12 Damson Plum

It is hardy cultivar and bears small, roundish, dark purple-black, with firm green or golden yellow flesh, semi clingstone fruits. The flavor is poor in fresh fruit, but excellent in jam. It is self-fruitful. It is recommended for cultivation in Canada.

6.1.13 Early Golden

A high quality early maturing plum with attractive red blush over golden yellow ground color, maturing 10–14 days before Shiro. Fruit is very sweet, small to medium in size. Tree are hardy, vigorous and productive. It needs cross pollination with another Japanese plum to ensure heavy cropping.

6.1.14 Early Laxton

It is a very early plum tree, producing ripe fruit from late July onwards. The plums have attractive yellow and light red skin. The flesh has a gage type flavor, yellow, full bodied and very juicy. It can be used for both eating and cooking. it was introduced in 1902 from Bedford, England. This variety ripens and ready for eating mid-August. It does require another plum tree for pollination and falls in pollination group. It is partially self-fertile, but will crop better with other plum trees nearby.

6.1.15 Early Transparent Gage

It is a regular and heavy cropper, prolific bearer with delicious fruits which are rather small and roundish. The color of the fruit is pale yellow with red dots and the fruit ripen in third week of July. It is a self-fertile variety.

6.1.16 Fortune

A Japanese type plum, trees are upright and vigorous in nature. The fruits are medium to large, reddish-purple and very firm, juicy. This attractive, good-tasting plum ripens in mid to second part of September. Cross pollination with other Japanese varieties is recommended.

6.1.17 Giant Prune

It is as Giant Plum which is a heavy cropping plum. Produces very large, dark red oval-shaped fruits that have medium blue bloom and some golden brown dots and patches on the fruits. The flesh is yellow, juicy, slightly sweet and peels away nicely from the the stone, freestone. It blooms late and is winter hardy.

6.1.18 Golden drop

It makes a spreading tree and is a shy bearer, blooms early and requires frost protection. It bears large fruits of quite exceptional flavor, pale yellow speckled with crimson. The fruit has rich apricot favor and characteristic sweetness.

6.1.19 Golden transparent

It is a Reine Claude like plum, which is vigorous, spreading with ovate to elliptic, toothed, mid to dark green leaves and white flower. It is self-fertile and blooms in the mid-season. The fruit is very delicious, small, oval to oblong, often red blushed, dull yellow to yellow green fruit and is late in maturity.

6.1.20 Green gage

It is a European cultivar and an old cultivar of the Reine Claude Group. It is one of the few green varieties of plums when they are ripe. The fruit is medium in size, roundish with greenish yellow skin. The flesh is firm, freestone and the flavor is sweet and very good. It tends to overset and bears biennially. The trees blossom in spring and the bumper crops are ready by the late summer.

6.1.21 Italian

A prune, it is also known as French Fellmbery Agen petite. Probably originated at Milan, Italy about 1800. The fruit is medium in size, oval and dark blue with a

heavy bloom. The flesh is firm, high in sugar content and good in flavor. Several early mutants such as, De Moris, Greata and Richards have been selected and grown commercially.

6.1.22 Kelsey

A Japanese plum, it produces a tree of moderate vigor. The fruit is heart shaped with greenish yellow skin and flesh. The flesh is juicy, firm and of good quality.

6.1.23 Mariposa

A Japanese plum, which is vigorous, upright growth habit, heavy fruiting in nature that requires cross pollination for adequate fruit set. The fruit is large heart shaped with greenish yellow fruit skin mottled with red. The flesh is red in color of excellent quality. It is self-fertile plum produces more fruit if a suitable cross pollinator is nearby planted. Excellent for eating fresh, preserves, jams and juices.

6.1.24 Methley

One of the first out of the orchard in mid-July, this is well known variety that has been present on the market stands for a long time. This variety is with fine quality and appearance. This fruit is harvested with a green shadow, but ripens to a vibrant purple with a deep red flesh, very juicy with a distinctive flavor. Methley is self-fruitful and a good pollinizer for Shiro.

6.1.25 President

It is a large blue/purple plum raised at the start of the 20th century by the Rivers Nursery, in Hertfordshire, England. The trees are adaptable to nearly any well-drained, loamy soil. It is an excellent dessert plum, being large, round in shape, rich purple with its deep yellow flesh, juicy and sweet. It blooms early and requires a polliniser.

6.1.26 Reine Claude

It is French variety of plum, the tree is compact, self-fertile and blooms very late. The richly flavoured fruit is large and have skin from pink to purple in color, speckled with white, flesh is crisp. It flavor and coloring are both unique with high quality. Susceptible to some pests and diseases.

6.1.27 Santa Rosa

A Japanese plum, it produces an upright tree. The fruit is large purplish crimson in color. The flesh is amber with red near the skin. In India, this cultivar has established itself primarily because of its self-fruitfulness, prolific bearing and characteristic flavor, not with standing the offending sour taste of relatively thick skin. A number of early and late maturing strains of this cultivar have been developed like the July Santa Rosa and Late Santa Rosa.

6.1.28 Satsuma

Satsuma plums are a Japanese variety of medium to small in size, red round plums are very sweet. It is also known as Blood Plum due to its deep red color of the

skin. The dark red (maroon) skins on this plum variety tend to be firm and tough with a sour flavor. However, the deep red-colored flesh is very sweet that offsets the bitter-tasting skin. It is a semi-clingstone variety, meaning that the flesh partially clings to the stone. The tree is upright and very productive. The fruit is susceptible to cracking in prolonged wet periods.

6.1.29 Shiro

The trees of this variety are spreading and very productive. A sweet, juicy yellow plum. Fruits are round, cling-stone, medium in size. It is a good pollinizer for Methley, Santa Rosa and Satsuma.

6.1.30 Stanley

A fine prune type plum with excellent quality suited for both home use or processing. Fruit is medium to large in size with a dark blue skin. Flesh is greenish yellow, juicy and fine grained. The tree is early bearing and a good pollinizer for other European varieties.

6.1.31 Starking delicious

A Japanese plum which ripens in the second week of August. The tree is upright, hardy and productive. The fruit is medium large with dark red skin and blood red flesh. The quality is good. This is a very good commercial Japanese type plum.

6.1.32 Valor

It ripens just a few days after Stanley. It was developed at Vineland, Ontario. The fruit has purple blue skin and yellow flesh, sizes medium large. It is a semi freestone and has great fresh market potential. The trees are healthy and productive.

6.1.33 Vanier

A hybrid originated from a cross of Wickson x Burbank, that matures one week after Wickson. Fruit is red medium size, yellow fleshed and clingstone. The trees are upright, vigorous and productive.

6.1.34 Victoria

One of the best known and popular of plum trees; produces pale red fruit. The fruit is ripe for eating around mid-August. It is a versatile plum tree, produces fruit which can be eaten, used for cooking and for jams. It produces a heavy crop which often needs to be thinned if fruits are to be produced each year. The victoria plums are produced in late autumn and it is self-sterile. This plum has stood the test of time, originally bred in Sussex around 1840.

6.1.35 Wickson

A large, greenish yellow heart shaped plum with yellow flesh. The tree is upright and vigorous and tends to be a shy cropper. Any of the Japanese plums will pollinate Wickson, however Wickson is not considered a good pollinizer.

6.2 Low chilling cultivars

6.2.1 Satluj Purple

The fruits are large, bright crimson having thick flesh and with good quality. The average yield is 40 kg per tree. It is self-incompatible and should be planted with pollinizer variety Kala Amritsari. Pollinizer should be planted as an alternate plant in alternate rows for maximum yield. Satluj Purple and Kala Amritsari plants should be planted in the ratio 85:25 in an acre. Trees are medium in vigor with upright growth habit. The fruit is medium large with average weight of 25–30 g, roundish, turns into crimson color on ripening. Fruits are thick skinned with yellow orange firm flesh. It is sweet in taste having 13–14 per cent TSS and 0.6–0.7 per cent acidity and is suitable for table purpose. It is an early variety ripening in the first week of May with average yield of 40 kg per tree.

6.2.2 Kala Amritsari

This is the most popular cultivar, particularly in Punjab. It is self-fruitful but yield improves if pollinated with Titron; high yielding indigenous variety with vigorous tree. Fruits are medium sized, round oblate depressed at both ends, on ripening turn dark purple. Flesh is yellow with moderately juicy pulp. Flesh is yellow, moderately juicy and excellent for jammaking. Fruits are somewhat acidic with 15.0 per cent TSS and 1.2 per cent acidity. It ripens in mid-May. Average yield is 45 kg/tree. Fruits are excellent for jam making.

6.2.3 Pant Plum 1

This is a selection from the seedling population raised at the Department of Horticulture, GBPUA&T, Pantnagar (Uttaranchal). It is dwarf and bears yellow and sub-acidic fruits. It can be a dwarfing rootstock for plum cultivars.

6.2.4 Alu Bokhara Amritsari

Large sized fruits of attractive red color having high TSS:acid ratio. Free stone, pink colored flesh with uniform sweetness. Free from sourness near the pit, the serious most drawback of Indian plums. The maturity period is also quite earlier as compared to those of temperate plums [37].

6.2.5 Titron

Fruit medium in size with deep purple, thin skin and yellow flesh. A self-fruitful variety and the yield improve when planted with 'Alucha Early Round' as pollinizer. The fruits ripen in second week of May. Average yield is 25–30 kg/tree.

6.2.6 Jamuni Meeruti

Fruits are small in size, dull yellow, thin skinned with soft melting flesh. The fruits ripen during end April. The average yield is about 28 kg per tree.

6.2.7 Kataru Chak

A partially self-fruitful variety but the yield improves when planted with 'Kala Amritsari'. Fruits are large, purplish with creamy flesh. They are good for jam and squash making.

6.2.8 Alu Bokhara

A self-unfruitful variety and should be planted in rows alternating with 'Howe'. Fruits are large, yellow in color sometimes having red over color.

6.2.9 Howe

The fruits are large, round, sweet juicy and red at maturity. Fruits ripen in second fortnight of May yielding on an average 30–35 kg fruit per tree. Interplanting of Alu Bokhara helped to increase yield.

7. Conclusion

Stone fruits or *Prunus* spp. are deciduous tree species originating from the temperate zone of the northern hemisphere. Plum, peach and nectarine, sweet and sour (tart) cherry, apricot and almond belongs to the group of stone fruits according to pomological classification. The genus *Prunus* has a vast species and varietal biodiversity expanded world over as wild, semi-wild and cultivated forms. Considering the significance in terms of its share to the plant biodiversity, genetic resources and horticultural utilization, it becomes very essential to create repositories, to generate data base and to adopt collaborative conservation strategies to mitigate the threats to this genus. Stone fruits typically have long breeding cycles; thus, developing a new cultivar through traditional breeding may require many breeding cycles and dozens of years. Major efforts in stone fruit breeding has been done in early twentieth century, however major progress was achieved in the second half of the twentieth century, with uneven results which differ from less effectual (in apricot) to extraordinarily successful (in peach and nectarine).

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Chapter 3

Expansion in Cultivating Almond Trees in Egypt

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Abstract

Egypt spends a lot of hard currency annually to import nut fruits (almond- walnut and pistachio) to apply market needs of these crops especially in Ramadan month. It is known that there are wide uncultivated areas in Sinai despite of its suitability for cultivation. Cultivating nut trees can share in development of Sinai. There are scarcity of these trees in Egypt in spite of the relevance of environmental conditions for growing almond trees in different regions. Since the last 25 years I and a group of scientists studied the possibility of achieving self sufficiency of almond by cultivating in Sinai Peninsula and different regions after carrying out climatic, economical, water requirements, nutrition and genetic studies. Many fruit trees require cold temperatures during the winter to overcome their seasonal dormancy. Most fruit species that evolved in temperate or cool subtropical climates have such chilling requirements that need to be fulfilled each winter to achieve homogeneous and simultaneous flowering and regular crop yields coldness. Monthly historical data of minimum temperature from Central laborator for Agricultural climate of four districts were analyzed in order to determine the changes in minimum temperature from October to February during the period from 2001 to 2010. Understanding monthly temperature changes from October to February during the period 2001–2010 was the first step in carrying out this study. The highest minimum temperature was found during 2010 year during the studied period in the October month for all districts except in November and December, the highest minimum temperature was observed in the year of 2009. Saint Catherine district was the lowest minimum temperature in all months during the studied period. Understanding average monthly temperature trends of the studied time serious from 2001 to 2010 was the second step in carrying out this study. October month was the highest values of minimum temperature and January was the lowest value of minimum temperature at the four districts. The highest and lowest values for temperature were found in Ras Sudr and Saint Catherine respectively. The third step in carrying out this study is to understanding the annual trend of minimum temperature for the period 2001–2010 at the Suez, Ras Sudr, El Tur and Saint Catherine districts. Data shows the average annual minimum temperature at the four districts during the years from 2001 up to 2010 and it can be observed that, Ras Sudr district has the highest average annual minimum temperature while Saint Catherine has the lowest one among the studied districts. It can be concluded that the carried out climatic studies, estimate the irrigation water requirements of almond trees and genetic studies help in solving the problem of achieving self sufficiency of almond fruits through expansion of cultivating almond trees in Egypt.

Keywords: Almond, Chilling Hours, Chilling model, Hard currency, Self sufficiency

1. Introduction

Many fruit trees require cold temperatures during the winter to overcome their seasonal dormancy [1, 2]. Most fruit species that evolved in temperate or cool subtropical climates have such chilling requirements that need to be fulfilled each winter to achieve homogeneous and simultaneous flowering and regular crop yields. In order to select appropriate fruit species and cultivars for the climate of a given site, researchers have developed chilling models, which convert temperature records into a metric of coldness [3, 4]. Chilling units are most meaningfully described and measured using an hourly time scale. Chill hours below a threshold are one of the most common methods for calculating chill. If the temperature is above 10°C then it is too warm for the plant to accumulate chilling. If the temperature is below 10°C then the plant is affected by the cold temperatures, with colder temperatures producing bigger effects. As soon as temperature drops below the base temperature for one hour, one chill unit is accumulated. Using the same chilling model to [5] quantify both a cultivar's chilling requirement and the amount of winter chill available at a given location enables growers to predict whether the cultivar will perform well under the specific temperature conditions of their sites. Chilling models also constitute tools to understand and manage the interannual variation in the time, at with tree crops complete their dormancy. The implications of climate change for winter chill have occasionally been investigated Baldocchi and Wong, [6] but no studies have compared the effects of temperature increases on winter chill, when quantified with different chilling models.

2. Material and methods

Depending on species cultivated and location of production, growers in Egypt use one of two different models to quantify chilling. The two most commonly used models are the Chilling Hours Model, developed in the 1930s and 1940s. The Chilling Hours Model (sometimes referred to as Weinberger Model; [7, 8]), as originally proposed, simply calculates the number of hours, when the temperature (T) is below 7.2°C. Other model when the temperature (T) is below 10°C.

3. Results

Tables 1–4 show the accumulation chilling hour from November 2014 to February 2015 for different location (Suze, Ras Sadr, Saintkatran and El Tore). The height chilling hour was found at Saintkatran followed by El Tore. The lowest chilling hour was found at Ras Sadr under the two chilling model 10 and 7.2.

The temperature changes over four districts in Egypt (Suez, Ras_Sudr, El_Tur and Saint Catherine) during the period from 2001 to 2010.

No.	Area	T < 10°C	T < 7.2°C
1	Suez	0	0
2	Ras Sudr	0	0
3	Saint katherin	208	90
4	El Tur	72	6

Table 1.
Chilling hour during November 2014.

No.	Area	T < 10°C	T < 7.2°C
1	Suez	152	0
2	Ras Sudr	36	0
3	Saint katherin	566	292
4	El Tur	272	57

Table 2.
Chilling hour from November to December 2014.

No.	Area	T < 10 °C	T < 7.2°C
1	Suez	250	20
2	Ras Sudr	169	28
3	Saint katherin	982	621
4	El Tur	594	267

Table 3.
Chilling hour from November 2014 to January 2015.

No.	Area	T < 10 °C	T < 7.2°C
1	Suez	514	44
2	Ras Sudr	252	48
3	Saint katherin	1335	882
4	El Tur	825	404

Table 4.
Chilling hour from November 2014 to February 2015.

3.1 Climate data

Monthly historical data of minimum temperature from Central laboratory for Agricultural climate of four districts were analyzed in order to determine the changes in minimum temperature from October to February during the period from 2001 to 2010.

3.2 Monthly temperature changes

Understanding monthly temperature changes from October to February during the period 2001–2010 was the first step in carrying out this study. **Table 5** shows the minimum temperature of the four studied districts (Suez, Ras_Sudr, El_Tur, and Saint Catherine) and it can be observed that the highest minimum temperature has been found in Ras Sudr district and the lowest minimum temperature has been found in Saint Catherine district among the studied districts in all months during the period 2001–2010. The highest minimum temperature was found during 2010 year during the studied period in the October month for all districts except in November and December, the highest minimum temperature was observed in the year of 2009. Saint Catherine district was the lowest minimum temperature in all months during the studied period.

Month	Year	Suez	Ras_Sudr	El_Tur	Saint Catherine
October	**2001**	18.0	19.6	13.6	13.3
	2002	18.6	20.3	14.4	14.6
	2003	19.6	21.3	15.9	14.8
	2004	19.1	20.8	15.2	15.2
	2005	19.1	20.9	15.4	15.6
	2006	18.6	20.4	14.2	14.0
	2007	19.0	20.6	14.8	15.0
	2008	19.0	20.8	15.6	15.6
	2009	20.0	20.8	16.5	14.4
	2010	20.4	21.9	16.2	15.7
November	**2001**	16.6	18.6	10.9	9.3
	2002	16.5	18.2	11.0	9.1
	2003	17.0	18.9	12.1	10.8
	2004	17.6	19.3	12.4	10.5
	2005	16.6	18.4	11.4	9.5
	2006	16.5	18.3	10.9	8.7
	2007	15.9	17.8	10.4	8.4
	2008	17.1	18.9	11.9	9.6
	2009	20.0	20.8	16.5	10.5
	2010	16.7	18.5	11.2	9.5
December	**2001**	13.4	15.2	8.0	5.6
	2002	14.2	16.1	8.1	5.7
	2003	13.5	15.5	7.8	5.9
	2004	13.3	15.1	7.7	5.3
	2005	13.6	15.5	7.8	4.2
	2006	15.3	17.3	9.9	7.2
	2007	12.6	14.6	6.9	3.6
	2008	13.7	15.7	8.3	4.7
	2009	20.0	20.8	16.5	6.4
	2010	15.0	16.7	9.1	6.6
January	**2001**	12.2	14.3	6.7	4.1
	2002	11.1	13.1	5.1	2.8
	2003	13.8	15.6	8.1	5.2
	2004	12.7	14.6	6.8	4.4
	2005	12.4	14.3	6.6	4.0
	2006	12.2	14.3	7.0	3.7
	2007	12.0	14.0	6.2	3.3
	2008	11.3	13.4	5.5	1.8
	2009	12.7	14.7	7.3	3.9
	2010	14.1	16.0	9.0	6.1

Month	Year	Suez	Ras_Sudr	El_Tur	Saint Catherine
February	**2001**	12.6	14.6	7.4	5.1
	2002	12.2	14.1	6.8	4.2
	2003	13.2	15.1	8.1	6.1
	2004	12.1	14.0	6.1	3.9
	2005	12.0	14.0	6.8	4.4
	2006	12.3	14.4	7.6	4.7
	2007	13.1	15.0	8.2	5.9
	2008	13.1	14.9	7.7	5.3
	2009	11.1	13.2	5.6	3.3
	2010	12.4	14.4	7.2	5.4

Table 5.
The monthly minimum temperature of the Suez, Ras_Sudr, El_Tur, and Saint Catherine for the months from October to February during the period from 2001 up to 2010.

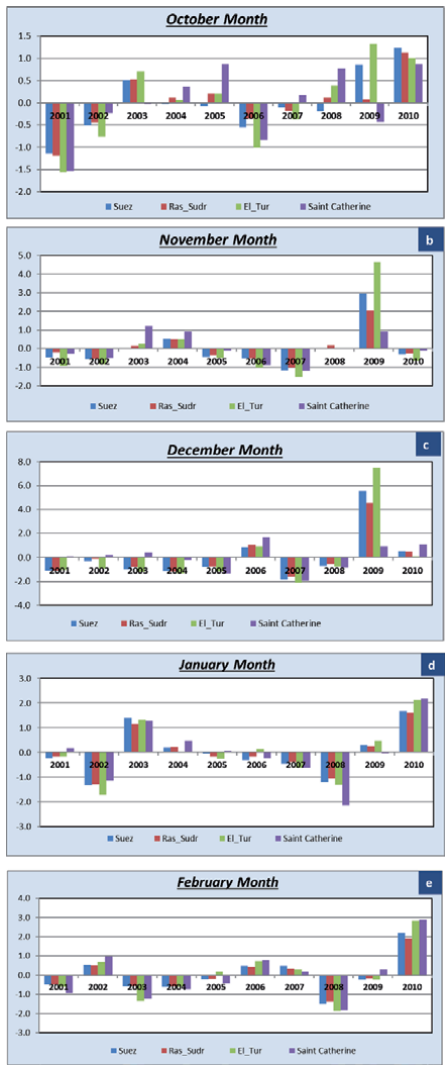

Figure 1.
The minimum temperature change from the normal minimum temperature of the Suez, Ras_Sudr, El_Tur, and Saint Catherine districts during the period from 2001 up to 2010 for the month of a) October, b) November, c) December, d) January, and e) February.

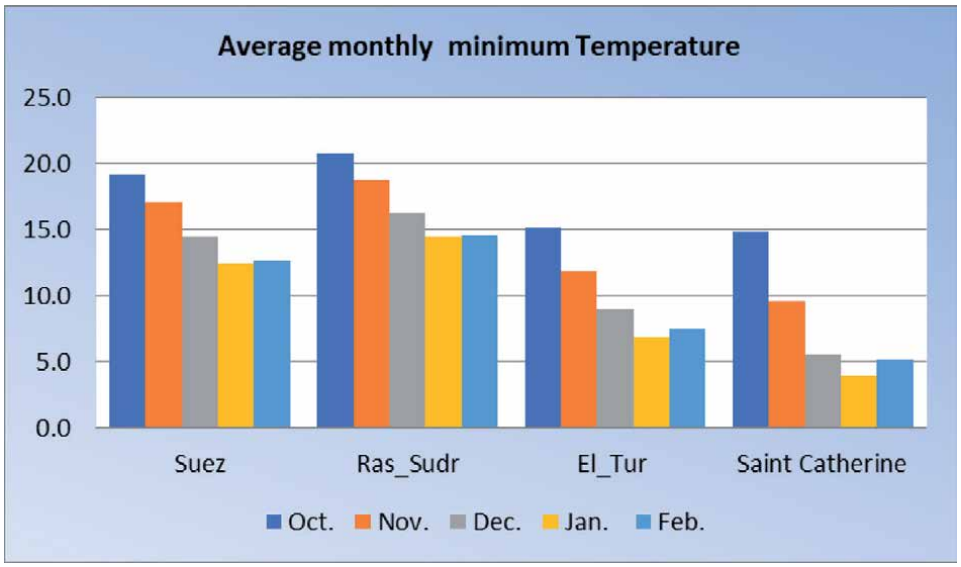

Figure 2.
Illustrate the comparison between historical minimum temperatures for the years 2001–2010 from October to February for four districts.

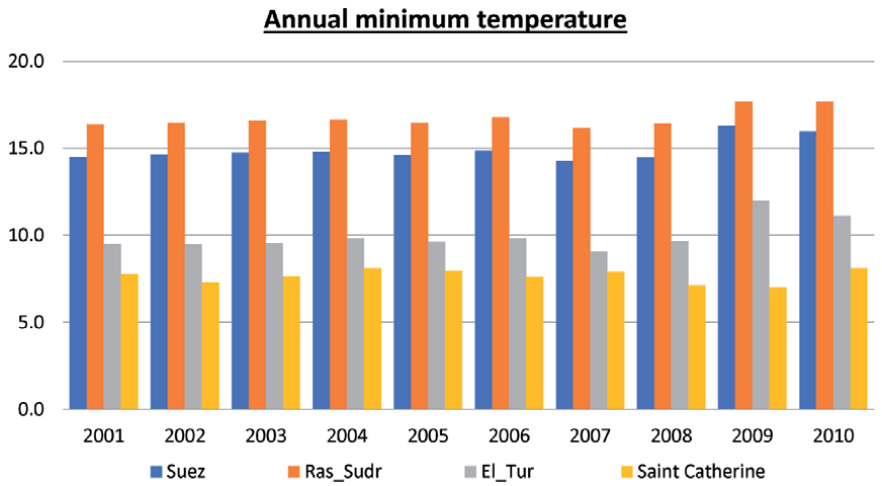

Figure 3.
The average annual minimum temperature e at Suez, Ras_Sudr, El_Tur and Saint Catherine during the years from 2001 up to 2010.

3.3 Average monthly temperature

Understanding average monthly temperature trends of the studied time serious from 2001 to 2010 was the second step in carrying out this study. **Figure 1** shows the average monthly minimum temperature from October to February during the period 2001–2010 at four districts (Suez, Ras Sudr, El Tur and Saint Catherine). October month was the highest values of minimum temperature and January was the lowest value of minimum temperature at the four districts. The highest and lowest values for temperature were found in Ras Sudr and Saint Catherine respectively.

The third step in carrying out this study is to understanding the annual trend of minimum temperature for the period 2001–2010 at the Suez, Ras Sudr, El Tur

and Saint Catherine districts. **Figures 2** and **3** shows the average annual minimum temperature at the four districts during the years from 2001 up to 2010 and it can be observed that, Ras Sudr district has the highest average annual minimum temperature while Saint Catherine has the lowest one among the studied districts.

4. Conclusion

Many fruit trees require cold temperatures during the winter to overcome their seasonal dormancy [1, 2]. In order to select appropriate fruit species and cultivars for the climate of a given site, researchers have developed chilling models, which convert temperature records into a metric of coldness [3, 4, 9].

Monthly historical data of minimum temperature from Central laboratory for Agricultural climate of four districts were analyzed in order to determine the changes in minimum temperature from October to February during the period from 2001 to 2010. Understanding monthly temperature changes from October to February during the period 2001–2010 was the first step in carrying out this study.

Understanding average monthly temperature trends of the studied time serious from 2001 to 2010 was the second step in carrying out this study. The third step in carrying out this study is to understanding the annual trend of minimum temperature for the period 2001–2010 at the Suez, Ras Sudr, El Tur and Saint Catherine districts.

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Chapter 4

Advances in Breeding of Peach, Plum and Apricot

Rimpika and DP Sharma

Abstract

Research on the expression of fruit specific genes may allow breeders in the future to selectively manipulate through gene transfer in certain aspects of fruit development/quality in their advanced breeding lines thus reducing the time necessary for cultivar development. This would be particularly useful in breeding programmes, hybridizing standard cultivars with exotic germplasm of low fruit quality. The use of exotic germplasm will be important for the expansion of the peach germplasm base and the development of stress resistant cultivars. More immediate results of research on fruit specific gene expression will provide a better understanding of fruit development and quality. It is required to learn how the differences at the gene level correlate with quality characteristics. With the continued cooperation of fruit biochemists it is expected to obtain a better definition of fruit quality and a better understanding of fruit biochemistry. The potential will exit to generate a range of "anti-sense mutants" i.e. transgenic plants expressing anti-sense gene contstructs that reduce or nullify the effects of the normal gene. The phenotypes of these mutants could help to define the biochemistry, genetics and quality of peach fruit. The development of efficient regeneration and transformation system in peach will be useful not only for the modification of fruit characteristics, but also for the transfer and manipulation of genes affecting stress resistance and other economically important characters.

Keywords: breeding methods, genetic resources, peach, plum, apricot

1. Peach

1.1 Introduction

The peach is an important fruit crop of temperature region all over the world. Important centers of commercial fruit production usually lies between latitudes 30° and 45° North and South. According to geographical distribution, the peach cultivars have been divided into three groups namely, Northern, Southern and European or Persian group. Peach cultivars can also be divided into two groups, high chilling and low chilling on the basis of their chilling requirements. Low chilling cultivars developed in Florida during last three to four decades have become very popular in the sub-mountainous Himalayan region. Peach is a temperate fruit rich in proteins, sugar, minerals and vitamins. At low latitudes the winter requirement is not met. Cultivars with less than 100 hours of chilling requirement are known. Strains of peach are grown throughout the subtropical and tropical zones, especially at higher elevations, where the heat of low-elevation tropics is ameliorated.

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1.2 Origin and distribution

The peach with its smooth skin mutant is nectarine. Nectarines can be used in the same way as peaches, and may be considered as substitutes for peaches. Peaches and nectarine [*Prunus persica* (L.) Batsch] are native to China and their culture dates back to at least 4,000 years and spread to the rest of the world by means of seeds [1]. It is a species well adapted to temperate and sub-tropical regions, between latitudes of 30° and 45° North and South [2]. Numerous forms and cultivars have been developed over the years. There are atleast 77 wild species of prunus and most of them found in central Asia. From China the peach reached Mediterranean basin from Persia was reiterated by early Greek and Roman writers. The Romans spread the peach throughout their realm. Its spread through the European Mediterranean countries was mainly by the Romans. The primary centre of peach diversity are believed to be the mountainous areas of Tibet and South west China while the secondary centres of diversity are Iron, Central Asia, Caucasus, Italy, Spain and California [3].

In India, high chilling peaches and nectarines are being grown in the mid hills of the Himalayan range in the states of Jammu and Kashmir, Himachal Pradesh and Uttrakhand, whereas, low chilling peaches are grown on a limited scale in foot hills of northern western plains of Punjab, Haryana. Uttar Pradesh and Nilgiri hills.

1.3 Evolutionary biology and taxonomy

The peach is the most widely grown species in.

A very important genus containing the European plum (*P. domestica* L.), Japanese plum (*P. salicina* Lindl.), apricot (*P. armeniaca* (L) Koatina), almond (*P. amygdalus* Batsch), Sweet cherry (*P. avium* L) and Sour cherry (*P. cerasus* L.). peach belongs to the family Rosaceae and the sub genus Amygdalus (**Table 1**).

1.4 Cytology

There are 17 species closely related to peach all belonging to same subgenus *amygdalus* that range in ploidy from 2x to 22x, all with basic chromosome number of x = 8. Peach is self pollinated diploid (2n = 16) and has small genome size of

Kingdom	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Rosales
Family:	Rosaceae
Genus:	*Prunus*
Subgenus:	*Amygdalus*
Species:	*Prunus persica*
Binomial name	*Prunus persica* (L.) Batsch

Table 1.
Taxonomy of peach.

approximately $5.9x10^8$ base pair/diploid nucleus (Barid *et al*. 1994). The chromosome number of peach is 2n = 16, X = 8. Other sub genres of *Prunus, Euprunus* and *Cerasus* exhibit naturally occurring euploid series (2n = 16, 32, 48 and even higher numbers), exceptioned to 2n = 16 are rare in subgenus *Amygdalus* and have been of no importance in development of cultivars.

1.5 Floral biology

The flowers of *P. persica* are commonly borne on one-year old shoots, with very few spurs forming. One or two flower buds form laterally to the vegetative buds ut some cultivars may form upto si or more flower buds per node. The flowers are mostly one per bud, anthesis is with or before leafing. The flowers are perfect, complete, perigynous, usually with a single pistil. There are five sepals and five petals, which are arranged alternately. Stamens number 30 or more. The filaments are long and thin, bearing the four loculed anthers which are reddish-yellow to yellow. The corolla cup is marked by the presence of nectarines, which vary in color from greenish through yellow to orange.

1.6 Genetic resources

The list of cultivars changes more rapidly than any other fruit tree

- Short span life of the tree
- Requirement of cultivars having hardiness to climate, soil, disease and pest reaction.
- Possibility of procuring improved cultivars through various breeding methods
- Less time required in establishing new peach orchard.

Choice of suitable cultivars for any region is governed by factors such as, type of market to be served, distance to market and adaptability to the local soil and climatic conditions. For table purpose, the cultivars should be yellow fleshed, freestone, regular producer and relatively free from fuzz. For canning purpose, the fruit should have yellow flesh, freestone, small non-splitting pit, good symmertrical size and should mature evenly.

1.7 Breeding objectives

Investigations in peach and nectarine breeding are concerned less with the inheritance of qualitative characters and more with an understanding of the transmission of quantitative traits. The manipulation of major genes to develop desired types of cultivars as free stones, canning cling stones or nectarines is basically understood. Development of efficient breeding system to maximize transmission of favorable variations is now of greater interest and importance. Extension of season of maturity makes an important objective in many breeding programmes. Improvement in flavor quality, both dessert and processed use, has become an important additional objective in most current breeding programmes. The main objectives in peach improvement and breeding programmes are:

1. High yield: Development of cultivars giving high yield at low cost production is essential in order to increase net returns that a triple income can be obtained by high density plantation than standard orchards.

2. Extension of season of maturity: Two factors account for this:

   a. Existing cultivars are deficient in desirable traits at the extreme of the seasons.

   b. Market opportunities are greatest at these times.

3. Processing purposes: Development of cultivars suitable for processing. Firmness of flesh, absence of tip on the pit, absence of split pits, freedom from loose fiber, fine texture, attractive color, non-browning of the flesh, flavor quality are all important traits needing improvement for processing outlets.

4. High fruit quality: It involves size, shape, skin color, flesh color, firmness, texture, freedom from loose fibers and non-browning of the flesh. Besides nutritive value, improvements of all these traits are needed to produce fruits of better quality.

5. Canopy modification: Increase in mechanization requires modification of tree structure and thus fruiting response. Therefore, canopy modification by controlling tree vigor to aid in mechanization and to lower the cost of manual labour in pruning, thinning and harvesting is a part of these changes.

6. Resistant varieties: Resistant varieties are the cheapest and most convenient methods of disease and insect control. The varieties resistant to disease (Leaf spot, Leaf curl, Blight, Crown ball, Brown rot, Mosaic etc.), pests (Fruit fly, Moth, Borer) and nematodes and to biotic and abiotic stress are needed to be evolved for successful cultivation. Recently, techniques like in vitro micrografting as a method for inoculation and slot-blot hybridization, with a digoxigenin (DIG)-labeled cRNA probe derived from PNRSV RNA3, for virus detection (Prunus necrotic ring spot virus) was evaluated and it was concluded that the system was suitable for rapid year round peach germplasm for resistance to PNRSV.

Similarly, the results of molecular hybridization have been effective for tomato ring spot virus detection in Prunus and substantiate the tomato RSV resistance of Marianna 2624 [4].

7. Broadening of genetic base: Reliance on distinctive gene source to meet the limiting requirement of environment conditions should be broadened so that the variants needed for diversity are available and the tendency of the breeding programmes to become highly inbred be stopped.

8. Evaluation of low chilling varieties: Main objectives of peach breeding programme for low chilling areas are:

   a. Low chilling requirement and tolerance to high summer temperature.

   b. Improvement of fruit quality for table use and processing.

   c. Varieties which mature early between 60 and 70 days after full bloom.

   d. Resistance to root knot nematode, water logging, pest and diseases.

   e. Dwarfness of scion and rootstock.

   f. Nutrient deficiencies like Fe-Chlorosis, Zn deficiency.

1.8 Heritability

Heritability can be considered as that portion of observed variability due to heredity. Its estimation requires the partition of observed variability between gene effects and environmental effects. Traits of high heritability are subjected to large genetic gain, under selection per generation and those with low heritability may not be capable of significant advance through selection. Such studies are common in agronomic crops and are sparse for tree crops. The heritability estimates are given for several of the traits in specific peach population. Ripe date had an extremely high heritability of .84, this indicate that the population contained a great deal of additive genetic variability for this trait. So the investigation of heritability in peaches to proceed by evaluation of measurement applied to characterize traits and that only those that are adequate be used.

1.9 Breeding methods

1.9.1 Introduction and selection

Selection of planting material as candidate for introduction as a new cultivar is largely subjective based on the experience of the plant breeder and the characteristics of the established cultivars against which the new selection must complete. It is usually based on the heritability studies, as phenotypes appear to be the best measure of the genotypes. So depending upon the climatic requirements and the performance desired, cultivars are introduced from time to time.

Eighteen indigenous and five exotic (Kanto 5, Somerset, Dawne from USA and Shimizu Hakuto and Okubo from Japan) cultivars were planned in H.P. Shimizu Hakuto and Kanto-5 were found promising and recommended for mid hills of H.P. [5]. Out of 34 introductions made from Australia, Bulgaria and other countries, four cultivars viz. Stark Earliglo, Stark Early White Giant, Starking Delicious and Candore were considered good for mid hills of H.P. as these mature by the first and second week of June escaping rainy season [6]. Flordasun, Sun Red, 16–33, Floradared, Florda Balle, Early Amber, 15–39 bred in Florda (USA), Bonita, Rochan and Vantura bred in California (USA) were introduced during late sixties at PAU, Ludhiana [7, 8]. Flordasun, Sun Red, 16–33 and Flordared were found to be successful and became more popular than Sharbati and Khurmani, TA 170 has been released in Punjab.

1.9.2 Outcrossing

Outcrossing in the peach is the range of 15–30%. The immediate goal was elucidation of the method of inheritance of several polymorphic traits really observable in the cultivars of that time such as foliar gland types, flower size, fruit flesh color etc. Selection of parents at these initial efforts relied on clones that were more or less unrelated, but that exhibited the contrasting characters to be studied. Later breeding programmes also relied on selection of unrelated parents but based on particular quantitative traits that, if combined would yield ultimate commercial variety. For eg: "Early Craw ford" used for its superior flavor, University of California, Davis through conscious outcrossing tried to incorporate higher processed fruit flavor in canning cling stone cultivars. Initially, a hybridization of identified sources of quality with so called conventional canning Cling stone was made and later selection of the improved quality derived from several such hybrid sources sill unrelated were combined with further hybridization. Finally the development of disease or pest resistant cultivars or seed sources, exemplified by the development of seed cultivars for root knot nematode resistant has relied on crossing of unrelated parents.

1.9.3 Inbreeding

The most peach breeding programmes involve outcrossing initially, but then resort to some form of inbreeding, usually through the use of related selections for continued advance through the selection procedure to maintain heterozygosity. Inbreeding is attained most quickly and in highest degree by continued selfing, and many peach breeding programmes have relied heavily on self-pollination following original crosses between more or less unrelated parents. Major disadvantage of inbreeding is that much of the cultivars improvement carried out traces back to a few clones and hence to a limited gene pool [9]. Because of the apparent homozygosity within lines. Lesley, 1957 proposed that new cultivar might be developed by crossing between such highly inbred selections. The seedlings of such crosses would presumably be so nearly homogenous that variability among them would be insignificance. The problem connected with Lesley's suggestion for the development of cultivars in this manner is the number of inbred lines needed to supply the type diversity required by the peach industry. Diversity could be generated only if additional inbred lines would develop.

1.9.4 Hybridization

In India, planned breeding programmes for developing varieties suited to sub-tropical region was initiated in 1957 at the Horticultural research institute, saharapur (up). As a result of crosses made between Sharbati, Elberta, Bidwill Warly, Tinston and Flordawon, 84 hybrids were evolved. But there is no record available about the release of any hybrid [10, 11].

Singh and Sirohi [10] suggested an improved technique for peach hybridization by emasculation and pollinating all the ready to emasculate buds on a branch followed by covering them in a large glassine bag as a faster method. By this method, success in crossing could be increased by 28% over the earlier method of single bud emasculation and pollination.

The varieties identified for use inhybridization were Gujrati for dwarf stature, SRE6, Safeda Early cream and happen for earliness and large fruits, sharbati for fruit quality and flordared, Flordawon and Okenawa for low chilling requirement [10].

Besides the study of Mendalian Characteristics in the above Table, there is today a renewal of interest for quantitative genetics which includes characteristics like ripening date, full bloom date, amount of bloom etc. the detail of heritability of which under different conditions is given in the Table below:

Interspecific hybrids involving the peach are summarized in table. Scorza and Okie [12] crosses between the peach like species *p.persica and pdavidiana, p.mira and p.kansuensis* have been reported. Crosses between *p.persica and p.davidiana* have been used in the development of nematode resistant rootstock. Hybrids between *p.persica Xp. Amygdalus* or the reciprocal have also been widely tested as rootstocks because of their vigor and graft compatibility with either peach or almond. Meader and Blake reported on F1 and F2 population of *p.persi Xp.kansuensis.* The F1 plants were vigorous and some what intermediate. F2 plants showed various segregations for some of the distinguishing characters.

1.9.5 Mutation breeding

Induction of mutations usually by X-rays or gamma radiations or by certain chemicall is termed as mutation breeding. With the objective to increase mutation rated over those observed naturally. Two aspects that need to be studied adequately are:

1. Production of micromutants with lower doses.

2. Irradiation of pollen.

Pollen irradiation requires the growing of second and third generation progenies to uncover recessive mutants. It may prove useful in the long run in the development of useful variations due to ability to sexually transmit the mutation. Nectarine rose as peach mutants and their inheritance pattern is consistent with glabrous skin characteristics controlled by a single recessive gene [13]. A nectarine mutant is having higher chilling requirements and shorter fruit development periods.

1.10 Morphological and physiological traits

Trees that have a prudent growth habit can easily be pruned in high density orchards are important goal in breeding programs. There are several single, recessive genes that cause extreme size reduction viz.; dwarf (dw, dw2, dw3), semi-dwarf (n).

1.11 Genetic mapping

Large number of molecular linkage maps have been identified for peach and its relatives. Five maps are identified for *prunus persica*, two for almond x *p.persica*, two of *P.persica* x *P.davidiana* and one each of *P.persica* x nectarine, *p.persica* x p.ferganensis and myrobalan plum x almond-p.persica hybrid. Molecular markers also been used to differentiate between peach cultivars, measure their relatedness and determine their origins. A genome-wide framework physical map of peach was constructed using high-information content fingerprinting (HICF) and FPC software by Zhebentyayeva et al.

1.12 Molecular markers

Modern breeding of the species *Prunus* is based on a very limited number of genotypes, because of this and its high degree of natural self-pollination, peaches are known to have a quite narrow genetic base [9]. To avoid misidentification of cultivars and to protect plant varieties, efficient tools are needed such as DNA fingerprinting. The large number of cultivars and their limited genetic diversity make cultivar differentiation and finger printing of this species particularly challenging [14]. Most of the work in peach has been done either to detect diseases, usually of viral origin or to conctruct linkage maps based on DNA banding patterns. The information can be used for the characterization of the genotypes, marker aided selection and for isolation of genes of interest

1.12.1 Genetic linkage and identification of genotypes

Jun-JH found that (SCAR) markers, Sequence Characterized Amplified Region to be adequate to identify the F/f gene (Flesh adhesion gene) in segregating progenies and commercial cultivars. Randomly Amplified polymorphic DNA (RAPD) were performed to detect markers linked to F/f gene and it is established that these markers can reliably be used in the marker assisted selection of peach cross seedling at an early development stages of a trees.

1.13 Somaclonal variation and in vitro selection

In vitro selection and somaclonal variation can be used effectively to obtain peach trees with increased levels of resistance to the bacterial spot. In vitro selection of peach

cells for insensitivity to pathotoxin produced by Xanthomonas compestris Pv. Pruni and subsequent regeneration of plants from the selected cells were carried out by Hammerschlag. Selection in vitro could be used in conjunction with mutagenesis to develop variants of established cultivars. For eg. Scorza and cordts found a differential response to cytokinin (benxyladenine) in vitro between 'Redhaven' and its compact mutant' Com-pant Redhaven'. Mutagenesis and selection on high cytokinin medium could produce compact mutants of other cultivars. Hammerschlag and Ognjanov screened peach varieties for resistnce to Xanthomonas compestris pv. Pruni and *pseudomonas syringae* pv. Syringae, the causal agent of bacterial cacker.

1.14 Rootstock breeding

The objectives of rootstock breeding:

- Resistant to nematodes, pest and soil borne diseases.
- Tolerant to heat, cold and drought conditions.
- Control of tree vigor for HDN system.
- Consistent, quality and quantity yield.
- Ease of propagation

Peach seedlings are the most usual rootstock for peaches. Generally peach seedlings are susceptible to nematode attack with the exception of "Nemaguard" and "Okinawa "seedling rootstock. "Nemared" is a descendant of "Nemaguard" are used specifically where root knot nematode are a problem [15]. Peach plum hybrid (*p. belsianaX yunam)* myran is tolerant to drough, poor soil, root knot nematodes and armillaria and vertilillarium diseases. Marianna GF 8–1 (*p. domestica Xp.munsoniana)* are better adapted to heavy soils [16]. Danis J et al developed a procedure for screening prunus species and related genotypes for resistance tolerance ot the root lesin nematode *pratylenchus vulnus allen and jensan*. However "Nemaguard" rootstock which was resistant to root knot nematode are highly susceptible to these root lesion nematodes, whereas plum root stock Marianna 2624 and Myrobalan 29C have been determined to be moderately to highly tolerant to lesion nematodes. Flordaguard is a root Knot resistant rootstock from the University of Florida [17]. "BY520–9 was released to provide resistance to peach tree to short life in SE-United States and Ausralia. Somaclonal variation as a result of tissue culture and its use in obtaining disease resistance variants at the cellular level with particular attention to the variants of peach with increased levels of resistance to bacterial spots *Xanthomonas campestris pv prunni,* bacterial canker and root knot nematode.

INRA peach interspecific breeding programme in France aimed at creating rootstocks with general grafting compatibility, waterlogging tolerance, Selection for resistance to root knot nematodes, tolerance of chlorosis, drought, salinity and crown gall. The studies reveal that the *myrobalan* plum having one dominant gene (Ma) resstance to melodiogyne species. A new interspecific breeding programme was initiated to combine the myrobalan nematode resistance source in a "Nemared" peach background to cerate highly resistant rootstocks. Massair R et al. tested the salinity to lerance on four different rootstocks (Mr S2/5, G.F. 655/2, G.F.677 and found that the rootstocks G.G. 655/2 were least affected by the

slts. Sirio (peach x almond rootstock Gf 677) is suitable for high density planting system even in fertile and calcareous soils [18].

1.15 Achievements and missing gaps

Peach and nectarine breeding is one of the brightest facet in all tree fruit breeding achievement.

- New producing regions have come to the fore through the efforts of peach and nectarine breeders.
- Fruits are available in the market for a greatly expanded period of time
- Cultivars for processing have been developed for regions not commonly recognized for such production.
- Some disease problems have been alleviated
- The genetics of the peach i.e. molecular mapping and gene transfer approaches to genetic improvement are being explored.
- Emphasis us given on the fundamental problems involved in such interrelated traits as climatic adaptation, tree and fruit disease and pest resistance, fruit quality and processing and cultural management and production.

1.16 Conclusions

Research on the expression of fruit specific genes may allow breeders in the future to selectively manipulate through gene transfer in certain aspects of fruit development / quality in their advanced breeding lines thus reducing the time necessary for cultivar development. This would be particularly useful in breeding programmes, hybridizing standard cultivars with exotic germplasm of low fruit quality. The use of exotic germplasm will be important for the expansion of the peach germplasm base and the development of stress resistant cultivars.

More immediate results of research on fruit specific gene expression will provide a better understanding of fruit development and quality. It is required to learn how the differences at the gene level correlate with quality characteristics. With the continued cooperation of fruit biochemists it is expected to obtain a better definition of fruit quality and a better understanding of fruit biochemistry. The potential will exit to generate a range of "anti-sense mutants" i.e. transgenic plants expressing anti-sense gene contstructs that reduce or nullify the effects of the normal gene. The phenotypes of these mutants could help to define the biochemistry, genetics and quality of peach fruit.

The development of efficient regeneration and transformation system in peach will be useful not only for the modification of fruit characteristics, but also for the transfer and manipulation of genes affecting stress resistance and other economically important characters. It is clear that an understanding of the genetic, molecular biology, and biochemistry of peaches and other perennial fruit crops along with a development of the technologies to manipulate these crops at the molecular level will be important for efficient progress in genetic improvement.

2. Plum

2.1 Introduction

Plum is an important temperate fruit, which is used both as fresh and in preserved form. Of the stone fruits it next to the peaches in economic importance. Plum belongs to family Rosaceae and sub-family Prunoideae. It require certain period of chilling during winter to break dormancy, thus grown in areas where winter is cold. Fruits are rich source of minerals, vitamins, sugars and organic acids in addition to protein fat and carbohydrates. Plums with high sugar content which can be dried with stones in them and without fermentation, are known as prunes. In India, plum was introduced by Alexander Coutts and two types i.e. European (*P.* domestica) and Japanese (*P. salicina*) were introduced during 1870 in Himachal Pradesh. After evaluation, only Japanese plum has been recommended for commercial cultivation in the temperate region of northwestern Himalayas. Most plums in commercial production today are classified as European (hexaploid) or Japanese (diploid) types. European plums *(Prunus domestica* L.) are adapted to cooler regions. Within *P. domestica,* several groups of cultivars are recognized, such as Green Gage or Reine Claude types, and Prunes.

European plums with a high enough sugar content so that they can be dried with the pit intact are referred to as prunes. Japanese plums (*Prunus salicina* Lindl., formerly *P. triflora* Roxb.) originated in China, but were further developed in Japan and the United States. The term "Japanese plum" now encompasses a wide range of fresh-market plums developed by intercrossing various diploid species.

2.2 Origin and distribution

There are at least five centres of origin for different species of plums. (1) *P. domestica*t (European plum) Euiope. (II) *P. salicina* (Japanese plum) China, (III) *P. inititia (*Damson plum) Western Asia and South Eastern Europe, (IV) *P.cerasifeni* (Cherry plum) Western and Central Asia, and (V) *P. americana* (American plum) North America.

Prunus domestica is believed to have originated by doubling of chtomosomes of a hybrid between *P. cerasifera* (2n 16) and *P. spinosa* (2n - 32); because it pos sesses 48 chromosomes.

The major plum producing countries are Germany, Yugoslavia, USA, Russia, Romania, France. Turkey and China, In India, plums are cultivated on a commercial scale in Himachal Pradesh, Jammu and Kashmir, hills of Uttar Pradesh and Arunachal Pradesh. It is also ultivated on a small scale in Nagaland, Sikkim and the Nilgiri Hills.

2.2.1 European plums

Historically, *Prunus domestica* has been the most important plum species. Crane and Lawrence suggested that it originated in Asia Minor as a hybrid between *P. cerasifera* Ehrh. (2n = 16, x = 8) and *P. spinosa* L. *(2n* = 32), which then doubled to produce a fertile hexaploid. Such natural hybrids could have been the progenitors of *P. domestica,* which may then have been spread by seed from Iran and Asia Minor across Europe. Several characteristics of *P. domestica* suggest such an origin, including skin and flesh colors and the presence of both citric acid (from *P. cerasifera)* and tannins (from *P. spinosa)* (Komarov et al. 1941). In Soviet Georgia, natural *P. spinosa* have been found with *In* = 16, 32, 48, 64, or 96. Natural hybrids *(2n* = 48) between *P. cerasifera* and *P. spinosa* were also found (Bajashvili 1991). Endlich and Murawski (1962) produced F_2 plants of this cross, some of which were hexaploids that

resembled *P.domestica.* Most of the wild *P. spinosa* fruit are black, bitter, and unpalatable, although some are used for drying and processing. Fossils of stones of this species have been found dating back to Neolithic period. It ranges from Scandinavia across Europe to Asia Minor. The northern forms are quite cold hardy, occurring up to 68°N in Norway.

Some authorities distinguish the wild forms as a separate species, *P. divaricata* Ledeb. Yoshida suggests that *P. cerasifera* is the progenitor of all plum species, because of its native range and cross- and graft-compatibility with many other species. The name "myrobalan" apparently resulted from resemblance of this plum to fruits of the tropical genus *Terminalia* (formerly *Myrobalanus),* which collectively are known as myrobalans and are used in tanning, dyeing, and medicine.

2.2.2 Asian plums

In China, *P. salicina* may have originated in the Yangtze River Basin (Yoshida 1987) but now is found across eastern China. The history of 'Zhui Li' cultivar goes back 2500 years. Numerous local selections have since been developed, but plum has never been as important in China as peach, either commercially or culturally. According to Zhang et al. plums in southern China are concentrated in seven provinces, but especially in Fujian and Zhejiang, where over 20 million plum trees and about 200 cultivars are found. Truly wild stands are rare but are reported to still occur in Hubei and Yunnan, where some trees in Zhongdian County are over 100 years old.

Plum stones have been found in Japan dating back to the Yayoi Era, about 2300 years ago. Japanese books dating back 1500 years mention cultivated plums. Plums have been common garden plants in Japan for centuries, but improvement efforts have occurred only in the last century. Low-chilling types are found in southern China and Taiwan. Cold-hardy plums in northern China have been classified as *P. ussuriensis* Kov. and Kost. and *P. gymnodonJa* Koehne, but are otherwise very similar to *P. salicina.* Modem breeding programs, especially in the former USSR, have utilized this source of hardiness. Western taxonomists have described other Chinese species, such as *P. thibetica* Franch., and *P. consociiflora* Schneid., but these are not listed in Chinese taxonomic references as distinct species and probably represent variants within *P. salicind.*

Prunus simonii Carr. was described by Western botanists based on cultivated specimens. This species (probably the same clone each time) was used in developing California cultivars because of its firm flesh and strong flavor. Chow describes it as native to north China, and occasionally cultivated. It has some characters reminiscent of apricot and was thought by some to have descended from a natural hybrid, but more likely is just an upright variant of *P. salicina.*

2.2.3 Japanese plums

Wilson's Early, Billington, Duffy's Early Jewel,Black Amber, Black Diamond, Fortune, Queen Rosa and Santa Rosa.

2.2.4 American plums

Native American plum species were already being grown by Native Americans. On the northeast coast, *P. maritime* Marsh, was grown. Across the eastern United States *P. americana* Marsh, was used. *Prunus angustifolia* Marsh, was widely grown by Native Americans, who may have extended its original range across the southeastern part of the country. The only edible plum native to the west coast,

P. subcordata Benth., was grown in northern California, where better quality forms occur. Apparently the xerophytic species were not used due to the lack of edible flesh.

2.3 History of improvement

Roach cites Gerard's report in 1597 that he had a collection of 60 of the best European plums, suggesting that slow but steady selection was occurring. Several cultivars known at that time are still grown, such as 'Reine Claude'. One of the earliest plum breeders was Thomas Andrew Knight in England, whose work encouraged two English nurserymen, Thomas Rivers and Thomas Laxton. Rivers released *Eaily Rivers' in 1834, followed by 'Early Transparent Gage', 'Czar', 'Monarch', and Tresident'. Laxton's cultivars were less enduring. Early settlers to North America brought European plums with them but the plums thrived only in more northern areas. A few selections were made but improvements were minor. Luther Burbank also developed European plums but only 'Giant', 'Sugar', and 'Standard' were important commercially.

2.4 Modern breeding objectives

The principal objective in a plum breeding program is the development of plums that can be grown successfully in a particular locality and that can be marketed profitably. A salable fruit must have an attractive appearance, adequate size and firmness, and acceptable flavor and texture. To be grown successfully, the trees must be productive and must be resistant or tolerant to local problems, that is, they must be hardy in northern regions, meet low chilling requirements for buds in southern regions, and be resistance to diseases and physiological problems. Suecessful marketing involves orchard location, proximity and types of markets, and the fruit's intended usage—shipping, canning, drying, or processing.

2.5 Breeding techniques

The techniques used in plum breeding are similar to those that are used for other deciduous fruits. They involve pollen collection, emasculation and pollination of flowers, seed collection and germination, and an evaluation study of the progeny.

2.6 Biotechnology

Because clonal rootstocks are being used for plum, particularly in Europe, tissue culture has been developed for commercial production. Much of the industry now uses rootstocks produced through tissue culture. Some plums, such as 'Santa Rosa', Marianna, and myrobalan, are relatively easy to multiply and root in vitro. Thermotherapy, meristem culture, and micrografting are commonly used to obtain virus-free propagating wood of both scions and rootstocks. Virus-free material is routinely available in North America and Europe. In some cases virus-free material grows much faster than infected plants. Micrografting may be more suitable than thermotherapy because it is less stressful to the plant material. In vitro methods have also been used to screen plums for resistance to crown gall caused by *Agrobacterium tumefaciens*. Druart and Gruselle summarize results in *Prunus* meristem and shoot-tip culture. Meristems can be obtained during the dormant or growing seasons. Rosettes and young shoots can then be multiplied

with the addition of a cytokinin such as 6-benzylaminopurine. Rooting is enhanced by the addition of auxin and vitamin E, and a dark period. European scientists have isolated plum pox (sharka) virus RNA, sequenced the virus coat protein gene, and constructed sense and antisense containing vectors. Scorza et al. reported the development of transgenic *P. domestica* plants from hypocotyls slices carrying the papaya ring spot virus coat protein gene. These plants are being tested for resistance to plum pox, which is a related potyvirus. Techniques for cryopreservation of dormant buds are being developed for long-term storage of genetic material.

2.7 Interspecific plum

"Cherry Plum" Hybrid myrobalan plum x Japanese plum (*P. cerasifera* and *P. salicina*).

Pluerry™ complex *Prunus* hybrid, primarily of plum and cherry (*P. salicina and P. avium*) with dominant parentage of plum and having fruit resembling plum.

Pluot® complex *Prunus* hybrid with dominant parentage of plum (*Prunus salicina*) and having fruit resembling plum.

Plumcot simple cross of plum and apricot (*Prunus salicina and P. armeniaca*).

2.8 Selection

The selection of parents in breeding new cultivars is most important. Parents should be strong in the particular characters desired in the progeny. Parents are often chosen to complement each other's deficiencies. The purpose is to recombine desired traits into a single individual. Small-fruited parents seldom give large-fruited progeny; thus at least one parent should be large-fruited. Parents should be selected for time of maturity, firmness of flesh, flavor, or other characters. Progeny will tend to be intermediate in various characters that are quantitatively inherited, with occasional seedlings possessing certain characters beyond the range of either parent.

2.9 Rootstocks

Mariana (uncertain origin, possibly *Prunus cerasifera* x *P. munsoniana*).
Pixie (*P. insititia*).
St.Julian X (*Prunus institia*).
St. Julian A (*Prunus institia*).
Myrobalan B (*Prunus cerasifera hybrid*).

3. Apricot

3.1 Introduction

Apricot belongs to the Rosaceae family, subfamily Prunoidese, genus Prunus L., subgenus Prunophora (Neck.) Focke, section Armeniaca. The Prunus genus comprises other tree crops of high economical importance in temperate regions, including peach, cherry, almond and plum. In term of economics, apricot is the third most important species of the stone fruit crops Apricot is diploid (2n = 16). Breeding programme of apricot has a long tradition in Europe and has achieved many very interesting results in some countries.

3.2 Origin and distributation

The apricot fruit moved westward from central Asia through Iran and transcaucasus region and reached Italy during first century, to England in 13th century and to North America by 1720. The major apricot producing countries are China, USSR, Turkey, Italy, Spain, Greece, France and USA. Commercial cultivation of apricot in India is at recent origin and was started by European settler and missionaries after 1870. there are three important regionsas origin of apricots although Armenia had been supposed apricot's origin and named as *Prunus armeniaca*, previously. These are;

a. The Chinese center (China and Tibet).

b. The Central Asian center (from Tien-Shan to Kashmir).

c. The Near-Eastern center (Iran, Caucasus, Turkey).

3.3 Floral biology

The apricot has a perfect, perigynous flower with a single pistil. The petals are usually white, though some are tinted and occasionally even deep pink in color. Pollen sterility occurs and is inherited as a single recessive (Hesse, personal communication). Cultivars may be either self-compatible or self-incompatible. Careful breeding work in the future may well demonstrate an allelic series forself-incompatibility like that known for sweet cherries, since T. Toyama, USDA, Prosser, Washington (personal communication), has observed that some crosses between self-incompatible selections have been unsuccessful.

3.4 Flowering, pollination and fruit set

In apricot, usually three buds develop in the axil of a leaf at each node on a shoot and spur. The central one being a vegetative bud, the two side buds are floral. Time of flowering and its duration varies with the variety and the prevailing weather conditions. Under mid hills condition the flowering in apricot comes in the month of March and higher hills in end of March & April. Most of the commercial as a apricot are self fruitful and set fruits without pollinizer. However, varieties Charmagz and Perfection have been reported self incompatible. There is generally a good fruit set in the apricot Cvs growing in appropriate climatic conditions. There is 40–60% fruit set in the cultivars commercially grown in mid hills, but fruit drop is to the extent of 79% in these cultivars, which occurs mostly in second week after fruit set.

3.5 Modern breeding objective

The major objective in most apricot breeding projects is climatic adaptation. The development of apricots with a long period of winter development of the flower buds (i.e., a long chilling period) that will withstand fluctuating temperatures in late winter is probably the most important objective for extending the region where apricots can be grown. In addition to the slow development of flower buds in mid-winter, it is important that the flower buds respond very slowly (require relatively high amounts of degree days of heat) to waning temperatures in the spring after their rest has been satisfied. The combination of these two characteristics will likely give the ultimate in late blooming so that the buds or flowers may escape spring frosts Apricots, because they are the earliest to bloom of the cultivated stone fruits, frequently encounter periods of cool, windy, cloudy, wet weather during or after

the bloom period, often with a negative effect on fruit set Later blooming would be helpful in avoiding late spring frosts. Apricot trees that grow well and ripen fruit under humid growing conditions are objectives of many breeding programs. Trees with a lower chilling requirement or trees with a high chilling requirement that is met at a higher threshold are needed to extend the production of apricots into warmer regions. Should be an objective:

- High yield
- Climate adaptability
- Extension of season of maturity
- Processing purposes
- High fruit quality
- Canopy modification
- Resistant varieties
- Broadening of genetic base
- Evaluation of low chilling varieties

3.6 Recurrent mass selection

This statement should provide the basic perspective for apricot breeders. In any breeding program at any one time it is necessary to budget the total resources of the *project* among the several objectives. Chances are increased for achieving a given objective, in the absence of progeny testing, when two or three phenotypes that seem equally promising for that objective are used. Consequently, after deciding on the budget (the total number of seedlings that can be afforded for the given breeding objective), a breeder raises several intennediate-sizea progenies, rather than trying to choose a single pair of parents for one large population. We agree with Hansche that progeny testing is an inefficient technique because of time. But where progeny test date are available, as is the case for many of the cultivars in the collection of the Nikitsky Botanic Garden, they should be considered in the choice of parents.

3.7 Interspecific hybridization

Aprium- complex Prunus hybrid primarily of apricot and plum (*Prunus armeniaca* and *Prunus salicina*) with dominant parentage of apricot and having fruit resembling apricot.

Color-Cot- complex Apricot hybrid (*Prunus armeniaca* hybrid) having fruit resembling apricot with pubescent skin strongly blushed red or orange-red.

Peacotum-complex apricot-plum-peach hybrid (*P. persica*, *P. armeniaca*, *P. salicina*) with dominant parentage of apricot and having fruit resembling apricot.

3.8 Modified backcrossing

Certain characters, such as *disease* resistance, cold hardiness, and late blooming, can be effectively incorporated with other desirable pomological characters through

a modified backcrossing program. Important considerations for the success of such a program are (1) an adequate screening procedure so that the desired phenotypes may be identified efficiently in each generation, and (2) the use of different high-quality backcross parents in successive generations. This practice allows the breeder to incorporate a greater variety of pomological characters into the breeding lines and at the same time to avoid the deleterious effects of inbreeding that are associated with repeated backcrossing to a single cultivar.

3.9 Biotechnology

Biotechnology has obvious implications as a new tool that can be employed in apricot breeding. Potential areas for its application include regeneration and micropropagation, virus elimination, and genetic improvement which could include somaclonal variation, protoplast culture and fusion, embryo rescue, and haploid induction. Recombinant DNA technology might also be employed to carry out genetic transformation and gene characterization. With the exception of micro-propagation, embryo rescue, and virus elimination Fridlund, biotechnology has not yet been fully employed as a tool in apricot breeding.

3.10 Mutation breeding

Early Blenheim' was selected following thermal neutron irradiation and was introduced by Lapins as an early ripening cultivar tor local markets. Lapin's work is the only report of achievement from mutation breeding with apricots.

3.11 Rootstock breeding

The objectives of rootstock breeding:

- Resistant to nematodes, pest and soil borne diseases.
- Tolerant to heat, cold and drought conditions.
- Control of tree vigor for HDP system.
- Consistent, quality and quantity yield.
- Ease of propagation
- Soil and Climate adaptability
- Stock-scion interaction
- Nursery handling feautures.

3.12 Genetic diversity of apricot based on molecular markers

Apricot is a temperate and subtropical zones fruit. China, the Irano-Caucasian region (Turkey and Iran), Central Asia, Europe and North America are the main producer regionsin the world. The Central Asia is the oldest and the primary genetic source of apricot groupis the Central Asian accessions are self-incompatible; the Irano-Caucasian apricots which aremostly the cultivated ones are mostly self-incompatible, with large fruits and low chillingrequirements. The European and the

North American apricots are originated from Irano-Caucasia has relatively narrow genetic diversity and are self-compatible with large fruits. For a long period, genetic diversity in apricot was studied with pomological, morphological and phenological characteristics. DNA-based markers that have been used in the last decade clarify the relationship among the apricot accessions. For breeding and commercialization of promising apricot cultivars, a precise characterization and discrimination of the cultivars are prerequest. Different types of markersuch as morphological, molecular, biochemical systems have been used for genetic analysisin horticultural plants. However, due to the effects of environmental factors, asessment of morphological and pomological traits may be ambiguous. Therefore, markers in dependent from the environment are necessary for reliable identification and discrimination of genotypes and cultivars. DNA markers are well known independent from environmental interactions and they show high level of polymorphism. Therefore, they are considered invaluable tools for determining genetic relationships/diversity. Various types of DNA markers are now available. Among them, RAPD developed by Williams et al. has been commonly used method in apricot to assess genetic variability and relationshipsamong cultivars techniques has also been used in apricot tocharacterize different cultivars belongs to diverse eco-geographical groups. The diversity determined between apricot cultivars was probably due to crosses betweenwild and cultivated apricots and cultivars from different eco-geographic origin. Microsatellite analyses suggested that European cultivars might have originatedthrough hybridization among Irano-Caucasian genotypes and also most of the European cultivars have originated by hybridization with genotypes from the Irano-Caucasian group. The heterozygosity of the apricotgenotypes narrowed while apricot transfer from China to Europe. Pedryc et al. show that Middle European and Chinese apricot are distantly related. Molecular markes have created new era in genetic diversity researches since early nineties. Restriction fragment length polymorphism (RFLP), and PCR based markers such asrandomly amplified polymorphic DNA (RAPDs), sequence-related amplifiedpolymorphism (SRAP), single nucleotide polymorphism (SNPs), microsatellites or simplesequence repeats (SSRs) are mostly used marker systems in plants and also in apricot genetic divesity researches. Microsattelites among all is a very useful tool for apricotdiversity studies, and most promising to cleary genetic relation among the apricots andtravel routes of apricots. Amplified fragment length polymorphism (AFLP) molecular markers assesment for thegenotyping of 118 commercial apricot accessions and some related apricot species. The researchers clustered the apricots into four groups corresponding to theirgeographic distribution; (1) Mediterranean apricots, (2) Chinese apricots, (3) apricots ofcontinental Europe and (4) Europe-North American apricots. Their data confirmed that themigration of apricot from the East to West. They also showed with molecular markers that*Prunus sibirica* and *Prunus mandshurica* are different from *Prunus armeniaca*, but they grouptogether with Chinese accessions. In anohter study Romero et al. studied apricots by using of SSR markers to determine the genetic relationships amonggenotypes from different eco-geographical groups. They observed that Western Europeanand North American subgroups clustered together in aggrement with their common originsfrom ancient European cultivars. However their study placed Hungarian cultivars closer to the Central Asian group than tothe other European cultivars.

Hayashi et al. studied Japanese apricot (*Prunus mume*) germplasm and reported thatthe genetic diversity and relationships among 127 Japanese apricot germplasms assessed by SSR markers. Their study supported the two hypotheses that Japanese apricot cultivated inJapan had been introduced from China and that fruiting cultivars had been selected fromflower-ornamentals.

Turkish germplasm was studied by Yilmaz and Uzun et al. and geneticdiversity and relationships among the accesessions were determined using RAPD, ISSR,

SRAP and SSR markers. The researchers reported the high genetic diversity in Turkishapricots. Four high chilling requiring cultivars originated from Eastern Turkey clustered apart from the rest. European, South African, North American and other Turkish cultivarswere not clearly grouped regarding to their geographic districts. Therefore the researcherssuggested that these cultivars, despite their different geographic origins, have similargenetic background.

3.13 Achievements and prospects

The first apricot cultivars from controlled pollinations were introduced in Russia Recently cultivars have been introduced in Canada, the United States, South Africa, Australia, Argentina, Romania, Hungary, France, and Czechoslovakia. A large proportion of these are from open pollination. Until now, such new cultivars have made no appreciable impact on production in established areas. Some of the cultivars from eastern North America, as well as some from the breeding programs in European Russia, seem to be more winter hardy and resistant to disease. Thus they give promise that the range of commercial apricot production can be extended into the humid, temperate fruit-growing regions that are close to concentrations of population.

The variability existing in desirable fruit characters assures the breeder that new cultivars can be produced that will be readily accepted in competition with other fruits, and the range in ecological adaptation indicates that apricots can be grown much more widely, so they certainly can become a greater part of the world's fruit production. But the limited ecological adaptation of any one genotype is the challenge to apricot breeders. Cultivars must be bred for each producing area and for each marketing opportunity. It is exasperating to realize that whenever a new character is introduced from another region into a breeding program it will likely be associated with unadapted attributes. With this perspective in mind, it becomes obvious that ambitious and persistent breeding programs are essential to expand the apricot industry throughout the temperate fruit regions.

The fruit qualities acceptable to consumers in the great centers of population will be quite similar, so the breeding programs will have similar objectives. Also, the ecological inflexibility of any genotype will require parallel bleeding programs. Certainly, it will be most efficient to have coordinated interregional and international breeding programs using a common gene pool and, in some cases, even common seedling populations that an understanding of the genetic, molecular biology, and biochemistry of peaches and other perennial fruit crops along with a development of the technologies to manipulate these crops at the molecular level will be important for efficient progress in genetic improvement.

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Chapter 5

Gene Editing in *Prunus* Spp.: The Challenge of Adapting Regular Gene Transfer Procedures for Precision Breeding

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Abstract

Successfully gene editing (GE) in *Prunus* spp. has been delayed due to its woody nature presenting additional difficulties in both, proper regeneration protocols and designing efficient gene transfer techniques. The availability of adequate, single cell culture techniques for GE such as protoplast regeneration, is a limiting step for the genus and for this reason, the improvement of regular regeneration protocols and finding more efficient techniques for the delivery of the "editing reagents" seem to be a reasonable strategy to incorporate GE in the genus. During the last 10 years, we have focused our efforts optimizing some previous regeneration and gene transfer procedures for Japanese plum (*P. salicina*), sweet cherry (*P. avium*) and peach (*P. persica*) to incorporate them into a GE technology on these species. In parallel, delivery techniques for the CRISPR/Cas9 editing components, i.e., guide RNA (gRNA) and Cas9, have been developed with the aim of improving gene targeting efficiencies. In that line, using DNA virus-based replicons provides a significant improvement, as their replicational release from their carriers enables their enhanced expression. Here, we make a brief overview of the tissue culture and regeneration protocols we have developed for *P. salicina*, *P. avium* and *P. persica*, and then we proceed to describe the use of *Bean yellow dwarf virus* (BeYDV)-derived replicon vectors to express the editing reagents *in vivo* and to evaluate their editing capability on individuals derived from *Agrobacterium*-mediated gene transfer experiments of these species. We show part of our characterization assays using new BeYDV-derived vectors harboring multiple gRNAs, the Cas9 gene, and the green fluorescent protein reporter gene. We also describe a dedicated genome analysis tool, by which gRNA pairs can be designed to address gene deletions of the target genes and to predict off-target sequences. Finally, as an example, we show the general results describing GE of the peach TERMINAL FLOWER 1 gene and some preliminary characterizations of these materials.

Keywords: LSL-based DNA replicons, geminivirus, gene editing, CRISPR/Cas9, Prunus genetic transformation

1. Introduction

The Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Cas9 system, discovered as an adaptive line of defense against viral infection in *Archaea* [1], is a common gene editing (GE) technique that allows for the direct generation of targeted sequence modifications in the genome. Its biotechnological use in plants represents a valuable gene expression tool that can contribute enormously to plant breeding. However, this so-called precision breeding, based on these GE techniques and its application in *Prunus* spp., requires developing three fundamental areas: a) tissue culture techniques, b) genome knowledge, and c) molecular biology tools for the delivery of GE components to the cell.

In *Prunus* spp., a huge amount of research has been carried out regarding regeneration and gene transfer protocols using different explants and strategies to improve these procedures [2]. From that research, current gene transfer procedures in the genus include some technical pipelines for whole plant production in species such as *P. salicina*, *P. domestica*, and certain genotypes in *P. avium*. In parallel, increased genome information for the genus is now available, and the advent of genome drafts for *P. persica*, *P. mume*, and *P. avium* have contributed to the feasibility of these new techniques, enabling the prediction and analysis of candidate genes [3]. Finally, improved molecular biology tools, including new expression vectors for GE components in the cell, can now be designed and built in an expedited manner, making the use of these technologies in the genus possible.

In the last decade, we have been immersed in implementing these components to enable precision breeding in the genus. Various tissue culture protocols for the regeneration of peach, plum, and sweet cherry genotypes have been assessed and improved. From that work, regeneration systems for diverse genotypes of these species have been deduced, achieving the first milestone in precision breeding. We also took the available genome information for some of these species and built a genome desktop for their analysis using dedicated bioinformatic components that allowed for the design of the GE key elements, i.e., the guide RNAs (gRNAs), and scored their effect for both on- and off-targets in those genomes. Finally, and due to the highly recalcitrant nature to regenerate found in all of these genotypes, we assumed the generation of new expression vectors for delivery of the GE elements to regular multicellular explants commonly used in our regeneration procedures; from this, traceable virus DNA-replicon derived vectors, with higher expression efficiency in the plant cell and offering the chance of generating either transgenic or non-transgenic edited individuals, have been developed.

2. Tissue culture systems in *Prunus* spp.

A considerable amount of the research carried out regarding *Prunus* spp. regeneration has been based on the use of multiple explant types and culture approaches. To date, most of the *Prunus* genotypes are still recalcitrant to transformation, either because of the lack of regeneration procedures or due to the absence of identified *Agrobacterium* genotypes that are competent for gene transfer.

Since we reviewed this topic 10 years ago [4], only a few examples of successful *Agrobacterium*-mediated transformation have been achieved in this genus and other fruit and nuts species [5]. These successful experiences include plum (*P. domestica*), Japanese apricot (*P. mume*), Japanese plum (*P. salicina*), and apricot (*P. armeniaca*). Whereas a remarkable cultivar/variety-dependence has been observed in these successful experiences, reliable and reproducible systems are yet to be developed for most *Prunus* spp. [2, 5].

Despite these technical constraints, the mentioned technical procedures in some *Prunus* species have resulted in the generation of a portfolio of interesting examples in the arena of genetically engineered trees, with improved traits deserving attention in agriculture. 'HoneySweet', one of the main products generated during the previous decade, came from a successful regeneration procedure described in *P. domestica*. 'HoneySweet' has been liberalized in the US by the Food and Drug Administration and the Environmental Protection Agency as a new *Plum pox virus*-resistant variety, considering trees safe for both the environment and consumers' health. In the last decade, derived from the same transformation system in *P. domestica*, the same group developed the "FasTrack" breeding approach, an advanced fruit tree breeding system that uses transgenic European plums that are continually flowering and, in that way, overcome the limitations of juvenility and dormancy processes producing the first generation in one year [6]. Also, in this period, primary tissue culture work in the sweet cherry rootstock 'Gisela 6′ [7] has led to improved gene transfer and regeneration methodologies for 'Gisela 7′, allowing for the generation of *Prunus necrotic ringspot virus* (PNRSV) resistant individuals through the use of RNA interference (RNAi) for viral sequences [8]. Interestingly, these materials have led to one of the few studies in woody plant species in which small RNAs have been demonstrated to be transported from rootstock to scion, making 'Emperor Francis' scions become resistant to PNRSV [9]. Finally, in 2019, we developed a transgenic Japanese plum tree exploiting the RNAi methodology to target a gene linked to *Plum pox virus* (PPV)-susceptibility, thus generating individuals resistant to PPV [10].

2.1 Regeneration in *Prunus salicina*

Since the first work on *in vitro* propagation described by Rosati *et al.* [11], Japanese plum has been demonstrated to be suitable for *in vitro* regeneration [12] and genetic transformation [13]. Based on the use of hypocotyls isolated from mature embryos, several varieties, such as 'Early Golden', 'Shiro', 'Angeleno' and 'Larry Ann', were responsive to culture treatment in the presence of thidiazuron (TDZ) and indole-3-butyric acid (IBA) as supplements of regular Murashige and Skoog (MS) [14] media. These procedures were derived from a previous description for European plum (*P. domestica*) reported by Mante *et al*. [15] and later improved by Padilla *et al*. [16].

In our hands, the use of hypocotyls has allowed for a reproducible gene transfer and regeneration procedure for several Japanese plum varieties (**Figure 1A–D**). Slight variations from the primary protocol described by Urtubia *et al.* [13] have allowed expanding this procedure to the use of epicotyl sections as starting explants, increasing the number of useful material when mature fruits are collected.

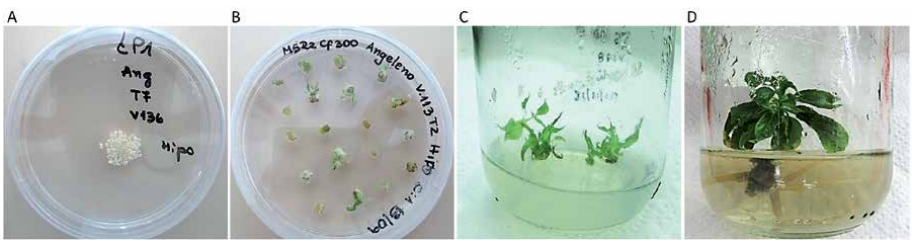

Figure 1.
In vitro Japanese plum (Prunus salicina cv. Angeleno) regeneration. After infection with A. tumefaciens GV3101, hypocotyl explants are subjected to regeneration procedures, achieving whole plant formation between 6 and 8 months of experimentation. A, plum explants (hypocotyls) in co-culture in LP1-medium. B, transformed explants in regeneration medium MSR2 for budding induction. C, shoots transferred to MS03 medium for improving shoot development. D, after elongation, shoots are transferred to WPM rooting medium.

Agrobacterium-mediated transformation of Japanese plum explants requires co-culturing (2–3 days in darkness, usually using strain GV3101) and regeneration on LP-derived medium [17] supplemented with picloram (LP1 medium). For shooting, explants are transferred to MS medium supplemented with variable ratios between TDZ and IBA (MSR2 medium), depending on the variety. Shoot elongation is achieved by cultivation in MS03 medium, i.e., MS-based medium supplemented with benzylaminopurine (BAP). Finally, rooting is achieved by culturing these shoots in a woody plant medium (WPM) [18] supplemented with 1-naphthaleneacetic acid (NAA) and kinetin (WPM rooting medium). Regarding the efficiencies, variable and acceptable values have been obtained depending on the starting explant and the variety assayed, for instance, whereas 'Larry Ann' hypocotyl sections reach about 3%, epicotyl segments of the same genotype have shown 17%.

2.2 Regeneration in *Prunus avium*

Very few reports have successfully achieved regeneration, and first procedures used expanded leaves of commercial varieties, including 'Lapins', 'Burlat', 'Napoleon', and 'Sweetheart' [19, 20]. These works used WPM supplemented with the growth regulators NAA, BAP, and TDZ. Later, improved regeneration efficiencies were described for 'Schneiders', 'Sweetheart', 'Starking Hardy Giant', 'Kordia', and 'Regina' using leaf and nodal segments, which were achieved by the combined use of WPM plus Driver and Kuniyuki medium (DKW) [21] or applying Quoirin and Lepoivre medium (QL) [22]. As best growth regulators, these experiments included TDZ and IBA. Using a different approach, mature cotyledons cultured in solid QL medium bound to a 10-day darkness incubation regime led to the genotypes 'Vista', 'Sunburst', 'Tehranivee', 'Vouge', and 'Heidelfingen' to be regenerated [23]. That shooting capability was lost by some varieties when darkness was eliminated.

Better results have been reported in other commercially important genotypes, including sour cherry (*P. cerasus* L.) [7, 24], black cherry (*P. serotina* Ehrh.) [25], and the cherry rootstocks 'Rosa' (*P. subhirtella* autumno) [26], 'Gisela 6' (*P. cerasus* x *P. canescens*) [7], 'Colt' (*P. avium* x *P. pseudocerasus*) [27]. In general, these protocols have cultured leaf explants in media, such as QL plus BAP, and WPM plus BAP and IBA, to produce whole plants in a 4–6 month period.

Based on those previous descriptions and considering our results in *P. salicina* regeneration, we developed a consistent procedure for sweet cherry varieties, such as 'Bing', 'Van', and 'Lapins'. The system is based on the use of epicotyl segments, which are first co-cultured with *Agrobacterium* (mostly strain GV3101) in MP2 medium (LP-derived medium supplemented with picloram) in darkness for 2–3 days. Subsequently, explants are transferred to B15-medium for budding induction; B15 medium is also based on LP micro- and macro-nutrients, supplemented with the growth regulators TDZ and 6-γ,γ-dimethylallylaminopurine (2iP). Shooting elongation is then achieved in D03 medium (DKW-based medium supplemented with BAP and IBA), first culturing the explants in Petri dishes and then using jars. Finally, elongated shoots are rooted in WPM medium (same as in plum regeneration) (**Figure 2A–D**). Observed efficiencies in this system reach about 4% (4.33% and 3.75% for 'Lapins' and 'Bing', respectively). Hypocotyls have also proven to be responsive to these procedures, although efficiencies are considerably lower.

2.3 Regeneration in *Prunus persica*

Peach highlights as one of the more recalcitrant species regarding the *in vitro* regeneration process. Although the first descriptions for *in vitro* propagation were

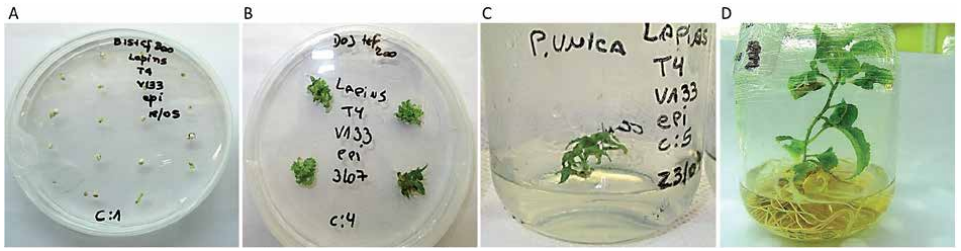

Figure 2.
In vitro sweet cherry (Prunus avium cv. Lapins) regeneration. After infection with A. tumefaciens GV3101, epicotyl explants are conducted to the regeneration procedures achieving whole plant formation between 6 and 8 months of experimentation. A, transformed explants in regeneration medium B15 for budding induction. B and C, early shootings transferred to D03 medium for elongation and development, first in Petri dishes and then in jars. D, elongated sweet cherry plantlets are transferred to WPM rooting medium.

described as early as 1982 [28], all the considerable research published to date pursuing the development of a reproducible regeneration system based on adult tissue as starting explants has shown this to be more complex than originally thought [2].

Considering those experiences and our own experimentation, we have been focused on improving efficiencies for the use of immature seeds as a source for explants [4], which in our hands represents the only reproducible procedure to achieve peach regeneration with a reasonable time frame. Since then, improvements on this pipeline have contributed to the generation of new individuals by an adequate protocol for the application of genetic engineering.

The protocol for peach regeneration using seed explants was formerly derived from descriptions found in the work by Gentile *et al.* [17], and it was initially applied on 'O'Henry' and 'Rich Lady' explants. One key point resulted in the use of immature cotyledons 70–90 days after bloom, which are cultured in LP modified medium. In general terms, after transformation and co-culture with *A. tumefaciens* strain GV3101, peach cotyledons are transferred to solid LP medium supplemented with BAP and NAA for callusing. Induced calli, usually observed after 3 to 6 months, are transferred to 03BAP-LP medium (LP medium supplemented with BAP and IBA) for budding induction. Shoot elongation is then achieved by transferring the shoots to CP2 medium (LP-derived medium supplemented with growth increased ratios of BAP/IBA). Finally, peach shoots are transferred to 03BAP-MP medium (LP medium using MS micronutrients plus growth regulators BAP and IBA) for elongation and rooting (**Figure 3A–D**). We have observed that embryo-derived explants from these immature fruits are also responsive to these treatments, and after infection, co-culture, and callusing in LP medium, explants must be cultured in 03BAP-MP medium.

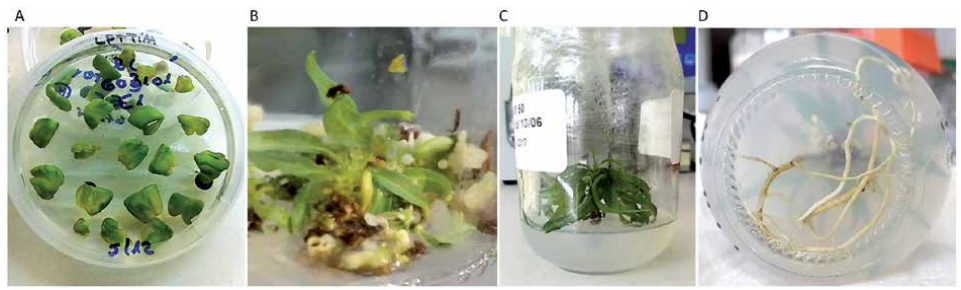

Figure 3.
In vitro peach (Prunus persica cv. Rich Lady) regeneration. After infection with A. tumefaciens GV3101, cotyledons are conducted to the regeneration procedures achieving whole plant formation after 8–12 months of experimentation. A, transformed explants are cultured in LP-derived medium for calli induction. B, Calli are then transferred to 03BAP-LP medium for budding. C, shoots are transferred to CP2 medium for elongation. D, shoots are transferred to 03BAP-MP medium achieving further elongation and rooting.

For rooting, plantlets regenerated from either cotyledon or embryo explants may need to be placed for 1 month in M1 medium (MS-derived medium supplemented with NAA); after this period, plantlets can be cultured back to 03BAP-MP medium. The procedure to achieve complete *in vitro* rooted plants can be as short as 8 months. However, longer times can be needed. In terms of regeneration efficiency, around 2% of embryo explants from 'Elegant Lady' can regenerate into plants. The regeneration rate for cotyledons in 'Elegant Lady' is in the region of 0.5–1%.

3. Genomes and CRISPR/Cas9 technology

3.1 Available genomes in *Prunus* and a CRISPR/Cas9 analysis tool for them

Prunus spp. belong to the *Rosaceae* family are among the most relevant groups in terms of genome drafts already available. The Genome Database for *Rosaceae* (GDR, https://www.rosaceae.org) provides public access to genomics, genetics, and breeding data for the family [29].

Peach was one of the first sequenced species in this group [30], and a reviewed version of the database (Peach v2.0) was recently made available [31]. The generated information was obtained from the doubled haploid cultivar 'Lovell', showing a relatively small genome size [265 Mb; diploid 2n = 16]. In addition to its short juvenile period (2–3 years) and self-pollination capacity, this condition makes this species a useful model for the *Rosaceae* family [32].

Genome assemblies for sweet cherry have been generated since 2017 for 'Satonishiki' [33], 'Karina' [34], and 'Big Star*' [35]. These works showed a slightly bigger genome size [353 Mb; diploid 2n = 16] compared to peach, although with a greater synteny between both genomes.

Just recently, a chromosome-level assembly for Japanese plum (*P. salicina*) has been described [36], revealing an intermediate genome size [284 Mb; diploid 2n = 16] relative to the other two species. Phylogenetic analysis showed *P. salicina* having a close relationship with the other two already sequenced species, *P. mume* [37] and *P. armeniaca* [38].

The availability of these genome drafts in *Prunus* enables advancement toward dedicated bioinformatics tools to carry out faster and safer analyses regarding the GE technology.

Targeted mutagenesis by CRISPR/Cas9 involves making guide RNAs (gRNAs) that target customized sequences in the genome of a cell to direct the Cas9 nuclease activity to generate double-strand DNA breaks close to the gRNA-joining location. Experimentally, this gRNA is a short synthetic RNA composed of a) a scaffold sequence that gives a secondary structure that is necessary for Cas9-recognition and assembly and b) a user-defined 20-nucleotide sequence (20-nt) spacer that determines the genomic target to be modified. Consequently, the target sequence recognized by the spacer will be a protospacer sequence contained in the target genome, located right next to a motif recognized by Cas9 for DNA cleavage, which is an NGG nucleotide arrangement called protospacer adjacent motif (PAM). Given its impact, the advent of CRISPR/Cas9 technology has led to the discovery and characterization of new nucleases recognizing different PAM motifs and even to Cas9 genetic engineering to develop modified enzyme activities [39].

Whereas the RNA scaffold sequence in the gRNA is operatively a regular, non-changing component in the system, the 20-nt spacer will be the key molecule to deduce from any available genome draft. As shown in **Figure 4**, from the genome information, it is possible to acquire the number of NGG motifs for each species, and from these data to consider the 20-nt adjacent to each one of these

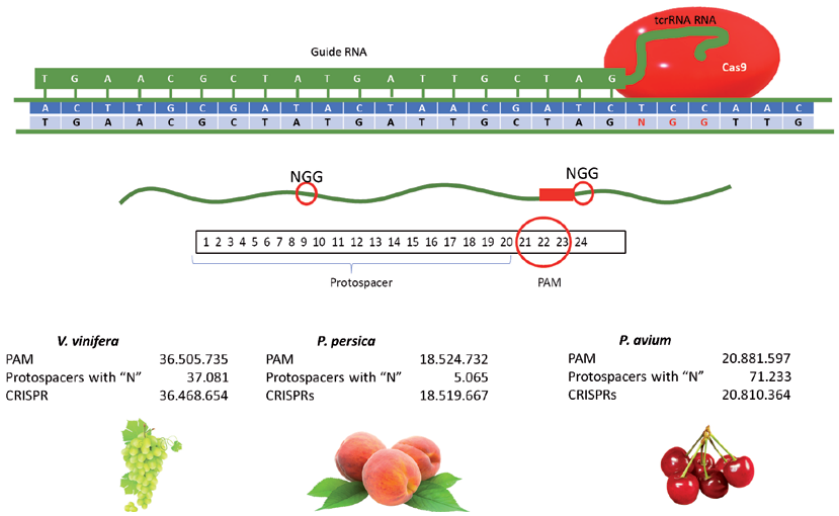

Figure 4.
Target sequences for the gRNA + Cas9 editing modules in some fruit tree crop species. Number of NGG motifs found in the sweet cherry and peach genome drafts from which data considering the 20-nt adjacent sequences are considered as all the putative CRISPR [gRNA + Cas9] recognition sites in each genome. Modules with unknown ("N") sequences are excluded from the CRISPR search database (see www.fruit/tree/genomics.com; "Biotools" bar). tcrRNA, trans CRISPR RNA.

motifs, creating a database for all the putative gRNA + Cas9 recognition site in each genome. This database will represent all the recognition sites for the CRISPR/Cas9 system in each genome and will allow us to predict both the on-target as the possible off-target sequences. As known for most of the genome information, several drafts still need to be confirmed due to sequencing errors or uncertainness and that information is filled with unknown nucleotides ("N"), information that will be removed from its corresponding protospacer databases.

Due to the probability of having nucleotide mismatches at the time of recognition between the gRNA and the protospacer, we can expand the 20-nt + PAM databases to consider the occurrence of mismatches, starting from one mismatch to as many pairing errors as we want to analyze. These expanded databases will increase the number of candidate off-targets in a particular genome, improving the predictive power of our *ex-ante* analysis for the right gRNA selection.

3.2 Vectors for gene editing

Despite its novelty, the delivery to the cell of CRISPR/Cas9 relies on regular and efficient gene transfer technology. The status of gene transfer procedures in tree fruit crops has been recently reviewed, including GE [5]. That work stressed the additional problems in using GE technologies in these species, considering their clonally propagated nature. In fact, the use of stable transformation in these species for gRNA + Cas9 delivery into the cell (explants) is not suitable because, unlike annual crops for which the transgenes can eventually be bred out after the mission of GE is achieved, breeding out these transgene sequences is a difficult, labor-intensive, and frequently time extensive process. Also, the use of crosses could lead to eliminating the identity and valuable traits of the variety.

For these reasons, efforts to improve the delivery of the editing components need to be considered in the case of fruit tree crops. One of the more convenient procedures to overcome these inconveniences has been the use of non-transgene-involved GE by using a gRNA-Cas9 ribonucleoprotein complex coupled to

protoplasts gene transfer and regeneration. This has been established as proof of concept in apple and grape cells with no final plant generation [40]. In this regard, massive use of this approach will depend on developing plant regeneration procedures from protoplast cells, which in tree fruit crops is quite limited [5].

Improvements in the delivery of GE tools have already been achieved by autonomously replicating viral vectors [41]. The single-stranded DNA (ssDNA) replicons from geminiviruses having been particularly useful. This group represents a large family of plant viruses with small (2.5–5.5 kb) genomes that replicate by rolling circle replication (RCR) in the plant cell nucleus. This process occurs through a double-stranded replication intermediate that also serves as a template for transcribing the viral open reading frames [42]. *Bean yellow dwarf virus* (BeYDV), the smallest member in the family, has been extensively explored as a molecular tool to improve gene expression by generating disarmed versions referred to as "LSL" (LIR-SIR-LIR) [43] or "deconstructed virus" vectors [44]. The three main elements from the virus replication machinery are retained in the LSL vectors, allowing virus replication by RCR to be emulated, thus enabling the transcriptional activation of the included expression cassettes. Two of these elements act in *cis*, the long and short intergenic regions (LIR and SIR, respectively) [45]. The SIR is the origin of replication for minus-strand synthesis and contains transcription termination signals. The LIR contains bidirectional promoter elements and provides a stem-loop structure, which is essential for initiating the RCR of the plus-strand [46]. The third element corresponds to the virus replication initiator protein (Rep/RepA), which acts in *trans* [47] and, therefore, must either be expressed by the viral replicon itself or be externally provided [43, 46].

In gene transfer experiments, the LSL vectors are activated by Rep's nicking function acting on the LIR components arranged in tandem in the delivered plasmid, which results in the replicative release of the recombinant viral DNA cloned between them [43]. The released DNA then replicates episomally in the nucleus, leading to the efficient expression of the encoded genes [48]. The shuttle capability of LSL vectors was demonstrated when T-DNAs released BeYDV-derived replicons in *Nicotiana benthamiana* cells, allowing for the expression of exogenes, including the green fluorescent protein (GFP) and human vaccine antigens for human papillomavirus and human immunodeficiency virus [49]. More recently, BeYDV-derived replicons have been used in T-DNAs in tobacco [41], tomato [47], and potato [50] to efficiently deliver plant genome editing machineries, such as zinc-finger nucleases, transcription activator-like effector nucleases, and CRISPR/Cas9.

3.3 Building a universal LSL-based vector for gene editing in plants

Considering our weakness in establishing protoplast systems in tree fruit crops, and particularly in *Prunus*, we decided to expand the geminivirus technology by designing and building general vectors that allow for efficient gene transfer experiments in these species that could lead to eventually edited individuals.

Formerly, we built a universal version of a BeYDV-derived LSL vector by assembling all the fragments containing the important components of a DNA replicon, as shown in (**Figure 5**, "*Agrobacterium* T-DNA + LSL components"). In this vector, named pGMV-Universal (pGMV-U), we incorporated all the elements required for both viral RCR replication and CRISPR/Cas9 gene editing. These include pGMV-U components arranged into multiple gRNA expression cassettes, allowing the individual expression of up to four gRNAs, the sequence comprising a SIR element, the coding sequence for Rep/RepA, and the LIR sequences adjacent to the right and left T-DNA borders, respectively. Finally, the vector included a Cas9 expression cassette (**Figure 5**, "pGMV-Universal vector"). Overall, a 15,657 bp vector for plant gene-transfer experiments was made (Addgene #112797).

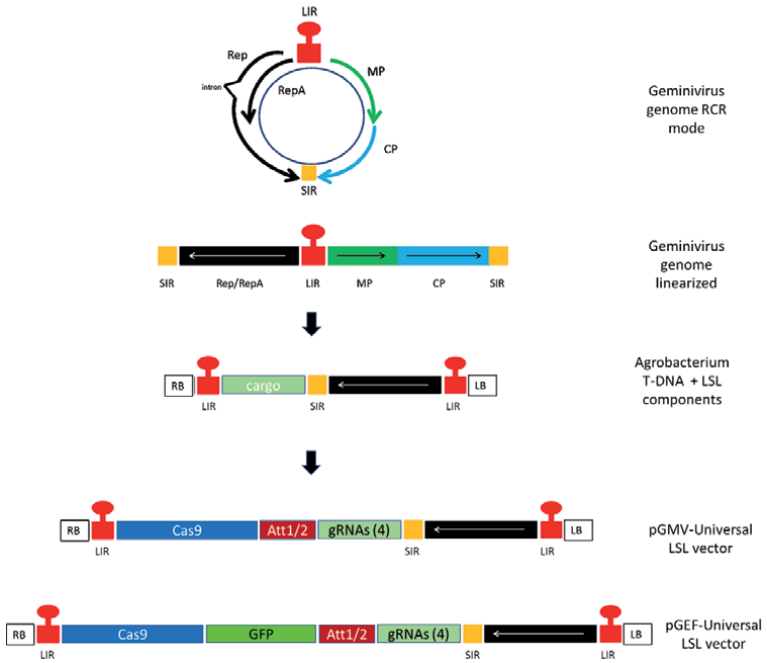

Figure 5.
Geminivirus-based vectors for plant gene editing. The genomic organization of geminiviruses is shown on top, including the circular and linearized versions (adapted from [51]). MP; movement protein, CP; coat protein, LIR and SIR; long and short intergenic regions; Rep/RepA, replication initiator protein. T-DNA-derived gene transfer vectors include key regulatory elements from these DNA viruses (Agrobacterium T-DNA + LSL components), a type of vector allowing for an important cloning capacity (cargo). Our first geminivirus-based vector (pGMV-Universal), based on BeYDV genome elements, consisted of the addition of multiple gRNA scaffolds that allow the expression of up to 4 different guide RNAs (gRNAs (4)) and an additional cloning site (Att 1/2). The second-generation (pGEF-Universal) contains the green fluorescent protein expression cassette (GFP).

In a second instance, considering that selection in the gene transfer process is a very limiting decision step in tree fruit regeneration processes, we improved pGMV-U into a traceable version of it by adding a GFP expression cassette (**Figure 5**, "pGEF-Universal LSL vector"). This vector has been named pGEF-U and retains all the previous functions for a geminivirus-based GE vector in plants.

4. Gene editing systems applied to regeneration protocols in Japanese plum, sweet cherry and peach

The regeneration protocols previously established for Japanese plum, sweet cherry, and peach allowed for the *Agrobacterium*-mediated delivery of the editing components through the pGMV-U and pGEF-U vectors.

The ability to replicate LSL vectors in the *Prunus* explants, a non-natural host for BeYDV, was first evaluated using an empty (i.e., with no gRNAs) version of pGEF-U, carrying out different time course analyses after gene transfer.

In *P. salicina*, our preliminary assays of transformation with *Agrobacterium tumefaciens* strain GV3101 carrying pGEF-U vector showed that both 'Larry Ann' and 'Angeleno' varieties responded well to infection. According to GFP expression detected 7 days post-infection (dpi), we observed that on all evaluated occasions, explants derived from both hypocotyls and epicotyls are infected at a rate greater than 90% for both varieties evaluated (**Figure 6A** and **B**).

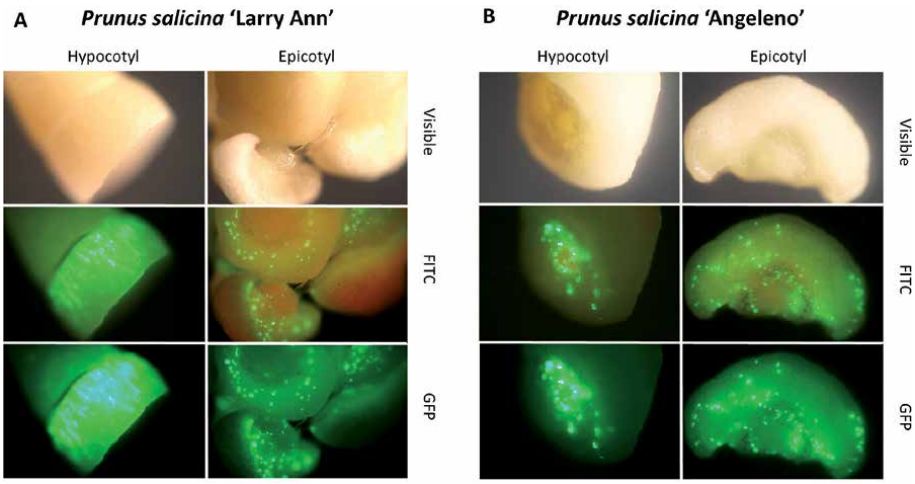

Figure 6.
Green fluorescent protein expression during gene transfer in Prunus salicina using the pGEF-U editing vector. Japanese plum explants were transformed with Agrobacterium tumefaciens strain GV3101 harboring the geminivirus-based vector, and GFP expression analyzed under epifluorescence microscopy at 7 days post-infection. A, P. salicina cv. Larry Ann; B, P. salicina cv. Angeleno. FITC, fluorescein isothiocyanate filter; GFP, green fluorescent protein filter.

In the same way, our preliminary assays of transformation with *Agrobacterium tumefaciens* strain GV3101 carrying pGEF-U vector evaluated in *P. avium* showed that both 'Lapins' and 'Bing' varieties also responded well to infection. According to GFP expression detected 7 dpi, we observed that explants derived from both hypocotyls and epicotyls are infected at a rate greater than 90% for both varieties evaluated (**Figure 7A** and **B**).

Evaluations of transformation with *Agrobacterium tumefaciens* strain GV3101 carrying pGEF-U vector carried out in *P. persica* showed that the three varieties assayed, 'Rich Lady', 'Elegant Lady', and 'Red Top', responded very well to infection,

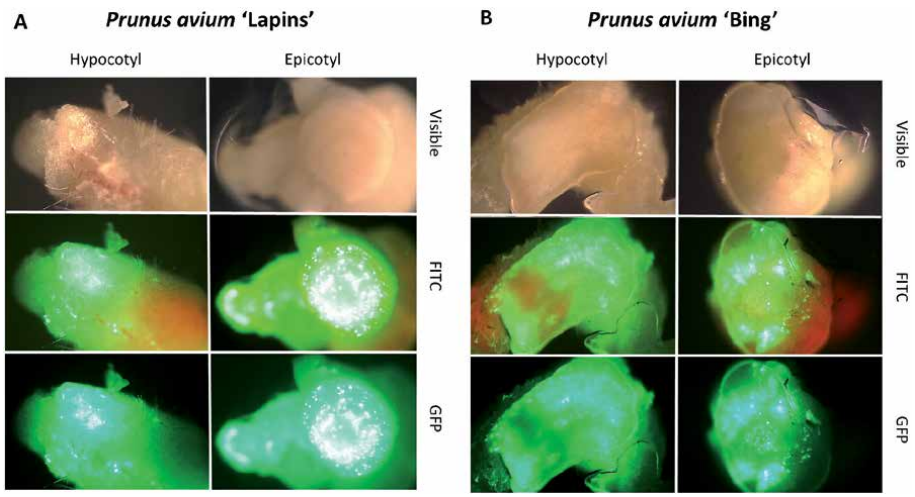

Figure 7.
Green fluorescent protein expression during gene transfer in Prunus avium using the pGEF-U editing vector. Sweet cherry epicotyls and hypocotyls are subjected to gene transfer experiments with Agrobacterium tumefaciens strain GV3101 harboring the geminivirus-based vector, and GFP expression analyzed under epifluorescence microscopy at 7 days post-infection. A, P. avium cv. Lapins. B, P. avium cv. Bing. FITC, fluorescein isothiocyanate filter; GFP, green fluorescent protein filter.

when we refer to explants derived from embryos, presenting an infection efficiency greater than 85% in all cases. The opposite occurs when cotyledon-derived explants are used, where we observed that, in general, the quantity and quality of the infection are quite variable. Whereas we have been able to obtain infections that reached 90% effectiveness (evaluated by GFP emission), these high rates in the gene transfer will depend on the proper physiological state when explants are collected, which takes place during a limited window of time after bloom (**Figure 8A–C**).

Tracking the GFP expression in these experiments allowed for analyses of pGEF-U at longer post-infection times. In that way, the interaction between explant cells and the vector beyond a stage of transient expression was observed over the regeneration protocols for these species. The longest GFP expression was observed in *P. salicina*, where it was detected even 42 dpi; nevertheless, this condition was observed in just a small number of the initial explants (**Figure 9**). In *P. avium*, the duration of GFP expression has a relatively shorter permanence (28 dpi) compared to Japanese plum and over a much shorter time in the tests carried out in *P. persica*, where GFP emission is detected only until day 21.

These results strongly suggested that our BeYDV-based vectors can successfully infect various types of *Prunus* spp. derived explants, and that they can generate multiple copies of DNA replicons that lead to the expression of GFP and the CRISPR/Cas9 system. Moreover, the screening of GFP expression during the proper time frame enables selecting the explants that bear the highest rates of infection, and consequently, are the most likely to generate successfully edited individuals for specific targets. In the coming sections, we will go over some preliminary results of our GE assays in *Prunus* spp. and the rationale behind our gene selection and experimental design.

4.1 An example: editing of the TERMINAL FLOWER 1 gene in peach

As perennial trees, *Prunus* spp. are grown in temperate climates. In the early fall season, the decrease in temperature and daylight hours promotes entry to the dormancy state, an evolutionary phenomenon relevant for survival during the adverse conditions in winter. In dormancy, certain requirements must be completed to resume growth. The first requirement corresponds to cold accumulation during winter (endo-dormancy) and then heat accumulation at the beginning of spring (eco-dormancy). After an adequate cold accumulation, flowering takes place.

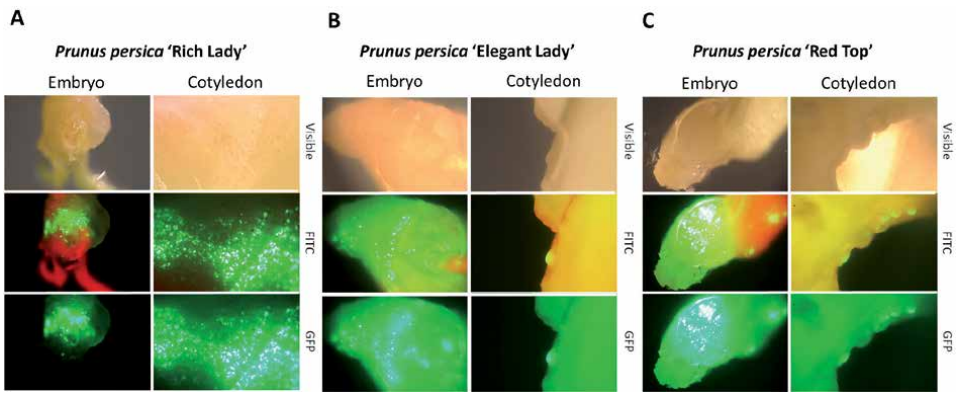

Figure 8.
Green fluorescent protein expression during gene transfer in Prunus persica using the pGEF-U editing vector. Immature peach embryos and cotyledons are subjected to gene transfer experiments with agrobacterium tumefaciens strain GV3101 harboring the geminivirus-based vector, and GFP expression analyzed under epifluorescence microscopy at 7 days post-infection. A, P. persica cv. Rich lady. B, P. persica cv. Elegant lady. C, P. persica cv. Red top. FITC, fluorescein isothiocyanate filter; GFP, green fluorescent protein filter.

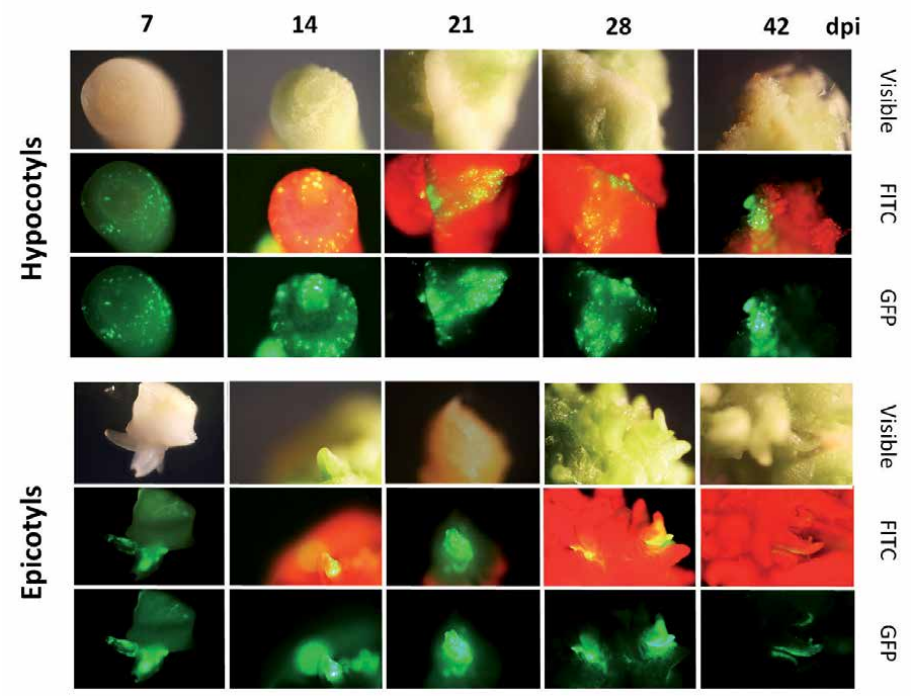

Figure 9.
Time-course analysis of the green fluorescent protein fluorescence from pGEF-U vector in Prunus salicina cv. Larry Ann transformed with Agrobacterium tumefaciens strain GV3101. FITC, fluorescein isothiocyanate filter; GFP, green fluorescent protein filter.

Plants continuously sense the environment (i.e., day and night conditions; seasonal cycles) to adapt their metabolism, growth, and development to these diverse conditions. These responses involve changes in gene expression programs to overcome a particular scenario until new conditions demand new responses. Diverse gene programs have been associated with dormancy and flowering signals and include cell cycle regulation, light perception, hormonal signaling, and stress response [52–54]. Two important genes participating at some level in these events are *FLOWERING LOCUS T (FT)* and *TERMINAL FLOWER* 1 (TFL1). Despite *FT* and *TFL1* share a high (~60%) amino acid sequence identity, they function in an opposite manner [55]. Whereas *FT* promotes the transition to reproductive development and flowering, *TFL1* represses this transition [56]. As already mentioned, transgenic European plum over-expressing the poplar *FT1* gene has led to altered dormancy requirement and continuous flowering, enabling what has been named "FasTrack" breeding technology [6]. On the other hand, the flowering repressive function of *TFL1* has been reported in many species of *Rosaceae*, including *Prunus* spp. [57–60]. In pear, silencing of *PcTFL1* and *PcTFL2* through RNAi resulted in individuals that started flowering as early as 4–6 months, with no adverse phenotypic effects [61]. Consequently, *TFL1* is an appealing candidate for producing fast-breeding trees through loss-of-function experiments in stone fruits using CRISPR/Cas9 edition.

4.1.1 Design and strategy

The *TFL1 locus* in *Prunus persica* comprises four exons and three introns and spans through 1.3 kb. We chose to simultaneously cleave two different located in exons 2 and 3, removing approximately half of the gene from the genome as a result (**Figure 10**).

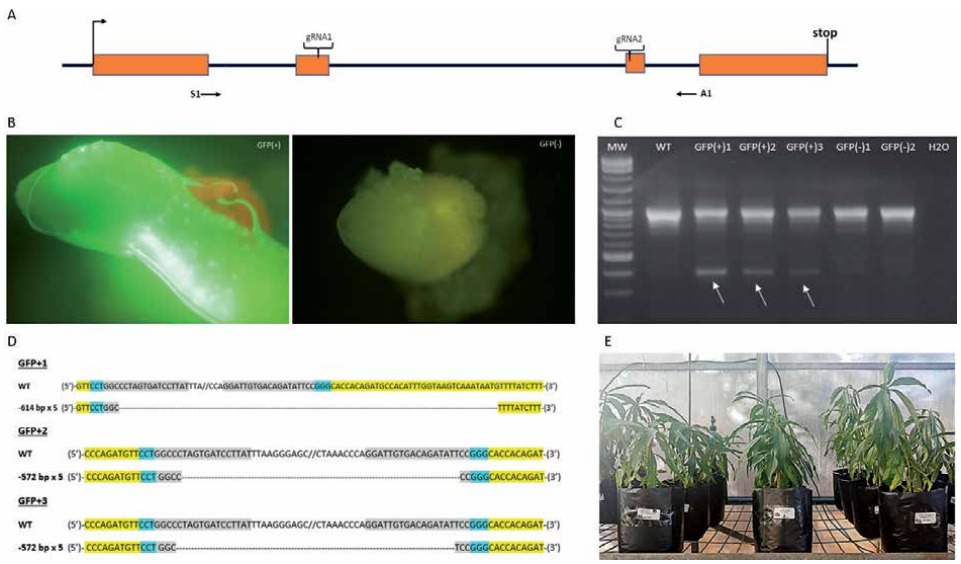

Figure 10.
CRISPR/Cas9 gene editing of the P. persica TFL1 gene. A, genomic structure of the locus (PRUPE_7G112600). Depicted are the UTR regions (black segments in the left and right ends), the translation starting site (arrow above the first orange rectangle), the exons (orange rectangles), introns (black segments between exons), and the translation stop site (stop). The approximate locations of the two gRNAs used to generate a 0.58 kb deletion (gRNA1, gRNA2) and the primers employed for deletion screening (S1 and A1) are also indicated. B, peach cotyledons were transformed with pGEF-TFL1 using agrobacterium tumefaciens strain AGL1; representative pictures of infected (GFP+) and uninfected (GFP-) embryo explants under an epifluorescence microscope 8 days post-infection. C, 1.5% agarose gel of PCR products from genomic DNAs derived from embryo explants transformed with pGEF-TFL1, 28 days post-infection. MW: Molecular weight ladder. GFP+: Embryo explants positive for GFP expression. WT: Untransformed, wild-type embryos. GFP-: Embryo explants negative for GFP expression. H2O: no DNA control. Amplicons corresponding to the expected size of the edited products are signaled with white arrows. D, sequencing analysis of amplicons derived from embryo explants infected with pGEF-TFL1. E, acclimatized individuals whose genotypic and phenotypic characteristics are under study.

Guide gRNAs were selected using the CRISPR-Search tool (freely available at https://www.fruit-tree-genomics.com/). This tool, generated by our group, searches for the best pair combinations of gRNAs to induce the deletion of a defined target area within a genomic sequence. As mentioned, one of the main characteristics of our pGMV-U and pGEF-U vectors is their cargo capability for up to four different gRNAs. Thus, both chosen gRNAs targeting *TFL1* were cloned into a single vector, named pGEF-TFL1. The "loss of a large gene fragment" approach has two distinct advantages: first, it allows us to maximize the chances of successfully inactivating our target genes by avoiding silent mutations, and second, it enables the screening of deletions through a straightforward PCR assay (**Figure 10A** and **C**).

We show results from the molecular analysis of embryo explants from *Prunus persica* transformed with the geminivirus-based GFP-Cas9 double gRNA vector pGEF-TFL1 (**Figure 10B**). Twenty-eight days post-infection, infected and uninfected embryo explants were processed for genomic DNA purification. DNA samples were analyzed through PCR, using primers located upstream from gRNA1 and downstream from gRNA2 (**Figure 10A**). As a result, we found an array of unedited and edited amplicons: the bigger PCR products, with a size around 950 bp, corresponding to the unedited form of the *PpTFL1* gene (**Figure 10C**). The second group, comprised of shorter amplicons with varying sizes close to 380 bp, originated from the deletion of the DNA segment between the two gRNAs. The cloning and sequencing of these amplicons confirmed the successful edition of *PpTFL1* (**Figure 10D**). These explants have already regenerated into several new individuals (**Figure 10E**) whose genotypic and phenotypic characteristics remain to be assessed.

5. Conclusions

The traditional breeding strategies mainly rely on natural genetic or induced variability for the incorporation of desired traits into crops. Individuals with interesting traits are selected as parental, and through controlled crosses, siblings with a combined genetic pool are obtained. Intense breeding programs based on the use of a narrow number of parentals have resulted in a considerable loss of genetic diversity. Also, old and newly released cultivars sometimes do not adapt well to the distinct environments in a demanding context characterized by global climate change.

We live today in a fantastic time of massive and increasing knowledge. In the last decade, an enormous amount of information has been made available from massive sequencing technologies in plants. This progress, fused to the biotechnological approaches based on RNAi and, more recently, CRISPR/Cas9 GE, has opened new alternatives in breeding and represent additional and available tools. Due to its commercial relevance and biological properties, the *Prunus* genus is part of these developments.

Today, we have genome drafts for diverse species in *Prunus*, which has opened an enormous opportunity to apply new breeding techniques in the genus. Ten years ago, the European and Japanese plums were successful examples in the genus, and these advances allowed for the current knowledge and possible expansion of tissue culture techniques to other species. As we show here, the need for consistent technical procedures in *Prunus* regeneration is a starting point for applying this "precision breeding" approach. It is also relevant to consider that few successful steps forward in the area are indeed huge advances. For instance, the generation of genetically engineered individuals – beyond a "proof of concept" – can also be considered potential parentals for breeding. This is also relevant when rootstock technology, based on these developments, can be projected.

The complementarity between RNAi and GE seems evident today. Whereas candidate gene functions have been clarified by the first, efforts in generating non-transgenic gene-edited individuals results are encouraged by those findings. In this regard, the strength of counting with reviewed genome drafts in these species is a requisite. It will be interesting to see how, in the future, the ever-increasing amount of genetic information will allow us to identify new targets for GE and perhaps "export" specific traits of interest from one cultivar to another in a straightforward manner, greatly speeding up the traditional breeding process.

As shown, our weaknesses in tissue culture systems have been overcome by improving the delivery procedures of the editing tools. We have built LSL-derived vectors to increase our relatively low regeneration efficiencies, opening a possibility of using regular multicellular explants in the generation of eventual edited non-transgenic individuals. Also, tracking of the processes during tissue culture has resulted in vital relevance, allowing us to prioritize explants in which the editing process is taking place.

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Section 2

Physiological and Nutritional Studies on Prunus Species

Chapter 6

Stock Influence on Growth, Morphological and Biochemical Leaf Parameters *Prunus domestica* L.

Svetlana Motyleva, Galina Upadysheva, Tatyana Tumaeva and Ivan Kulikov

Abstract

Vegetation growth peculiarities and morphological and physical-biochemical features of *Prunus domestica* L. Utro and Yaichnaya Sinyaya varieties leaves grafted on different growing strength rootstocks were studied. Low-growing clonal rootstocks 140–1 and Novinka decreased the trees growing strength on 15–20% in comparison with strong-growing stocks; medium-growing rootstocks OPA-15-2 and OP-23-23 reduced it on 10%. The longest growing activity and the largest sprouts length was stated on these rootstocks as well, i.e. 1.3–1.4 times more than on other ones. Stable sprouts average length decrease was registered on grafted stocks 140–1 and Novinka. Leaf surface index value on the trees grafted on clonal rootstocks OPA-15-2 and OP-23-23 was on 40% higher than on control, i.e. 4.3 leaves m2/crown projection area m2. Optimal values of total increment, sprouts average length, leaves area and the largest part of physiological-biochemical parameters were stated at medium-growing clonal rootstocks OPA-15-2 and OP-23-23 use. Plum leaves blades were hypostomatic; numerous stomata were located on the abaxial (bottom) side of leaves. Stomata were located in interveinal space irregularly. Stomata length size varied from 14.6 μm (Utro/seedlings) to 22.1 μm (Yaichnaya Sinyaya/OP-23-23). The rootstock has influence on the process of photosynthesis, antioxidant activity, accumulation of minerals and metabolic answerin the leaves.

Keywords: *Prunus domestica* L., scion-stock combinations, growth and development, leaf, scanning electronic microscopy, physiological parameters

1. Introduction

The popularity of domestic plum (*Prunus domestica* L.) in different horticultural zones of Russia is connected with ecological plasticity, winter resistance, early maturity and stable productivity of cultivated varieties [1, 2]. New clonal stocks are used to duplicate new valuable varieties of fruit crops and to create intensive plantations because they favorably influence adaptivity, early maturity and productivity of grafted plants [3, 4]. The spread, the start of fruiting and the productivity of grafted plants depends on the rootstock. Seedling rootstocks (seedlings of *Prunus domestica* L. and *Prunus cerasifera* Ehrh.) and clonal ones of different growing spread, i.e. strong-growing – 13-113, medium-growing – OPA-15-2, OP-23-23,

SVG-11-19 and low-growing – 140-1, Novinka, VVA-1 are used for plums cultivation in the Central region of Russia [5].

Many questions devoted to plum varieties propagation and scion-stock combination selection are successfully solved in the world; at the same time, the life length of grafted plants and the harvest quality depending on a variety and a rootstock are not studied enough. The major part of the researches in this branch of study were held by [6–8], who found out that photosynthesis intensity varies depending on a variety and scion-stock combination. The influence of rootstock on the quality of plum fruits is shown in the works [9, 10].

This present study was planned to analyze the least studied morphological and biochemical characteristics, i.e. – growing activity and the sprouts length, lea fsurface index on the trees, Stock influence on leaf morphological features and parameters, on photosynthetic pigments synthesis, on antioxidant activity and phenol compounds sum accumulation in plum leaves, on ash composition and on *Prunus domestica* L. leaves metabolic answer.

2. Studies results

2.1 Studies place, objects and methods

The field researches were held in 2018–2020 on the experimental *Prunus domestica* L. plantations, located at laboratory plot of Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery (FHR CBAN) in Moscow region (55° 56′ of North latitude, 37° 64′ East longitude). The garden was planted in 2010. The plantation overall area was 0.5 ha. The garden of intensive type was set out using the scheme 5 x 2.5 m. The soil in the row spacing was agrogenic (**Figure 1**).

The leaves of *Prunus domestica* L. Utro and Yaichnaya Sinyaya varieties on 5 stocks, i.e. seedlings *Prunus domestica* L., Novinka (*Prunus bessyi* L.H. Bailey x *Prunus ussuriensis* Kovalev&Kostina), OP-23-23 (*Prunus pumila* L. x *Prunus salicina* L. x *Prunus persica* Stokes), OPA-15-2 (*Prunus pumila* L. x *Prunus salicina* L. x

Figure 1.
Plum plantations: 1-blossoming garden, row spacing – Agrogenic soil.

Prunus cerasifera Ehrh.) and 140–1 (*Prunus bessyi* L.H. Bailey x *Aflatunia ilmifolia*) were the object of the scientific studies, as well as the understudies rootstocks leaves. The leaves samples were taken from the medium part of the formed one-year-old sprouts (the beginning – mid July).

The biochemical researches were held in the Laboratory of Biochemistry and Physiology of FHR CBAN.

The understudied parameters included field registration (the trees growth, the crown volume, the total sprouts increment, leaf surface area) and the leaves laboratory studies (comparative micromorphology of the leaf adaxial and abaxial sides, stomata number and size, photosynthetic pigments content, antioxidant activity, phenolic compounds sum, ash composition and quality content of the leaves main metabolites). The leaves microsculpture and ash composition were determined on analytical REM JEOL JSM – 6010 LA (JEOL Ltd., Japan). Photosynthetic pigments Chl a and b and total carotenoids (Car) were studied on spectrophotometer Helios ϒ UV–vis (USA) in accordance with the method [11], total phenolic amount was determined with Folin–Ciocalteu reagent in accordance with the method [12] and total antioxidant capacity the scavenging activity for the 2,2-dipheny l-1-picrylhydrazyl (DPPH) radical was determined in accordance with the method [13].

Metabolites quality composition, contained in plum leaf extracts was analyzed on JEOL JMS-Q1050GC (JEOL Ltd., Japan) via the method of gas chromate-mass-spectrometry in accordance with the method [14].

2.2 Stock influence on growth parameters

Stock influence on trees growth parameters were studied using tree height, crown volume, one-year-old sprout length, leaf surface area. Utro variety 8-year-old tree height was within the range of 2.8 m (140–1) – 3.2 m (seedlings). Yaichnaya Sinyaya variety height differences determined by stock were 0.6 m, and height varied from 3.1.m (140–01) to 3.7 m (seedlings). In comparison with strong-growing seedlings low-growing rootstocks 140–1 and Novinka reduced tree growing strength on 15–20%, medium-growing stocks OPA-15-2 and OP-23-23 – on 10%.

Depending on a rootstock, plum trees crown volume was within the range of 11.5 m3 (Yaichnaya Sinyaya/140–1) – 14 m3 (Yaichnaya Sinyaya/OP-23-23). Utro variety crown volume varies insignificantly on different rootstocks (12–13 m3).

Plum trees growing process intensity are characterized by active sprout growth duration and total one-year-old sprouts length. Domesticated plum active growth duration was from 32 days (Utro variety) to 40 days (Yaichnaya Sinyaya variety), and depending on a rootstock the fluctuations were 8–11 days. The longest active growth duration was registered on 140–1 and OP-23-23 rootstocks.

Average sprouts length that characterizes tree general state was within wide ranges, i.e. from 9.0 cm (Utro/Novinka) to 22.8 cm (Yaichnay Sinyaya/OP-23-23) and was 25.0 cm at average. At Yaichnaya Sinyaya variety this parameter was stable depending on a rootstock (18–22 cm) and 1.5 times higher than at Utro variety (**Figure 2**).

Stable reduction of average sprouts length was stated on grafted 140–1 and Novinka rootstocks. On OPA-15-2 and OP-23-23 rootstocks this parameter was 1.3–1.4 times higher (**Figure 3**).

The main indicator of growing processes activity is total sprouts length. This parameter was significantly less at the trees grafted on clonal rootstocks, i.e. 140–1 (110 m) and Novinka (120 m). On medium-growing OPA-15-2 and OP-23-23 sprouts buildup was 170–180 m and 1.2 times bigger than on seedlings.

The main indicator that characterizes plum tree crown leaf coverage level is leaf surface area. The size of the leaves varied significantly depending on the scion-rootstock combination (**Figure 3**).

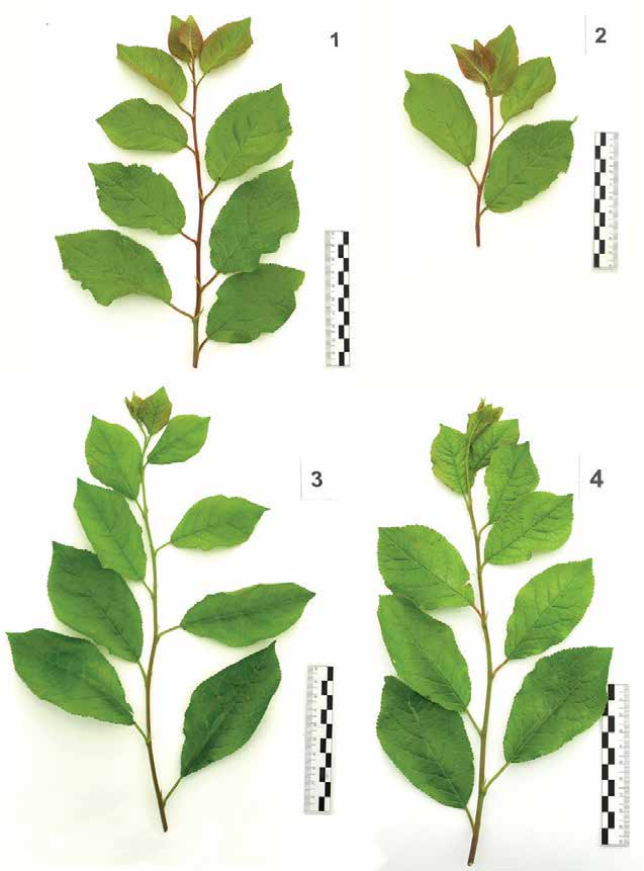

Figure 2.
Length of Prunus domestica *L. shoots depending on the rootstock.*

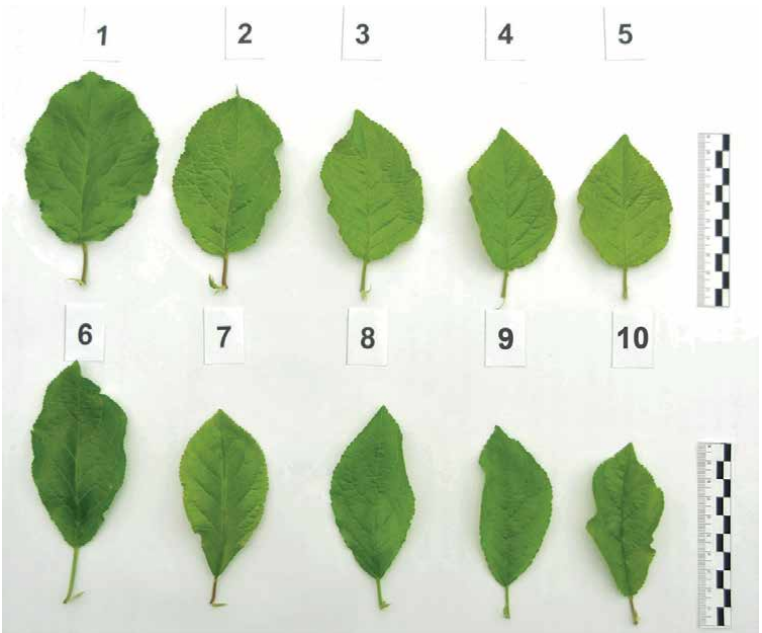

Figure 3.
The size of the leaves of Prunus domestica *L. depending on the rootstock.*

Leaves area was within the range of 21.4 m^2/tr. (Yaichnaya Sinyaya/seedlings) – 36.2 m2/tr. (Utro/OPA-15-2). At Utro variety the differences between low and strong-growing rootstocks were 9–11 m2/tr., and at Yaichnaya Sinyaya variety – 6-8 m^2/tr. More higher values at that were closer to physiological optimum. i.e. more than 30 m2/tr., were registered on grafted OPA-15-2 and OP-23-23 rootstocks. Less assimilation apparatus was formed on low-growing 140–1 rootstock than on control.

Plum trees leaf surface index depending on a combination was within the range of 4.3–7.2 leaves m2/crown projection area m2. Leaf surface index maximum values, i.e. 7.2 leaves m2/crown projection area m2, were registered at scion-stock combinations Utro/OPA-15-2 and Yauchnaya Sinyaya/OP-23-23. Leaf surface index value was on 40% at the trees grafted on OPA-15-2 and OP-23-23 rootstocks than on control, i.e. 4.3 leaves m2/crown projection area m2.

The main indicators of domesticated plum trees vegetative productivity, i.e. crown volume, total sprouts buildup, leaf surface area, depended not only on a variety, but also on a rootstock. Optimal values of total buildup, average sprouts length, leaves area were registered on medium-growing clonal OPA-15-2 and OP–23–23 rootstocks.

2.3 Stock influence on leaf morphological features and parameters

Morphological differences of various varieties leaves were studied using scanning electron microscopy (SEM). Adaxial epidermis consists of thick cells layer. Their surface is covered with firm cuticle with numerous folds or in the form of long stripes and colpi, microsculptural differences are presented in **Figure 4**. Plum leaves blades were hypostomatic; numerous stomata were located on the abaxial (bottom) side of leaves (**Figure 5**). Grafted plants leaves, as a rule, had well-developed rollers around stomata. Stomata were located in interveinal space irregularly. Stomata length size varied from 16.42 μm (Utro/seedlings) to 22.11 μm (Yaichnaya Sinyaya/OP-23-23). It is typical that the ratio of stomata length to their width, at variety-stock combinations was larger than at a rootstock. Such regularity was stated for all variety-stock combinations. Stomata density varied significantly from 436 ± 9

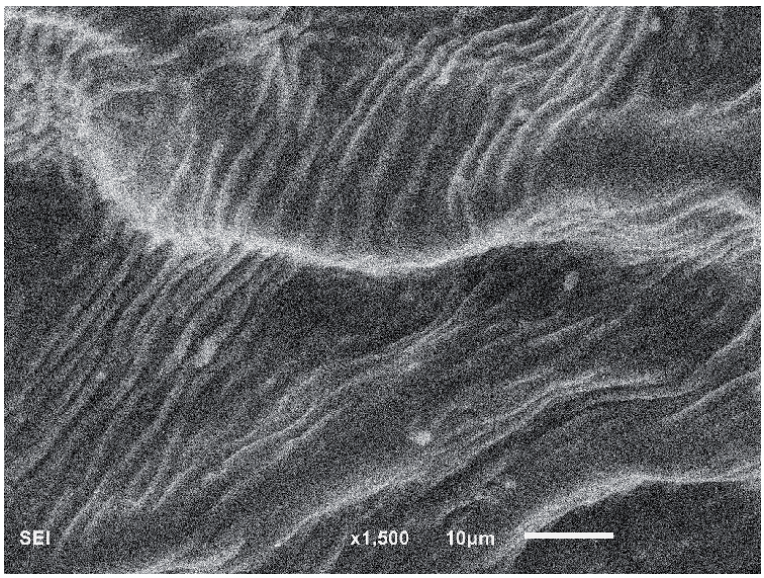

Figure 4.
Prunus domestica *L. leaf adaxial side.*

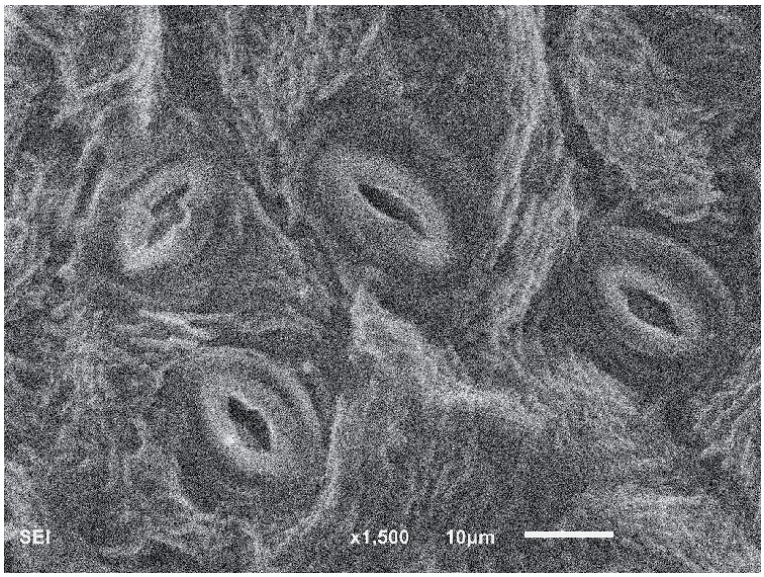

Figure 5.
Prunus domestica *L. leaf abaxial side.*

(Yaichnaya Sinyaya/OP-23-23) to 1000 ± 17 (Utro/seedlings) stomata on mm^2 at. Variation of mean indicators that characterize stomata and the density of stomata location on leaf surface are shown in **Table 1** using the example of the Utro variety.

2.4 Stock influence on photosynthetic pigments synthesis

Chlorophylls Chl a and Chl b content is one of the main indicators of domesticated plum trees vegetative productivity. Chl a content is 3–4 times higher than Chl b one at average and varies from 4.12 mg/ml (seedlings) to 13.71 mg/ml (OP-23-23). Chl a + Chl b sum content increase and the highest ratio Chl a/b were registered in Yaichnaya Sinyaya and Utro leaves on OPA-15-2 rootstocks and OP-23-23, that lead to higher intensity of photosynthesis process (**Figure 6**). The highest carotenoids content was registered in OP-23-23 rootstock leaf extracts (1.26 mg/ml), Utro/Novinka (0.9 mg/ml), Utro/OP-23-23 (0.79 mg/ml) and Yaichnaya Sinyaya (0.75 mg/ml) combinations (**Figure 7**). Consequently, photosynthetic pigments synthesis depended not only on a genotype, but also on a used rootstock.

2.5 Stock influence on antioxidant activity and phenol compounds sum accumulation in plum leaves

The capacity of plum leaf extracts to scavenge DPPH+ free radicals, which has been used as a measure of total antioxidant capacity (AA), and total phenolic

Stick-stok kombination	Number of stomata/mm^2	Stomata length	Stomata width	Stomatal index
Utro/seedlings	1000 ± 17	17.59 ± 0.14	9.57 ± 1.11	1.8
Utro/Novinka	800 ± 13	19.22 ± 1.24	11.23 ± 1.11	1.7
Utro/140–1	689 ± 21	16.42 ± 2.11	8.32 ± 1.14	1.9
Utro/OP–23–23	556 ± 22	18.26 ± 2.11	8.94 ± 0.85	2.0

Table 1.
Variation of mean indicators that characterize stomata and the density of stomata location on leaf surface.

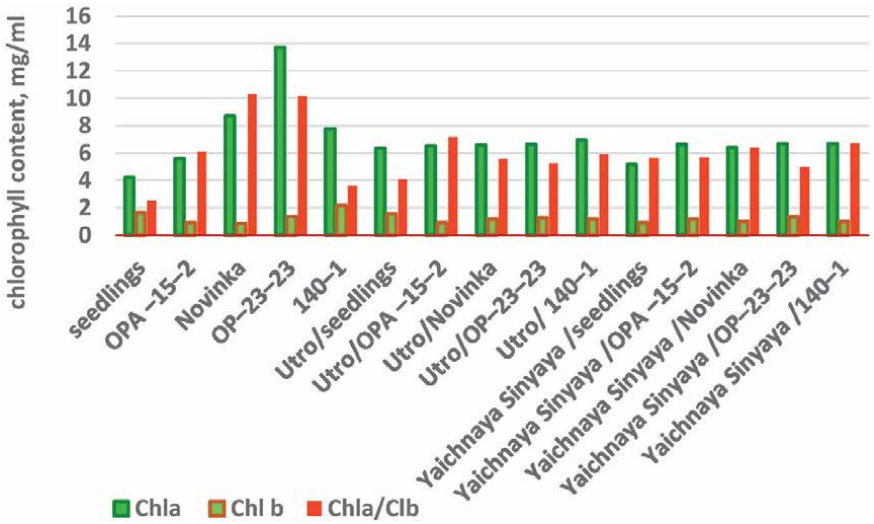

Figure 6.
Chlorophylls Chl a and Chl b and ratio Chl a/Chl content in the leaves of Prunus domestica rootstocks and scion-stock combinations.

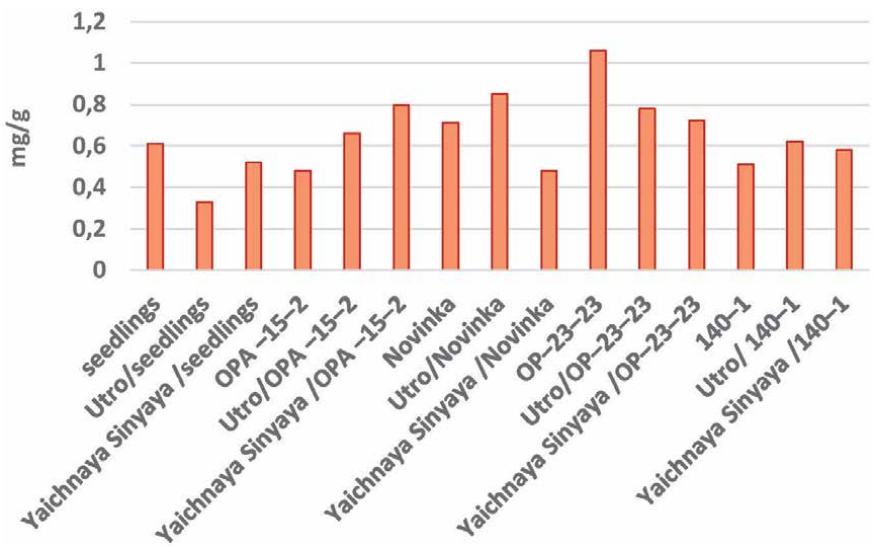

Figure 7.
Carotenoids content in the leaves of Prunus domestica *rootstocks and scion-stock combinations.*

content (TPC) are shown in **Table 2**. AA speaks about the presence of biologically active substances-antioxidants that are synthesized in plum leaves. Average AAA and AAM of ethanol extracts was 88.7%; average AA of water extracts was 73.8%. The maximum AAA (90.72%) and AAM (92.12%)values were registered in the leaves of Yaichnaya Sinyaya/Novinka combination; the minimal values were stated in the leaves of strong-growing *P. domestica* L. seedlings AAA (88.14%). Among the rootstocks the maximum AA values were marked in the leaves of Novinla and OP–23–23. The leaves of scion-stock combinations on these rootstocks showed high AAA and AAM values in comparison with other combinations. Consequently, a rootstock has influence on the synthesis of substances-antioxidants in the scion leaves. TPC in all the scion-stock combinations leaves is higher than in rootstocks leaves on 3–12 mg/g. The comparison of ethanol leaf extracts spectra of rootstocks

Samples	Determined indicators		
	AAA	AAM	TPS
Strong-growing *P. domestica* L. seedlings	88.14 ± 1.14	57.08 ± 1.11	13.68 ± 0.24
Yaichnaya Sinyaya/strong-growing *P. domestica* L. seedlings	89.76 ± 2.01	68.11 ± 1.21	16.48 ± 0.31
OPA–15–2	89.71 ± 1.14	79.15 ± 1.08	6.23 ± 0.21
Yaichnaya Sinyaya/OPA–15–2	88.55 ± 2.11	47.61 ± 0.89	11.42 ± 0.22
Novinka	90.72 ± 1.46	82.38 ± 1.24	9.83 ± 0.11
Yaichnaya Sinyaya/Novinka	88.51 ± 1.34	79.19 ± 2.01	14.63 ± 0.21
OP–23–23	90.55 ± 1.51	75.77 ± 2.12	9.19 ± 0.31
Yaichnaya Sinyaya/OP–23–23	90.72 ± 1.34	92.12 ± 2.13	14.51 ± 0.42
140–1	89.71 ± 1.24	79.31 ± 1.06	14.93 ± 0.61
Yaichnaya Sinyaya/140–1	89.36 ± 1.40	77.35 ± 1.42	23.48 ± 0.42

Table 2.
The antioxidant activity of water (AAA) and methanol (AAM) extracts, expressed in % and the total content of polyphenols (TPC), expressed in mg equivalent of gallic acid (mg/g TW) in the leaves of Prunus domestica *L.*

and scion-stock combinations was fulfilled in the range of 350–700 nm. The spectra profiles of Yaichnaya Sinyaya/seedlings, Yaichnaya Sinyaya/Novinka and Yaichnaya Sinyaya/OP-23-23 combinations are lower in the understudied wavelength range than the rootstocks profiles. The leaf extracts spectrum of Yaichnaya Sinyaya/ OPA-15-2 combination in 200–350 nm range significantly varies from OPA-15-2 rootstock profile that speaks about absorbent substances presence in this spectrum region (**Figure 8**).

2.6 Stock influence on plum leaves ash composition

12 ash elements, i.e. P, S, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, were studied. The decreasing row of the elements content in the plum leaves ash is the following: K > Ca > Co > Mg > P ≈ S > Cu ≈ Zn > Fe > Mo > Cr ≈ Ni.

The principal proportion of the leaves ash composition was K, which was accumulated up to 25 mass % and Ca up to 10 mass % in ash (**Figure 9**). The maximum content of K is noted in the leaves of Yaichnaya Sinyaya and Novinka on OP-23-23

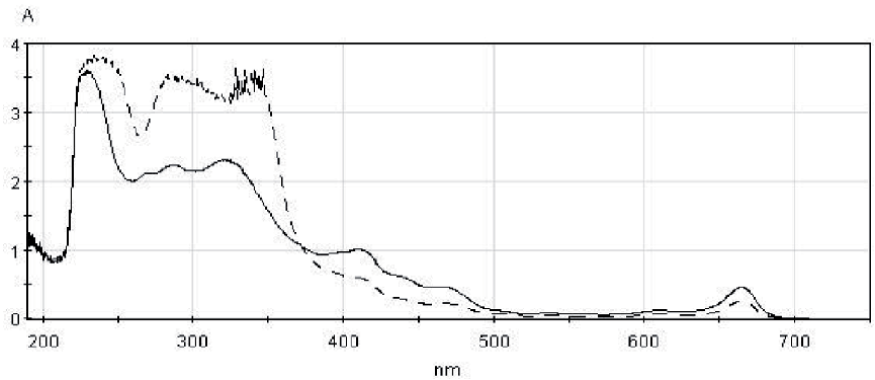

Figure 8.
Comparative spectra of Prunus domestica seedlings and Yaichnaya Sinyaya scion-stock combinations leaf extracts (____ - OPA–15–2; - - - - - Yaichnaya Sinyaya/OPA–15–2).

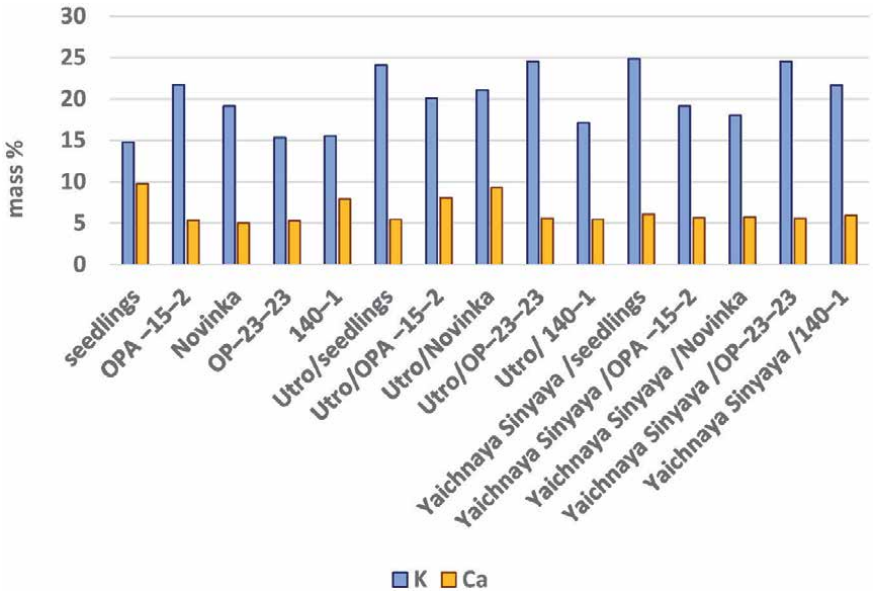

Figure 9.
The content of K and Ca in the leaves Prunus domestica *L.*

combination; the minimal values were stated in the leaves of strong-growing *P. domestica* L. seedlings, OP-23-23 and 140–1. The scientific literature shows that K is very mobile in the plants, and it is known that K plays the role of osmotic agent in the stomata opening and closing processes. Ca is found in cell walls in the form of calcium pectate which affects cell walls elasticity. The important role of these ash elements was also noted in plants adaptive processes [15, 16]. Co is involved in the processes of nitrogen uptake by plants. The maximum content of Co is noted in the leaves of Yaichnaya Sinyaya on of strong – growing *P. domestica* L. combination and Novinka; the minimal values were stated in the leaves of Utro on of OP-23-23 and Novinka combination. Mn is a part of the chlorophyll molecule and is involved

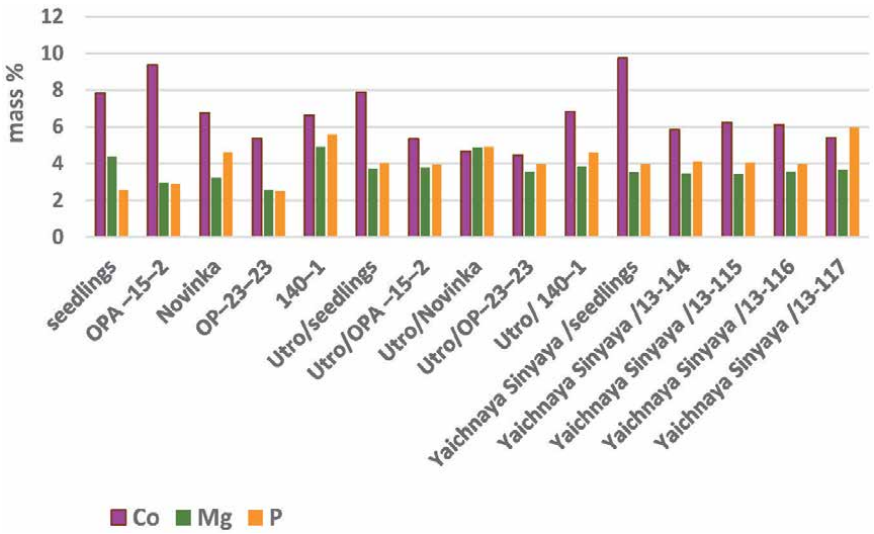

Figure 10.
The content of Co, Mg and P in the leaves Prunus domestica *L.*

in a number of enzyme systems. P is found in phospholipids and nucleoproteins; macro-energy connections between phosphate groups and serves as the main energy transfer agent in plants. The content of Mg and P ranges from 2.3 mass % (OP–23–23) to 4.8–5.8 mass % (140–1), **Figure 10**. The highest content of Cu, Zn and Fe in plum leaves was noted in the combination Utro/140–1 (**Figure 11**).

The certain oligo-elements Mo and Ni weare contained with in the range of from 1.2 (140–1) to 3.2 (Utro/OP–23–23) mass % – Cu and Ni from 2.1 (Yaichnaya Sinyaya/140–1) to 5.6 (Utro/140–1) mass % (**Figure 12**).

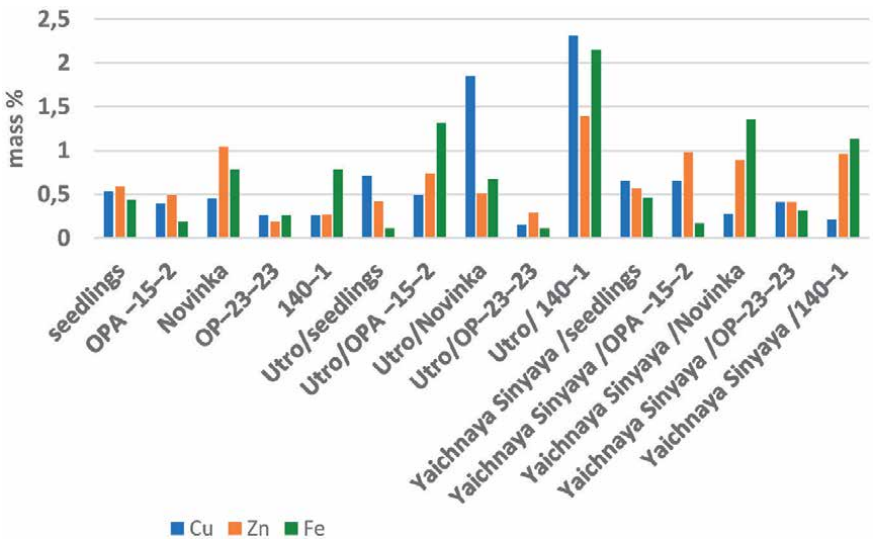

Figure 11.
The content of Cu, Zn and Fe in the leaves Prunus domestica *L.*

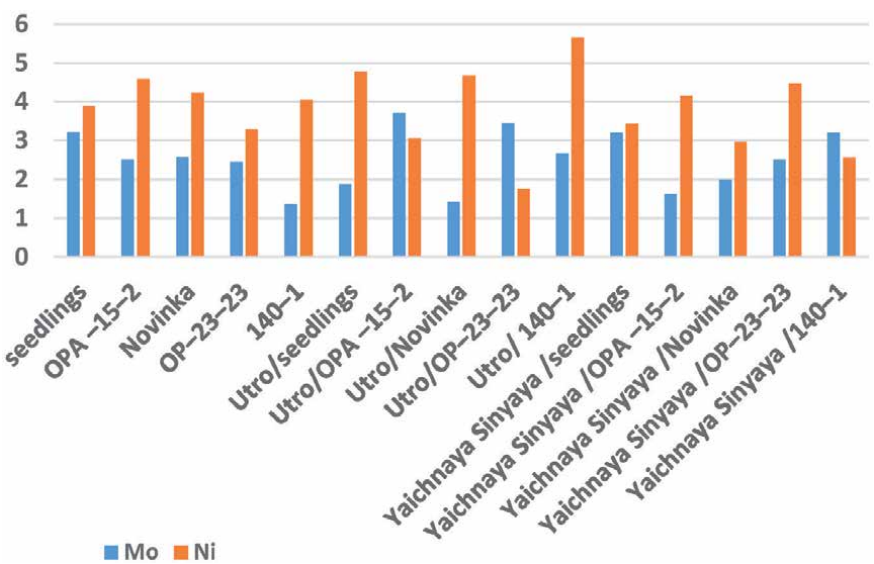

Figure 12.
The content of Mo and Ni in the leaves Prunus domestica *L.*

2.7 Stock influence on plum leaves metabolic answer

Metabolites component composition of rootstocks and scion-stock combinations leaves was fulfilled using the method of gas chromate-mass-spectrometry. Comparative chromatogram of Yaichnaya Sinyaya/seedlings combination with other combinations are given in **Figure 13**. In the whole, chromatographic profiles are alike and are characteristic for *Prunus domestica*. The main differences may be found in quantitative content of the substances that are identified by the peaks with the following retention time: 13.50 min (Erythronic acid), 13.43 min (glycerol), 14.27 min (Hellenic acid), 16.37 min (Lactic acid), 19.53 min (levoglucosan), 21.02 min

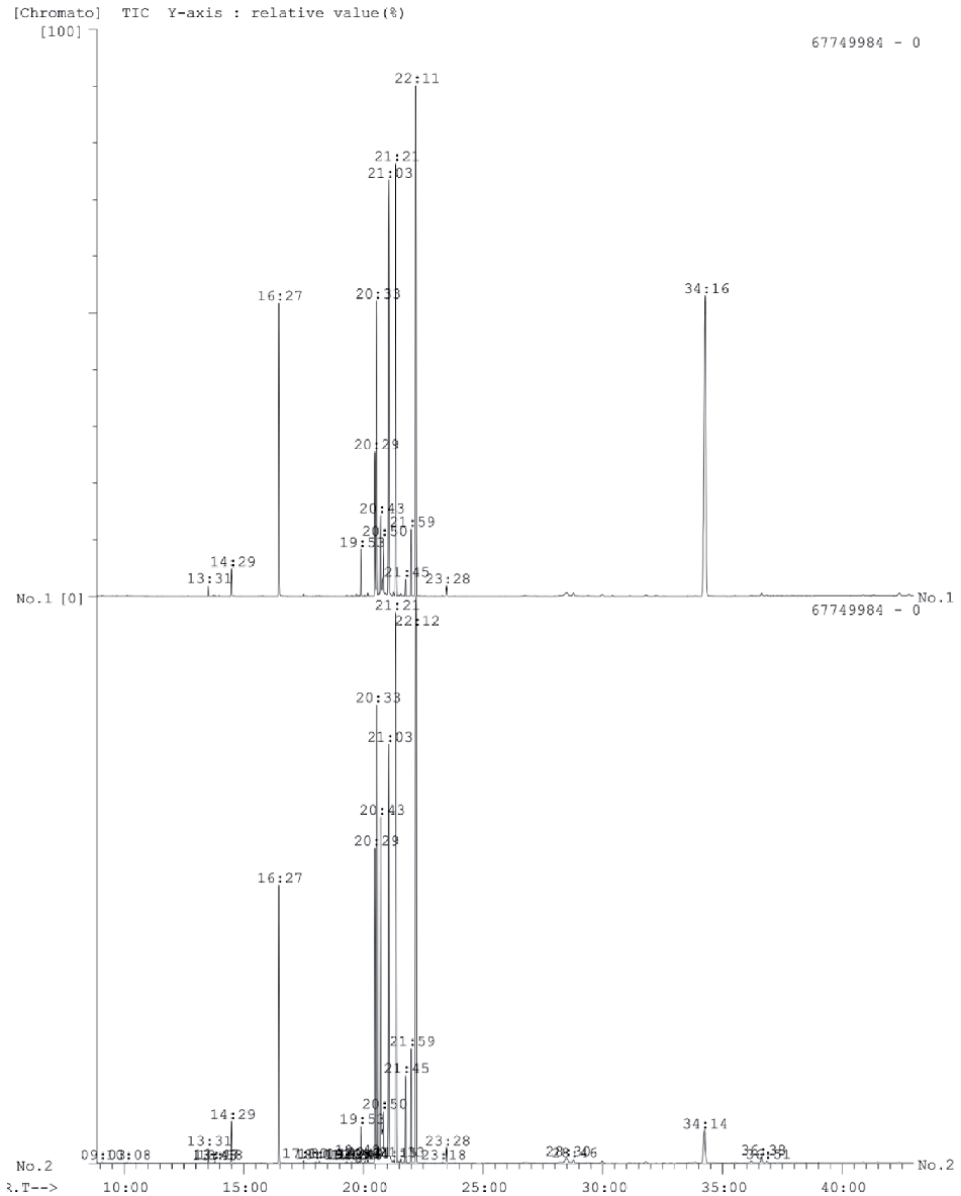

Figure 13.
Comparative chromatograms of Novinka rootstock (above) and Yaichnaya Sinyaya/Novinka (below) combination leaf ethanol extracts.

(Quinnic acid) and 23.27 min (antioxidant mio-inositol). The highest peak of mio-inositol and its content respectively were registered in the leaf extracts of Yaichnaya Sinyaya/OPA-15-2 combination). Our studies showed that in the leaves of Yaichnaya Sinyaya variety on OP-23-23 rootstock Quinnic acid and Chlorogenic acid that play a great role in adaptive processes [16] are synthesized on 15% and 10% more respectively than in a rootstock leaves. Sucrose, Fructofuranose and Fructose content in Yaichnaya Sinyaya variety leaves was 2.5–3 times more than in a rootstock leaves.

3. Conclusions

Understudied *Prunus domestica* varieties demonstrated different growth character and morphological-biochemical peculiarities depending on the used rootstock. While analyzing the data, it was stated that growth parameters, photosynthetic processes and metabolites synthesis in plum leaves differed in similar experimental environmental conditions. They depended on a variety and a rootstock inside the variety. Both the variety and the rootstock have special influence on photosynthesis process intensity, moreover the received results varied from one combination to another one. For example, Yaichnaya Sinyaya variety on OPA-15-2 and OP-23-23 rootstocks were the highest and showed the largest sprout formation. These differences have influence on plum trees light regime that causes the changes in morphology and leaves chemical composition (photosynthetic pigments and metabolites synthesis) and mineral substances accumulation.

Such information is useful for the grafted plants physiology evaluation and scion-stock combination choice.

Acknowledgments

We would like to thank Mariya Mertvishcheva and Darya Panishcheva for assistance in determining photosynthetic pigments, antioxidant activity and phenolic compounds sum and for metabolites analysis probes preparation.

Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

None.

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Chapter 7

Recent Techniques and Developments on Cherry Growing in Turkey

Ali Küden, Ayzin B. Küden, Songul Comlekcioglu, Burhanettin Imrak and Muhsin Bag

Abstract

The wild cherries are mostly seen in the North Anatolian and the Taurus mountains of Turkey. Cherry cultivation is concentrated on the slopes of 1000–1500 m where wild cherries are grown, also at the river valleys or in Izmir and Manisa provinces at the western parts of the country over 100 m elevation with very high quality. Turkey is the leading country in the world on cherry production. Cherry production in Turkey has been performed mostly with '0900 Ziraat' cherry cultivar, which was known as Turkish cherry in Europe. As a result of the studies carried out for fruit cracking and self fertility, Regina, Kordia, Sweet Heart and Lapins cherry cultivars were selected as alternative cherry cultivars to '0900 Ziraat'. In 1997, "Turkish National Cherry Working Group" was founded and organized 20 working group meetings until today to solve all the problems of cherries. In these meetings, all the research results were shared and discussed among cherry scientists. Recently, studies on prolonging the cherry season with early and late cherry cultivars to increase the amount of cherry exportation was achieved. For this aim, Sweet Heart at high elevations, Royal Lynn® and Royal Tioga® at subtropical climatic conditions were found to be suitable.

Keywords: cherry, *Prunus avium* L., growing techniques, Turkish cultivars

1. Introduction

The origin of the cherry (*Prunus avium* L.) is in the southern Caucasus, the Caspian Sea and north-eastern Anatolia. Cherry has spread from the center to the east and west and has covered a wide area in the world. Considering cherry germplasm in the world, the wild forms of cherry are grown in the northern Anatolian mountains and at the Taurus mountains of the South Anatolia in Turkey [1].

Cherries grow large trees up to 15 m in height upright and scattered, the branches are smooth, the growth tips are sticky when the leaves open. The flowers form white double or triple bunches. The fruits are in different shapes and colors and the core is semiadherent to the flesh. The fruit is colorless in some cultivars and very dark red in some cultivars [1, 2].

Cultivation of cherry is naturally and wildly grown on the slopes and river valleys of the North Anatolian Mountains, Western and Central Taurus Mountains and at the 1000–1500 m elevations of the Mediterranean side of Eastern Taurus

Mountains with very good quality cherries. However, rainfall during the flowering period negatively affects fertilization by preventing bee flight. Also, excessive and long-lasting rainfall at harvest time of '0900 Ziraat' cherry cultivar causes cracking and decreases the cherry production. For this purpose, cracking resistant cultivars have been introduced with TÜBİTAK (The Scientific and Technological Research Council of Turkey) and DPT (State Planning Organization) supported projects which were carried out at the Pozantı Agricultural Research and Application Center of the University of Çukurova. As a result of these experiments, 'Regina' and 'Kordia' cherry cultivars were determined to be resistant to cracking, and grown and exported besides '0900 Ziraat' national cultivar and 'Sweetheart' and 'Lapins' self fertile cultivars.

"Turkey's National Sweet Cherry-Sour Cherry Working Group" was founded in 1997, under the leadership of Prof. Dr. Dr. Nurettin Kaska. Later on, T.C. The General Directorate of Agricultural Research and Policies (TAGEM) of the Ministry of Agriculture and Forestry undertook this organization. All the problems and solutions related to national cherry production were discussed in these working group meetings held 22 times so far and shared with the stakeholders.

1.1 Cherry production and export

Cherry production of Turkey has increased to 732.000 tons in 2020 from 215.000 tons in 1997. Turkey usually exceeded the estimates of the cherry marketers. However, considering the exportation, Turkey ranks third or fourth and most of the exportation has been traditionally performed to Russia and the European Union. Fruit prices in the world cherry market rise to the highest levels both in the early season in April and late season in August. Among world cherry producer countries, USA, Chile and Turkey take the first three places in exportation. Turkey's cherry exportation in 2020 was 87.944 tons and ranked 4th in cherry exports. This is because Austria and Hong Kong (China) play an important role in the cherry trade, although they do not have a say in cherry production. Although cherry ranks 4th, it is the most valuable crop in our exports in terms of income.

2. Cherry cultivar adaptation studies

The first trial in Turkey on cherry cultivar adaptation experiments was carried out in Yalova Atatürk Horticultural Central Research Institute on "Selection of local and foreign sweet cherry and sour cherry cultivars" by Dr. Fahrettin Oz. In this study, 51 sweet cherries, 7 sour cherries and 1 sweet cherry-sour cherry hybrid of domestic and foreign cultivars were selected in 1974 and 1975 for their fruit quality characteristics. Yalova Horticultural Central Research Institute carried out cherry adaptation trials in different parts of Turkey from 1982. With these studies '0900 Ziraat' major sweet cherry cultivar and pollinators ('Lambert', 'Bigarreau Gaucher' and 'Starks Gold') became popular.

S alleles of '0900 Ziraat'cultivar are S3/S12. Another German cherry cultivar with the same S allele is Nordwunder (Schneiders Späte Knorpel), 'Princess' (Prinzesskirsche) and in Italian cherry cultivar 'Ferrovia'. Therefore, there are various opinions that these two cultivars are the same. The cherry cultivar 'Schneiders Späte Knorpel' was discovered in 1850 in Guben, Germany, by co-producer Schneider. Today, it is still one of the most produced cherry cultivars in Germany. Another cherry cultivar found in Guben is 'Noir de Guben'. This cultivar is produced in our country under the names of 'Kemalpaşa Napoleon' in Kemalpaşa, 'Erkenci Napoleon' in Bursa and '0900 Ziraat' was also grown under the name of 'Napoleon' [3–6].

Although '0900 Ziraat' national sweet cherry cultivar has a high fruit quality such as resistance for transportation, long shelf life, good fruit flesh firmness, very good taste and aroma, it has also some inadequate features such as low yield caused by the rainfall during the pollination period. Many studies on these problems and similar issues on cherries have been carried out in the Faculty of Agriculture of the University of Cukurova. These studies include; adaptation of cherry cultivars to subtropical conditions [7, 8]; 'Aksehir Napolyonu' cherry cultivar packaging and storage in a modified atmosphere, developments on pre-cooling and cold transportation of cherries [9]; clonal micro propagation of clonal cherry rootstocks, investigations on new cherry cultivars adaptable to cold regions of our country, classification of cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) cultivars by DNA fingerprinting method [10, 11].

Another project was carried out during 1995–1996 by Dr. Nurettin Kaska and his colleagues at Ulukışla and Pozantı villages to increase the economic levels of export-oriented cherry growing potential by modern methods'. With this project, the first pruning was applied on cherry trees.

Newly introduced foreign and local cherry cultivars were used in this project such as Regina', 'Venüs', 'Summit', 'Lapins', 'Na-478′, 'Na-474′, 'Noir de Guben', 'Van', 'Larian', 'Akşehir Napoleon', 'Starks Gold', 'Octavia', 'Bigarreau Gaucher', '0900 Ziraat', 'New Star', 'Durono-3′, 'Tardie de Vignola', 'Na-1(Nafrina)', 'Early Burlat', 'Van Compact', 'Bing Spur', 'Sunburst', 'Fercer Arciana', 'Meckenheimer', 'Hedelfingen', 'Nadino', 'E. Rivers', 'Kordia', 'Precoce de Bernard', 'Garnet', 'Telegal', 'Cristobalina', 'Namosa', 'Lamida', 'New Star', 'Prima Giant', 'Rainier', 'Early Lory', 'Big Lory', 'Late Lory', 'Sweet Heart', 'Ferrovia', 'Tieton' and 'Staccato' [12].

Orchards were established with these sweet cherry cultivars at different institutes and locations such as Uludağ University Faculty of Agriculture, Yalova Atatürk Horticultural Central Research Institute, Eğirdir Horticultural Research Institute, Malatya Apricot Research Institute, Ordu University Faculty of Agriculture, Çukurova University Faculty of Agriculture and Pozantı Agricultural Research and Application Center. As the result of these studies, 'Regina' and 'Kordia' cherry cultivars were found to be the best quality cherries in the country [13]. New cultivars were added to those sweet cherry cultivars in Yalova and Eğirdir in 1999 which were 'Precoce de Bernard', 'Techlovan', 'Sylvia', 'Summit', 'N. de Meched', '0900 Ziraat', 'Octavia', 'Belge', 'Sweetheart', and 'Regina'. The best results were obtained from 'Veysel', 'Noir de Meched', 'Ranier', '0900 Ziraat', 'Octavia', 'Belge', 'Lapins' '0900 Ziraat' and 'Sweetheart' cherry cultivars in Eğirdir and Yalova ecological conditions considering the harvesting time [13].

In a study carried out by Bas et al. [14] on determination of self fertile and exportable cherry cultivars by cross breeding and mutation methods, '0900 Ziraat' and self-fertile 'Stella' and 'Sweetheart' cultivars were used as parents. Ten of them were taken to the second stage of the selection. Cherry breeding studies have been continued in Yalova and Eğirdir research institutes.

As a result of the experiments carried out at Çukurova University on cherry adaptation in early ripening cherries, these cherry cultivars were detected (end of April-May) 'Cristobalina' (self fertile), 'Prime Giant' (Pollinators; 'Brooks', 'Lapins'), for mid season cherries, 'Lapins' (self fertile), 'Regina' (Pollinators; 'Skeena' and 'Durone 3 Nero' and 'Kordia' (Pollinators; 'Summit', 'Skeena' and 'Regina for late ripening cherries 'Sweet Heart' (self fertile) cherry cultivars were found. 'Regina' and 'Kordia', resistant cultivars to fruit cracking and 'Sweetheart', late season cultivar were found to be suitable for exportation. The very early cherry cultivar 'Cristobalina' was found to be more suitable for the domestic market.

3. Morphological and biological characteristics of cherries

Cherry trees usually form a pyramid-shaped crown that rises up to 20–25 m. The trunk of the trees is upright and smooth, and the trunk is grayish-black or dull black with transverse stripes. Cherry branches are smooth, internodes are long in standard cultivars. Flower buds generally begin to form bouquet flowers at the bottom of the branches in the 2nd year. The buds form two types of buds as wooden buds and fruit buds. Wooden buds are thinner and smaller than fruit buds. Fruit buds are large and plump, and they are found in the twigs as side buds. In bouquet branches, there is a shoot bud in the middle and 5–6 fruit buds around it. In fruit buds, the flowers are not one by one, but many. The number of flowers also increases up to 6. The flowers have 5 sepals and 5 petals and up to 30 stamens. Flowers normally have one pistil. Some cultivars have double pistils. The formation of multiple pistils is related to climatic conditions as well as a kind of feature. During flower bud formation in summer, high air temperatures increase the number of double pistil flowers. Multiple pistil flowers reduce the market value of the fruits as they cause twin fruit formation [15].

4. Fertilization biology of cherries

In terms of fertilization biology of cherries, self incompatibility and cross incompatibility may occur so this situation should be taken into account in orchard plantations [16, 17]. Most of the local cherry cultivars grown in the Marmara Region were found to be self incompatible. Thus, 'Starks Gold', 'Bigarreau Gaucher', Merton Late' and 'Lambert' cultivars are recommended as pollinators for our important export cultivar '0900 Ziraat'. The reason for the low yield of '0900 Ziraat' sweet cherry cultivar was found to be the unfavorable weather conditions during the pollination and fertilization period [18]. As a result of these studies in 2017, the pollinators for '0900 Ziraat' were determined and given in **Table 1**. According to the results, the best pollination in '0900 Ziraat' cherry cultivar was obtained with 'Merton Late'. However, Stark's Gold, Lambert and Bigarreau Gaucher could only pollinate '0900 Ziraat' 50% even if the flowering times coincide. In this case, the reason for the low yield of '0900 Ziraat' in 2017 was the use of 'Stark's Gold' cherry cultivar in most of the cherry orchards which have only 50% of pollination ability (**Figure 1** and **Table 1**). When the unsuitable weather conditions were also considered, the yield was very low.

a. Self incompatible

b. 50% pollination

c. 100% pollination

Many nurseries recommend '0900 Ziraat', 'Regina', 'Sweet Heart' and 'Kordia' cherry cultivars to establish cherry orchards. Self fertility, flowering periods and pollination ability of cherry cultivars in nurseries and in orchard plantations should be taken into account (**Table 2**).

On the other hand, cherry orchards are also established with self fertile cultivars. In this case, although the cultivars are self fertile, bee activity and little windy weather are needed in the orchards for a better pollination at the flowering period. There is no bee activity at temperatures below 10°C and above 38°C. Generally, bee flights are very low at temperatures below 12–14°C. Also, in heavy windy, rainy and

Pollinators	S allelles	Pollination rates (%)
Starks Gold	S3S6	50
Bigarreau Gaucher	S3S5	50
Merton Late	S1S4	100
Lambert	S3S4	50

Table 1.
The pollinators and the pollination rates of '0900 Ziraat' (S3-S12).

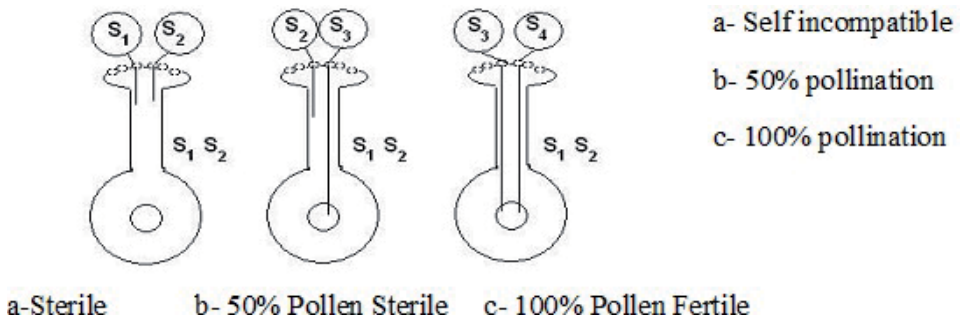

Figure 1.
The pollination ability of similar and different alleles. a. Sterile. b. 50% pollen sterile. c. 100% pollen fertile.

cloudy weather bee flight is very weak. For this reason, wild bees are used for fertilization in cold and rainy climates. For this purpose, bee hotels are made or bunches of reeds at different diameters hang on the row of cherry trees.

Thousands of wild bees can fly in unsuitable weather conditions where honey bees can not fly and provide fertilization. Each alone female makes her own nest and finds food for herself and her offspring. Ninety percent of wild bee species live alone. Seventy percent of the 20,000 bee species in the world live underground. Therefore, to keep the bee nests intact, covered soil tillage is becoming forward in cherry orchards.

Cultivars	Flowering periods	S alleles
Prime Giant	Mid early	S1S9
'Lapins'	Early	S1S4 (Self-fertile)
'Brooks'	Mid early	S1S9
'Burlat'	Mid early	S3S9
'Regina'	Late	S1S3
'Kordia'	Mid late	S3S6
'Sweetheart'	Mid late-late	S3S4 (Self-fertile)
'Rainier'	Early	S1S4
'0900 Ziraat'	Mid season	S3S12
'Ferrovia'	Mid season	S3S12
'Staccato'	Mid season	S3S4 (Self-fertile)

Table 2.
Flowering periods and S-alleles of some important cherry cultivars.

5. Sweet cherry cultivars

Early Burlat (S3S9); Early Burlat ripens in the early season. It is firmer than Black Tartarian, softer than Bing and known for its consistent productivity of medium-sized, Bing-like cherries. Early Burlat is resistant to bacterial canker and cracking. The tree is moderately vigorous and spreading. Early Burlat pollinated by Rainier.

Merchant (S2S4); Origin - United Kingdom; fruit size medium (fairly large for season); good quality fruit; hangs well on tree; picks with dry stem scar; cracks quite badly; vigorous, health tree. Comments: Worthy of trial as early fresh market cherry, but cracking may be too severe. It is a universal pollen donor, blossoms mid-season, and is one of the earlier cultivars to mature. Pollinated by Burlat, Stella, Lapins, Sunburst. Pick time early July. Merchant is early mid season cultivar with very large, black fruits. Very heavy cropping.

Tieton (S3S9); Very large fruit (10–14 g) with mild flavor and export quality firmness. Dark red skin. Very early harvest. Gisela rootstocks are suggested to increase cropping potential. Tieton™ is an early-ripening mildly sweet cherry with a beautiful, glossy mahogany red finish over medium red flesh. The fruit is very large with excellent firmness and a susceptibility to rain cracking similar to Bing. To encourage productivity, Tieton™ should be planted with more than one cultivar of pollinizer and/or more than 10% pollenizer density. Pollination Rainier, Bing, Van, Lapins and Sweetheart™; use of multiple pollinizers recommended.

Brooks (S1S9); Developed by the University of California, Brooks is a large, firm red cherry that tolerates hot climates. This cultivar is very susceptible to cracking in rain. Brooks, ripens 10–14 days ahead of Bing. A very large fruit that is firm with an exceptional flavor. There are very few doubles in the fruit set in hotter climates. It is susceptible to severe cracking in the rain. Pollinators for Brooks are Bing, Rainer, Early Burlat and Tulare.

Prime Giant (S1S3); This cherry ripens with Brooks about 7–10 days after Burlat. It is very firm and large and has an excellent, sweet-tart taste. Seven days before harvest this cherry had a much stronger and better flavor than a ripe Burlat. Sugars and flavor are produced early, before the cherry is ripe. Marvin Niece in California developed prime Giant. I thought this was a good cherry but I have two major concerns about this cherry. First, it is susceptible to rain cracking, and second, trees in Europe had a virus that was killing the trees. It needs to be cleaned up before it is of interest, but otherwise a great-looking cherry. The trees are a very fertile cultivar that thrives in the mid trough. Even though it is early, the fruit is 10–11 g. Heavily weighted, heart shaped. The outer diameter of the stem is middle length fruit is bright red color. Flesh is pinkish, juicy and aromatic. It is susceptible to cracking. The harvest time is at the end of May, 10 days after Early Burlat.

Lapins (S1S4); Very large fruit is the hallmark of the Lapins cherry. Lapins cherries are firm and crack-resistant with a mahogany-red skin and lighter red flesh. Self-fruitful, Lapins ripens 10–14 days after Bing in the Northwest and 5–7 days after Bing in the Central Valley of California. Lapins is crack resistant, similar to Van in color and resembles Stella in shape. The estimated chilling requirement 500 hours.

Rainier (S1S4); The Rainier title has become well established as a promise of unmatched flavor. Rainier is a large, yellow cherry with a red blush and light yellow flesh. Exquisitely flavored with high sugar levels, this is a premium niche cultivar that ripens just after Bing. The tree is vigorous, early bearing and very productive with excellent cold weather hardiness. Rainier requires cross-pollination and is inter fruitful with Sweetheart and Lapins. Pollinators for Rainier Lapins, Sweetheart,

Early Burlat. Estimated chilling requirement is 700 hours. In addition, Rainier is highly susceptible to powdery mildew and rain cracking.

Attika (Kordia) (S3S6); A Czeck raised cultivar. In Europe, Skeena and Regina® is a good pollinator for Kordia. Kordia is a mid season sweet cherry producing a heavy yield of large, firm, black fruit with a good flavor. It has some resistance to cracking, is not self fertile, and is ready to pick in mid summer.

Regina (S1S3); Good quality (13 g), highly crack resistant. It was developed in an area with high bacterial canker pressure, so should have good bacterial canker tolerance. Very large cartilage cherry, solid, reddish brown, sweet, rich. Regina is a high quality, late season cherry that exhibits excellent rain crack resistance. The fruit is very large and firm, with a mild, pleasant flavor. When ripe, this cherry is darker than most. For peak flavor, it is important to delay harvest until total soluble solids reach 20–22%. In order to ensure high quality yields, a regular cut in autumn or early spring, mandatory! In this context and due to different educational forms, the final height can be determined by fruit trees, depending on the amount of space required. Large shrub or small tree, taut-broad erect, pyramidal crown, loosely branched, 3–5 m high and 2–4 m wide. Pollinators for Regina, Sweetheart and Attika-Kordia.

Sweet Heart (S3S4); Sweetheart™ is a large, bright red heart-shaped cherry. Sweetheart™ matures at the end of the season, about 5–7 days after Lapins and remains firm after picking. This self-fruitful cross of Van and Newstar is productive with good firmness, size and flavor. The tree is spreading and precocious, yielding heavy crops on all rootstocks. Sweetheart™ shows moderate cracking. Very late productive, medium to large fruit, very good firmness and good flavor. Tree survival was reported poor from northern Germany where bacterial canker is a serious problem. As with Lapins, trial plantings should only be made on excellent sites, but may have a niche for late season local sales (though cracking is a problem).

0900 Ziraat; It is the most cultivated cultivar of Anatolian origin known as Salihli, Akşehir Napoleon, Uluborlu and Dalbastı, and this cultivar supplies the 90% of our exports. 0900 Ziraat cherry is in demand from the foreign markets due to its fruit size and other quality features. The trees grow very vigorously, semi upright and form a wide crown. It is incompatible with itself. Its fruits are large (8-9 g), dark bright red, hard, crispy, long stemmed. Fruit flesh is juicy, very resistant to cracking and transportation. Ripening is in the last week of June. Fertilizers, Lambert, Starks Gold, Regina, B. Gaucher, Lapins, Metron Late. Noble varieties.

0900 Ziraat cultivar S alleles are S3/S12. Another cultivar with the same S alleles is the German cherry cultivar Nordwunder ('Schneiders Späte Knorpel). Therefore, there are various opinions that these two cultivars are the same. The cherry cultivar 'Schneiders Späte Knorpel' was found by the producer Schneider on the banks of the Neisse River in Guben, Germany, as a random seed in 1850. This cultivar was recommended and planted everywhere from the early 19th century until the 1960s as the queen of cherries sprung from Guben city, the cherry growing center in Germany. Today, it is still one of the most produced cherry cultivars in Germany. Other cultivars with the same S alleles (S3/S12) are Ferrovia grown in Italy and Princess (Prinzesskirsche) in Germany.

6. Ecological requirements of cherries

In our country, cherries grow naturally between Artvin and Kocaeli in North Anatolia and Taurus Mountains in the south. Culture cherries are also concentrated on the slopes and river valleys of these mountains at an altitude of 1000–1500 m. However, in recent years, with appropriate rootstock and cultivar selection, the

growing areas have spread to lower regions such as Bursa, Iznik, Çanakkale, Izmir (Kemal Paşa) and Manisa in the Aegean region.

Cherry is a high chilling requiring species. The chilling requirements of the cherry cultivars grown in our country are between 500 and 1500 h. Winter cooling is necessary to break dormancy and continue the development in spring. If this requirement is not satisfied, irregular flowering and flower drops are seen. In this regard, approximately 1000 m altitudes are ideal regions for cherries. Cherry trees can damage at low temperatures below −20 and − 24°C. One of the most important factors limiting cherry cultivation is late spring frosts. The flower buds usually die at −4°C, although it also depends on some other factors. Opened flowers are damaged at −2°C. Extreme summer temperatures are undesirable because it promotes double pistil formation and twin fruits and such fruits have no market value.

7. Cherry growing techniques, orchard management

Cherries are propagated by budding and grafting on several rootstocks. In Turkey, cherries and sour cherries are generally propagated by dormant budding. Although, this budding period varies according to the climatic conditions, it is mostly carried out between July and September, usually by classical "T" budding method.

In subtropical climatic conditions with a long vegetation period, the most suitable budding period is the spring budding period in February-March. Budsticks can be taken directly from the trees for chip budding. There are negative points for classical "T" budding at spring growth season in April. First of all, it is necessary to wait for the removal of the bark of the rootstocks and the budsticks have to be kept at low temperatures until mid-April for the most suitable period for budding. In April buddings, the bud union may be delayed, the growth season shortens and the seedling quality may decrease. For this reason, the most suitable propagation period for cherries and sour cherries is in winter with chip budding under controlled or outdoor budding conditions.

The natural growth habit of cherry trees is upright and vigorous, forms very high trees. The formation of large trees also affects the planting distances and the cherry orchards are planted at intervals of 7 × 7–8 × 8 m, even 10 × 10 m. The height of them should be reduced by using dwarf rootstocks. Recently, dwarf rootstocks, spur and compact cultivars have been used in modern fruit growing. In traditional cherry cultivation in our country, medium strong *P. mahalep* in calcareous soils and very strong *P. avium* rootstocks are used in low calcareous soils.

Today, cherry production in many European countries is declining in favor of pome fruits. The reasons for this are; very high forms of the trees, difficulty in mechanization, high labor costs in harvest and in other cultural techniques. Tree heights can be kept at 3.5–4 m by using dwarf rootstocks with low crown. Planting intervals can be reduced from 9 × 7 or 8 × 6 m to 5 × 5, 5 × 2.5 and 4 × 1.6 m.

8. Cherry rootstocks

Belgium: Three commonly used rootstocks; Inmil (G.M.9), Damil (G.M.61) and Camil (G.M.79). Germany: 12 rootstocks under the name of Gisela were obtained during the hybridization studies of cherry rootstocks in Giessen. Apart from Germany, these rootstocks have started to spread in Europe and America. 5 sour cherry selection was obtained from the rootstock in M. Freising Weihestephan

Tree form	Rootstocks	Planting distances (m)	Tree per/ha
Spindel	Maxma14	4.5 × 3.5	593
Spindel	Gisela 6	4.5 × 2.5	831
Drapeau	Maxma14	4.5 × 3.5	593
Drapeau	Gisela 6	4.5 × 2.5	831
Bush	Maxma14	5 × 3.5	534
Bush	Gisela 6	5 × 2.5	748
Mikado	Maxma14	5 × 2.5	748 (4 main branches)
Drilling	Gisela 6	5 × 2	935 (4 main branches)

Table 3.
Different training, planting distances and number of trees per hectare in Turkey.

University. These are W-10, W-13, W-53, W-72 and Weiroot 158. In the hybrid studies conducted by H. Fisher at the Pillnitz Research Institute in Dresden, PiKu 422 and Pi-Ku 483 rootstocks were obtained. Italy: CAP rootstocks were obtained by selection from cherries at the University of Bologna. USA: MM series or MaxMa rootstocks were obtained from *P. avium* and *Prunus mahaleb* hybrid studies. These; MxM2, MxMI4, MxM34, MxM60 and MxM97. Czech Republic: PHLA and PHLB rootstocks were obtained by sour cherry selection. Spain: MM9, MMP12 rootstocks by selection from sour cherries and Adora rootstock were obtained by selection from plums. France: Mahaleb SL-64 rootstock selection and Tabel Edabriz selection were obtained from sour cherry. Among these rootstocks, mostly MaxMa is used in our country (**Table 3**).

Another problem in cherry seedling production is the inoculation of cultivars suitable for rootstocks. This problem was encountered on Giesela rootstocks. In the orchards established with self fertile Sweetheart and Lapins cherry cultivars on dwarf Giesela-5, the fruit size did not reach the export size due to excessive fruit set.

9. Pruning of cherry trees

No pruning was applied on cherry trees before 1996 in Turkey except cutting dry branches. In a cherry meeting with cherry growers and technicians in Pozanti Agricultural Research and Application Center, under the leadership of Prof. Dr. Nurettin Kaşka in 1996, Prof. Dr. Ali Küden [19] gave a seminar and a training course on pruning and showed pruning applications on cherry trees at the orchard conditions at the first time in the country. After this activity, several pruning seminars and applications were held in cherry regions, such as training of 30 Agricultural Engineers from various institutes of the Ministry of Agriculture in Pozanti Center. In this way, new cherry cultivars and new pruning methods of cherries were spread in important cherry production regions.

The experimental cherry trees established at Pozantı Agricultural Research and Application Center were given a different upright branching shape and pruning was done in the following years. As in other fruits, pruning in cherries can be divided into three groups which were: training, yield pruning and rejuvenation pruning. Generally, the training shapes given to cherry trees were; Modified Leader, Spindel, Super Spindel, Super Spindel Ax, Solax, V system, bush, KGB, Spanish bush system, Fan, Upright Fruiting Offshoots (UFO), Drapeau and Bibaum. Recently,

the Super Spindel Ax and UFO in USA, the Fruit Wall system in France and the 3-branch Fruit Wall system in Italy are getting popular.

These pruning forms have been developed in different countries by considering their soil and climatic conditions such as humidity, lightening and common cherry diseases and economical causes. In Belgium, compared the classical system, the British system, the Spanish bush system, the V-system and the UFO-systems in the European project on 'Kordia' and 'Sweetheart' cherry cultivars grafted on Gisela-5 rootstock. At the end of this study, the classical system was found to be the best in terms of efficiency and quality [20].

According to the rootstocks, pruning and planting systems were changed in cherries, for early fruit set, dwarf rootstocks came forward. In another study carried out at Çukurova University to get early fruit set from the cherries grafted on semi-dwarf and strong rootstocks, short cuts in winter pruning (**Figure 2**) and shoot breaking method.

9.1 Short cuts

In a study carried out in Ulukışla, short cuts of 5–10–15 cm in winter were applied to 1–2 years old branches of 'Lapins', 'Summit', 'Sweetheart' and '0900 Ziraat' cultivars grafted on *Purunus. mahaleb* rootstock and the amount of carbohydrate in the cut branches was higher than the uncut branches [21].

Nitrogen contents of the branches were found to be higher in the uncut branches and lower in the cut branches. On the cut shoots, fruit bouquet, called the 1st summer may bouquet, was formed, and immediately the second summer fruit was taken. In this case, the branches should be cut and discarded in the shape of the pruning and short cuts caused early fruit set. At the end of this research, short cutting was accepted to be a good method to break juvenility period [21].

The shoot tips of cherries get too much nitrogen. When the shoots are cut, nitrogen uptake decreases and carbohydrate (Ch) accumulation increases (**Table 4**). According to these results, in winter pruning, 1–2 years old shoots are cut from 40 to 50 cm in standard cultivars and from 25 to 30 cm in self-fertile cultivars. As a result of this study, shortening the juvenility period of the young cherry trees by shoot cutting was determined to be possible (**Figure 3**).

9.2 Breaking shoots

Dwarf rootstocks in cherries as well as expanding branch angles, hanging various weights and semi horizontal planting accelerates the formation of fruit buds. However, the branch breaking method previously applied in apples, also gives very positive results in cherries (**Figure 3**).

Figure 2.
In winter pruning, with short cuts of 5-15 cm in annual shoots fruit set in the same summer and the second summer fruit production.

Cultivars	Control (no pruning)			5 cm cut			10 cm cut			15 cm cut		
	Fruit bud	CH (%)	N (%)	Fruit bud	CH (%)	N (%)	Fruit bud	CH (%)	N (%)	Fuit bud	CH (%)	N (%)
Sweet Heart	—	9.3	0.86	3	9.6	0.53	4	10.7	0.75	5	16.7	0.80
Lapins	—	5.0	0.82	8	11.5	0.43	2	11.1	0.68	6	6.3	0.52
Summit	—	8.1	1.07	7	9.0	0.69	6	4.9	0.71	5	14.5	0.75
0900 Ziraat	—	3.2	0.85	10	6.9	0.48	11	6.5	0.37	8	14.4	0.52

Table 4.
The effect of different short cuttings of the shoots on the nitrogen and carbohydrate accumulation.

In the branch breaking method, ¼ of the branches are cut from the top and the branches are half broken. As a result, fruit buds are formed in the broken branches in 1–2 years (**Figures 4** and **5**).

In cherry trees, 40–60 cm cuts are made in the whole crown according to the development of the shoots from outside to inside. In cherry trees, especially the injured and cut areas are the entry points of the bacteria that cause branch cancer, cutting thick branches in winter pruning increases the risk of branch cancer in the trees.

Figure 3.
In winter pruning, with short cuts of 20–25 cm in annual shoots fruit set in the same summer and the second summer fruit production.

Figure 4.
Tying the broken branches after winter pruning, formation of flower buds, flowering and fruit set.

Figure 5.
Tying the broken branches, this application is done in February – March.

Bacteria, which are branch cancer agents and cause gluing at these points, are rarely found in nature, especially in August. For this reason, shape prunings should be done in early July, and thick branches in yield prunings should be done in August. In winter pruning, the thickness of the cut branches should not exceed finger thickness. Thicker branches should be made at 15–20 cm lengths in thick branch sections in areas where the disease is very common. As a result, the pruning of cherry trees should be done in winter and July, while in winter prunings yield pruning in thin branches and short cuts should be done in these branches, especially in areas where branch cancer is common, thick branch segments should be left to the end of summer.

- Standard tree pruning (mature tree) 40–60 cm
- Spur and selfertil cultivars 20–30 cm
- Bush tree 5–15 cm
- Apical Pruning: Nitrogen uptake stops when branches are cut, carbohydrate is accumulated.

10. Cherry growing under subtropical climatic conditions with low chilling cultivars

Generally, sweet cherries in Turkey are midseason cherries and grown in June and July. To extend the growing season of cherries and widen the exportation period, cherries were begun to be produced at the subtropical region of Turkey at the Mediterranean coastal line, since the prices at early and late season cherries are very high.

Under subtropical climatic conditions, cherry cultivation experiments started in 1990 with a self-fertile cultivar Stella. The main problem in cherries in this area was not only the insufficient chilling, but also the fertilization problems. The studies began on apple and pear in 1984, and on cherries in 1990 [6]. For this purpose, cherry collection orchards were established in Adana. In the meantime, the chilling requirements of the cultivars and the chilling duration of the area were determined.

Küden et al. [7], determined the performances and the chilling requirements of 'Stella', 'Noir de Guben', 'Van' and 'Bing' cherry cultivars and 'Kütahya' sour cherry cultivar under subtropical conditions with the classical and chill unit methods. In the study, the chilling requirements were determined as 600–1200 h for 'Stella', 700–800 h for 'Noir de Guben' and 1000–1200 h for 'Van'. Considering these results, it was reported that 'Stella' and 'Noir de Guben' cherry cultivars can be grown under subtropical conditions with some cultural practices. KNO_3, thiourea and hydrogen cyanamide (Dormex) were applied to break the dormancy of the buds in cherry cultivars. Among chemical applications, KNO_3 + thiourea (2% + 1%) combination gave the best results. The bud broke dormancy 100%, 96%, 92% and 77% respectively on 'Stella', 'Van', 'Noir de Guben' and 'Bing'.

Küden and Küden [22], stated that 'Cristobalina', "Temprano de Sot', 'Precoce de Bernard', 'Sunburst', 'Lapins', 'Chelan' and' 'Na-1'were found to be the promising cultivars and adaptable to subtropical climatic conditions.

Imrak et al. [15] studied on 'Na-1', 'Early Van Compact', 'Bing Spur', 'Lapins' and 'Cristobalina' cherry cultivars under subtropical conditions to prevent or decrease the multiple fruit formation that occurred at the differentiation period of the buds over 30°C. They found the use of green net with a shading feature of 55% used as

Figure 6.
The fruit set of new cherry cultivars at low altitudes.

a cover system to reduce the air temperature values between 1.9°C and 3.1°C and reduced double pistil formation ranging from 60.87% to 27.81% percentages.

Another issue in warm regions is that the cover materials used in cherry orchards are not collected during the winter. In this way, the trees are kept in a cooler environment with the shade effect on sunny days and help to satisfy chilling.

The studies on cherry growing under subtropical conditions continued in three locations began in 2013: 1—Çukurova University, Sarıçam/Adana, 2—Bilici Farm, Ceyhan/Adana, 3—Özler Abdioğlu Farm, Yüreğir, Yakapınar/Adana) with 15 low chilling cherry cultivars planted on 17, 30 and 50 m altitudes, respectively.

Recently, prolonging the cherry season with early and late cherry cultivars extend the cherry exportation season. As a result of the studies carried out at the University of Cukurova, Sweet Heart at high elevations, Royal Lynn® and Royal Tioga® at subtropical climatic conditions were found to be suitable (**Figure 6**).

11. Use of plant growth regulators in cherry

Recently, use of plant growth regulators in cherry cultivation is increasing. Gibberellic acid applications are used in our country to delay ripening period and to increase the fruit size. GA applications on cherry fruits at color changing stage delayed the harvest for 8–10 days. It is better to prune cherry trees together with GA applications to get bigger fruit size as well as delay harvest.

Erger Applications: Manisa Province Sweet Cherry Altitude: 214 m. Cultivar: 0900 Ziraat, Rootstock: Giesela 6.

In this study, chemical applications were found to be effective on breaking dormancy of '0900 Ziraat' sweet cherry cultivar. The chilling duration of the experimental areas were found to be 586 chill units and 1225 h in 2011–2012 winter period while it was 453 chill units and 819 h in 2012–2013 winter period. All treatments were applied (KNO_3 8%, Erger 6%), on December 15 (45 days before the end of dormancy duration) using 20 L Knapsack Sprayer. The experimental winter period of 2012–2013 was warmer and had lower chilling accumulation. Therefore, no yield could be obtained from the orchards at 150–200 m height. This study was

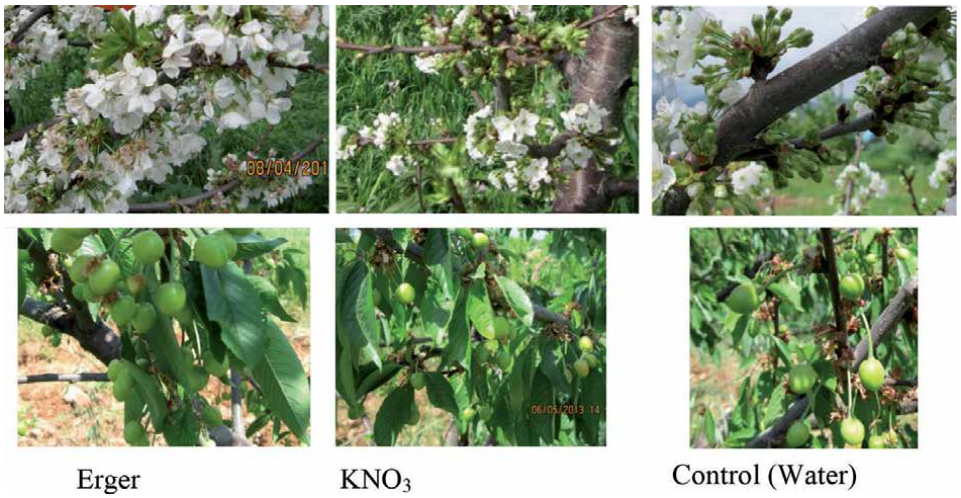

Figure 7.
Comparison of the flowering times of the applications.

carried out for 2 years (2011–2013). The capacity of Erger (total nitrogen 15.0%, ureic nitrogen 6.1%, nitric nitrogen 5.8%, ammoniacal 3.1%, water soluble calcium oxide 4.7%), Dormex (hydrogen cyanamide) and potassium nitrate (KNO3) for breaking of dormancy in buds of '0900 Ziraat' sweet cherry cultivar trees were determined (**Figure 7**).

12. Cherry harvest, storage and marketing

Cherry fruits do not continue ripening after harvest. Therefore, the right harvest time should be determined carefully. Generally, the harvest starts after the coloring of the fruits.

Fruit cracking in cherries is an important problem in rainy regions. Also, excessive irrigation of the orchards and prolonged stay in a humid environment increase the rate of fruit cracking. Cracked fruits lost their market value, fungal infections occur in fractured parts. Cracking occurs when water enters into the fruit peel and the fruit swells rapidly. In rainy weather, the fruit volume can increase by 10% as the water enters into the ripen fruit.

There are differences between cultivars in terms of susceptibility to cracking. Bing, Van, Karabodur, Early Burlat are sensitive cherry cultivars to cracking. Generally, cherry cultivars with firm fruit flesh are more susceptible to cracking. It was determined that fruit cracking was decreased with the application of burgundy slurry and copper sulphate on trees before harvest. If 450 g borax/decare was given to the cherry orchards that show boron deficiency, it was found that the cracking rate of cherry cultivars was decreased for about 25–50%. Giberellic acid applications to increase the fruit load and fruit set reduce Ca content of the fruit. The use of plant growth regulators that reduce shoot growth and Giberellin synthesis can increase Ca content of the fruit. Spraying Ca 10 days before the harvest decrease cracking and increase the fruit flesh firmness [23].

Various parameters are used to determine the right harvest time in cherries. Among them, the size, fruit color and amount of Brix value are the most commonly used parameters. Generally, the minimum size for exportable cherry fruit is 26 mm. Cherries are nonclimacteric fruits and they do not ripen after harvest. At the harvest time, cherries should contain at least 14–15% Brix value.

Cherry fruits are very sensitive to mechanical damage and deterioration after harvest. For this purpose, cherries should be precooled quickly after harvest and should be packaged properly by using the correct package products. Continuity of the cold chain is also mandatory during the storage and marketing of packaged products [9, 24].

13. Conclusion

Cherry (*Prunus avium* L.) is originated from South Caucasus, Caspian Sea and North-East Anatolia and the wild cherries are mostly seen at the North Anatolian and the Taurus mountains of Turkey. Turkey is the leader country in the world on sweet cherry production (732.000 tons). Cherry production in Turkey has been performed mostly with '0900 Ziraat' local cherry cultivar, It is heart shaped with pink red fruit flesh, bright, firm, juicy, very large and high-quality fruits, suitable for transportation and long shelf life.

In 1997, "Turkish National Cherry Working Group" was founded and organized 22 working group meetings until today to solve all the problems of cherries. In these meetings, all the research results were shared and discussed among cherry scientists. These problems were pruning, cultivars, fertilization, rootstocks, irrigation, harvesting, pre-cooling, storage, packing, disease and pest control. Under this working group studies, seminars and conferences were organized at the most important cherry producer regions on training, pruning, rootstocks and growing techniques. With the widespread use of yield pruning in grown cherry trees, fruit yield and quality have increased besides the use of dwarf and semi-dwarf cherry rootstocks.

Some favorite and promising foreign sweet cherry cultivars were introduced in the country alternative to 0900 Ziraat cultivar. Especially with the spread of the late maturing Sweet Heart cultivar the cherry season, which ended at the end of July was extended until mid-August.

Many studies have been carried out on the chilling requirements of sweet cherry cultivars and chilling durations of Çukurova region which has a subtropical climate. As a result of working on low chill cherry cultivars, cherry orchards have started to be established in the subtropical regions.

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Chapter 8

Logistics Chain and Cost Assessment of Pruning-to-Energy Value Chains: Application of Life Cycle Cost Analysis Approach

Techane Bosona and Girma Gebresenbet

Abstract

Biomass from agricultural residue has significant potential as renewable energy resource. Therefore, cost-efficient processing and supply of agricultural residues are important to strategically plan and utilize this energy resource. This chapter describes the agricultural pruning to energy (PtE) value chains and presents the life cycle cost analysis (LCCA)-based cost assessment results, focusing on almond and peach tree pruning data obtained from Spain during 2015–2016. Along the main life cycle stages of PtE system, costs of harvesting, off-farm storage, transport, biomass loss, and management of biomass supply chain were considered. In terms of functional unit cost, the life cycle cost (LCC) was calculated to be about 126 €/t for almond PtE and 115 €/t for peach PtE value chain. In both cases, the harvesting stage was found to be cost at hot stage followed by the storage stage. The cost at harvesting stage was about 83% (of 126 €/t) and 82% (of 115 €/t) in the case of almond and peach cases, respectively. Similarly, the share of operational cost was about 74% and 76% for almond and peach cases, respectively. Therefore, more efforts should be made to improve the performance of logistics operations and management of such PtE initiatives.

Keywords: pruning-to-energy, life cycle cost analysis, almond tree pruning, peach tree pruning

1. Introduction

Reducing the use of fossil fuels and increasing renewable energy use contribute to sustainable energy use. In this regard, biomass from pruning residue could contribute a lot as an energy resource [1]. Due to some restrictions on nonrenewable energy sources such as fossil fuels and nuclear fuels, the use of renewable energy is expected to expand. Bioenergy is one of such renewable energy sources, and it is derived from biomass such as forests, municipal solid waste, and agricultural residues. Bioenergy could provide heating and cooling energy, electricity, and transport fuel [2]. About 60% of renewable energy sources in EU is bioenergy. However, there are challenges in production and supply of bioenergy in relation to: cost and efficiency of technologies; development of effective bioenergy supply chain from feedstock production to conversion into heat, electricity, and transport fuels; and how to integrate the bioenergy in the overall energy system [3].

Pruning is an important management practice for almond and peach trees [4, 5]. Globally, the land under almond tree cultivation in 2016 was estimated to be more than 1.8 million hectares while in Mediterranean areas, almond-tree pruning is one of largely available agricultural wastes as a fuel [5]. For example, Spain has devoted the largest area for almond tree cultivation and annually produces about 7.3×10^8 kg of almond tree pruning.

Even though agricultural pruning has a significant potential, it is not being utilized much for energy production because of several constraints such as cost and lack of technologies. Biomass-based energy production systems such as pruning-to-energy (PtE) initiatives should be designed well in order to reduce the financial and environmental cost [6]. Therefore, cost-efficient biomass supply is very important to strategically plan and implement more sustainable energy production from biomass including agricultural residues [7]. In such cases, cost models could include costs of harvesting, processing, transportation of biomass, procurement and supply chain management of biomass product, and the installation and management cost of power plants. In order to establish more optimized system, different methods such as location analysis (e.g., determining best location of biomass storage, power plant), transport route analysis, and integrated management systems could be applied [7–9].

This chapter presents part of the study that investigated the costs of fruit tree PtE value chains focusing on almond and peach tree pruning. The main objective of the cost assessment was to assess costs at different life cycle stages along PtE value using Life Cycle Cost Analysis (LCCA) approach. This enables to facilitate sustainable utilization of agricultural pruning as a source of renewable energy. It also facilitates the decision-making regarding PtE initiation in Europe and enables entrepreneurs to identify the type of logistics and process chains of lower investments.

2. Pruning-to-energy value chain and logistics configurations

The core processes in the PtE value chains include pruning, harvesting (collecting), processing, storage, transport, and energy production (see **Figure 1**). The main actors are farmers, harvesters (processors), traders, transporters, end users (owners of power plants), and administrator of the entire pruning supply chain. Different actors have different activities in the PtE value chain as indicated in **Figure 1**.

In the supply of pruning biomass, different logistics configuration types (LCT) could be designed depending on geographical conditions, biomass availability, and demand (see **Table 1** and **Figure 2**). For instance, if the pruning biomass quantity is limited, it could be used only for self-consumption. The storage facilities could be established on farm, off-farm, and at power plant. In most cases, off-farm

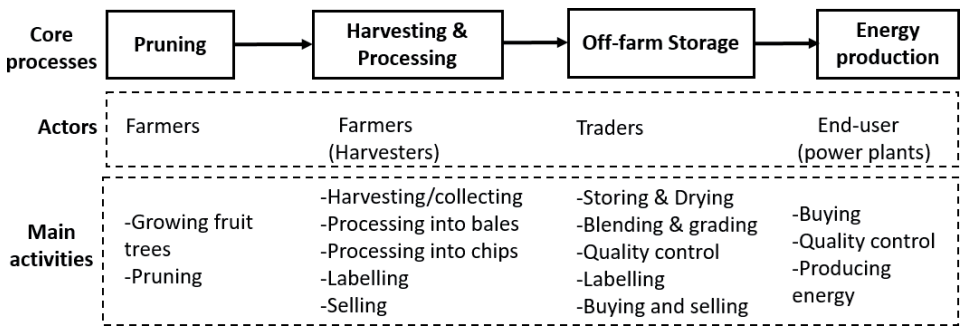

Figure 1.
Mapping of core processes, main actors, and activities along PtE value chains.

Type	Description
LCT1	Self-consumption (with or without storage), no significant transport
LCT2	On-farm storage, then direct delivery to final user
LCT3	Intermediate storage
LCT4	Direct delivery and storage at final user
LCT5	No-storage but direct delivery

Table 1.
Major logistics configuration types (LCT) pruning-to-energy value chain.

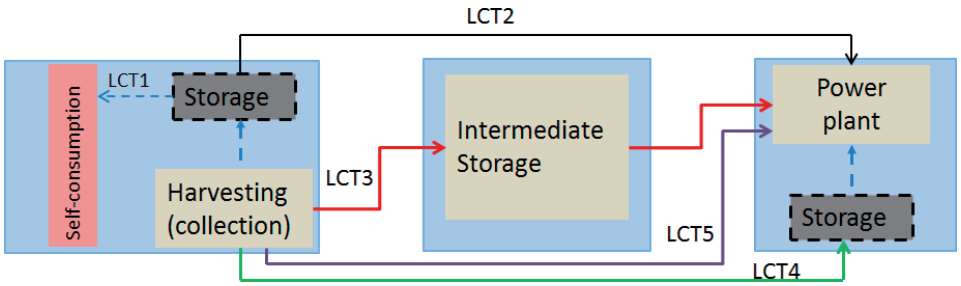

Figure 2.
Major logistics configuration types identified in the investigated pruning-to-energy (PtE) chains [10].

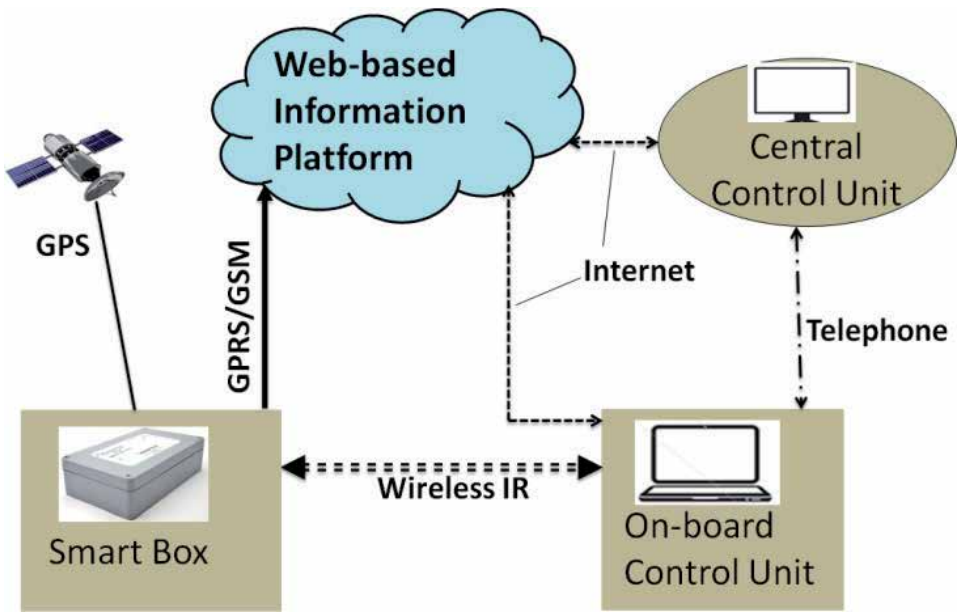

Figure 3.
Major components of smart system for coordinated management and monitoring of pruning biomass supply chain [11].

storage is used. Pruning biomass could be used as energy source at farm or could be sold to power plants either directly by farmers or through traders. Even though farmers, biomass traders, and end users are the major actors in these PtE initiatives, an independent management unit could be introduced as an actor, which could promote such PtE initiatives through application of smart systems for its

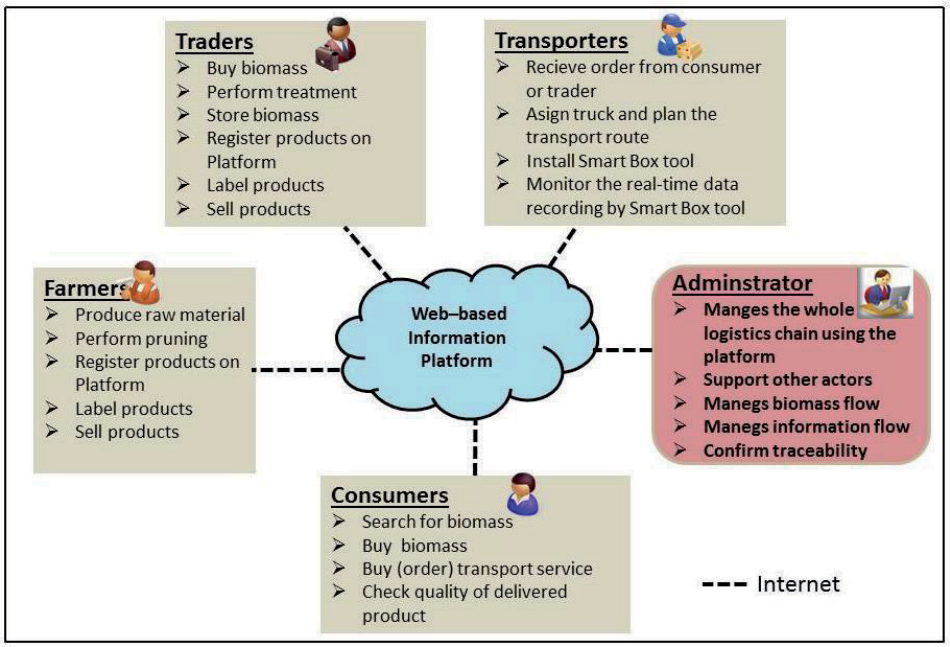

Figure 4.
Major activities of different users of the digital platform.

integrated management (see **Figures 3** and **4**). In relation to using smart system for coordinated management of the PtE initiatives, more actor-specific activities have been described in **Figure 4**.

3. Cost assessment of PtE value chain using LCCA

3.1 Life cycle cost analysis

Cost assessment is one of performance measurement areas, which include cost, asset assessment, productivity, customer service, and logistics quality [12]. In this chapter, life cycle cost analysis (LCCA) approach and its application to evaluate the economic performance of PtE initiative have been presented focusing on almond and peach tree pruning. By definition, life cycle thinking about a product considers the product from its inception to disposal [13]. Life cycle cost (LCC) is the total cost of owning and operating a product, facility, or a system over a period of time or its entire life span depending on the system boundary defined within the scope of the planed study [14].

Although LCCA was a tool originally developed by US Department of Defense, it is now applied in many industrial sectors [13]. It enables to make important decision in design, development, and implementation of projects or products with clear identification of cost distribution over the useful life span. To determine LCC value of a product or system, a bottom-up approach (e.g., engineering technique) and/or top-down approach (e.g., analogy method to make cost estimate using costs of similar existing products or system) [14, 15] could be used. In LCCA, short-term costs such as design and establishment of the initiated project or product and long-term costs such as operations, utilities, and maintenance costs should be considered appropriately [16].

3.1.1 Scope, purpose, and system boundary of LCCA

In an LCCA study, the scope, purpose, and system boundary of the system to be studied should be defined. In this chapter, the scope of LCCA study was limited to PtE value chains of almond and peach tree pruning. The purpose was to assess the cost effectiveness of pruning biomass supply chains with almond and peach tree pruning supplied in the form of chips in Spain. It was intended to develop LCCA methodology for evaluation of PtE initiatives, identify the cost-efficient design, development, and implementation of pruning biomass logistics configurations that could be benchmarked and applied different PtE initiatives in the same region or beyond.

The system boundary of LCCA of almond and peach tree pruning biomass includes core stages such as harvesting (collecting the pruning residue), processing, storage, transport, and management of the entire pruning biomass supply chain (see **Figure 5**). Almond and peach tree growing and pruning activities were not included due to limitation of data. Similarly, at power plant, power plant installation and plant operation costs were not included. The system boundary could be considered also from period over which an investment or LCC assessment is to be analyzed. The economic life (life span) of farm machines such as tractors is often considered to be 10–12 years during cost estimation [17]. Therefore, 10 years was considered for LCCA of almond and peach PtE value chain under consideration. Defining functional unit cost (FUC) is also important during defining the system. FUC is important for LCCA in order to harmonize cost data obtained from cost data inventory (expressed in different units). In case of PtE value chain, FUC could be expressed in Euro per ton of biomass (€/t) over a wet basis (w.b.). This should be done with caution, because the weight of biomass varies along the supply chain.

LCCA considers both capital cost (initial expense) and future expenses. Initial expenses are one-time start-up costs (initial investment costs). The future expense consists of different operational (e.g., labor, maintenance and repair), disposal cost at the end of life span and contingency costs. The disposal cost could be incurred at different stages of PtE value chain (e.g., harvesting, storage, transport) and management activities. For instance, there could be disposal of machineries and tools at farm or storage, dismantling of structures such as storage site, disposal of equipment from power plant after useful life span. It is important to note that, in some cases, the machineries, equipment, and facilities could have

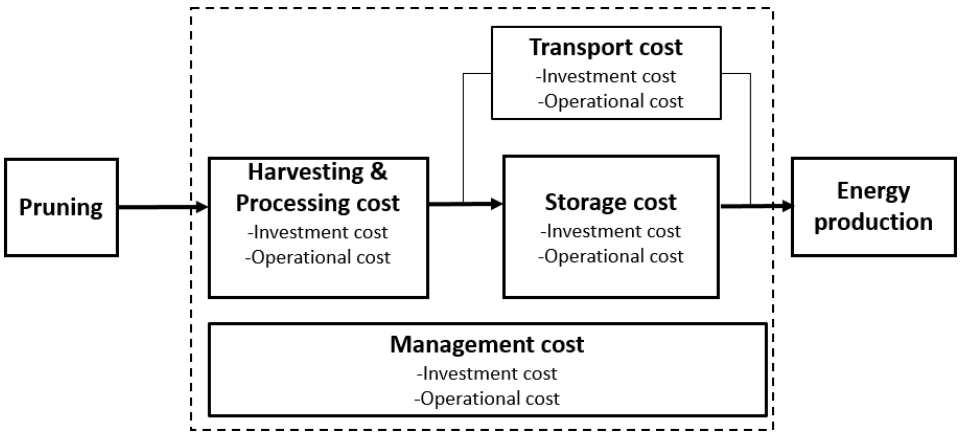

Figure 5.
Illustration of system boundary defined for this LCCA study of almond and peach PtE value chain.

some salvage value rather than incurring disposal cost, which could be considered as income (i.e., selling at price equal to estimated salvage value). Future expenses could also include environmental damage costs (e.g., emission costs) when standard monetary expressions (e.g., € per ton of CO_2) of environmental impacts are available. In this study, environmental damage costs were not considered as it requires more data and time to deal with its complexity, especially which environmental parameters to consider and which ones to omit is more complex, and was out of the scope of this study.

3.1.2 Remaining value

The purchasing price (list price) of machineries and equipment (see **Table 2**) could be considered as investment cost at the beginning of the PtE initiative, while the remaining value (RV) after depreciation over project life span could be considered as an income at the end of project service life. In order to estimate the RV at the end of project lifetime, i.e., 10 years, depreciation estimation approach can be used. In this study, remaining value and salvage value are not necessarily equal. Salvage value refers to actual economic service life of a machinery (or other equipment), say 15 years, while RV refers to only project lifetime e.g., 10 years of service life of the PtE initiative under consideration. Therefore, RV could be greater than salvage value in some cases. Eqs. (1) and (2) could be used to calculate the economic depreciation rate and RV.

$$D = \frac{LP - SV}{Ys} \quad (1)$$

$$RV = LP - 10 * D \quad (2)$$

where D is the depreciation cost in €/year; LP is the list price (purchase cost); SV is the salvage value at equipment economic life span of Ys; and RV is the remaining value at 10 years of project life span.

Item description	Quantity	Purchase price	Annual working time	RV
	Number	€	h	€
Harvester/chipper	1	30,000	800	9000
Harvester/baler	1	28,000	800	8400
Windrower	1	2700	800	756
Tractor (for chipping/baling activities)	1	61,000	800	21,960
Trailer	1	14,000	1000	0
Chipper (at storage site)	1	150,000	800	37,500
Tractor for loading and other activities (with shovel for loading biomass)	1	61,000	1000	21,960
Regular telescopic handler	1	58,000	1400	14,500
Truck with 90 m^3 mobile floor (trailer)	1	144,000	2000	28,800
Smart system for coordinated management	1	5800	1400	0

Table 2.
Investment cost and some related basic data of machineries and equipment.

3.2 Identification and description of costs along PtE value chains

The major components of operating costs to be considered in this study have been depicted in **Figure 5**. These include collection (harvesting) cost, storage cost, transport cost, and management (coordinated administrating and monitoring the PtE system) cost. The biomass production (cultivation) and pruning activities were not part of cost assessment in this study. Rather, the focus was how to use the pruning residues for energy generation instead of leaving them on soil or being mulched. Therefore, biomass production and pruning activities are out of the scope of this cost analysis. Costs of operational activities at the power plants are also out of the scope of this analysis (see **Figure 5**).

3.2.1 Harvesting and processing cost

The harvesting and processing (chipping) cost includes costs incurred during harvesting of pruning from arm field using machine. Costs related to on-farm activities such as chipping, on-farm transport, and on-farm temporary storage are considered as part of harvesting and processing cost. If the pruning should be collected from different fields, the average of costs from at each field could be considered.

3.2.2 Pruning biomass storage cost

Costs incurred at off-farm storage stage could be the initial cost such as construction of the storage and land cost while future variable costs at storage could comprise costs of handling and processing (e.g., chipping process at off-farm storage), truck unloading and loading, pile construction, and dismantling activities. In some cases, costs of sampling and quality analysis could be added (but not included in the case of almond and peach tree PtE value chain under consideration). In the case where the trader (owning the storage) could have multiple suppliers and receivers of pruning biomass, optimal location of storage site is required to reduce logistics costs. Storage cost depends on volume of pruning biomass stored and operational activities at the site. For the almond and peach pruning case, land cost is excluded from storage cost assuming that land can be obtained freely to promote renewable energy development from biomass.

3.2.3 Pruning biomass transport cost

The transport cost consists of fixed transport costs (e.g., investment on truck and trailers) and variable transport costs (e.g., operating cost during transport from farm to off-farm storage and from storage to power plant). The FUC of transport was euro per ton of biomass transported (€/t), and the FUC value could vary for different transport distances. From the survey of 25 PtE value chains [10], it was learnt that the average distance between farm and off-farm storage is about 14 km while the average distance between off-farm storage and power plant (end users) is about 116 km. As a basic scenario, the FUC was determined for 50 km average transport distance (i.e., 100 km round trip) from farm to power plant. The off-farm storage was assumed to be placed in middle way between farm and power plant. The truck facility location and off-farm biomass storage were assumed to be the same, and the truck starts from its location (storage) and returns back to storage after each trip. In addition, the capacity of truck used to transport pruning biomass was assumed to be 90 m^3 with payload of 24 t. The influence of transport distance on cost could be investigated through sensitivity analysis. Sensitivity analysis could be done also to investigate the influence of discount rate and truck capacity (volume and/or load) on LCC values.

3.2.4 Cost due to pruning biomass loss

Pruning biomass loss could occur during harvesting, storage, loading, and unloading activities. However, at the farm level, only biomass losses after the harvesting activity (e.g., handling during on-farm storage and loading for transport) were considered in the case of almond and peach. At off-farm storage site, all biomass losses have been considered. Except the biomass loss during loading, material loss during transport was considered to be negligible. For pruning in form of chips, the values used in calculation were 3% and 10% for on-farm loss and loss at storage stage, respectively. The loss at on-farm level is material loss at delivery to off-farm storage in reference to amount harvested. The loss at off-farm storage is the loss at the end of storage in reference to biomass weight at the start of storage. For pruning biomass processed in the form of bales, the loss is often less than the case of chips, i.e., about 2.5% and 1.5% for on-farm and storage stages, respectively. Due to the reduction of biomass weight at end of storage, it is important to consider the bulk density difference.

3.2.5 Pruning biomass supply chain management cost

In the case of almond and peach PtE value chains under consideration, the pruning biomass supply chain was managed by central management unit supported with smart system (see **Figures 3** and **4**) [11, 18]. Therefore, the management cost includes costs related to the management of biomass flow and product traceability information flow including marketing cost (e.g., cost of procurement and order management). The average cost can be estimated in €/t considering the total management cost during the year and the product delivered to end user during the same year. The management cost includes also the investment and maintenance cost of smart system and cost of providing appropriate training and technical support to all actors using the platform integrated in the smart system.

3.2.6 Calculating life cycle cost

For this coordinated management system, the total cost including cost of harvesting, storage, transport, and management could be modeled as indicated by Eq. (3).

$$LCC = H_c + S_c + T_c + M_c \tag{3}$$

where LCC is life cycle cost in €/t; H_c is harvesting cost in €/t; S_c is storage cost in €/t; T_c is transport cost in €/t; and M_c is management cost in €/t. For the case of almond and peach, costs due to biomass losses have been included into harvesting cost and storage cost. During cost calculation, FUC should be determined with caution due to variation of biomass moisture content. This model (Eq. (3)) could be used to determine either only the total operating cost or include the investment costs incurred at each stage of life cycle and expressed in €/t.

LCC values were calculated in the present money value, considering project lifetime of 10 years. Present value (PV) of payments to be made at future times can be determined using discount rate as given below (Eq. (4)) [19].

$$PV(i,N) = \sum_{i=1}^{N} \frac{C}{(1+r)^y} \tag{4}$$

where PV is the present value of total expenditure over N-year period; C is expenditure during year y; N is the lifetime of the system (years); and r is (real) discount rate (e.g., 5%).

Species	Country	Logistics description			
		Harvesting stage	**Transport to storage**	**Storage**	**Transport to end user**
Almond	Spain	Chipping in big bags	Chips in big bags	Pile of chips	Loose chips
Peach	Spain	Chipping in big bag	Chips in big bag	Pile of chips	Loose chips

Table 3.
Almond and peach pruning biomass and description of processing and handling.

Species	Country	Pruning potential prior to harvesting	Average material capacity*	Harvester working hours	Yearly potential [t]
		(t/ha)	**t/h**	**h/year**	**t/year**
Almond	Spain	0.62	0.63	800	504
Peach	Spain	2.64	0.77	800	616

**Harvesting capacity of the harvester in ton per hour.*

Table 4.
Harvestable quantity of pruning biomass during 1 year.

Where income data is available, net present value (NPV) could be determined (i.e., inflow cash minus outflow cash). In this case, the cost calculations were done for almond and peach tree PtE value chains for clearly defined system boundary. The processing and handling of biomass and harvestable quantity have been presented in **Tables 3** and **4**, respectively.

4. Results and discussions

Table 5 presents the calculated LCC values for almond and peach PtE value chains within the defined scope. In the analysis, weight over wet basis at the moisture content of starting of the storage construction was considered. Based on the bulk density at different duration along the pruning biomass supply chain, the weight at different stages has been converted to weight at the beginning of off-farm storage (in wet basis). The analysis results indicate that the harvesting stage was cost of hot-spot stage of the pruning biomass value chain, followed by storage stage. However, this was only for the case of 50 km transport distance considered. Therefore, if the transport distance increases, the transport cost could exceed the storage costs due to the increased logistics cost. For almond case, the cost at harvesting stage was about 83% of the total LCC (126 €/t) while it was about 82% (of 115 €/t) in the case of peach pruning. The main cause of high harvesting cost could be the poor harvesting performance for almond and peach tree pruning, i.e., 0.63 t/h and 0.77 t/h, respectively, when expressed in harvestable biomass quantity per hour (see **Table 4**).

Even though project lifetime of 10 years was considered, the calculated LCC values were presented in present money value at base year of 2016. In this type of cost assessment, besides the costs, income and net values (cost less income) could be determined. The main sources of income include the selling of pruning biomass in terms of chips, the avoided cost (i.e., cost of pruning handling by farmers, e.g., avoided mulching cost), and residual value at the end of project time. During the period when data of this study was gathered, the average selling price of chips was

Life cycle stage	Almond tree pruning			Peach tree pruning		
	Investment cost	Operating cost	Sum	Investment cost	Operating cost	Sum
Harvesting	30.92	74.09	105.01	25.44	69.03	94.47
Storage	0.46	8.60	9.06	0.52	8.92	9.44
Transport	0.58	3.51	4.09	0.47	4.30	4.76
System management	1.15	7.04	8.19	0.94	5.76	6.70
Total	33.10	93.25	126.35	27.36	88.01	115.37

Table 5.
LCCA results in €/t including the investment and operational costs.

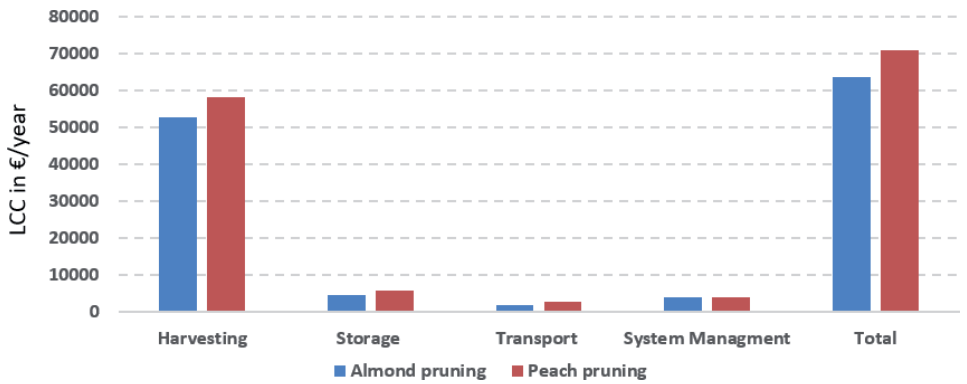

Figure 6.
LCC values in € per year, i.e., considering 504 t almond and 616 t of peach pruning biomass per year, 50 km transport distance from farm to power plant, and 90 m³ truck capacity.

about 64 €/t for both almond and peach pruning chips while the avoidable cost at farm was estimated to be 75 €/t on average.

When the quantity of almond and peach pruning biomass to be handled at off-farm storage during a year is known as indicated in **Table 4**, the LCC values could be determined in €/year as indicated in **Figure 6**. In this case, the yearly costs included both investment and operational costs (see **Figure 6**). From **Table 5** and **Figure 6**, it is clear that the operating cost is higher than investment cost in both almond and peach pruning cases. Considering the final LCC values, the share of operating cost was 74% and 76% for almond case and peach case, respectively. Therefore, more attention should be given to improving the operating (e.g., efficient harvesting machines) and management systems (e.g., smart tools for management and monitoring biomass flow) to reduce the operational costs, increase the economic performance, and promote the PtE initiatives.

5. Conclusion

Biomass from agricultural residue such as almond and peach tree pruning has significant potential as renewable energy resource, which enables to reduce the use of fossil fuels and contributes to sustainable energy use. Therefore, cost-efficient biomass processing and supply are very important to strategically plan and implement more sustainable energy production from biomass including agricultural

residues. In this chapter, the cost of pruning to energy (PtE) value chains has been investigated using the case of almond and peach tree pruning with data from Spain. The life cycle cost analysis (LCCA) approach was used considering both investment and operating costs over the project lifetime of 10 years, while 2016 was the base year. First, the typical PtE value chain has been described. Then the scope and framework of LCCA study have been defined. In this case, harvesting (collection of tree pruning from field) and chipping, off-farm storage of chips, biomass loss at harvesting and storage, transport, and coordinated management of the entire pruning biomass supply chain have been considered. In the cost estimation, the pruning (cutting branches) activity and costs at power plant stage have been excluded due to lack of appropriate data. As a basic scenario, 50 km transport distance (from farm to power plant) and 90 m^3 truck capacity have been considered for both almond and peach cases. In the investigated cases, the yearly harvestable quantities have been 504 t and 616 t per year for almond and peach pruning, respectively. Accordingly, the yearly cost was calculated to be 63,680 € and 71,070 € per year for almond and peach cases, respectively. In terms of functional unit cost, the life cycle cost was calculated to be about 126 €/t for almond pruning and 115 €/t for peach pruning. In both cases, harvesting stage was found to be cost of hot-stage followed by the storage stage. The cost at harvesting stage was about 83% of the total LCC (126 €/t) in the case of almond, while it was about 82% (of 115 €/t) in the case of peach pruning. Similarly, the share of operational cost was found to be higher than investment cost. Considering the final LCC values, the share of operating cost was 74% and 76% for almond case and peach case, respectively. Therefore, at strategic level, more attention should be given to improvement of logistics operations and management in order to increase the economic performance of such PtE initiatives.

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Chapter 9

Nutritional and Antioxidant Values of the Black Plum (*Vitex doniana*)

Koba Fatou Traore, Kisselmina Youssouf Kone, Amédée Pascal Ahi, Doudjo Soro, Nogbou Emmanuel Assidjo and Marianne Sindic

Abstract

This study was conducted to first determine the nutritional potential and the antioxidant activity of black plum fruit pulp and peel. For these characterizations, classic methods were used. The results indicated high fibre and ash contents of black plum from all localities studied, ranging, respectively, from 34.79 ± 0.07–39.83 ± 1.85% and 4.91 ± 0.45–5.91 ± 0.41% for pulp, and 46.38 ± 0.09–50.21 ± 1.07% and 4.16 ± 0.81–4.28 ± 0.20% for peel. The mineral analysis revealed that Black plum pulp and peel are high in potassium (1863.00 ± 1.4–2584.55 ± 3.54 mg/100 g dry weight [DW]) and calcium (355.30 ± 2.52–389.52 ± 3.54 mg/100 g DW). Both the peel and pulp are characterised by a good essential amino acids profile of the protein. The total polyphenol, flavonoid and anthocyanin contents of pulp and peel ranged from 202.51 ± 4.19 to 463.45 ± 6.85 mg gallic acid equivalent (GAE)/100 g of Dry Weight (DW), 75.71 ± 1.03 to 145.55 ± 1.03 mg quercetin equivalent (QE)/100 g DW, and from 1.91 ± 0.08 to 8.28 ± 0.83 mg cyanidin 3-O-β-D-glucoside equivalent (C3GE)/100 g DW respectively. Thus, these fruits constitute a good source of important nutrients for health.

Keywords: Nutritional values, antioxidant, black plum, valorization

1. Introduction

Fruits and vegetables have always been considered as essential sources of micronutrients and dietary fibre for the body to function properly. Moreover, regular fruit consumption is recommended for disease prevention and health benefits due to the nutrient composition of fruits, which includes vitamins, minerals, fibre and bioactive compounds [1]. In Côte d'Ivoire, these nutrients are generally delivered by some cultivated tropical fruit, including mangoes, papaya, citrus fruit, as well as imported temperate climate fruit, such as apple, pear and grape [2]. These tropical fruits are often not readily accessible to the populations beyond production time, especially in the developing countries due to their high cost or scarcity in the local market [3, 4]. However, these shortages correspond to the production stage of some wild fruit-bearing species, underutilised because of the limited knowledge

regarding their nutritional values. Assessing the quantitative and qualitative value of this fruit (including peel, pulp and seed) could reveal novel food sources with potential health-protective properties.

Among these wild plant resources, black plum (*Vitex doniana*) is an abundant and widespread species in tropical countries, such as Côte d'Ivoire [5–8]. In Côte d'Ivoire, it is found, for example, in Pakobo, Ahiérémou and Zougoussi, located in the centre of the country [9], Seguela in the west–north, Korhogo and Tingrela in the north [10], and Bondoukou in the east–north region [11]. Various parts of the plant, including the leaves and bark, have been used for many decades by some rural populations to treat diabetes, ulcer, diarrhoea, dysentery, asthma, insomnia and various other illnesses. The fruit of this species is an ellipsoid drupe of about 2.5–3.0 cm in height and 2.0–2.5 cm in diameter, and rich in sugar [12, 13]. The unripe fruit is green but turn brown–black when ripe, and are eaten raw [9, 14]. Nutritional analyses indicate that black plum is a good source of vitamins, such as calcium, iron, magnesium, phosphorus and vitamin C [15–18]. In addition, the pulp provides useful quantities of flavonoids with strong antioxidant properties [19].

Interestingly, no comparative study between the nutritional composition of black plum pulp and peel has yet been carried out. This study sought to show the nutritional potential and antioxidant properties of black plum peel and pulp, by analysing the physicochemical and nutritional properties in order to contribute to the valorization of this fruit, produced in large quantities, even in some remote areas of Côte d'Ivoire, particularly in the regions of Ferke, Tiebissou and Yamoussoukro.

2. Materials and methods he entirety

2.1 Sample preparation

The ripe black plum were harvested in three Côte d'Ivoire localities: Ferkéssédougou (Ferke), Tiébissou and Yamoussoukro. The fresh fruit was washed with tap and distilled water, sequentially. After drying the fruit with a paper towel, the pulp and peel were separated by hand using a stainless-steel knife, dried in an oven (Memmert U30-Gemini BV, GmbH, Germany) at 50°C for 24 h, then milled using a miller (ZBK220077–88 LW74d(B) A, China. Milled pulp and peel were packaged in polyethylene bag using a vacuum packager (NG 10121 MULTIVAC, Belgium) and frozen at –19°C until analysis. All tests were realised in triplicate.

2.2 Chemical and biochemical analyses

2.2.1 Ash, protein, fat, carbohydrate and dietary fibre contents

Ash, crude protein, fat and total carbohydrate were determined by standard methods AOAC [20]. Dietary fibre was analysed by AOAC 991.43. [21], and total carbohydrates by the protocol reported by Bertrand and Thomas [22]. The energy value (kcal/100 g) was calculated by multiplying the protein, fat and carbohydrate contents (g) by factors of 4, 9 and 4 kcal/g, respectively.

2.2.2 Minerals analysis

Dried pulp and peel (0.5 g in each case) of Black plum were transferred to digestion flasks containing 5 mL of HCl/HNO3 (1:3). After 2 h of slowly boiling, the mixture was cooled at room temperature, then 50 mL of distilled water was added

and followed by filtration. The cooled solution was placed in flasks and transferred to the atomic absorption spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) for mineral determination. The phosphorous was measured spectrophotometrically. Standard calibration curves constructed for each element were used for direct quantification.

2.2.3 Amino acid analysis

The black plum pulp and peel amino acids composition was determined based on a previous method [23]. Duplicate samples were hydrolysed by transferring around 50 mg of sample weighed accurately in a 15 mL flask in which 5 mL of HCl (6.0 N) was added. The flask was closed under vacuum, nitrogen-purged and digested at 110°C for 24 h. Sulphur-containing amino acids were determined by using performic acid. The amino acids were analysed using a Biochrom 20+ amino acid analyser, Cambridge, United. Kingdom).

2.2.4 Phenols extraction

Phenols were extracted by the method of Soro et al. [19]. Dried V. doniana pulp and peel samples (1 g) were homogenised in 10 mL solution of 80% methanol and 2% formic acid, using an Ultra TurraxT25 basic homogeniser (Heldoph Instruments D-91126, Schabach, Germany) at room temperature. The homogenate was sonicated for 30 min in a Bandelin electronic RK 541 H sonicator (Heinrichstrasse 3–4 D-12207, Germany) and then centrifuged at 9400 × g for 25 min in a DBS centrifuger (PCB 1500, Italy). The supernatant was collected and the precipitate extracted again with 10 mL of 80% methanol, under the conditions previously described. The two supernatants were mixed and filtered using Whatman filter paper No.1. The final methanolic extract was stored at 25°C to be used in determination of total phenols, flavonoids and anthocyanin contents.

2.2.4.1 Determination of total phenols

Total phenolic compounds (TPC) determination was performed as described by Gao et al. [24] Phenolic extract (100 μL) was mixed with 0.2 mL Folin–Ciocalteu reagent (Sigma), 2 mL of H2O and 1 mL of 15% Na2CO3 and the absorbance measured at 765 nm in a spectrophotometer (Thermo Scientific™ 75003631, ThermoFisher Scientific SAS, Strasbourg, France) after 2 h incubation at room temperature. Gallic acid was used for the calibration curve with a concentration range from 0 to 200 mg/L. Total phenols were expressed as mg gallic acid equivalent (GAE)/100 g DW.

2.2.4.2 Determination of total flavonoids

Total flavonoid (TF) contents were determined according method used by Meda et al. [25], but slightly modified. A volume of 0.5 mL of sample methanolic extract was diluted in 0.5 mL of distilled water. Then, 0.5 mL of aluminium chloride 10% (w/v) and the same volume of 1 M sodium acetate was added. Finally, 2 mL of distilled water was added and absorption reading at 415 nm was taken after 30 min against a blank sample consisting of a 4 mL methanolic extract without aluminium chloride. Quercetin was used for the calibration curve with a concentration range from 0 to 3.125 mg/mL. Results were expressed as mg quercetin equivalent (QE)/100 g DW.

2.3 Statistical analysis

All data were analysed using ANOVA based on Tukey's HSD multiple comparison test at $p < 0.05$. Software RStudio version 1.2.1335 2009–2019 was used, and triple analyses were performed. The results were presented as mean ± standard deviation.

3. Results and discussion

The nutritional composition of black plum pulp and peel harvested in three localities of Côte d'Ivoire (Ferke, Tiébissou and Yamoussoukro) are given in **Figures 1–3**. Values are compared on a dry weight (DW) basis, except for moisture content.

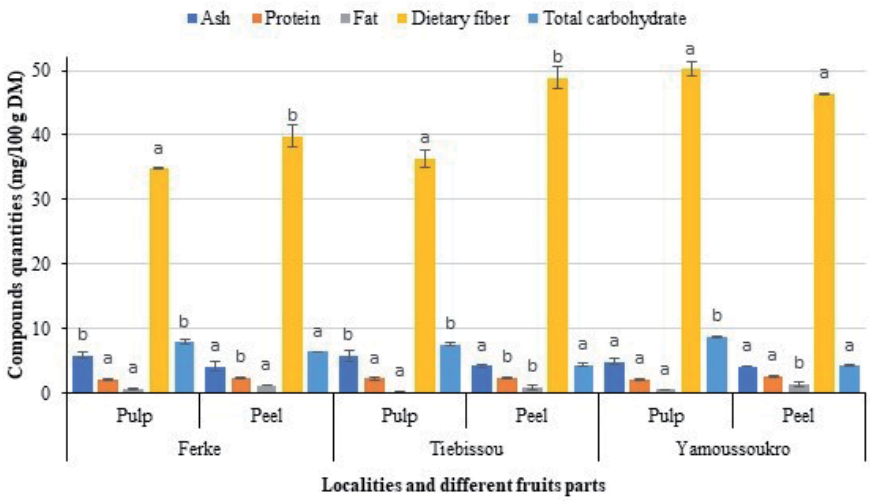

Figure 1.
Comparison between the nutritional composition of pulp and peel of Vitex doniana fruit harvested in Ferke, Tiebissou and Yamoussoukro.

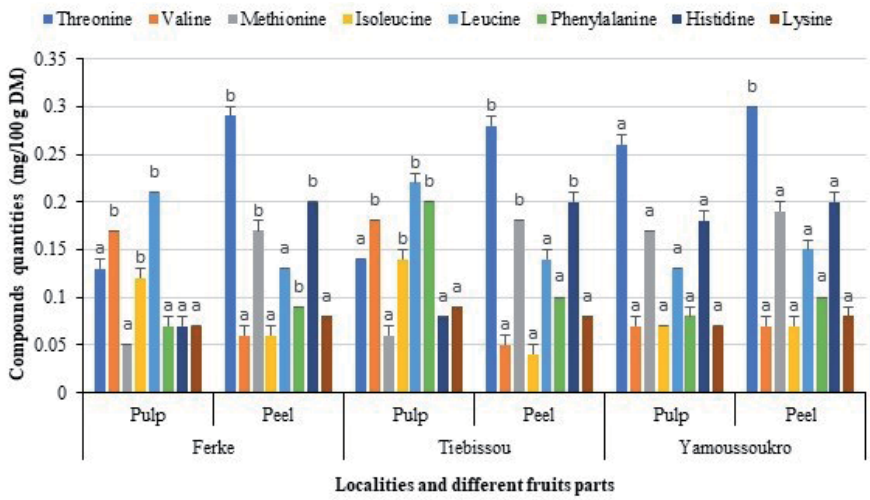

Figure 2.
Comparison between pulp and peel amino acid contents of black plum harvested in Ferke, Tiebissou and Yamoussoukro.

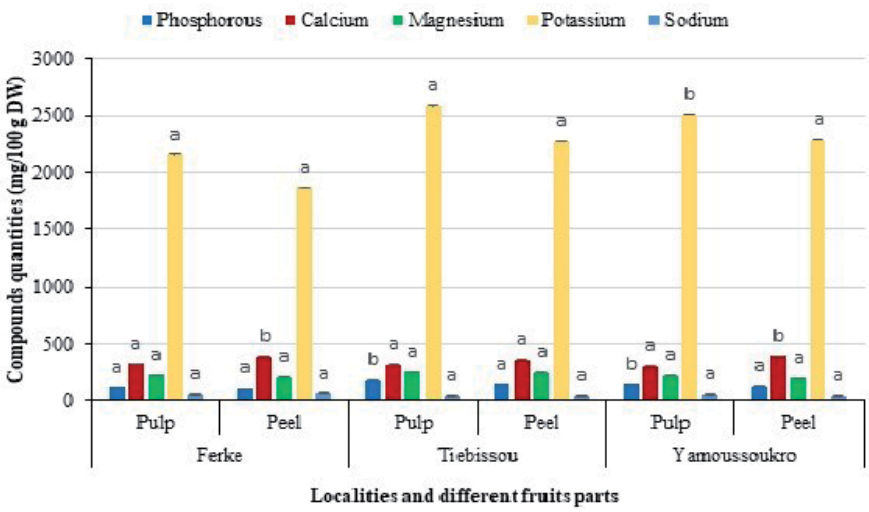

Figure 3.
Comparison between pulp and peel micromineral contents of Vitex doniana fruit harvested in Ferke, Tiebissou and Yamoussoukro.

3.1 Ash content

There was no significant difference between pulp and peel of fruit among the localities, but the ash contents of Ferke and Tiebissou fruit pulps were significantly higher ($p < 0.05$) than those of Yamoussoukro (**Figure 1**).

These results contrast sharply with those of previous authors, notably, Soro et al. [19], who obtained average ash contents of 1.4% and 3.53%, respectively, for the fruit pulp of V. doniana harvested in V-Baoulé and Nord of Côte d'Ivoire. Comparatively higher ash contents of 11.50% were reported by Vunchi et al. [18], whereas Agbede and Ibitoye [16] stated levels (5.1%) similar to the current work. In addition, compared with the pulp from fruit of the same genus, the ash content found here is higher than that of baobab (4.5 ± 0.2%; Magdi, 2004) and avocado (2.1 ± 0.6%; [26]).

This ash content variation within the same fruit and between the same species may be due to the water content and soil properties [27]. The ash content of food reflects its total mineral content, and so Black plum would be a valuable source of minerals.

3.2 Protein content

Pulp and peel protein contents of Black plum ranged from 2.11 ± 0.15 to 2.28 ± 0.16% (**Figure 1**). A markedly higher protein content (2.61 ± 0.07%) occurred in the peel of fruit from Yamoussoukro than Ferke and Tiebissou, respectively, of 2.35 ± 0.10 and 2.40 ± 0.13%, which had more protein in their pulps than peels (**Figure 1**). In comparison to these data, higher protein values (3.04%) have been recorded for Black plum in Uganda by Acipa et al. [15], and in the work by Jacob et al. [28] for *Strychnos spinosa* (8.72 ± 0.02%), *Diospyros mespiliformis* (6.99 ± 0.02%) and *Dialium guineense* (5.23 ± 0.01%). The fruit pulps studied have a low protein content, as per most fruits [26]. Protein contents are known to influenced by the soil composition, including nitrogen content [29].

3.3 Fat content

Black plum pulp has a markedly lower fat content (0.20 ± 0.03–0.66 ± 0.16%) than the pulp of strawberry (2.1 ± 0.16%; [30]), as well as mango (5.9 ± 0.05%), passion fruit (0.8 ± 0.4%) and pineapple (1.8 ± 0.03%) studied by Martinez et al. [31], Although black plum peel (0.97 ± 0.37–1.46 ± 0.33%) had a higher fat content relative to the pulp, it is still low in fat (**Figure 1**), particularly when considering the fat content of tomato peel (6.01 ± 0.13%; [32]. From these results, Black plum is not a useful source of lipids.

3.4 Total carbohydrates content

Both the pulp and peel studied in this work, are low in carbohydrate. Yamoussoukro pulp samples were the highest in carbohydrate compared with the other samples (**Figure 1**), while fruit from Ferke showed intermediate carbohydrate contents (7.94 ± 0.36%). Nonetheless, both values were lower than those found in the pulp of Black plum from Burkina Faso (19.68–20.25%; [33]) and Nigeria (28.40 ± 1.06%; [16]), as well as the pulp of some other fruits, including mango (11.9 ± 0.41% DW) but close to the fruit of *Adansonia digitata* (8.25 ± 0.03–9.25 ± 0.21%; [33]). The age of the tree, and environmental factors, such as climate and soil, could be the basis of the observed differences because Ferke is located in a dry savanna zone, with a very hot and dry climate (Sudanese climate) and ferralitic soils [34, 35], while Yamoussoukro and Tiebissou, located in mesophilous and savanna wooded areas, respectively, have a Baoulean climate, both with moderately desaturated ferralitic soils [36].

Among the localities, Ferke (6.49 ± 0.00%) corresponded to fruit peel with the highest total carbohydrate content, while an average of 4.36% carbohydrate occurred in the peel of fruit harvested in Tiebissou and Yamoussoukro. Comparatively richer sources of carbohydrate are the peels of mango Raspuri (28.20 ± 0.60% DW), mango Badami (20.80 ± 0.20% DW; [37]) and tomato (32.16 ± 1.11% DW; [38]). However, fruit pulp contained the most carbohydrates whatever the locality (**Figure 1**). The high carbohydrate content of Ferke fruit peel would be linked to the climate, as this region is located in the northern part of Côte d'Ivoire where a generally high temperature occurs. Given the Black plum are low in carbohydrate, it can be concluded that regular consumption of this fruit would be beneficial for people with obesity, who are most often subject to a diet restricted in carbohydrates.

3.5 Dietary fibre

This study showed that dietary fibre is a main component in the pulp (34.79 ± 0.07–39.83 ± 1.85%) and peel (46.38 ± 0.09–50.21 ± 1.07%) of Black plum harvested in the three localities of Côte d'Ivoire. The dietary fibre levels in the pulp of Black plum are high when considering the amounts present in the pulp of white guava (3.50 ± 0.01%; [39]) and Deglet-Nour and Allig date varieties (14.4 ± 1.12% and 18, 0.45%; [40]. Moreover, the values obtained in this work approximate those recorded by Grigelmo-Miguel and Martín-Belloso [41] for the three orange varieties, Navel (35.4 ± 1.4%), Salustiana (35.9 ± 0.5%) and Valencia Late (36.9 ± 0.3%), which are well-recognised as sources of dietary fibre.

In addition, black plum peel displayed a much higher level of dietary fibre than some other fruit peels too, especially avocado (43.9 ± 2.7%), pineapple (16.3 ± 2.5%) and papaya (16.6 ± 2.2%) [42]. Interestingly, the peel fibre contents were significantly higher for the fruit harvested in Yamoussoukro (46.38 ± 0.09–50.21 ± 1.07%)

than Ferke and Tiebissou (**Figure 1**). This trend could be explained as a fruit defence against heat stress. Ferke and Tiebissou fruit pulp results agree well with results found by Lamghari et al. [43], Gorinstein et al. [44] and Morais et al. [26]. Similar to the present study, these authors also obtained significantly higher ($p < 0.05$) dietary fibre values in the studied peels than pulps. It is well known that diets high in dietary fibre are associated with the prevention and treatment of various diseases, such as diverticular and coronary heart disease, colon cancer and diabetes, besides contributing to weight loss in individuals with obesity [45, 46].

Based on the results observed, black plum is a potentially good dietary fibre source and could contribute towards the prevention and treatment of several degenerative diseases. Therefore, these fruits could be included in some food formulations for possible fibre enrichment.

3.6 Amino acids

Similarly to most fruits, such as apple [47], medlar [48], strawberry [49] and tomato [50], the main amino acids present in Black plum pulp were glutamic acid and aspartic acid, which had averages of 0.27 mg/100 g DW (**Table 1**). Conversely, the peels were particularly high in threonine and proline, with mean contents of 0.29 ± 0.01 and 0.27 ± 0.01 mg/100 g DW, respectively. Furthermore, the percentages of essential amino acids in the protein of black plum pulp and peel compare well with standard protein, according to the World Health Organisation (WHO; **Table 2**). In pulp protein, only two amino acids or amino acid pairs (methionine/cysteine and lysine) had scores below 100%, of a few percentage points only or around 50% (lysine). However, only isoleucine (47.82–80.42%), lysine (41.60–51.62%) and valine (30.80–41.43%) percentages characterised the peel protein. In addition to Yamoussoukro fruit, Ferke and Tiebissou fruit pulps contained higher threonine, histidine and methionine amounts than their peels (**Figure 2**). The peels were rather rich in valine and leucine. Despite the low protein content, black plum protein has a good amino acids profile.

3.7 Mineral contents

Regarding the macrominerals, all samples contained high amounts of potassium (K), followed by calcium (Ca), magnesium (Mg), phosphorus (P) and sodium (Na). In addition, the main micro-minerals were iron (Fe), followed by manganese (Mn) and Zinc (Zn). The concentration ranges of K, Ca, Mg, P and Na in Black plum, were respectively, 1863.00 ± 1.41–2283 ± 1.41, 355.30 ± 2.52–389.52 ± 3.54, 221.00 ± 1.41–255.5 ± 2.12, 118.00 ± 0.71–179.00 ± 2.83 and 38, 30 ± 7.77–53.33 ± 1.53 mg/100 g DW for the pulp, and 2155.50 ± 4.95–2584.55 ± 3.54, 301.50 ± 2.12–325.50 ± 0.00, 199.67 ± 1.15–250.00 ± 2.83, 105.50 ± 2.12–142.00 ± 1.41 and 39.70 ± 5.69–62.31 ± 2.08 mg/100 g DW for the peel (**Table 3**).

The pulp and peel of fruit collected in Tiébissou and Yamoussoukro exhibited the highest levels of K, while the lowest K levels (2155.50 ± 4.95 and 1863.00 ± 1.41 mg/100 g DW, respectively) occurred in those from Ferke. The K content of the fruit pulp of V. doniana in this work is higher than that obtained by Ladeji and Okoye [51] of 127.2 mg/100 g DW, and Vunchi et al. [18] of 15.70 ± 0.26 mg/100 g DW. In addition, the observed Black plum pulp and peel K contents are higher than those in banana (382 ± 15 and 337 ± 7 mg/100 g DW; [52]) and mango (185 ± 11 and 444 ± 13 mg/100 g DW; [53]. The pulps Ca concentrations were significantly similar ($p > 0.05$) among the localities, whereas Ca concentration was highest in Yamoussoukro fruit peel (389.52 ± 3.54 mg/100 g DW), and lowest in Tiebissou fruit peel (355.30 ± 2. 52 mg/100 g DW). In addition, except for Tiebissou

Amino acid	Pulp			Peel		
	Ferke	Tiebissou	Yamoussoukro	Ferke	Tiebissou	Yamoussoukro
Essential amino acids						
Threonine	0.13 ± 0.01a	0.14 ± 0.00a	0.12± 001a	0.29 ± 0.02a	0.28 ± 0.01a	030 ± 0.00a
Valine	0.17 ± 0.00a	0.18 ± 0.00a	0.17 ± 0.01a	0.06 ± 0.01a	0.05 ± 0.02a	0.07 ± 0.03a
Methionine	0.05 ± 0.00a	0.06 ± 0.02a	0.07 ± 0.00a	0.17 ± 0.01ab	0.18 ± 0.00a	0.19 ± 0.01b
Isoleucine	0.12 ± 0.01a	0.14 ± 0.01a	0.13 ± 0.00a	0.06 ± 0.01ab	0.04 ± 0.01a	0.07 ± 0.01b
Leucine	0.21 ± 0.00a	0.22 ± 0.01a	020± 0.00a	0.13 ± 0.00a	0.14 ± 0.01a	0.15 ± 0.02a
Phenylalanine	0.07 ± 0.01a	0.20 ± 0.00c	0.08 ± 0.01b	0.09 ± 0.00a	0.10 ± 0.00a	0.10 ± 0.00a
Histidine	0.07 ± 0.01a	0.08 ± 0.00a	0.18 ± 0.04b	0.20 ± 0.00a	0.20 ± 0.01a	0.20 ± 0.01a
Lysine	0.07 ± 0.00a	0.09 ± 0.00b	0.07 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.01a
Non-essential amino acids						
Aspartate	0.26 ± 0.01a	0.29 ± 0.01a	0.26 ± 0.00a	0.04 ± 0.00a	0.02 ± 0.02a	0.04 ± 0.00a
Serine	0.14 ± 0.00b	0.16 ± 0.00b	0.12 ± 0.00a	0.14 ± 0.00a	0.14 ± 0.00a	0.14 ± 0.01a
Glutamate	0.26 ± 0.01a	0.30 ± 0.01b	0.26 ± 0.00a	0.17 ± 0.00a	0.17 ± 0.00a	0.17 ± 0.01a
Proline	0.15 ± 0.04a	0.14 ± 0.01a	0.15 ± 0.01a	0.28 ± 0.01a	0.29 ± 0.01a	0.29 ± 0.00a
Glycine	0.13 ± 0.00a	0.14 ± 0.00a	0.15 ± 0.01a	0.16 ± 0.00a	0.15 ± 0.03a	0.15 ± 0.02a
Alanine	0.13 ± 0.00a	0.16 ± 0.00b	0.12 ± 0.00a	0.15 ± 0.01a	0.16 ± 0.00b	0.15 ± 0.00ab
Cysteine	0.08 ± 0.01a	0.07 ± 0.02a	0.13 ± 0.00b	0.14 ± 0.00a	0.15 ± 0.00a	0.15 ± 0.00a
Tyrosine	0.10 ± 0.01a	0.10 ± 0.00a	0.20 ± 0.00b	0.22 ± 0.01a	0.23 ± 0.01a	0.24 ± 0.00a

Amino acid	Pulp			Peel		
	Ferke	Tiebissou	Yamoussoukro	Ferke	Tiebissou	Yamoussoukro
Lysine	0.07 ± 0.00a	0.09 ± 0.00b	0.07 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.00a	0.08 ± 0.01a
Arginine	0.11 ± 0.01b	0.12 ± 0.01b	0.08 ± 0.00a	0.09 ± 0.00a	0.08 ± 0.01a	0.07 ± 0.00a
ΣProtein (mg/100 g DW)	2.25	2.59	2.26	2.47	2.46	2.56

Results are expressed as mean ± standard deviation of three separate extractions and determinations. Data were analysed by ANOVA. In each column of each group, different letters indicate statistically different values according to Tukey's HSD comparison at p <0.05. DW: dry weight.

Table 1.
Amino acids contents (mg/100 g DW) of pulp and peel of black plum harvested in Ferke, Tiebissou and Yamoussoukro.

Pulp							Peel						
	Ferke		Tiebissou		Yamoussoukro		Ferke		Tiebissou		Yamoussoukro		
Amino acid	% Total AA	(%AA) ×100% ideal	% Total AA	(%AA) ×100% ideal	% Total AA	(%AA) ×100% ideal	% Total AA	(%AA) ×100% ideal	% Total AA	(%AA) ×100% ideal	% Total AA	(%AA)×100% ideal	WHO Ideal Protein
Thr	5.78	206.35	5.41	193.05	11.50	410.87	11.74	419.32	11.38	406.50	11.72	418.53	2.8
Val	7.56	114.48	6.95	105.30	3.10	46.93	2.43	36.81	2.03	30.80	2.73	41.43	6.6
Met+ Cys	5.78	99.62	4.63	79.88	7.96	137.32	7.69	132.63	8.13	140.17	7.81	134.70	5.8
Phe + Thr	8.89	355.56	8.11	324.32	14.60	584.07	14.57	583.00	14.23	569.11	14.45	578.13	2.5
Lys	3.11	49.38	3.47	55.16	3.10	49.16	3.24	51.41	3.25	51.62	3.13	49.60	6.3
Ile	5.33	156.86	5.41	158.98	3.10	91.10	2.43	71.45	1.63	47.82	2.73	80.42	3.4
Leu	9.33	266.67	8.49	242.69	5.75	164.35	5.26	150.38	5.69	162.60	5.86	167.41	3.5

*AA: amino acid; Thr: threonine; Val: valine; Met + Cys: methionine + cysteine; Phe + Thr: phenylalanine + threonine; Lys: lysine; Ile: isoleucine; Leu: leucine.*WHO (1985)*

Table 2.
Pulp and peel amino acid contents of black plum harvested in Ferke, Tiebissou and Yamoussoukro compared with the "ideal" amino acid profile of the protein according to the World Health Organisation.

Mineral (mg/100 g dry weight)	Pulp			Peel		
	Ferke	**Tiebissou**	**Yamoussoukro**	**Ferke**	**Tiebissou**	**Yamoussoukro**
Phosphorous, P	118.00 ± 0.71a	179.00 ± 2.83c	146.00 ± 1.00b	105.50 ± 2.12a	142.00 ± 1.41b	127.00 ± 1.00b
Calcium, Ca	325.50 ± 0.00a	316.00 ± 3.61a	301.50 ± 2.12a	387.30 ± 3.06ab	355.30 ± 2.52a	389.52 ± 3.54b
Magnesium, Mg	228.50 ± 2.12a	255.5 ± 2.12a	221.00 ± 1.41a	207.00 ± 1.41a	250.00 ± 2.83a	199.67 ± 1.15a
Potassium, K	2155.50 ± 4.95a	2584.55 ± 3.54b	2505.00 ± 2.83b	1863.00 ± 1.41a	2270.50 ± 2.12b	2283.00 ± 1.41b
Sodium, Na	53.33 ± 1.53b	38.30 ± 7.77a	52.76 ± 0.58b	62.31 ± 2.08b	41.71 ± 1.53a	39.70 ± 5.69a
Zinc, Zn	1.11 ± 0.06a	0.97 ± 0.06a	1.00 ± 0.00a	1.07 ± 0.15a	1.00 ± 0.00a	1.03 ± 0.06a
Iron, Fe	19.00 ± 0.10b	15.30 ± 0.42a	46.75 ± 0.92c	9.20 ± 0.57a	42.77 ± 0.57c	21.33 ± 0.76b
Manganese, Mn	3.43 ± 0.07b	2.23 ± 0.25a	2.87 ± 0.35ab	3.57 ± 0.64a	2.93 ± 0.51a	3.17 ± 0.47a
Ca/P	2.7:1	1.8:1	1.8:1	2.0:1	3.8:1	2.5:1
K/Na	40.28:1	67.3 :1	47.21:1	30.83:1	55.24:1	56.40:1

The results are expressed as mean ± standard deviation of three separate extractions and determinations. Data were analysed by ANOVA. In each column of each group, different letters indicate statistically different values according to Tukey's HSD comparison at $p < 0.05$.

Table 3.
Mineral composition of pulp and peel of Vitex doniana fruit harvested in Ferke, Tiebissou and Yamoussoukro.

fruit pulp and peel, which showed no significant difference ($p > 0.05$), there was more Ca in the Black plum peel than pulp (**Figure 3**). Moreover, Black plum pulp and peel possess remarkably higher Ca contents than stated by Manzoor et al. [52] and Singh et al. ([53] for mango pulp and peel (105 ± 3 and 60 ± 0), banana (52 ± 2 and 55 ± 4 mg/100 g DW) and apple (19.8 ± 0.41–48.9 ± 0.99 and 35.6 ± 0.78–72.1 ± 1.47 mg/100 g DW).

Thus, the consumption of Black plum could contribute to the proper functioning of the body, the constitution of bones and teeth, and the regulation of nerve, muscle and hormonal functions through its high levels of K and Ca [54–58].

Yamoussoukro fruit pulp (46.75 ± 0.92 mg/100 g DW) and Tiebissou peel (42.77 ± 0.57 mg/100 g DW) had the highest levels of Fe. However, no significant variation was observed between fruit pulp and peel from these localities with regards to Zn and Mn contents (**Figure 4**).

Singh et al. [59] analysed important micronutrients in various plants, including mint, coriander, spinach, amaranth, cauliflower and carrot, and found, respectively, Fe, Mn and Zn concentration ranges of 7.7 ± 0.01–84.4 ± 0.08, 1.8 ± 0.01–10.2 ± 0.05 and 2.4 ± 0.05–6.0 ± 0.04 mg/100 g DW. These data indicate that Yamoussoukro and Tiebissou Black plum pulps and peels are, respectively, richer in Fe than coriander, spinach, amaranth and carrot, while the pulp and peel of Black plum collected in Ferke contains less Fe than all these plants. Given the amounts of Fe in the different parts of Black plum, it is concluded that Black plums could be excellent sources of Fe. This element is an integral part of important enzymatic systems in various tissues, facilitates oxygen transport from red blood cells and to the lungs (as part of haemoglobin) and acts as a carrier for electrons within cells [60, 61]. Variations in the contents of these different minerals in fruit from region to region and from one part of the fruit to another could be explained by maturity of the fruit, fruit conditions, root system, fruiting position and the leaf area of forest cover [62].

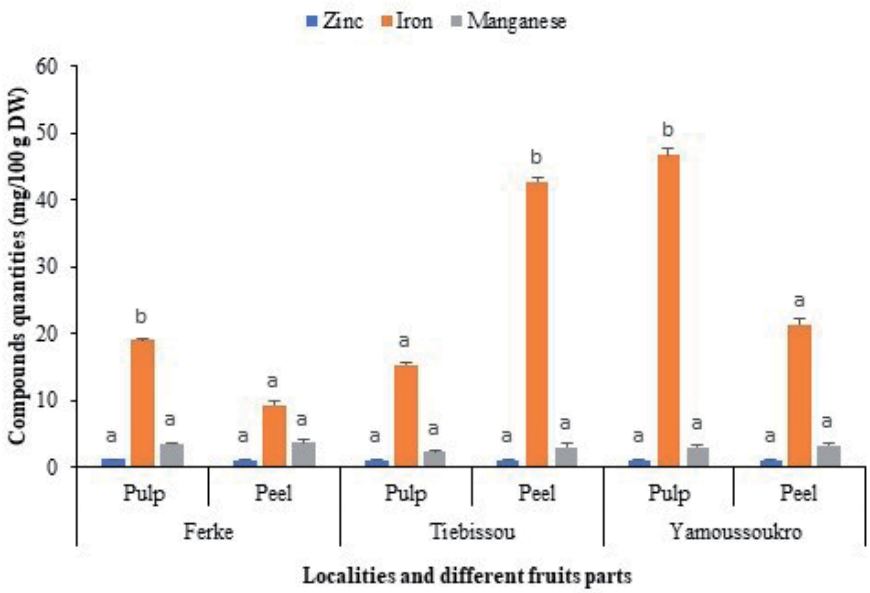

Figure 4.
Comparison between pulp and peel micromineral contents of Vitex doniana fruit harvested in Ferke, Tiebissou and Yamoussoukro.

Samples	TPC (mg GAE/100 g MS)		TF (mg QE/100 g MS)		TF / TPC	
	Pulp	Peel	Pulp	Peel	Pulp	Peel
Ferkéssédougou	227.47 ± 3.35b	225.84 ± 5.89a	79.43 ± 1.13b	103.86 ± 0.66a	0.35	0.46
Tiébissou	202.51 ± 4.19a	383.03 ± 6.54b	75.71 ± 1.03a	145.55 ± 1.03c	0.37	0.38
Yamoussoukro	259.75 ± 2.81c	463.45 ± 6.85c	77.95 ± 0.72ab	141.48 ± 0.66b	0.30	0.30

The results are expressed as mean± deviation of three separate extractions and determinations. The data were analysed by ANOVA and in each column of each group (pulp and skin) different letters indicate statistically different values according to Tukey's HSD test at p <0.05. TPC total phenolic compounds, TF total flavonoids.

Table 4.
Content of antioxidant compounds in black plum fruit pulp and peel.

An excess of a mineral can be antagonistic for other minerals absorption and their proper utilisation. For this reason, mineral constituent ratios are important for good nutrition. Our study shows that the Ca amount contained in Black plum pulp and peel is at least twice that of P and K, and at least 40-fold greater than that of Na. The Ca:P ratios of Black plum pulp and peel ranged between 1.8:1–3.8:1 and 2:1–3.8:1, respectively, and the K:Na ratios ranged from 40.28:1–67.35:1 for the pulps and from 30.83:1–56.40:1 for the peels (**Table 3**). It is well known that a Ca:P ratio greater than or equal to 1:1 is favourable for mineralisation and bone turnover [63–65]. Previous research [63–66] reported that blood pressure reduction was strongly correlated with decreased Na:K ratio and increased K in hypertensive patients. As a result, regular consumption of Black plum could contribute to mineralisation, bone turnover, and the prevention of high blood pressure and cardiovascular disease.

3.8 Total phenolic compound and total flavonoid

The results (**Table 4**) showed that total phenolic compounds (TPC) and total flavonoids (TF) contents, extracted from Black plum pulp and peel, differed considerably ($P < 0.05$) from one locality to another. This corroborates other results that suggested that TPC content of Black plum in Côte d'Ivoire varied from region to region [19]. Thus, Black plum pulp and peel TPC contents were between 202.51 ± 4.19 and 259.75 ± 2.81 mg GAE/100 g DW and 225.84 ± 5.89 and 463.45 ± 6.85 mg GAE/100 g DW, respectively. The pulp and peel of fruit collected in Yamoussoukro exhibited the highest levels of TPC (259.75 ± 2.81 and 463.45 ± 6.85 mg GAE/100 g DW, respectively), while the lowest TPC levels occurred in those from Tiébissou and Ferke (202.51 ± 4.19) and 225.84 ± 5.89 mg GAE/100 g DW, respectively (**Table 4**). In addition, except for Ferke fruit pulp and peel, which showed no significant difference ($p > 0.05$), there were higher concentration of TPC in the Black plum peel than in pulp (**Table 4**). This result is consistent with results obtained by several authors [67–69] whose reported a higher phenolic compound content in fruit peel such as orange, grape and mango. For TF levels, significant variation was observed ($P < 0.05$) between samples (**Table 4**). The TF of Black plum pulp and peel ranged from 75.71 ± 1.03–145.55 ± 1.03 mg QE/100 g DW. The highest amounts of TF were also found in peel (**Table 4**). Tiébissou peel (145.55 ± 1.03 mg QE/100 g DW) and Ferke pulp (79.43 ± 1.13 mg QE/100 g DW) showed high TF content. Those results agree well with results reported by Levaj et al. [70] and Reza et al. [71]. **Table 4** shows that the TF content of Black plum pulp and peel represent 34% and 38% of TPC, respectively. In this study, the fruit pulp and peel TF/TPC ratios are higher than those of carrot (0.28), tomato (0.17) and red pepper (0.08) and similar to okra (0.32) for the pulp [72]. In addition, these ratios are also higher than those of nine varieties of grenadine (0.114–0.288) for peel [71].

4. Conclusion

Black plum pulps and peels collected in Ferke, Tiebissou and Yamoussoukro (Côte d'Ivoire) areas were characterised by high contents of dietary fibre (34.79–50.21%) and minerals, including K (2415.01 and 2138.83%), P (147.66 and 124.83%) and Ca (314.33 and 377.37%), besides displaying a good essential amino acids profile (threonine, leucine, isoleucine) of the protein, compared to kiwi, fruit reference rich in ash and polyphenols. However, the fruit peels are the parts rich in dietary fibre, Ca, total polyphenols and total flavonoids. Thus, these fruits constitute a good source of important nutrients (fibre, potassium, calcium and phosphorus)

and compounds (antioxidants) beneficial for health. It is recommended that both parts of this fruit (pulp and peel) are consumed. Therefore, we propose that these fruit parts are integrated into the human diet in order to contribute to good health. Furthermore, incorporation of the fruit parts in some foods (like flour of wheat, maize, millet, etc.,...) could be of great interest in the valorization of this species. Especially this incorporation would allow the fight against malnutrition in rural areas where food is less rich in minerals and polyphenols.

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Chapter 10

Behavior of *Prunus persica* as Green and Friendly Corrosion Inhibitor for Corrosion Protection

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Abstract

Prunus persica (peach) is a delicious and juicy fruit, making a valuable and healthy food. *P. persica* is an interesting specie that have been studied in different ways, one of them is as green corrosion inhibitor to protect metals. From this specie, it has been studied as juice, seeds, pomace of fruit and leaves on alloy steels immersed in acids (HCl, H_2SO_4 and H_3PO_4) and salts (NaCl, Na_2SO_4). This chapter explains briefly global importance of corrosion, how corrosion occurs and how to protect metals with corrosion inhibitors, including examples about the studies of green corrosion inhibitors and the results of *Prunus* species. The phytochemicals mixture was extracted from different tissues of peach (leaves, fruits, seeds, peels, and pomace) through different methods. All these extracts were studied to protect steel alloys immersed in different aggressive environments (acids and salts) and showed good and high corrosion inhibitions using low quantities of phytoextract (0.5 g/L) as corrosion inhibitors reaching more than 87% of corrosion inhibition efficiencies. Leaves of *P. persica* containing flavonoids like fruits and is possible to use leaves or pomace to produce green corrosion inhibitors.

Keywords: Corrosion, Green corrosion inhibitors, *Prunus persica*, Peach, metal protection

1. Introduction

The use of metals is growing up faster due to the different applications they have. Currently, different objects have been built using metals. Unfortunately, the deterioration of metallic materials is impossible to stop or avoid occurring, because the reaction of metallic materials with the present oxygen in any environment is energetically favorable and oxidation occurs spontaneously [1]. Industrially many serious and economic problems are produced for corrosion, it produces material loss on surface, and it conduce that the materials loss their mechanical properties and the structures fail making the industrial process shutting down.

In 1998 was estimated by the National Association of Corrosion Engineers (NACE) that the total annual cost generated to attend and prevent the corrosion problems in U.S.A. was closer to US $276 billion, it was approximately equivalent to the 3.1% of the Gross Domestic Product. This inversion has been growing since

2011, becoming more than US $2.2 trillion. While, in the same year was mentioned in the 1st Global Corrosion Summit that the corrosion cost in India was around US $45 billion [2]. However, these estimated data are outdated, and recently closer investigation of the NACE reported that the annual global cost of corrosion has been increased approximately to US $2.5 trillion and is equivalent to 3.4% of the global GDP [2–4]. According to a new report data, it was mentioned on 2019, it is expected that the global corrosion inhibition market will reach USD $ 9.6 Billion by 2026 [5].

The metals importance in society through the years has raised the search for efficient alternatives to protect them. Corrosion inhibitors are one of the most used methods, as they act by decreasing the corrosion velocity and the metallic surface is protected [6]. Synthetic corrosion inhibitors is the most used in industry [6–8]. However, the excessive use of inorganic and organic synthetic corrosion inhibitors during years have been produced pollution and environmental damage [9].

Different hazards occur naturally and deliberately (such as industrial pollution, transportation accidents and damage, radioactive pollution, water pollution, petroleum pollution, etc.). The hazards of most synthetic organic compounds are commonly known, and the restrictive environmental regulations of many countries forced researchers to focus on developing cheap, nontoxic, and environmentally acceptable products [10, 11].

The metallic protection or the use of corrosion inhibitors cause significatively pollution. Therefore, environmental protection legislation raised to prevent using the environmentally unacceptable materials such as the use of chromium salts is now restricted because chromium (Cr^{+6}) is highly toxic and carcinogenic [12, 13]. Every year, billions of dollars are spent on capital replacement and methods for corrosion control in infrastructure [14, 15].

Since the nineties researchers have been searched and studied new and different alternatives for metal protection because the corrosion has drawn considerable academic and industrial attention [10, 16, 17]. Various researchers keeping these ideas in mind have been focused on studying the corrosion inhibitors activity of expired nontoxic medicines, natural molecules that come from microorganisms [18], and others have been oriented to study plant extracts which contain many natural and eco-friendly organic compounds [19]. An appropriate green corrosion control can help to avoid many potential disasters that can cause serious issues including life-loss, negative social impacts, water resource and environmental pollution. In this way green corrosion inhibition studies have become oriented towards human health and safety considerations [14, 20]. However, different studies have shown that green corrosion inhibition effect is usually found to be very low compared to synthetic organic inhibitors [10].

Likewise, it has been suggested to focus the studies in plant extracts or single natural products as corrosion inhibitors until reaching their application. Accordingly, there is an increasing demand to protect the environment by decreasing and controlling all causes, which pollute the environment, damage society's health, and affect the economy [21].

2. Corrosion

Metal corrosion is an unavoidable chemical process; it is defined as the deterioration of desired metal properties on interaction with certain elements that are present in the environment. Material's degradation is associated with the term erosion which means *"a progressive loss of original material from a solid surface due to mechanical interaction between the surface and a fluid, a multicomponent fluid -or impinging liquid or solid particles"* [22].

Chemically, corrosion is an oxide-reduction reaction; it commonly occurs an interchange or transferred the valency electrons between metal and the major element present in their environment, e.g., oxygen in air or water [23]. In the industrial practices certain processes like acidizing, acid cleaning, pickling, etc. facilitate metal corrosion, because the metal surface reacts quickly with pH solution, temperature, ions, etc. However, corrosion rate depends on the cell or environmental conditions. In the **Figure 1** is represented the electrochemical process that is carried on in the oxide-reduction reaction of steel (**Figure 1**) under aqueous system.

The corrosion occurs, in an aqueous or wet environment, when the metal is in contact with an electrolytic conducting liquid or when two dissimilar metals or alloys are either immersed or dipped partially in the electrolytic conducting solutions [20].

At anode (oxidation reaction)

$$M\,(metal) \rightarrow M^{n+} + ne^{-} \tag{1}$$

Where M^{n+} (metallic ion) dissolves in solution and forms compounds such as oxide.

At cathode (reduction reactions).

In acid solution

$$2H^{+} + 2e^{-} \rightarrow H_2 \uparrow \tag{2}$$

In alkaline solution

$$2H_2O + 2e^{-} \rightarrow H_2 \uparrow + 2OH^{-} \tag{3}$$

In neutral solution

$$O_2 + 2H_2O + 4e^{-} \rightarrow 4OH^{-} \tag{4}$$

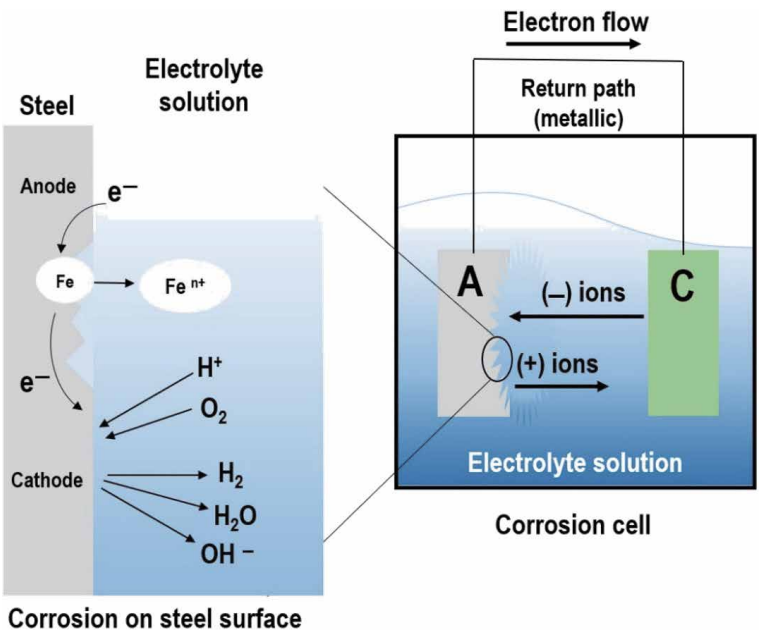

Figure 1.
Metallic corrosion (cell and metal surface).

These reactions are always associated with standard conditions to explaining the phenomenon of metal corrosion degradation (**Figure 1**).

3. Green corrosion inhibition

Metal corrosion rate could be reduced or mitigated through different strategies such are to cover the metal surface with painting or coatings; with the respective oxide that is formed through passivation or by reducing the aggressivity of the medium [24]. Those are to protect the metal and to reduce the damage.

Various authors reported the successful corrosion inhibition activity (CIA) exhibited by organic compounds, it depends on the presence of functional groups in their chemical structure, those containing elements such as ***O***, ***N*** or ***S*****;** such as hydroxyls (-OH), carboxyl (-COOH), carbonyl (>C=O; -CH=O); amine (-NH_2), thiols (-SH), or these elements that are included as heteroatoms (**Figure 2**).

On the other hand, other reports mentioned that aromatic rings contain double bonds and π electrons, those are important as they do favorable chelator

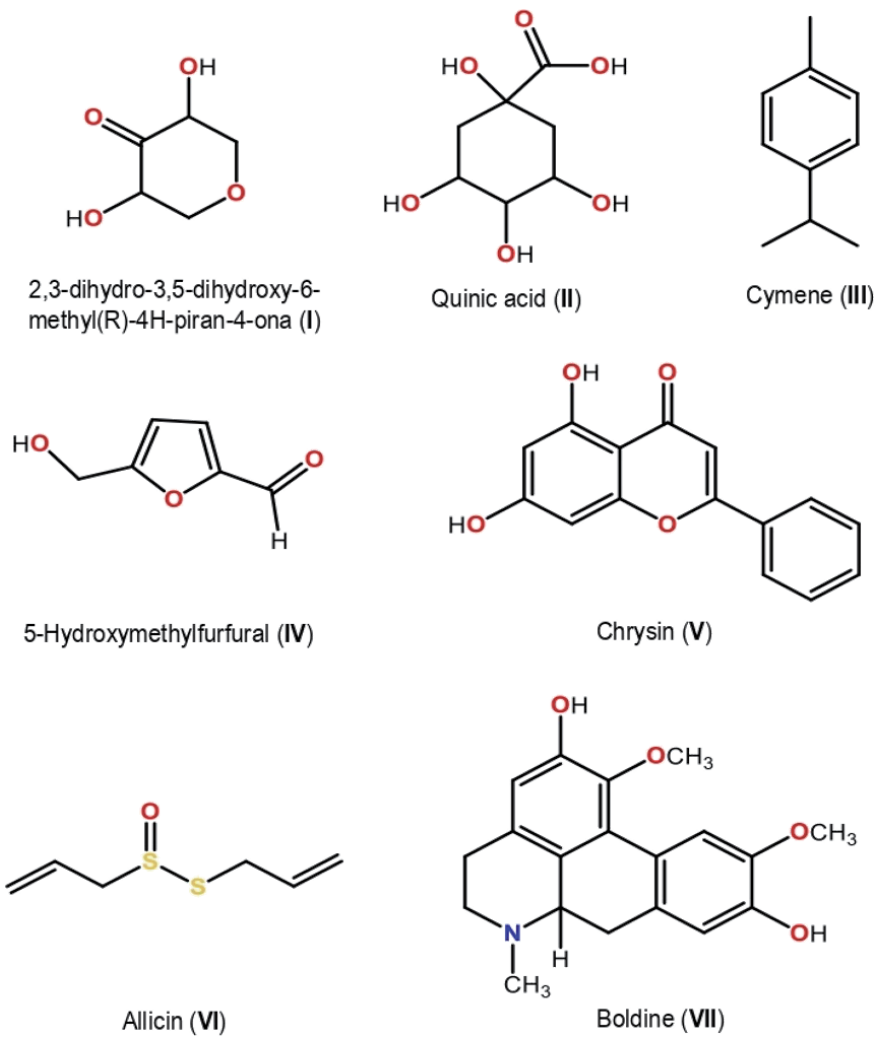

Figure 2.
General representation of organic chemical structures included heteroatoms.

interactions on the metal surface too [25, 26]. Compounds which frequently depends on the presence of functional groups in their chemical structure and electron pairs provided by heteroatoms such as N, O, P or S; these features and the presence of pi electrons as double or triple carbon–carbon bonds enable this compounds to form favorable interactions with the metal surface (**Figure 2**).

Some interesting aspect is that the nature provides different natural organic compounds, in which those chemical characteristics are present. Additionally, several of these compounds have antioxidant activity which has been related with anticorrosion activity or corrosion inhibition [27]. The interference with the flow of electrons reduces the reaction rate and gives protection to the metal.

All previous aspects motivate to perform the study of corrosion inhibition activity and to observe how the corrosion rate is reduced and how the inhibitor act and how much time gives protection to the metal surface [26–28]. In **Table 1** it is shown

Source of green corrosion inhibitor **Natural specie** **Type pf extract** **Natural products reported**	**Experimental conditions** **Metal or alloy** **& Electrolyte** **& [Green corrosion inh conc]**	**Inhibitive performance**	**Ref**
Argania spinosa Cosmetic oil Oleic acid (fatty acids) and Schottenol (terpene)	Mild steel 1.0 M HCl 3.0 gL^{-1}	CIE = 81.0% By weight lost in 8 h as residence time	[29]
Borassus flabellifer Methanol and Water N. M.	Aluminum 1.0 M H_2SO_4 4.0 gL^{-1}	CIE_M = 65.78% CIE_W = 50.88% By weight lost in 3 h as residence time	[30]
Khaya senegalensis Acid aqueous extract N. M.	Carbon steel 1.0 M HCl 2.0 gL^{-1}	CIE = 91.1% By weight lost	[31]
Lavandula multifida L Essential oil Carvacrol	C38 steel 0.5 M H_2SO_4 2.0 gL^{-1}	CIE = 72.2% By weight lost in 6 h as residence time	[32]
Lemon balm Powder extracted using water Caryophyllene, Germacrene, Chlorogenic acid, Luteonin, Rosmarinic acid, and Citral	Mild steel 1.0 M HCl 0.8 gL^{-1}	CIE = 95.0% By weight lost in 12 h as residence time	[33]
Mangifera indica Powder extracted using ethanol Gallic acid, Iriflophenone, and Mangiferin	Mild steel 1.0 M HCl 1.0 gL^{-1}	CIE = 92.0% By weight lost in 8 h as residence time	[34]
Salvia aucheri var. mesatlantica Essential oil Camphor	Steel 0.5 M H_2SO_4 2.0 gL^{-1}	CIE = 86.12% By weight lost in 6 h as residence time	[35]
Silybum marianum Methanol extract Flavonoids: Silybin, Silychristin, Isosilybin and Silydanin	304 stainless steel 1.0 M HCl 0.6 gL^{-1}	CIE = 89.7% By weight lost in 6 h as residence time	[36]
Tragia plukenetii Methanol N. M.	Mild steel 1.0 M HCl 0.5 gL^{-1}	CIE = 88.0% By weight lost in 3 h as residence time	[37]

CIE, Corrosion inhibition efficiency; CIE_M, Corrosion inhibition efficiency of methanol extract; CIE_W, Corrosion inhibition efficiency of water extract; N. M., Non mentioned.

Table 1.
Studies of green corrosion inhibitor reported.

some examples of green corrosion inhibitors for some species. Green corrosion inhibitors have been positioned as a good alternative because they have come from natural sources, so they are considered less toxic, biodegradables, ecofriendly, and sustainable [1, 10, 26, 38, 39].

Traditionally the plants are used in infusion or tee, it could be the reason why in many studies have been performed water infusion as green corrosion inhibitors, however the corrosion inhibition efficiencies reported show low percentage of inhibition, one of the strategies observed in the reports is using more quantity of phytoextract to increase the corrosion inhibition efficiencies [30]. In another hand, the wide way to employ plants is in cosmetic, many works reported the study of essential oils as green corrosion inhibitor [32]. Other studies reported acid or basic extraction, it is depending on the electrolyte used, for example if the study is made in hydrochloric acid the extraction of inhibitor is made in HCl and heat [31]. These kinds of extractions are stronger and exhausted, it destroys not only the tissue, frequently modified chemically the compounds.

Organic and natural compounds are chemically sensible and thermolabile and through their functional groups reacts with acid or bases, for example the functional group alcohol in acid environment is going to transform into carboxylic acid, and tannins are going to separate into their monomeric units; other groups such as ring with oxygen as heteroatom like lactones tend to open the ring and the compounds interchange in a different position to the original. In the same way the thermolabile compounds with the heat suffer inactivation and chemical destruction [40]. The phytochemicals extraction method is an important step to recover the green corrosion inhibitor because extraction separates the desired natural products from the raw materials and follows to continue the study of chemical structure of the molecule responsible of the inhibition activity. The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ratio, the extraction temperature, and the extraction duration will affect the extraction efficiency [41].

The plants are considered and the diversity of phytochemical compounds could be different for the same species of plant that grew in different locations and in different tissue of the same plant biosynthesize can present different kind of natural compounds, and the kind of phytochemical biosynthesize depend on the age or stage, because it is not the same old barks than mature leaves or flowers than suckers [42]. In this way different tissue of plants have been processed, extracted, studied and reported as green corrosion inhibitors employing different alloys and electrolytes. In the **Table 2** are shown examples of specified tissue plant extracted and studied as green corrosion inhibitors.

3.1 Rosaceae family

Rosaceae is a moderately large angiosperm family in Rosales order, with about 3000 species, 3 subfamilies, 16 tribes, and 88–100 genera [55–57]. Rosaceae is a family plant producing seeds within the fruits. The fruit protects the seeds against damage from pathogens, water loss, and the other stresses. Rosaceae family have an important commercial fruit species, fruits are highly nutritious and are consumed by humans, they have benefited greatly by freshy or drier fruits including woodland strawberry (*Fragaria vesca*) [54], domesticated apple (*Malus_domestica*) [58], pear (*Pyrus bretschneideri*) [59], Mei (*Prunus mume*, related to apricot) [59] and peach (*Prunus persica*) [60] and others more [61].

Different documents mentioned that peach was originated in China and then spread westward through Asia to the Mediterranean countries and later to other parts of Europe. The Spanish explorers took the peach to the New World, and in the early 1600 the fruit was found in Mexico. The large-scale commercial peach

Source of green corrosion inhibitor **Specie** **Tissue** **Solvent employed by maceration extraction** **Natural product reported**	**Experimental conditions** **Metal or alloy** **Electrolyte** **Inh conc [g/L]**	**Inhibitive performance weight lost technique**	**Ref**
Allium sativum Bulbs Hexane Allicin	Carbon steel 0.5 M H_2SO_4 0.4 gL^{-1}	CIE_H = 96.0%	[43]
Brugmansia arborea Flowers Hexane, ketone, methanol individual maceration Hyoscyamine; Anisodamine, and Scopolamine	1018 steel 0.5 M H_2SO_4 0.4 gL^{-1} (all extracts)	CIE_H = 90.0% CIE_k = 80.0% CIE_M = 78.0%	[44]
Chenopodium amborsioides Stems and leaves Hexane N. M.	1018 steel 0.5 M H_2SO_4 0.25 gL^{-1}	CIE_H = 80.0%	[45]
Curcuma longa Turmeric Hexane, methanol individual maceration Mainly Curcuminoids	1018 mild steel 3.5% NaCl 0.1 gL^{-1}	CIE_H = 62.6% CIE_M = 93.7%	[46]
Cynara scolymus Fruit Methanol 2,3-dihydro-3,5-dihydroxy-6-methyl(R)-4H-piran-4-on; Quinic acid; Octadecanoic acid; 9,12-Octadecadienoic acid; 9,12-Octadecadienoic acid, methyl ester; Hexadecenoic acid and Hexadecenoic acid, methyl ester; Stigmasterol, and β-Sitosterol	1018 steel 0.5 M H_2SO_4 1.0 gL^{-1}	CIE_M = 89.0%	[47]
Equisetum arvense Leaves Methanol Hexadecenoic acid; 9,12-octadecadienoic acid; oleanolic acid, methyl ester; Campesterol, and β-Sitosterol	A36 steel 0.5 M H_2SO_4 0.4 gL^{-1}	CIE_M = 78.0%	[48]
Medicago sativa Stems and leaves Methanol Hexadecenoic acid; 9,12-octadecadienoic; oleanolic acid; Cymene, and Limonene	1018 steel 0.5 M H_2SO_4 0.5 gL^{-1}	CIE_M = 92.0%	[49]
Mentha spicata Stems and leaves Methanol Oleic acid; Stearic acid, Limonene, Cymene, and 5-hydroxymethylfurfural	1018 steel 0.5 M H_2SO_4 0.6 gL^{-1}	CIE_M = 88.0%	[50]
Peumus boldus Leaves Methanol Chrysin; Boldine, and α-Tocopherol	Carbon steel 0.5 M H_2SO_4 1.0 gL^{-1}	CIE_M = 73.0%	[51]
Prosopis laevigata Branches Methanol Prosopine and Prosopinine	Aluminum 0.5 M H_2SO_4 0.2 gL^{-1}	CIE_M = 40.5%	[52]
Rosmarinus officinalis Stems and leaves Hexane, ketone, methanol individual maceration Carnosol, Carnosic acid, and Rosmarinic acid	Carbon steel 0.5 M H_2SO_4 1.0 gL^{-1}	CIE_H = 62.5% CIE_k = 50.0% CIE_M = 42.6%	[53]

Source of green corrosion inhibitor **Specie** **Tissue** **Solvent employed by maceration extraction** **Natural product reported**	**Experimental conditions** **Metal or alloy** **Electrolyte** **Inh conc [g/L]**	**Inhibitive performance weight lost technique**	**Ref**
Salvia officinalis Stems and leaves Methanol Carnosol, Limonene, and Rosmarinic acid	Carbon steel 0.5 M H_2SO_4 0.3 gL^{-1}	CIE_M = 80.0%	[54]

N. M., Non mentioned in the paper; CIE_H, Corrosion inhibition efficiency of hexane extract; CIE_k, Corrosion inhibition efficiency of ketone extract CIE_M, Corrosion inhibition efficiency of methanol extract.

Table 2.
Tissue of plants to obtain green corrosion inhibitors.

growing did not begin until the 19th century, in the United States. The early plantings were seedling peaches, inevitably variable, and often of poor quality. The practice of grafting superior strains onto hardy seedling rootstocks, which came later in the century, led to the development of large commercial orchards [61–65].

Peach trees are relatively short-lived as compared with some other fruit trees. In some regions orchards are replanted after 8 to 10 years, while in others trees may produce satisfactorily for 20 to 25 years or more. Trees are usually pruned annually to prevent them from becoming too tall; the upright shoots are pruned back to outgrowing laterals to produce a spreading tree and keep it open to sunlight. Small to medium-sized peach trees seldom reach 6.5 meters (21 feet) in height. Under cultivation, however, they are usually kept between 3 and 4 meters (10 and 13 feet) by pruning. The leaves are glossy green, lance-shaped, and long pointed; they usually have glands at their bases that secrete a fluid to attract ants and other insects. The flowers, borne in the leaf axils, are arranged singly or in groups of two or three at nodes along the shoots of the previous season's growth. The five petals, usually pink but occasionally white, five sepals, and three whorls of stamens are borne on the outer rim of the short tube, known as the hypanthium, that forms the base of the flower (**Figure 3**) [61, 62].

Figure 3.
Peach tree without and with fruit and flowers.

Thousands of varieties of peach have been developed. Varieties may be freestone types, which have stones that separate easily from the ripe flesh, or clingstones, which have flesh that adheres firmly to the stone. The skin of most ripe peaches is downy or fuzzy; peaches with smooth skins are called nectarines (**Figure 4**) [65]. Worldwide, the peach is one of the most important of the deciduous-tree fruits, and China, Italy, Spain, and the United States are major producers.

Peaches and apricots belong to the same family, *Rosaceae*, also known as the rose family. Although closely related, peaches and apricots are not from the same regions. The scientific name for the peach, *Prunus persica*, denotes its abundance in Persia - modern-day Iran - despite having originated in Asia. Meanwhile, apricots (*Prunus armeniaca*) are also called armenian plums because they are known to have grown historically in this region [61]. Peach fruits come from the same family, they contain similar nutrients, including potassium, vitamin C, and beta carotene [63, 64].

However, peaches provide higher amounts of these nutrients in a single serving because of their larger size.

3.2 *Prunus persica*

Different efforts have been oriented in the search and study of species that could be applied as green corrosion inhibitors. And the peach does not an exception because has antioxidant activity and the compounds have been reported as responsible from this activity are polyphenols and carotenoids [63, 64, 66]. Likewise, the peaches are widely produced around the world because they are good and harmless for the health [66], and many products are derived from their industrializing, and it generates high quantities of agro-waste, which can be used to produce green corrosion inhibitors [67, 68]. In this way, we are interested to aim and analyze the different results reported over the activity of *P. persica* as green corrosion inhibitor.

The scientific reports on *P. persica* related to studies as green corrosion inhibitor have been organized and presented on **Table 3**.

Different tissues of peach have been used and extracted by different ways to recover natural and organic compounds to study as green corrosion inhibitors. The fruits, seeds, pomace, and gum of *P. armeniaca* have been extracted and studied

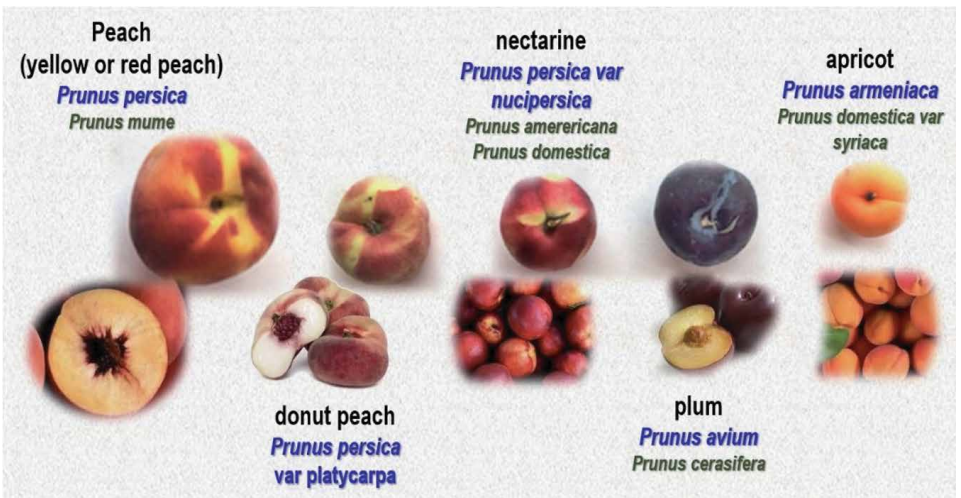

Figure 4.
Peach fruits commercially available. Common name in black color, scientific name in blue color, synonym un green color.

Specie (common name)	Experimental conditions Alloy Electrolyte Inh conc [g/L]	Inhibitive performance	Ref
Prunus armeniaca L. (peach) Tissue Extracted technique Natural product reported			
Fruits Soxhlet extraction using 2-propanol/ethanol Octadecanoic acid; (9Z)-octadec-9-enoic acid; Linalool; α-terpineol; geraniol; nerolidol	Mild steel St7–2 0.5 M Na_2SO_4, pH 7.2 100 gL^{-1}	CIE by WL 97.6% in 21 days as residence time CIE by PPC 89.0% in 72 h as residence time	[69]
Fruits Soxhlet extraction using 95% Ethanol N. M.	Mild steel 0.5 M NaCl 0.5 gL^{-1}	CIE by WL 94.6% in 26 days as residence time	[70]
Fruits Mechanical juice N. M.	Mild steel 1.0 M H_3PO_4 40.0 gL^{-1}	CIE by WL 75.0% in 2 h as residence time	[71]
Seeds Essential oil Oleic acid; Linoleic acid; Palmitic acid; Stearic acid	C38 Carbon steel 1.0 M HCl 0.5 gL^{-1}	CIE by WL 84.0% in 6 h as residence time CIE by PPC 84.0% CIE by EIS 83.5%	[72]
Gum N. M. N. M.	Carbon steel 0.5 M H_3PO_4 0.5 gL^{-1}	CIE by PPC 86.0% CIE by EIS 81.0%	[73]
Pomace Soxhlet extraction using 2-propanol β-cyclocitral; octadecanoic acid; hexadecanoic acid; (9Z)-octadec-9-enoic acid; Terpineol; Geraniol; Nerolidol	Mild steel 0.5 M NaCl 0.5 gL^{-1}	CIE by WL 94.6% in 26 days as residence time	[74]
Prunus avium (sweet cherry)			
Leaves Reflux distillation with water Phenolic compounds and anthocyanins	Mild steel 0.5 M HCl 0.40 gL^{-1}	CIE by PPC 61.0% CIE by EIS 55.0%	[75]
Prunus cerasus (cherry)			
Fruits Mechanical compressed to obtained juice N.M.	St-37 Steel 1.0 M HCl ~40* gL^{-1}	CIE by PPC 94.1% CIE by EIS 92.7%	[76]
Prunus dulcis (almond)			
Fruit peels Methanol and water Catechin, Chlorogenic acid, and Isorhamnetin-3-o-rutinoside	Mild steel 0.1 M HCl 0.12 gL^{-1} 0.24 gL^{-1}	CIE by WL 93.0 and 85%, respectively CIE by PPC 09 & 83.0%, respectively CIE by EIS 92.0 and 88.0%, respectively	[77]
Seeds Soxhlet extraction using hexane Phenolic compounds, Saponins, Tannins, Flavonoids, and proanthocyanidins	Mild steel 1.5 M HCl 150 gL^{-1}	CIE by WL 68.0% in 20 h as residence time	[78]
Prunus persica (Peach)			
Leaves Methanol maceration	1018 Carbon steel 0.5 M H_2SO_4 0.60 gL^{-1}	CIE by WL 80.0% CIE by PPC 92.0% CIE by EIS 97.0%	[79]

Specie (common name)	Experimental conditions Alloy Electrolyte Inh conc [g/L]	Inhibitive performance	Ref
Kaempferol, Quercetin, Rutin, Ursolic acid, Daucosterol, and β-Sitosterol			
Pomace Ultrasound dissolved in 2-propanol/ethanol/ water Cinnamaldehyde, Thymol, Decosanal, a-Terpineol, and Linalool	Carbon steel 0.5 M NaCl 0.8 gL^{-1}	CIE by WL 80.0% in 45 h as residence time	[80]
Fruits Mechanical compressed to obtained Juice N.M.	Mild steel 1.0 M HCl 0.05 gL^{-1}	CIE by WL 88.0% at 50° C CIE by PPC 90.0%	[81]

N. M., Non mentioned in the paper; CIE_H, Corrosion inhibition efficiency of hexane extract; CIE_k, Corrosion inhibition efficiency of ketone extract CIE_M, Corrosion inhibition efficiency of methanol extract.

Table 3.
Species of Prunus studied as green corrosion inhibitors.

as green corrosion inhibitors [72–74] against mild and carbon steel using salts [69, 70, 74] and acids as electrolytes [71–73]. The corrosion inhibitor efficiencies reported are between 75 to 94 percent [69–74]. The fruit and pomace extracted by Soxhlet using ethanol and isopropanol reaching high corrosion inhibition efficiencies [69, 70, 74]. The natural compounds reported for the active extract are fatty acids and terpenes [69, 72, 74].

The leaves of *P. avium* extracted by reflux distillation using water, was tested as green corrosion inhibitor against mild steel immersed in hydrochloric acid, the corrosion inhibition efficiency reported were 55 percent when 0.4 g/L of the extract was used [75]. The juice of fruits of *P. ceracus* obtained by mechanical compressed was tested against St-37 steel immersed in hydrochloric acid. The electrochemical test permitted to observe that the corrosion inhibition efficiency upper 90 percent when 40 g/L of juice was used [76].

The fruit peels and seeds of *P. dulcis* have been extracted using methanol and water individually and studied as green corrosion inhibitors against mild steel in hydrochloric acid, the corrosion inhibition efficiencies reported were high for fruit peels [77] compared those reported for seeds [78].

The leaves, fruits, and pomace of *P. persica* have been extracted individually by maceration, ultrasound and compressed mechanical and studied individually against carbon and mild steel immersed in sulfuric acid [79]; sodium chloride [80] and hydrochloric acid [81]. The corrosion inhibition efficiencies reported were more than 80 percent.

The phytoextracts from *Prunus* show they can inhibit the corrosion rate of steel alloys in acid and saline environments. These reports shown the importance of continue studying peach as green corrosion inhibitor to find the organic molecule responsible of the inhibition activity, and search to modeling isotherms, obtain Gibbs free energies and Density functional theory (DFT) studies to understanding the involved mechanisms on the green corrosion inhibition.

The best natural material of peach to obtain and for particular interest as green corrosion inhibitor could be the leaves, because these are not commercial than fruits, and in orchards many threes are pruned, and the leaves are disposable as residues. Other kind of residues from peaches are produced by the food industry or

jam and juice factories, they discarded and waste dispose peels, seeds, and pomace of peaches (**Figure 5**).

Recently was reported the use and approval of peaches waste to produce green corrosion inhibitors with prolongated activity on mild steel [82]. Different compounds are chemically detected and reported from *Prunus* genus like sterols, phenolics, flavonoids and polyphenols (**Table 3**, **Figure 6**), some characteristic compounds are studied as corrosion inhibitors.

The inhibition performance of loquat (*Eribotrya japonica*) leaves extract for the corrosion of mild steel in 0.5 M H_2SO_4 were investigated. The weight loss method shown a corrosion inhibition efficiency of 91.0% using 50% (V/V) of extract. High performance liquid chromatograph (HPLC) of loquat extract shows the presence of Ursolic acid (**VIII**) as chemical component in the green inhibitor [83].

β-Sitosterol (**IX**) inhibiting the corrosive effect that has produced 1 M H_2SO_4 solution on the mild Steel. The inhibition efficiency increased with increasing inhibitor concentration and decreased when increasing temperature. The maximum inhibition efficiency of β-sitosterol (500 ppm) was 95%. Electrochemical studies indicated that isolated β-sitosterol acted as a good corrosion inhibitor [84].

In another hand, the potential of flavonoid extract of *Erigeron floribundus* was studied as green inhibitor for the corrosion of mild steel in 2 M HCl solution using gasometric method. The results indicated that the extract acts as corrosion inhibitor by adsorption on mild steel surface. The inhibition efficiency of the flavonoid extract was 93.1% using 2.0 g/L of the inhibitor [85].

The *Hemerocallis fulva* extract obtained using methanol as solvent was tested as corrosion inhibition on aluminum, in a 1 M H_2SO_4 solution. Results obtained show a maximum inhibition efficiency of about 89% at 600 ppm and 303°K. Kaempferol (**X**) and other phytochemicals were detected by ultra-high-performance liquid chromatography as the major components of *Hemerocallis fulva* methanol extract [86].

According to the literature flavonoids were found in the infusions of *Baccharis trimera*. Quercetin (**XI**) was used representative of flavonoids and was studied as

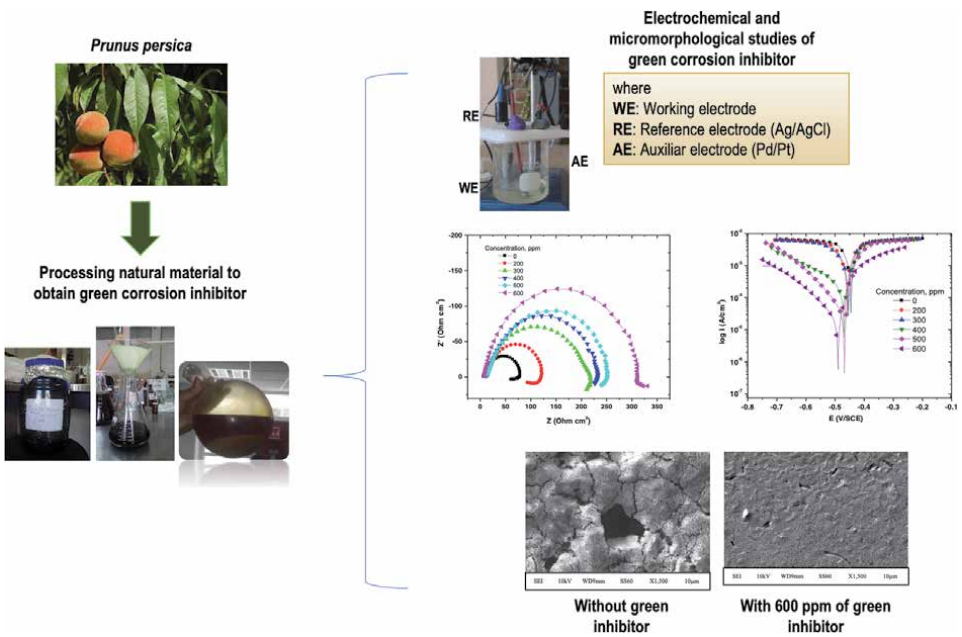

Figure 5.
Obtaining and studying the green corrosion inhibitor of Prunus persica.

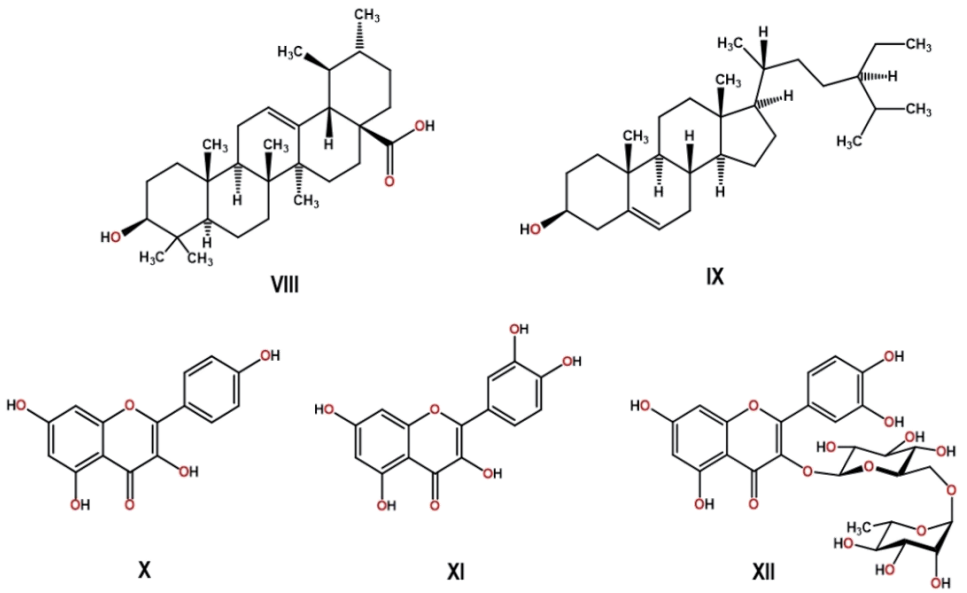

Figure 6.
Natural compounds reported for Prunus persica.

corrosion inhibitor on mild steel immersed in 1 M HCl solution, the results shown that Quercetin gives protection when it was employed at 100 mg/L [87].

Cryptostegia grandiflora leaf extract was evaluated for its anti-corrosion property on mild steel in 1 M H_2SO_4, reported that 500 ppm of the inhibitor achieved a maximum corrosion inhibition efficiency of 87.54%. One of the flavonoids present in green inhibitor was Rutin (**XII**) [88].

Folin–Ciocalteu reagent was used to assess total phenolic content present in apricot samples and were compared with the blue complex formed of gallic acid using as a standard. Aluminum chloride method was used for the detection of total flavonoid content producing a yellow color due to the presence of flavonoids; in this case Quercetin was used as a standard for the measurement of flavonoids. The results shown that fruits of *Prunus armeniaca* and leaf of *Prunus persica* contained flavonoids, phenolic compounds, and tannins [89]. Showing the chemical structures of flavonoids, they contain more oxygen atoms through them, these compounds made a greater number of interactions with metal surface.

Phytochemicals frequently are considered "organic, natural and safe compounds". However is adequate, proper, and scientifically correct to determine that green corrosion inhibitors are not toxic substances at the concentration are active as corrosion inhibitors, and at the same time to confirm they are safe to the human and environment. Are few reports of green corrosion inhibitors with good corrosion inhibition efficiencies that included the determination of toxicity.

However, for *P. persica* green corrosion inhibitor was determined there toxicity by the *Artemia saline* lethality and toxicity against *Lactuca sativa* (Lettuce) seeds germination. Both bioassays give valuable information to determine that the active substances as green corrosion inhibitors have not an environmental impact on their application. The results showed that methanol extract *P. persica* leaves produced the LC_{50} (necessary concentration to produce 50% of the deaths) for *A. salina* with 1568 ppm of green corrosion inhibitor. The active concentration of *P. persica* as a corrosion inhibitor was 600 ppm, this concentration not produced toxicity for *A. salina*. However, at the same concentration *P. persica* extract produced 45% of the

germination index of *L. sativa*. Indicating that lettuce seeds were more sensible than *A. salina* to methanol extract of *P. persica* leaves [79].

4. Conclusions

The phytoextracts extracted from *Prunus persica* shown the capacity to protect alloy steels of corrosion immersed in acid or saline aggressive environments. The flavonoids could be the responsible compounds to metal protection because they contain oxygen atoms in their chemical structure, each oxygen atom has two lone pairs of electrons through made favorable physical interactions on the metal surface to establish metal and protect it.

The high content of flavonoids was found in leaves and pomace of *P. persica*, they are taking advantage of making novel green corrosion inhibitors and open a new window to study chemically and electrochemically the present compound in leaves as corrosion inhibitors. The Phytochemicals contains in the methanol *P. persica* leaves used as a green corrosion inhibitor at 600 ppm are highly toxic for lettuce.

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

M	Molar
H_2SO_4	Sulfuric acid
HCl	Chloride acid
gL^{-1}	grams per liter
ppm	parts per million
N. M.	Non mentioned in the paper
CIE_H	Corrosion inhibition efficiency of hexane extract
CIE_k	Corrosion inhibition efficiency of ketone extract
CIE_M	Corrosion inhibition efficiency of methanol extract.
WL	weight lose
PPC	Potentiodynamic polarization curves
EIS	Electrochemical Impedance Spectroscopy
HPLC	High performance liquid chromatograph
LC_{50}	Lethal concentration, 50%

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