IntechOpen

Potato

From Incas to All Over the World

Edited by Mustafa Yildiz

POTATO - FROM INCAS TO ALL OVER THE WORLD

Edited by **Mustafa Yildiz**

Potato - From Incas to All Over the World
http://dx.doi.org/10.5772/intechopen.68698
Edited by Mustafa Yildiz

Contributors

Chandrama Prakash Upadhyaya, Deepak Singh Bagri, Devanshi Chandel Upadhyaya, Manuel Muñoz, Carolina Folch, Julio Kalazich, Sandra Orena, Annelore Winkler, Andrzej Zbigniew Wójtowicz, Marek Wójtowicz, Maciej Zacharczuk, Henryk Ratajkiewicz, Maria Pasternak, Shashank Shekhar Solankey, Meenakshi Kumari, Manoj Kumar, Mehi Lal, Sanjeev Sharma, Saurabh Yadav, Santosh Kumar, Fábia Lima, Valdir Correa, Edgar Rueda, Carmen Muller, Hettie Schönfeldt, Nicolette Hall

Notice

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Potato - From Incas to All Over the World
Edited by Mustafa Yildiz

p. cm.

Print ISBN 978-1-78923-254-7

Online ISBN 978-1-78923-255-4

eBook (PDF) ISBN 978-1-83881-344-4

Meet the editor

Mustafa Yildiz obtained his MSc degree in Agricultural Sciences at Ankara University in 1996. He had been at Osaka Prefecture University (Japan) for training in Plant Biotechnology for 5 months in 1998. He received his PhD degree in 2000 from Ankara University, Turkey. He is currently working as a professor at Ankara University, Faculty of Agriculture, Department of Field Crops. Prof. Yildiz's research areas include plant tissue culture, plant biotechnology, molecular markers, *Agrobacterium tumefaciens*-mediated gene transfer, plant stress physiology, plant immune system, plant defense mechanism, and plant breeding. He has over 150 scientific publications, 2 books and 7 book chapters. He was awarded the first place in the "International Sunflower Project Market" by "International Sunflower Association" for his project titled "A New Environmental Friendly Production Method in Sunflower for High Seed and Crude Oil Yields."

Contents

Preface

Thousands of people die every year in many parts of the world due to hunger and malnutrition. It is necessary to increase crop production so that human beings can feed on a sufficient and balanced diet to sustain their existence on Earth. This can only be achieved by increasing the amount of yield obtained from each unit area of land, since it is not possible to further increase existing cultivating areas. In parallel with increasing population, agricultural areas are being used for other nonagricultural purposes (settlement, road, factory, etc.) or are shrinking rapidly due to erosion, salinization, acidification, intensive agriculture, and overgrazing.

The yield in agricultural production declines due to biotic and abiotic stress factors. Developing a resistant or tolerant cultivar against stress factors is the main goal of plant breeding. It is possible to increase the production to a certain degree by using high-yielding cultivars, fertilizing and applying chemicals where necessary. Chemical methods are commonly used to combat biotic stressors (diseases and pests) that reduce crop production. However, herbicides and insecticides have been shown to cause the emergences of new diseases and pests. In addition, unconscious use of fertilizers and chemicals applied in plant production has negatively affected long-term ecological balance.

Potato (*Solanum tuberosum* L.), a very important crop, is the world's fourth largest food crop production following maize, wheat, and rice with approximately 390 million tons on 19 million ha. Potato is a staple crop in many diets worldwide, and the underground swollen tubers of the plant are a rich source of proteins, carbohydrates, minerals (K, Mn, Mg, Fe, Cu, and P), and vitamins (C, B1, B3, B6, K, folate, pantothenic acid). Global average potato yield, 12 tons/da, is below its yield potential because of biotic (diseases and pests) and abiotic (salinity, drought, high temperature, etc.) stress factors. That is why improvement of new cultivars resistant to stress factors by conventional and biotechnological methods is extremely important. The most important factor in production increase is the use of healthy seed tubers along with using drought-, heat-, and salt-tolerant cultivars. On the other hand, protection and storage of surplus crops, which are the most important stage in its marketability, are the main problems in potato. It is hoped that this book will help growers and researchers in solving problems in potato cultivation.

Prof. Dr. Mustafa Yildiz
Ankara University, Faculty of Agriculture
Department of Field Crops
Ankara, Turkey

Section 1

Potato Breeding

Chapter 1

The Use and Impact of Biotechnology in Potato Breeding: Experience of the Potato Breeding Program at INIA, Chile

Manuel Andrés Muñoz, Julio César Kalazich, Carolina Verónica Folch, Sandra Valeska Orena and Annelore Winkler

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72961

Abstract

The potato breeding program of Instituto de Investigaciones Agropecuarias (INIA) Chile has developed and released 11 commercial varieties of potato. It is estimated that these varieties have 50% of the Chilean potato market and are being evaluated in seven foreign countries. The aim of this work is to summarize the current importance and scope of biotechnology in breeding in Chile, by presenting a program that has generated widespread material among farmers and consumers. The germplasm bank is the source of genetic diversity for controlled crosses. Techniques to introduce the material to in vitro conditions and thermotherapy to obtain pathogen-free in vitro plants are applied. The material is characterized by SSR markers. There is a flow of material from gene bank to the annual scheme of controlled crosses and selection in the plant breeding program. In the selection plots, molecular markers associated with one or few genes that have a large and heritable effect in important traits are used: golden nematode resistance, virus resistance, and late blight resistance. Then, in the early stages of seed production, all the material of the new varieties is checked by fingerprint and molecular and ELISA test for pathogen, to assure the identity and pathogen-free status of the starting seed material.

Keywords: breeding, germplasm bank, molecular markers, varietal fingerprinting, varietal development, seed production

1. Introduction

The potato (*Solanum tuberosum* L.) is one of the three most commonly consumed crops along with wheat and rice. The annual worldwide potato production is approximately 330 million tons [1],

and the annual Chilean production is 1 million tons including 50,000 ha with 60,000 farmers. Therefore, potato has a strong economic and social importance. For this reason, Instituto de Investigaciones Agropecuarias or the Agricultural Research Institute (INIA) established a potato breeding program. In Chile, official statistics indicate that among the eight potato varieties mostly sold in wholesale markets, three correspond to varieties developed by INIA [2].

Potato varieties must fulfill the requirements of the market and consumer preferences, as well as to show good agronomic performance in several environments and wide adaptation to productive systems. Traits as high yield, tuber conformation, early maturity, and resistance to biotic and abiotic stresses are the most important goals for potato breeding in Chile.

The breeding of potatoes needs to deal with some complicated issues that make potato breeding a special case in genetic improvement of crops:

- Most of the cultivated potatoes are tetraploid and show tetrasomic inheritance.
- Tetraploidy, together with severe inbreeding depression upon repeated selfing, renders the generation of pure lines, recombinant inbred lines (RILs), or near-isogenic lines (NILs) impractical.
- Tetraploid potato genotypes are therefore highly heterozygous. The heterozygous genotypes are fixed and maintained by vegetative propagation via tubers.
- Current breeding of marketable varieties comprises the generation of genetic variation by crossing elite tetraploid parents, usually varieties and advanced breeding clones.
- Evaluation and selection of approximately 13 main characters of plant and tuber in the recombinant F1 generation via multiyear and location trials. The selection cycle from crossing to variety release requires approximately 10–12 years.
- As potato is clonally propagated, diseases are accumulated and transmitted to descendant tubers; therefore, a system for cleaning and maintaining virus-free stocks of seed is essential.

These issues make potato breeding to concentrate a large effort in developing a system of controlled crosses that generate seeds from several families producing a large F1 population that will be the source of new breeding lines with potential to become varieties. Additionally, during the process, a big expense of resources is destined to rouging and maintaining of clean seed stocks of seeds.

Under optimized agricultural practices, potato production can yield more than 40 tons per hectare within 4 months from planting to harvest. To achieve this yield, it is essential to have high-quality seeds and improved cultivars as well as good agronomic practices and pest and disease control. With low technology, average yields are much lower ranging from 5 to 20 tons per hectare.

It is expected that through applications of biotechnology such as tissue and cell culture, genetic engineering, marker-assisted technologies, genome-assisted technologies, or a combination of

all the technologies for the improvement, potato has the potential to provide an increased proportion of the food intake required for the anticipated population expansion over the coming decades. Access to these biotechnological techniques is of vital importance for developing countries. However, the highly heterozygous genotypes produce a strong segregation in the progeny from controlled crosses; therefore to obtain a precise combination of characters or the improvement of some specific traits without losing other relevant genetic controlled traits is a difficult task. Genetic engineering could be the key to reach some specific gain in a particular trait preserving good genetic background to address better development of varieties. Nevertheless, GMOs are questioned for public opinion and even forbidden in numerous countries, as the case of Chile. In this way, the role of biotechnology is an assistant for the processes of classical breeding to make them more effective and to know in a better way the plant material at the genetic level.

So how is the experience of potato breeding program in Chile by using biotechnology to assist the development of Chilean varieties, specially adapted to local environments and productive systems?

Presently, 11 varieties have been released and inscribed in official system of seed certification. With the 11 varieties, it is possible to obtain 40 tons per hectare of yield in dryland conditions and 80 tons with irrigation, a good yield for Chilean conditions.

2. Breeding schemes of potato at INIA Chile

The mission of the potato breeding program in Chile is to develop potato varieties for different uses and productive systems to meet the Chilean market demand, with international projection.

The INIA uses a breeding scheme that is similar to classical potato breeding programs [3–5] with modifications according to local requirements. The potato breeding program begins with the selection of a large number of genotypes to be used as crossing parents. In the early steps of selection, around 100 crosses are made and 30,000 genotypes are evaluated. Selection of F1 progeny at early breeding stages (i.e., the primary individual selection of seedlings and the secondary individual clonal selection) is based on characteristics with high heritability and little annual variation, such as skin color, flesh color, and tuber shape, according to Chilean consumer preferences. The elimination of progeny with severe defects that can devastate potato production (e.g., hollow heart, growth cracks, and brown spots) also occurs at these stages. Progeny that is extremely susceptible to diseases as PVY, PVX, common scab, and late blight is discarded based on visual inspections of the field, although molecular markers are also available for genotype analysis. In later stages (i.e., line selection and the performance yield test), selection is carried out based on quantitative characteristics, such as yield, maturity, cooking qualities, and aptitude for chips or French fries. Molecular markers are applied in all the advanced breeding lines in order to confirm combinations of several resistance genes for diseases. A fixation process is unnecessary as they are clonally propagated.

The main objectives of the program are:

- Good performance for different end uses (fresh market and processing) for national or international demand
- Conformation and appearance of tuber
- Good agronomic characteristics: high yield and wide adaptation to agro-climatic zones
- Industrial uses
- Specific objectives:
    - Late blight resistance
    - Golden nematode resistance
    - PVY resistance

To achieve these objectives, the activities of the program involve controlled crosses every year (around 100), with 30,000 novel genotypes that are evaluated in multiyear and locations in field conditions.

3. Participation of biotechnology to support and improve the breeding process in the INIA potato program

Figure 1 shows an organization chart about the role of biotechnology in the potato breeding program in Chile. In first place, the germplasm bank is the source of genetic diversity for controlled crosses. This in vitro gene banks hold the varieties developed, advanced breeding lines, and imported breeding material that can be used of donor of some characters and native landraces. With this system, the material is preserved free from pathogens and suitable to be transferred to foreign countries in case of any need of varieties and breeding lines.

Techniques to introduce the material to in vitro conditions and thermotherapy to obtain pathogen-free in vitro plants are applied. Thermotherapy in combination with previous chemotherapy can be employed successfully, but efficiency is variable depending on virus types to remove. Results of DAS ELISA test before and after thermotherapy of a group of potato accessions from the field strongly infected by different viruses are shown in **Table 1**, indicating that in the case of PVY, 51% of the materials could be cleaned after two rounds of thermotherapy.

The materials stored in germplasm bank are analyzed by SSR markers to characterize them by molecular fingerprint. Currently, eight SSR markers are used. These markers are employed as a routine test for varietal identification since all the varieties are released with a described molecular profile that allows tracking of the varieties in the markets after available to farmers and to solve problems as mixture of varieties. These markers have been used in an overview of the genetic diversity and genotype numbers in germplasm bank of Chilean collection,

http://dx.doi.org/10.5772/intechopen.72961

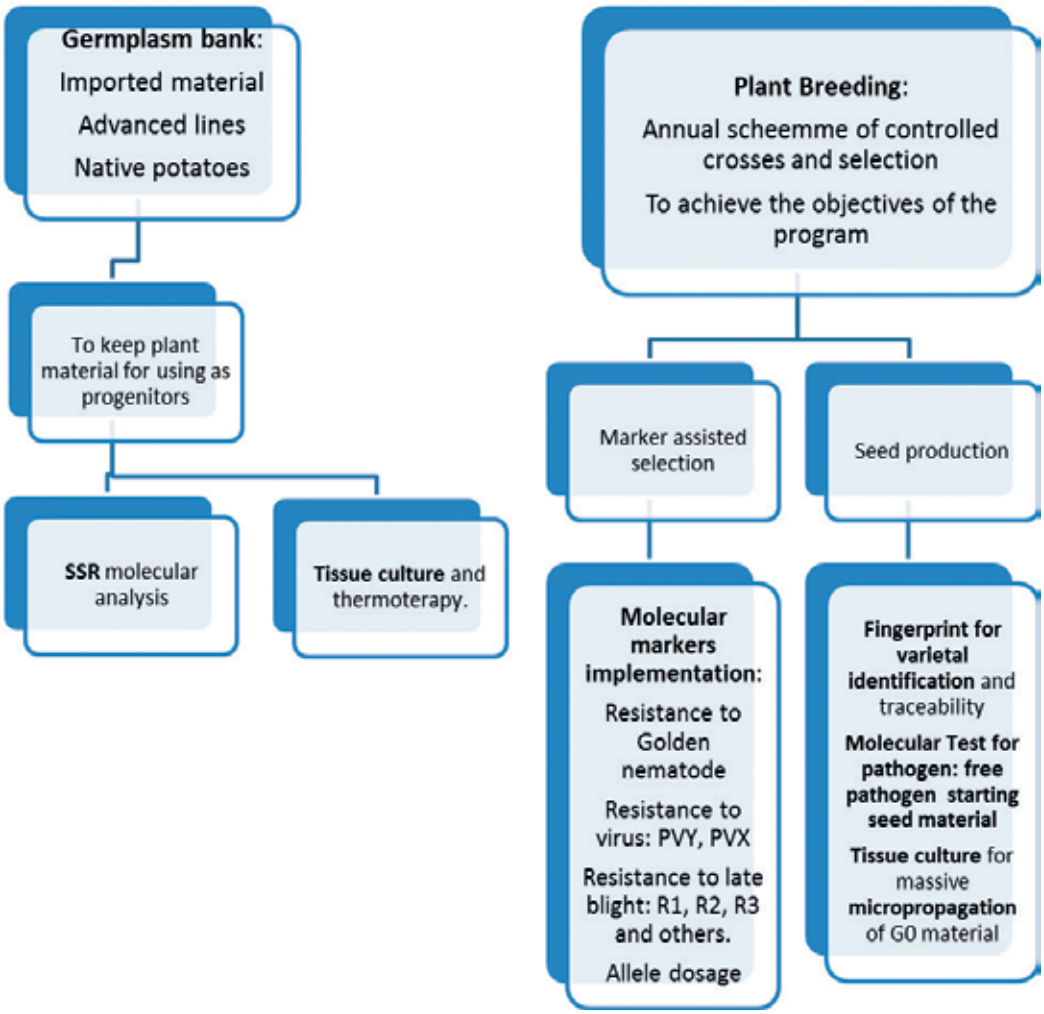

Figure 1. Organization chart about the role of biotechnology in the Chilean potato breeding program.

including native potatoes, commercial varieties, and valuable breeding lines. It is important to notice that SSR markers must be polymorphic enough to distinguish between the different varieties that it is necessary to discriminate. In the case of using molecular markers in the process of plant propagation of potato, as a tool to assure the identity of the commercial varieties that are being propagated in vitro, it is convenient to determine the set of markers that allow to produce different allele phenotypes (band patterns) for all the commercial varieties that are multiplied by the seed program, in order to differentiate them. In the case of wild material collected and kept in germplasm bank, or breeding lines with unknown pedigree, the SSR markers could discriminate different allele phenotypes, but it depends on the numbers of markers and polymorphism detected. Always it is possible that different genotypes could not be differentiated because no polymorphism in the regions of the genomes are being analyzed, but once a different band pattern is found between some plant materials, it is proven that they

Situation prior to thermotherapy treatment	Number or %	Situation after in vitro thermotherapy treatments	Number or %	Efficiency of thermotherapy (INI-FIN)/ FIN × 100
Number of accessions subjected to in vitro thermotherapy	157	Number of accessions cleaned after two rounds of thermotherapy	66	42%
Number of accessions infected with at least one virus	157	Number of accessions infected with at least one virus	91	
% incidence of PVX	26	% incidence of PVX	3.2	88%
% incidence of PVY	69	% incidence of PVY	33	51%
% incidence of PVS	78	% incidence of PVS	7	91%
% incidence of PLRV	86	% incidence of PLRV	21.7	75%
% incidence of PVA	8.9	% incidence of PVA	0.6	93%
% incidence of PVM	1.2	% incidence of PVM	0	100%
Average number of viruses present per infected accession	2.6	Average number of viruses present per infected accession	0.63	

Table 1. Efficiency of thermotherapy treatments for virus cleaning in a group of potato accessions from the field strongly infected by different viruses.

are different genotypes. This is very useful when studying collections of material with similar morphological features or when not all the descriptors are available to be examined (collections of tubers, in vitro plants or others where no flowers or leaves are available, or material affected by virus that affects the phenotype). In the case of Chilean collection of native potatoes, we have found 320 different allelic phenotypes using four SSR markers, indicating that there are at least 320 different genotypes in the collections. Of these, 158 belonging to the INIA collection were not found in another collection belonging to other Chilean institutions. As expected, different genotypes were known under the same popular name by the farmers. The molecular information is useful to know the genetic structure of the material preserved or used for breeding. For more details of our results, please see [6].

There is a flow of material from the gene bank to the annual scheme of controlled crosses and selection in the plant breeding program. Some genotypes are selected in order to combine characteristics in the progeny through controlled crosses and grown to obtain flowers and used as donor of valuable traits. On the other hand, promissory breeding lines from the field are introduced to in vitro culture and kept in the bank.

During the phases of selecting/discarding clones in field plots, molecular markers are implemented. Molecular markers associated with one or few genes that have a large and heritable effect in important traits are used (e.g., disease resistance in gene per gene model). Molecular markers for golden nematode resistance, virus resistance, late blight resistance, and some markers for flesh color are involved in the battery of markers to assist the selection and verify the combination of several resistance genes (**Table 2**). We investigate the allele dosage in some

	Gene	Marker	Resistance to	Reference
Routine markers for resistance genes implemented in PMGP-INIA	*H1*	57R	*G. rostochiensis*	[7] Finkers-Tomczak et al.
		TG689		De Jong, W. Cornell University, (unpublished); [8] Galek et al.
	Gro1–4	Gro1–4		[9] Paal et al.
	GroV1	U14		[10] Jacobs et al.
	Ry_{adg}	Ry3.3.3S/RyADG23R	PVY	[11] Kasai et al.
	Rx2	AC15	PVX	[12] Bendahmane et al.
	Rx1 y Rx2	Ask		[13] Bendahmane et al.
	R1	R1	*P. infestans*	[14] Ballvora et al.
	R2	R2		[15] Kim et al.
	R3a	R3a		[16] Huang et al.
	R3b	R3b		[17] Rietman
N°	10	11		

Table 2. Molecular markers used for routine assays to select breeding lines with resistance genes to potato diseases.

parents by means of study segregation of the marker in an F1 population in order to know the frequency of progeny that can hold the desired character and recognize most efficient parents for controlling crosses.

Once the new variety is ready to enter to the market, it is necessary to produce the stock of seed to support the entrance in the seed certification system.

Then, in the early stages of seed production in certification system, all the mother plant materials for the new varieties are checked by molecular fingerprint, PCR, and ELISA test for pathogen diagnosis, to assure the identity and pathogen-free status of the starting seed material and then micropropagated before entering to the certification system in the field. PVY and *Pectobacterium* are tested by PCR (**Table 3**), and PVX, PVM, PVS, PVA, PLRV, and PVY are diagnosed by DAS ELISA test.

In this stage, tissue culture for massive micropropagation of new varieties is still a pivotal biotechnological technique:

- Today, in the official certification system, all the varieties are propagated via in vitro culture before being multiplied in the field.
- Introduction of new varieties to in vitro condition is essential to produce certificated seed and makes possible that the varieties be distributed in the market.

Test	Marker	Gene target	Reference
Pectobacterium spp.	Y1/Y2	*Pel*	[18] Darrasse et al., modification of protocol
PVY	PVYF/PVYR	Capsid protein, strain: N	[19] Du et al., modification of protocol

Table 3. Procedures used for molecular diagnosis for ***Pectobacterium*** spp. and PVY in potato plants.

Currently our program keeps in vitro the 11 INIA varieties, 134 advanced breeding lines from INIA program, and 32 foreign varieties and breeding lines with research purposes.

Molecular fingerprints have been done to characterize 61 varieties, 25 advanced breeding lines, and 823 native landraces. We use the CIP identity kit for molecular profiling of the most valuable material for reliable identification and traceability during breeding process and seed production and in the future to track the presence in the market.

We use 11 molecular markers for marker-assisted selection, and at the date, we have analyzed 461 breeding lines and 33 varieties. The most important advantage of applying these markers is to allow more precision to choose parents for crossing in order to combine or pyramiding genes.

In **Table 4**, we can see the markers associated with resistance genes and light yellow flesh color present in the released varieties. It is possible to see that many varieties hold markers associated to golden nematode resistance, a quarantine pest in Chile but present in some

Variety	Golden nematode resistance	PVX resistance	Late blight resistance	PVY resistance	Light yellow flesh/ white flesh
Karú-INIA	*H1*; *Gro VI*				*Allele 3 BCH 2*
Patagonia-INIA	*Gro VI*				*Allele 3 BCH 2*
Pukará-INIA	*Gro VI*; *Gro 1–4*	*RX2*			*Allele 3 BCH 2*
Puyehue-INIA	*H1*; *Gro VI*; *Gro 1–4*		*R3a*		*Allele 3 BCH 2*
Yagana-INIA	*H1*; *Gro VI*	*RX2*			*Allele 3 BCH 2*
Fueguina-INIA	—		*R3a–R3b*		—
Ona-INIA	—		*R1*		*Allele 3 BCH 2*
Pehuenche-INIA	*Gro VI*; *Gro 1–4*		*R3a*		*Allele 3 BCH 2*
Purén-INIA	*Gro VI*		*R1*; *R3a*; *R3b*		—
Kuyén-INIA	—	*RX2*	—		
Rayún-INIA	*H1*; *Gro VI*		*R3b*		
R87009–28				Ry_{adg}	

Table 4. Molecular markers associated with resistance genes and light yellow flesh color present in the released varieties.

areas with potato cultivation in northern part of the country. For this pest, it is not possible to conduct field trials in south of Chile, so molecular marker implementation is crucial to track resistance in the progeny from controlled crosses with appropriate donor parents able to produce offspring with different resistance genes.

4. Conclusions about the role of biotechnology in Chilean potato breeding program

The program has implemented six biotechnological techniques; these are applied in the stages of characterization of the gene bank, selection of parents, marker-assisted selection, characterization of varieties, and propagation of material for seed production. One hundred percent of the varieties have been released involving biotechnology, especially by the use of in vitro culture techniques to produce pathogen-free material for initial stages of seed production of advanced lines. Biotechnological techniques have participated in the improvement of 2 of the 13 main characteristics associated with the program objectives. Two of the 11 varieties were characterized by molecular fingerprint at the time of their release. Biotechnological techniques such as in vitro culture, molecular fingerprint, and molecular diagnosis of diseases are used to produce primary multiplication of reproductive material for 100% of the varieties released by INIA currently present on the market.

Some important facts about the use of biotechnology in breeding and development of varieties are:

- Tissue culture is essential in the maintenance of varieties with the same genotype and initial steps of seed production system.
- Molecular fingerprint is important for varietal identification: vital in traceability of stock plants during micropropagation, and it has possibilities to be used to track the presence of varieties in the market.
- Molecular markers associated with one or few genes that have a large and heritable effect in important traits are used (e.g., disease resistance in gene per gene model).
- Molecular markers allow to have more precision to choose parents for crossing in order to combine or pyramiding genes.
- Markers for multigenic traits such as stress tolerance or cold tolerance have not been developed yet and remain as a big challenge to develop molecular genetic tools for multigenic traits.

In potato breeding, the selection of desirable phenotypes from a large breeding population will remain essential.

- Automatic, low-cost, and high-throughput phenomic technologies would be a valuable tool for massive screening of phenotypes.

- Screening methods based on next-generation sequencing technologies promise to revolutionize screening for desired genotypes, but it is necessary to solve the problem of distinguish between three different heterozygous genotypes (AAAB, AABB, and ABBB) in traits where plex number affects the character under selection.
- In order to make more precise the addressing of breeding procedures to improve specific traits (i.e., compounds with nutritional value or to eliminate undesired characters), methods as the new biotechnological techniques (NBTs) could be promising in countries where GMOs are not allowed. These new technologies as CRIPSR/Cas can be used to develop a genetic engineering with no transgenic status of the final product.

5. Biotechnology techniques to support clone selection procedures to pyramiding resistance genes to late blight

Biotechnology can be easily combined with classical breeding methods with the objective to pyramiding resistance genes (R genes) to avoid the breakdown of resistance in the case of fast evolving pathogens. We will describe below our experience in developing a strategy to pyramiding R genes for late blight resistance in breeding lines.

For this purpose, we are using the Ma*R8* and Ma*R9* genotypes as sources of resistance to late blight in the Chilean potato breeding program.

5.1. Late blight as a major threat for potato production and food security

The potato late blight caused by *Phytophthora infestans* (Mont.) de Bary is a major challenge to potato production worldwide [20]. Reliance on susceptible potato cultivars in commercial agriculture has meant that fungicides are widely used to control late blight. However, such materials have significant monetary and environmental costs to society [21–23]. To control this disease, up to 14 applications of fungicides may be needed for a crop season.

P. infestans can infect the entire plant, including the stems, leaves, and tubers. When left unchecked, it can quickly destroy a potato crop within a few days. The success of this pathogen is not only due to its elevated virulence but also to its remarkable capacity of rapidly adapt to resistant plants. Therefore, new resistant potato varieties with multiple resistance genes must be produced, as the use of varieties with genetic resistance to late blight is essential for growing low-cost, healthy, and environmentally sustainable potatoes.

However, only three of the 25 potato varieties used in Chile have some intermediate level of resistance to late blight. Notably, while *S. tuberosum* lacks significant resistance, wild potato species are rich sources of late blight resistance genes. *Solanum demissum*, a hexaploid Mexican wild *Solanum* species, is an important source of resistance to late blight. The major resistance (R) genes from *S. demissum* have late blight race specificity.

Eleven R gene differentials containing R genes introgressed into *S. tuberosum* from *S. demissum* were collected by Mastenbroek [24] and are referred to as the Mastenbroek differential set: Ma*R1*

to Ma*R11*. In Ma*R8* and Ma*R9*, at least four (*R3a*, *R3b*, *R4*, and *R8*) and seven (*R1*, *Rpi-abpt1*, *R3a*, *R3b*, *R4*, *R8*, and *R9*) R genes were present, respectively [15, 25]. This set can be used to simultaneously introduce multiple R genes. However, since this set has a low agronomic value, crosses must be made with elite breeding material to obtain breeding lines suitable for propagation.

Significantly, the resistance provided by the R genes is background dependent as genes that suppress R genes can also be segregated into F1 offspring plants [26]. Thus, more knowledge is needed about how R genes perform in different genetic backgrounds. Research has also focused on creating GM organisms carrying constructions with the described R genes [27]. However, this class of plant material is not allowed in many parts of the world for human or animal consumption including Europe and Chile. Furthermore, society is suspicious about its sustainable utilization because of the health, environmental, and social implications of GMOs. For this reason, it is necessary to investigate the applications of the natural stacking of several R genes to overcome *P. infestans*.

The objectives of our work are:

a. To combine multiple R genes that confer resistance to late blight in new lines of the potato breeding program of INIA Remehue, Chile

b. To evaluate the level of resistance to *P. infestans* in genotypes carrying different R genes inherited from hybridization between Ma*R8* and Ma*R9* with elite breeding material

5.2. Materials and methods

Controlled crosses were performed between five commercial varieties and the genotypes Ma*R8* and Ma*R9* that hold four and seven genes of resistance, respectively.

Ten progenies from each cross were randomly selected for molecular analysis and phenotypic evaluations in order to assay for the presence of R genes. The performance of these genotypes was monitored under pathogenic pressure in field conditions.

During the 2013–2014 season, 90 randomly selected progenies were evaluated for pathogen resistance. We calculated the area under disease progress curve (AUDPC) in individual plants under natural infections in the field. To promote infections, the progeny was watered by a spray system twice a week although the natural inoculum was high in Osorno, Chile.

For the molecular analysis, DNA extractions and PCR amplification were performed using genetic markers as described in **Table 2** for *P. infestans* to track the presence or absence of R genes in the Ma*R8* or Ma*R9* crossed with elite material progeny.

Progeny was also phenotypically evaluated. We determined the percentage of leaves affected by late blight during plant development by visually estimating the green and non-green portions of the leaves. The estimations were integrated into the AUDPC or area under the disease progress curve. AUDPC is obtained by the repeated visual inspections and estimation of the percentage of the leaf affected in a set of plants. The value is calculated by the formula used by Jo et al. [28]. Percentages of damaged foliage are plotted through a period of time. AUDPC was calculated for the 10 randomly selected individuals from each cross in the field.

For the following 2014–2015 season, the tubers of 71 genotypes that were not damaged by late blight were harvested. The experiment was designed with three replicates in randomized blocks. We planted three plants of each genotype that hold R genes in front of three plants of the susceptible Atlantic cultivar (susceptible control) per each replicate.

Rows of the susceptible Atlantic cultivar were also planted in the border and interspersed in the complete area of the assay. AUDPC values were calculated for all genotypes and susceptible control plots. A pairwise comparison was performed between each genotype and the respective control plot. We calculated the AUDPC from the visual estimation of the percentage of infected foliage.

5.3. Results

In the 2013–2014 season, an evaluation of randomly selected individual plants from the progeny of Ma*R8* and Ma*R9* crossed with the commercial varieties indicated that progeny carrying the *R2* gene had less foliage damage represented by lower AUDPC values. Furthermore, higher AUDPC values were found in plants that did not contain R genes. We were not able to perform statistical analysis as there were different numbers of clones holding R genes.

In the second season, we utilized undamaged plants from the first season. Most of the plants carrying R genes again had lower AUDPC values than the control plots. Interestingly, plants carrying the *R3* gene did not have different AUDPC values compared to the susceptible control plots.

In conclusion, we found that plants carrying R genes were only slightly affected by late blight in conditions of high pathogenic pressure, with the exception of the *R3a* and *R3b* genes (**Table 5**). Out of the 11 genotypes that did not show differences compared to the susceptible control, nine were holders of genes *R3a* and *R3b*, suggesting that these R genes are less resistant to late blight and, therefore, should not be used in breeding programs in Chile.

	Number of genotypes tested	**% genotypes significantly different to susceptible**
Genotypes carrying 1 R genes	5	60
Genotypes carrying 2 R genes	21	71
Genotypes carrying 3 R genes	18	94
Genotypes carrying 4 R genes	13	92
Any genotype carrying R1	27	96
Any genotype carrying R2	17	88
Genotypes carrying only R3a or R3b	20	55

Table 5. Genotypes harboring different numbers of R genes and % showing an AUDPC value significantly lower than the susceptible control plots.

5.4. Conclusions about pyramiding R genes for resistance to late blight

The Ma*R8* and Ma*R9* crosses were successful and generated hybrid genotypes harboring at least four different R genes that are now available for breeding. The progeny carrying R genes has been selected as parents for backcrosses or early clonal selection step and entered to the scheme of the potato breeding program.

A major challenge remains to develop an efficient and reliable system of phenotyping for late blight damage and for large-scale screening of breeding lines.

Acknowledgements

Financial support: Subsecretary of the Chilean Ministry of Agriculture through Potato Breeding Program project and Conservation of Genetic Resources program (501453-70). CORFO (Corporación de Fomento a la Producción) through INNOVA-Public Goods 14BPC4-28525.

Author details

Manuel Andrés Muñoz*, Julio César Kalazich, Carolina Verónica Folch,
Sandra Valeska Orena and Annelore Winkler

*Address all correspondence to: manuel.munozd@inia.cl

Institute of Agricultural Research INIA, Regional Center Remehue, Chile

References

[1] Monneveux P, Ramírez D, Pino MT. Drought tolerance in potato (*S. tuberosum* L) can we learn from drought tolerance research in cereals? Plant Science. 2013;**205-206**:76-86

[2] Oficina de estudios y políticas agrarias ODEPA. Boletin de la papa. Ministerio de Agricultura del gobierno de Chile; 2017. 17 p

[3] Carputo D, Frusciante L. Classical genetics and traditional breeding. In: Genetics, Genomics and Breeding of Potato. Enfield, New Hampshire: Ed: Braaden and Kole, Science Publishers; 2011. pp. 20-40

[4] Mori K, Asano K, Tamiya S, Nakao T, Mori M. Challenges of breeding potato cultivars to grow in various environments and to meet different demands. Breeding Science. 2015;**65**:3-16

[5] Eriksson D, Carlson-Nilssen V, Ortiz R, Andreasson E. Overview and breeding strategies of table potato production in Sweeden on the fennoscandian region. Potato Research. 2016. DOI: 10.1007/s11540-016-9328-6

[6] Muñoz M, Folch C, Rodriguez F, Kalazich J, Orena S, Santos J, Vargas R, Fahrenkrog A, Puga A. Genotype number and allelic diversity overview in the national collection of Chilean potatoes. Potato Research. 2016;**59**(3):227-240

[7] Finkers-Tomczak A, Bakker E, de Boer J, van der Vossen E, Achenbach U, Golas T, Suryaningrat S, Smant G, Bakker J, Goverse A. Comparative sequence analysis of the potato cyst nematode resistance locus H1 reveals a major lack of co-linearity between three haplotypes in potato (*Solanum tuberosum* ssp.). Theoretical and Applied Genetics. 2011;**122**:595-608

[8] Galek R, M, Rurek De Jong, W.S, Pietkiewicz G, Augustyniak H, Sawicka-Sienkiewicz E. Application of DNA markers linked to the potato H1 gene conferring resistance to pathotype Ro1 of *Globodera rostochiensis*. Journal of Applied Genetics. 2011;**52**:407-411

[9] Paal J, Henselewski H, Muth J, Meksem K, Menéndez CM, Salamini F, Ballvora A, Gebhardt C. Molecular cloning of the potato *Gro1-4* gene conferring resistance to pathotype Ro1 of the root cyst nematode Globodera rostochiensis, based on a candidate gene approach. Plant Journal. 2004;**38**:285-297

[10] Jacobs J, van Eck HJ, Horsman K, Arens P, Verkerk-Bakker B, Jacobsen E, Pereira A, Willem Stiekema J. Mapping of resistance to the potato cyst nematode *Globodera rostochiensis* from the wild potato species *Solarium vernei*. Molecular Breeding. 1996;**2**:51-60

[11] Kasai K, Morikawa Y, Sorri VA, Valkonen JPT, Gebhardt C, Watanabe KN. Development of SCAR markers to the PVY resistance gene Ry*adg* based on a common feature of plant disease resistance genes. Genome. 2000;**43**:1-8

[12] Bendahmane A, Querci M, Kanyuka K, Baulcombe D. *Agrobacterium* transient expression system as a tool for the isolation of disease resistance genes: Application to the Rx2 locus in potato. The Plant Journal. 2000;**21**(1):73-81

[13] Bendahmane A, Kanyuka K, Baulcombe D. The *Rx* gene from potato controls separate virus resistance and cell death responses. The Plant Cell. 1999;**11**:781-791

[14] Ballvora A, Ercolano MR, Weiû J, Meksem K, Bormann CA, Oberhagemann P, Salamini F, Gebhardt C. The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. The Plant Journal. 2002;**30**(3):361-371

[15] Kim HJ, Lee HR, Jo KR, Mortazavian SM, Huigen DJ, Evenhuis B, Kessel G, Visser RGF, Jacobsen E, Vossen JH. Broad spectrum late blight resistance in potato differential set plants Ma*R8* and Ma*R9* is conferred by multiple stacked R genes. Theor. Appl. Genet. 2011;**124**:923-935

[16] Huang S, van der Vossen EAG, Kuang H, Vleeshouwers VGAA, Zhang N, Borm TJA, van Eck HJ, Baker B, Jacobsen E, Visser RGF. Comparative genomics enabled the cloning of the R3a late blight resistance gene in potato. The Plant Journal. 2005;**42**(2):251-261

[17] Rietman H. Putting the *Phytophthora infestans* genome sequence at work; identification of many new *R* and *Avr* genes in *Solanum* [PhD thesis]. Wageningen University; 2011

[18] Darrasse A, Priou S, Kotoujansky A, Bertheau Y. PCR and restriction fragment length polymorphism of a pel gene as a tool to identify *Erwinia carotovora* in relation to potato diseases. Applied and Environmental Microbiology. 1994;**60**(5):1437-1443

[19] Du Z, Chen J, Hiruki C. Optimization and application of a multiplex RT-PCR system for simultaneous detection of five potato viruses using 18S rRNA as an internal control. Plant Disease. 2006;**90**:185-189

[20] Termorshuizen AJ. Fungal and fungus-like pathogen of potato. In: Vreugdenhil D, editor. Potato Biology and Biotechnology. Advances and Perspectives. Oxford, UK: Elsevier; pp. 643-665

[21] Mayton H, Rauscher G, Simko I, Fry W. Evaluation of the *Rpi-ber* late blight resistance gene for tuber resistance in the field and laboratory. Plant Breeding. 2011;**130**:464-468

[22] Rodewald J, Trognitz B. Solanum resistance genes against *Phytophthora infestans* and their corresponding avirulence genes. Molecular Plant Pathology. 2013;**14**:740-757. DOI: 10.1111/mpp.12036

[23] Tiwari J, Siddappa S, Singh B, Kaushik S, Chakrabarti S, Bhardwaj V, Chandel P. Molecular markers for late blight resistance breeding of potato: An update. Plant Breeding. 2013;**132**:237-245

[24] Mastenbroek C. Investigations into the inheritance of the immunity from *Phytophthora infestans* de Bary of *Solanum demissum* Lindl. Euphytica. 1952;**1**:187-198

[25] Jo KR. Unveiling and deploying durability of late blight resistance in potato. From natural stacking to cisgenic stacking [PhD Thesis]. Netherlands: Wageningen University; 2013. 168 p

[26] Ordonez ME, Forbes GA, Trognitz BR. Resistance to late blight in potato. A putative gene that suppresses R genes and is elicited by specific isolates. Euphytica. 1997;**95**:167-172

[27] Jo KR, Kim CK, Kim SJ, Kim TY, Bergervoet M, Jongsma M, Visser R, Jacobsen E, Vossen J. Development of late blight resistant potatoes by cisgene stacking. BMC Biotechnology. 2014;**14**:50

[28] Amin M, Mulugeta N, Thangavel S. Field evaluation of new fungicide, Victory 72 WP for management of potato and tomato late blight (*Phytophthora infestans* (Mont) de Bary) in West Shewa Highland, Oromia, Ethiopia. Journal of Plant Pathology and Microbiology. 2013;**4**:1-8

Chapter 2

Genetically Modified Potato as a Source of Novel Carbohydrates

Chandrama Prakash Upadhyaya,
Deepak Singh Bagri and
Devanshi Chandel Upadhyaya

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71079

Abstract

Significant progress has been made in understanding of carbohydrate (starch) biosynthesis through molecular biology and genetic engineering techniques. Genetic modification of plants has a great potential to produce novel carbohydrates with unique properties that cannot be generated by conventional breeding approaches. Starch is the predominant carbohydrate in potatoes and serves as an energy reserve for the plant. Genetic engineering of potato (*Solanum tuberosum* L.) tuber can revolutionise the synthesis of unique starches with altered physical and chemical properties that are engineered to meet the specific industrial requirements. In addition to expression of foreign genes involved in carbohydrate biosynthesis, genes regulating the carbohydrate metabolism, transport and resource partitioning have also been achieved. Here we summarise the recent progress made towards modifications of the biosynthetic pathways by which potato can produce novel carbohydrates. Further, we discuss the prospects of engineering potatoes for production of structural and non-structural carbohydrates.

Keywords: carbohydrate metabolism, starch, genetic engineering, novel carbohydrates, *Solanum tuberosum* L., sucrose transport

1. Introduction

The most abundant bio-compounds on our planet are the carbohydrates which is synthesised by green plants during the process of photosynthesis. More than 100 billion metric tons of CO_2 and H_2O per year are converted into carbohydrates during the process of photosynthesis [1]. The carbohydrates converted into the starch in the plant plastids which is the major storage

carbohydrate found in various types of green plant tissues and organs. Two types of starch are reported to be present in the plants which are distinguishable as the 'transitory starch' and the 'storage starch'. The transitory starch is synthesised and accumulated in chloroplasts of green leaves during photosynthesis and degraded throughout the night to provide substrates for respiration and continued sucrose synthesis to supply to sink tissues [2]. The storage starch is synthesised in amyloplasts and typically associated with sink organs, such as stems, seeds, roots, and tubers. This type of starch is accumulated during different developmental stages and utilised during various periods of dormancy, germination, growth, or other specific processes. The storage starch is deposited in granules rich in amylose content than that observed in the transitory starch [3, 4].

According to the recent reports published by the Food and Agricultural Organisation Statistics (FAO statistics), the potato (*Solanum tuberosum* L.) stands at the world's 4th major crop in terms of yield after rice, wheat and corn, and in terms of area under cultivation, its stands at the 8th position. The potato is grown for its tuber which is considered as is a high-energy staple food around the world and high productivity per unit area due to its intense cultivation. Thus, the potato represents one of the best candidates for alleviating food shortages. Potato belongs to *Solanaceae* family, is the perennial herbaceous plant having white to purple flowers with yellow stamens. Some potato cultivar bears small green fruits, each containing up to 300 seeds. Potato is grown from the botanical seeds or usually propagated vegetatively by planting pieces of tubers containing eyes or dormant buds which develop into new shoots (sprouts) when grown under suitable conditions. Potato tubers are rich in starch, storage proteins and develop by the morphological changes of the underground stem into stolon bearing auxiliary buds and scars of scale leaves. The total carbohydrates in potato tuber range from 1.0 to 7.0 g/kg. The reducing sugar (glucose, fructose) concentrations are higher in young tubers and reduced significantly near the end of the cultivating season. Starch is the prime carbohydrate component of the potato dry matter containing the amylose and amylopectin. Starch has conventionally been used in the food industry to augment the functional properties of various foods. The physicochemical and functional properties of starch system vary with the starch biological origin. The structural characteristics and amylose-to-amylopectin ratio of potato starch also vary among cultivars. Nutritional and processing quality of potato products (frozen and dry) are greatly affected by their starch characteristics and content. Several chemical, physical, and enzymatic modifications have been accomplished to improve the processing operation of potato starch. Potato starch can be used in other industrial applications as a gelling agent, thickener, bulking agent, colloidal stabiliser and water-holding agent. However, due to low shear and thermal resistance and high bent towards degradation hinder its use in some industrial food applications. These limitations are generally overcome by starch modification, which can be achieved through derivatization, such as etherification, esterification, cross-linking, and grafting; decomposition (acid or enzymatic hydrolysis and oxidisation); or physical treatment of starch using heat or moisture or pressure, etc. Most of these modifications are usually recognised as non-toxic by the safety authorities. Several modified potato starches with slow digestibility are being developed that may provide nutritional benefits for humans. These starches have the potential to be used for the treatment of certain medical conditions (e.g., glycogen storage disease and *Diabetes mellitus*). The Food and Drug Administration (FDA) controls and emphasises the type and amount of each chemical used in starch modification, as well as the percentage of the substitution.

Starch which is known as the product of the plant photosynthetic carbohydrates, is commercially isolated from a wide range of sources including cereal grains such as corn, wheat, rice, and sorghum; roots and tubers such as potato, sweet potato, cassava, and arrowroot; and stem and pith such as sago. The composition of naturally occurring starch is universal, irrespective of its source, with the main component as amylopectin (75%) and a minor component as amylose (25%). Amylose and amylopectin are synthesised in the plastids, where they assemble into a semi-crystalline granule. The starch found in potato tuber is distinct granules approximately 10–100 μm in diameter [5]. Compared with other commodity starches, the potato starch granules are relatively larger, smooth with a high content of covalently linked phosphate, long amylopectin chains and high-molecular weight amylose. The granules are synthesised by two polysaccharides consisting exclusively of glucose as the monomer component. The glucopyranosyl residues are connected through α-d-(1,4)-linkages forming chains through α-d-(1,6)-branches at the reducing end side linked to similar other chains. The industrial application of starch includes the manufacture of high-quality paper [6] and generation of viscous hydrocolloid systems to be used for food processing [7]. However, the well-ordered and dense structure of the native potato starch granule renders it resistant to enzymatic degradation by hydrolytic enzymes such as amyloglucosidases and α-amylases [8], which is very important in industrial applications. Amylopectin is the major component of starch in general, and in potato it normally constitutes 70–80% by weight [9] regardless of the size of the granules [10]. Approximately 4–6% of linkages are of the α-d-(1,6)-type, making it extensively branched. The weight-average molecular size of amylopectin is on the order of 107 Da [11, 12]; as a result, the macromolecule consists of a huge number of relatively short chains with an average degree of polymerisation (DP) of 21–28 residues [13, 14]. The amylose is considerably smaller than amylopectin and is basically a linear polymer comprising of 2000–5000 residues [5]. This composition may affect the physicochemical properties, such as gelatinization, texture, moisture retention, viscosity, and product homogeneity that are determinants for its industrial applications. Besides the polysaccharide components, potato starch consists of low amounts of material of a non-carbohydrate nature. Less than 0.5% of the granules are proteins [9], apparently involved in starch synthesis. Potato starch also contains phosphorus in the form of phosphate covalently linked to the amylopectin component. It is considered an important factor contributing to potato starch properties.

2. Mechanism underlying starch biosynthesis in potato

The photosynthesis is the primary metabolic mechanism for the carbohydrate biosynthesis which is well-known phenomenon of the plant that takes place with the help of the chlorophyll and light. However, the conversion of carbohydrates to starch granule is a subtle equilibrium between proficient packing of the glucan chains and the prospect of breaking these structures during degradation. Hence, a series of enzyme catalytic activities are required to complete this process in the starch biosynthesis. These include three steps, the first is the activation of the major carbohydrate, the glucose molecule, second step is the elongation of the glucan chain, and the final step is the transfer of linear backbone chains forming branched structures (**Figure 1**). The activation of the glucose residues to form adenosine diphosphoglucose

(ADP-glucose) takes place with the help of enzyme ADP-glucose pyrophosphorylase (AGPase) using the ATP and a molecule of glucose 1-phosphate [15]. This reaction is the rate-limiting step in the starch biosynthesis. In the next step, the elongation of the chain takes place which constitute the amylopectin and finally the starch granule is synthesised with the help of soluble starch synthase (SS) and starch-branching enzymes (SBE). The soluble SS catalyses the elongation of the chain at the non-reducing end in a reaction in which ADP of the ADP-glucose molecule is replaced by the terminal hydroxyl group of the growing glucan chain, creating an elongated linear α-(1,4)-glucan chain. However, only one enzyme is essential recognised as granule bound (GB)-starch synthase (SS) for amylose synthesis. The formation of branched

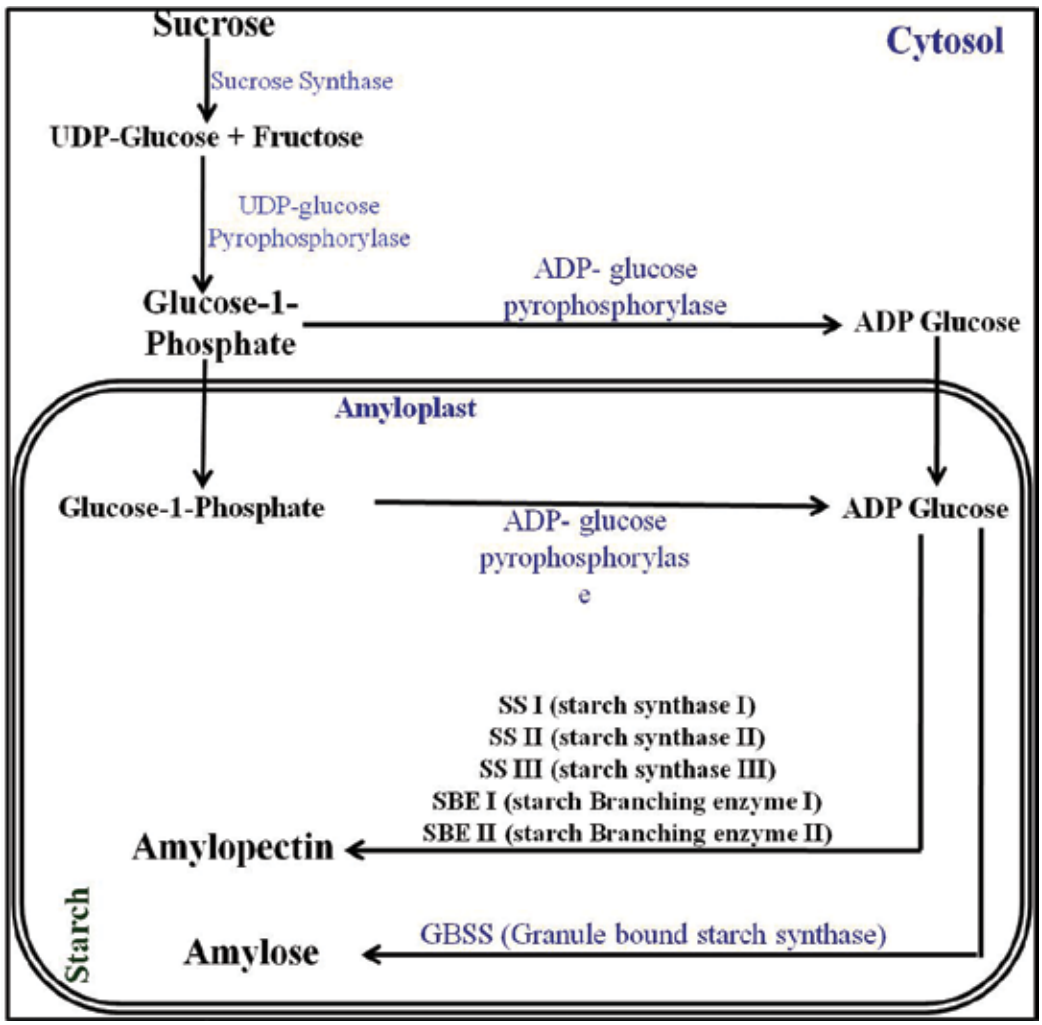

Figure 1. Pathway of starch synthesis. ADP-glucose (ADP-Glc), the donor substrate for both amylose and amylopectin, is synthesised by the ADP-Glc pyrophosphorylase (AGPase). The combined action of different starch synthases (SSI, SSII, and SSIII), branching enzymes (BEI and BEII), and the debranching enzyme (DBE) is necessary for the synthesis of amylopectin. Granule-bound starch synthases (GBSSI and GBSSII) use amylopectin as the acceptor substrate to synthesise amylose, which is formed down-stream of amylopectin.

α-(1,6)-linkages in starch is catalysed by the SBE. In this reaction, an α-(1,4)-linkage within the chain is cleaved and an α-(1,6)-linkage is formed between the reducing end of the cleaved glucan chain and a C-6 linked oxygen of an adjacent chain. The starch-branching enzyme has been reported to exist as multiple enzyme isoforms. The action of debranching activities during biosynthesis seems to be important for correct assembly of the starch granule [16]. This process is generally more complicated as compared to glycogen biosynthesis involving a multitude of different homologous enzymes probably responsible for synthesising specific structures of the starch granule in different tissues and at different developmental stages. Apparently, many of these enzymes interact to form enzyme complexes or metabolomes to channel and direct substrates and products.

New cultivars of potato with better yield, disease resistance, have been developed since long time with the help of breeding techniques. Following the advancement of genetic engineering tools, several other potato cultivars with desired yield, dry matter, protein and antioxidant quality, cooking texture (such as waxy, floury), flesh colour, and abiotic stress tolerant plant have also been developed. The demand for starches with special properties useful for industrial food processing has led to the introduction of modified starches using the genetic engineering techniques. Though, there are a lot of information available in the literature on chemical modification of starch, however, genetically modified potato with altered carbohydrates, starch or amylose/amylopectin content, have also been developed. Some of the genetically modified potato starches are being used in the industry under strict control, however, these transgenic varieties of potatoes are not permitted for food use in several countries because of the concerns related to consumer health and the environment. Until these genetically modified potatoes have been given proper clearance by the food authorities and acceptance by the consumers, they may have a good scope for their use in non-food or other industrial applications. We will first describe attempts to alter starch structure to improve starch functionality, then explain how increasing knowledge of the regulation of starch biosynthesis is being used to increase starch production, and finish by summarising new methods for increasing the genetic diversity in crops as well as methods for fine-tuning gene expression in plants in order to bring improved starch-based products with value-added consumer benefits to the marketplace.

3. Genetic engineering of potato for starch modification

Starch modification involves efforts to both achieving enhanced starch production and modifies the composition or component structure to impart specific properties to suit final product. The most widely referred target for starch modification is the alteration in the amylose-amylopectin ratio. Several plants have been genetically modified in their starch biosynthetic pathway to yield high-amylose and high-amylopectin starches.

3.1. Genetic engineering of potato for starch synthase enzyme

It is evident that the starch synthase (SS; ADP-glucose:α-1,4 glucosyl transferase) catalyses transfer of the glucosyl moieties from ADP-glucose to the non-reducing end of an α-1,4-glucan [17]. The potato SSs enzymes catalyse the same reaction represented as

ADP-glucose + (1,4-alpha-D-glucosyl) (n) = ADP + (1,4-alpha-D-glucosyl) (n + 1). Potato is reported to contain four different SSs isoforms known as SSI, SSII, SSIII and GBSSI. The SSI does not have multiple isoforms in plants while other SSs are present in multiple isoforms, suggesting a presumably unique and important role of SSI in starch biosynthesis [18]. However, the precise role of SSI is still not well-defined. Though the activity of SSI enzyme was repressed to non-detectable quantity in transgenic potato plants silenced for SSI gene, neither amylopectin structure nor starch granule morphology was changed. The reason for no detectible changes might be because SSI is mainly expressed in the potato leaf tissue and mainly involved in the synthesis of transitory starch in the leaves [19]. The SSI prefers the shortest amylopectin chains as substrate, and it is particularly responsible for synthesising amylopectin short chains [20, 21]. It was found that a short glucose chain of 6–7 residues are apparently the substrates for SSI enzyme, which then extend to a length to 8–12 glucose residues inside the amylopectin cluster [22]. Despite these findings, antisense down regulation of SSI in potato tubers did not alter the starch structure, signifying other SSs may fairly balance for the lack of SSI in tubers. The SSIII is the key SS in potato tubers and accounts for just about 80% of soluble starch synthase activity; although a minute fraction of the SSIII activity is found there to the starch granules [23, 24]. Down regulation of SSIII via antisense techniques in transgenic potato tubers led to about 80% loss of SSIII activity, and therefore, alterations in the morphology of starch granule were observed [25]. However, the significance of the individual SS isoforms and their distribution between stroma and starch granules within the plastids is very species-dependent and these differences probably provide to deviations seen in the structure of starches created from diverse plant species [26]. Interestingly, the apparent redundant function of SSII and SSIII in amylopectin biosynthesis is also reported by researchers [27]. The exact role of other SS, the SSIV is still not yet known, however, the development of *Arabidopsis* mutants lacking SSIV and abnormalities in their granule initiation is well studied [28]. The investigations have revealed two additional discrete isoforms of SSIV, that is, SSIVa and SSIVb, which are present in cereal endosperms and leaves, respectively [29]. To the best of our knowledge, an enzyme like any known SSIV has not been reported/or characterised in potato. Blast information using potato genome sequence database (www.potatogenome.net) and sequence based comparison showed that this putative enzyme belongs to the same family as other SSs, the GT5 glycosyltransferase family [30].

Genetic modification in SSs expression reported to an impact on the yield, fine structure and physical properties of the starch. Typically, downregulation of synthase activity does not have much impact on the starch yield possibly because reduced activity in one SS isoform may result in compensatory increases in the activity of another isoform. In case of the transgenic potato with antisense expression of the SSII + SSIII, surprisingly the potato tuber's capability to polymerise glucans from ADP-Glc was mainly compromised. The downregulation of only one SSI activity in potato resulted in reduction of the amylose content. As a result, the physical properties of the starch were also changed, the melting temperature of the granule was increased, and the constancy of the starch solution after gelatinization enhanced due to lack of retrogradation. When SSII was down-regulated in potato, small changes were observed in the chain length distribution. This lends some support to the hypothesis that each synthase may

play a distinct role in elongating side chains of a specific length. The physical properties of the potato starch down-regulated in the SSII activity were also altered, both the melting temperature of the granule and the peak viscosity were decreased. Furthermore, the phosphate content of the antisense SSII starch was also decreased [19]. This is most probably associated with the strictly lowered peak viscosity. This is in consistent with the result that inhibition of an enzyme involved in starch phosphorylation (R1) may also lead to a crumple of the peak viscosity [31]. When SSII plays a more predominant role in assembling the starch granule in potato (for example, when SSIII expression was inhibited, then an increase in the phosphate content, as well as in peak viscosity is also observed) [3]. Thus, there seems to be a relationship between SSII and starch phosphorylation. It is tempting to speculate that the N-terminal extension of SSII may interact with a starch-phosphorylating enzyme. Another possibility is that SSII can introduce phosphorylated glucose residues into nascent glucan chains or preferentially synthesises branch lengths more suitable for phosphorylation. The heterologous gene expression of SSs was also reported in transgenic potato tubers. The cassava granule bound (GB)-SSI was expressed in the amylose-free (amf) potato mutant. Although the GBSSI activity in these starch granules was comparable to that of wild-type granules, the amylase content was only restored to 60% of that of wild-type ones. The fine structure and the physical properties of potato starch can be altered by introduction of a glycogen synthase A (glgA, EC 2.4.1.21) gene. It is worth noting that both the degree of phosphorylation and the peak viscosity were lowered, which agreed with the data for antisense SSII starch.

3.2. Genetic engineering of potato for starch-branching enzyme

Differences in amylopectin branching affect granule crystallinity, which together with differences between species in granule size and shape result in altered thermal, pasting and biophysical properties [32, 33] The two starch-branching enzyme (SBE) isoforms, SBEI and SBEII have been reported in the potato. Amylopectin branching by SBEI and SBEII enzymes form branch points by cleaving α-(1 → 4)-linkages and reattaching the glucan chain via an α-(1 → 6)-linkage. The changes in SBE activity change the number and size distribution of amylopectin branches. SBEI is the major form of SBE in the potato tuber; however, antisense downregulation of SBEI did not change the amylose content, although small changes in the physical properties of the starch such as the gelatinization onset could be measured in differential scanning calorimetry [34]. The second form, SBEII was subsequently discovered, and when expression of this gene was reduced in the potato tuber, the amylose content was elevated to about 35% even though this SBE isoform made up less than 2% of the SBE activity of the tuber [3]. A combined downregulation of both these SBE isoforms directed to a noteworthy further increase up to 70% or higher [35]. It is now well known that the starch biosynthesis takes place by a group of enzymes including different starch synthases and starch-branching and -debranching enzymes. The role of all these enzymes has been investigated using the gene silencing or genetic knockouts techniques have been used to analyse the role of all these enzymes; however, there are only some example of over-expression existed which is probably due to the problems in cloning large genomic fragments for over-expression or severe toxicity of functional cDNAs to bacteria during cloning. A promising study on the function of potato starch-branching enzyme (SBEII) using over-expression in potato tubers was done recently

[36]. The transgenic potato lines with SBEII over-expression were generated. Compared with wild-type, starch from these tubers possessed an increased degree of amylopectin branching, with more short chains of degree of polymerisation (DP) 6–12 and particularly of DP6. Further, the increased ratio of short to long amylopectin branches facilitated gelatinisation, which occurred at a reduced temperature (by up to 3°C) or lower urea concentration.

3.3. Genetic engineering of potato for modification of carbohydrates (starch) in storage organs

The initiation of starch biosynthesis in storage organs inevitably requires the mobilisation of sucrose into glucose-6-phosphate (G6P), import of G6P into the amyloplast through inorganic phosphate (Pi) exchange, and subsequent conversion of G6P into glucose-1-phosphate (G1P) by plastidial phosphoglucomutase. The first committed step to starch synthesis is the formation of ADP-glucose (ADP-Glc) through ATP activation of G1P, catalysed by ADPG pyrophosphorylase (AGPase). AGPase has a heterotetrameric structure with two small subunits and two large subunits. The AGPase enzymes are reported to be allosterically regulated with 3-phosphoglyceric acid (3PGA) being the main activator and Pi the main inhibitor.

The AGPase enzymes were reported to present in several plant tissues and bacterial extract [37]; however, the localisation and regulation of ADP-Glc synthesis and import of Glc-1-P are highly variable among species and in different organs within a species [38, 39]. As a key factor of this rate-controlling step, AGPase was extensively used to develop different transgenic plants including potato where the rate of ADP-Glc formation and starch accumulation is under tight control of the gene expression. The amylose content was severely reduced in transgenic potato that contained a lower expression of AGPase [40, 41]. The transgenic potato lines were developed using the *Agrobacterium* mediated transformation method where the expression of ADP-glucose pyrophosphorylase (AGPase) was inhibited by introducing a chimeric gene with the coding region of one of the subunits of the AGPase linked in an antisense orientation to the *CaMV 35S* promoter. Limited inhibition of the AGPase enzyme was achieved in leaves and almost complete inhibition in tubers. This resulted in the lowering of starch formation in tubers, which also proved that AGPase has a distinctive role in starch biosynthesis in plants. Biochemical analysis of these tubers revealed a reduction (up to 30%) in the dry weight of tubers and the accumulation of soluble sugars in tubers resulting in a significant increase of the total fresh weight tuber. However, the tuber induction enhanced with increase in the number of tuber per stolon. The molecular analysis of these antisense lines showed that there was no significant change in the RNA level of other starch biosynthetic enzymes, except an increase in the RNA level of the major sucrose synthesising enzyme known as the sucrose phosphate synthase. In addition, the inhibition of starch biosynthesis was complemented by a massive reduction in the expression of the major storage protein species of potato tubers, supporting the idea that the expression of storage protein genes is in some way connected to carbohydrate formation in sink storage tissues. There are some reports also exist where a mutant *Escherichia coli* AGPase gene (glgc16) were over-expressed in some plants for enhancing the starch accumulation, such as potato [17] and maize [42].

Transgenic potato lines were developed with collective expression of invertase and glucokinase which indicated an intense decrease in starch accumulation and a stimulation of glycolysis [43]. The aim was to surge starch increase in potato tubers by enriching their capacity to metabolise the sucrose. As a first step, the precise expression of a yeast invertase in the cytosol of transgenic tubers led to a 95% decrease in sucrose content that was complemented by a larger accumulation of glucose and a reduction in starch. In the next step, a double transgenic potato lines were developed where the bacterial glucokinase from *Zymomonas mobilis* was introduced by the transformation method into an invertase-expressing transgenic potato aiming to transport the glucose into the metabolism. The double transgenic lines obtained showed up to three folds glucokinase activity than in the parent invertase transgenic line and which did not accumulate glucose. Surprisingly, there was an additional intense reduction (up to 35%) in starch content was observed in the transgenic lines than the wild-type control plants. The biochemical analysis of growing tuber tissue revealed great expansions in the metabolic intermediates of glycolysis, organic acids and amino acids, 2–3 folds increases in the maximum catalytic activities of key enzymes in the respiratory pathways, and 3–4 folds increases in carbon dioxide production. These variations occur in the lines expressing invertase, and are highlighted following introduction of the second transgene, glucokinase. It was determined that the expression of invertase in potato tubers leads to an improved flux via the glycolytic pathway at the expense of starch synthesis and that heterologous over-expression of glucokinase augmented this change in partitioning.

The investigations were done to analyse the extent to which starch synthesis in potato tubers is controlled by the activity of AGPase [44]. The transgenic potato was developed with the down regulation of AGPase gene. In the biochemical assay, the fluxes of carbohydrate metabolism were measured in tubers. It was found that the reduction in AGPase activity led to a reduction in starch accumulation, and an increase in sucrose accumulation. The control coefficient of AGPase on starch accumulation in intact plants was estimated to be around 0.3. The fluxes of carbohydrate metabolism were measured in tuber discs from wild-type and transgenic plants by investigating the metabolism of [U-(14) C] glucose. In tuber discs, the control coefficient of AGPase over starch synthesis was estimated to be approximately 0.55, while the control coefficient of the enzyme over sucrose synthesis was −0.47. The values obtained suggested that AGPase activity shows substantial control over tuber metabolism in potato.

3.4. Genetic engineering of potato to modify the starch granule size

Starch granule size is an important feature that determines the suitability for many of the food and non-food uses. Starch granule size is highly species-specific and starch granules can appear in many different forms. The large sizes of starch granules in potato tubers are advantageous in application demanding the high viscosity but limit its suitability for noodle making. In order to alter the granule size using the genetic engineering approaches, the transgenic potato lines were developed by over-expression of the *cyclodextrin glycosyltransferase* gene isolated from *Bacillus circulans* bacterial strain which is a multiple tandem starch-binding domain (SBD). Biochemical analysis of transgenic potato tuber starch revealed large number of small starch granules without affecting the starch yield [45, 46].

Two starch phosphorylases in plants, plastidic phosphorylase A (Pho 1a) and cytosolic phosphorylase (Pho 2), catalyse reversible transfer of glucose from glucose-1-phosphate to a-glucan chain-releasing phosphorus (pi). A gene was cloned which translated protein counterpart was involved in starch metabolism identified by its ability to bind the potato starch granules [31]. The gene was introduced as RNAi construct in the potato using the *Agrobacterium* mediated transformation method. Biochemical analysis revealed that the reduction in the protein level of transgenic potato augmented with reduction in the phosphate content of the starch. The complementary result is obtained when the same gene was expressed in *E. coli*, as this leads to an increased phosphate content of the glycogen. It was assumed that this protein might be responsible for the incorporation of phosphate into starch-like glucans, a process that is not understood at the biochemical level. The reduced phosphate content in potato starch have some secondary effects on its degradability, as the respective plants show a starch excess phenotype in leaves and a reduction in cold-sweetening in tubers. In a mapping study in potato, Pho 1a emerged as a candidate gene linked to starch gelling and starch granule size [47]. In another study, it was found that the antisense inhibition of isoamylase in potato induces massive numbers of small granules in tubers, suggesting that the debranching activity is necessary to prevent excessive granule initiation [48]. On the other hand, it has been observed that mutation of starch synthase IV in *Arabidopsis* increases the starch granule size in leaf [28]. However, despite these observations, we really have a poor understanding of what controls granule size and shape.

4. Potato starch engineering for industrial application

The principal industrial productions of starches are based only on four main resources such as maize, cassava, wheat, and potatoes, which represent 76, 12, 7, and 4%, respectively. The other botanical resources represent less than 1%. The main production areas are North America, China, Europe, Southeast Asia, and South America with 33, 33, 18, 11, and 5%, respectively. Along with the better understanding of starch structure and enzymes involved in starch biosynthesis, many of the genes that encode these enzymes have been cloned and transformed into plants using *Agrobacterium tumefaciens* to modify the starch metabolism. Transgenic plants have been generated by down regulation (antisense or co-suppression approaches) or over-expression of endogenous gene or expression of heterologous genes, where starch properties and morphology have been altered. The possibility to produce tailor-made starches in planta has broadened the functionality of starches in industrial applications. The in planta modified starches, such as the amf starch, are often of better quality relatively to the chemically derivative which excludes the use of hazardous chemicals and leads to energy savings in the production process (of up to 60% for, e.g., synthetic polymer replacers). Potato starch shows a naturally high degree of phosphorylation compared to starches from other crops. It is universally acknowledged that starch with longer polymer chains tends to contain higher levels of phosphate because the longer chains provide a better substrate for the phosphorylating enzyme. The presence of phosphate in potato starch results in the stable-paste properties and transparent gels. Hence, potato starch is preferred for use in paste products and as an

ingredient in noodles. The transgenic potato developed by simultaneous inhibition of starch-branching enzymes (SBE A & B) [35] revealed that the phosphate content of high-amylose starches increased up to five fold as compared to the wild-type plant starch. A crucial enzyme glucan water dekinases (GWD) responsible for phosphorylating starch has been identified in potatoes, and regulating the expression of this gene could change the phosphate content and viscosity of potato starch [49].

5. Stress tolerant transgenic potato over-expressing modified carbohydrates as signalling factors

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stresses are serious threats to agriculture besides their deteriorative impact to the environment. Drought and salinity are the most important environmental stress factors that limit food production worldwide, and may cause a serious salinisation on more than 50% of all arable lands by the year 2050 [50]. In China, almost half of the land is arid or semi-arid and the crop production is strongly affected by drought and salinity seasonally even if in the irrigated farm land. Trehalose is a non-reducing disaccharide of glucose. A plant that produces trehalose is often highly tolerant to desiccation stress. Genetic engineering of potato has done for trehalose biosynthesis in potato by introducing the *otsA* and *otsB* genes from *E. coli*, which encode trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, respectively [51]. The plants also report to contain sucrose-non-fermenting-1- (SNF1) linked protein, analogous to that of the protein kinase (SNF-) yeast-signalling pathway. The role of SNF1related protein in sugar signalling has been analysed experimentally by developing the transgenic potato expressing an antisense SNF1-related protein kinase. Analysis of these plants revealed that the SNF1-linked protein plays a role in transduction of the sugar signal, triggering the initiation of sucrose synthase in potato leaves [52]. It has been well established that the plants share with few yeast elements involved in sugar sensing, however, several aspects of sugar receptors are likely to be unusual to higher plants. It has been reported that the abiotic stress factors may bring forth the synthesis of stress-related hormones particularly ABA and ethylene, which appear to be implicated in sugar-sensing mechanisms [53, 54]. The level of complexity in sugar signalling and protein involved depicted that despite the successful explanation of some sugar-sensing signalling mechanisms in recent years, added efforts are required to achieve a complete picture of sugar sensing in plants and thus, increase our knowledge of the mechanisms for plant abiotic stress tolerance and adaptation.

6. Perspectives of GMO starches

In an era towards a bio-based economy, the knowledge on how to improve complex carbohydrates such as starch is essential. A deeper understanding of the starch biosynthetic pathway, how storage starch granules are formed and how the composition, size, and shape can be changed and optimised for different bio-products, is of great importance for food and

non-food applications. Despite its great importance, the development and commercialisation of crops with altered starch properties using biotechnological approaches is being hampered by regulatory hurdles. The very high costs and the great deal of time needed, associated with the regulation of genetically modified crops (GMOs), are major problems. Although there is currently one GMO potato variety in the market, the *Amflora*, the commercialisation of this variety has been challenged by farmers and environmental organisations. The development of new methods in plant breeding that would circumvent these regulatory problems would be of greatly stimulated the development of novel starches [55]. The identification of genetic marker associated with starch properties and the exploitation of new mutations in tilling populations are other tools with great potential for uncovering key genes determining starch properties [47]. Another bottleneck to produce improved starches is associated with the difficulties in predicting beforehand the effect of a (trans) gene. The understanding of mechanism by which starch granules are made in the form of dense granules would be a great step forward in the synthesis of tailored starches for different bio-based applications.

Acknowledgements

Authors are gratefully acknowledging the contribution of Ms. Neha Joshi, Department of Biotechnology for critically evaluating and correcting the manuscript. The DCU acknowledge the Department of Science and Technology (DST-WOS-A/LS/426/2013) for the financial assistance to conduct the experiments on similar aspect in the laboratory of Plant Molecular Biology at Department of Biotechnology, Dr Harisingh Gour Central University, Sagar, India.

Author details

Chandrama Prakash Upadhyaya*, Deepak Singh Bagri and Devanshi Chandel Upadhyaya

*Address all correspondence to: cpupadhyay@gmail.com

Department of Biotechnology, Dr Harisingh Gour Central University, Sagar, Madhya Pradesh, India

References

[1] Nelson DL, Cox MM. Chapter 7: Carbohydrates and glycobiology. In: Nelson DL, Cox MM, editors. Lehninge principles of biochemistry. 4th ed. New York, USA: W.H. Freeman; 2004. p. 238-272

[2] Zeeman SC, Ap Rees T. Changes in carbohydrate metabolism and assimilate export in starch-excess mutants of Arabidopsis. Plant Cell and Environment. 1999;**22**:1445-1453. DOI: 10.1046/j.1365-3040.1999.00503.x

[3] Jobling SA, Westcott RJ, Tayal A, Jeffcoat R, Schwall GP. Production of a freeze-thaw-stable potato starch by antisense inhibition of three starch synthase genes. Nature Biotechnology. 2002;**20**:295-299. DOI: 10.1038/nbt0302-295

[4] Matheson NK. The chemical structure of amylose and amylopectin fractions of starch from tobacco leaves during development and diurnally nocturnally. Carbohydrate Research. 1996;**282**:247-262. DOI: 10.1016/0008-6215(95)00381-9

[5] Hoover R. Composition, molecular structure, and physicochemical properties of tuber and root starches: A review. Carbohydrate Polymer. 2001;**45**:253-267. DOI: 10.1016/S0144-8617(00)00260-5

[6] Blennow A, Bay-Smidt AM, Leonhardt P, Bandsholm O, Madsen HM. Starch paste stickiness is a relevant native starch selection criterion for wet-end paper manufacturing. Starch. 2003;**55**:381-389. DOI: 10.1002/star.200300169

[7] Wiesenborn DP, Orr PH, Casper HH, Tacke BK. Potato starch paste behavior as related to some physical/chemical properties. Journal of Food Science. 1994;**59**:644-648. DOI: 10.1111/j.1365-2621.1994.tb05583.x

[8] Sun T, Lærke HN, Jørgensen H, Bach Knudsen KE. The effect of heat processing of different starch sources on the in vitro and in vivo digestibility in growing pigs. Animal Feed Science and Technology. 2006;**131**:66-85. DOI: 10.1016/j.anifeedsci.2006.02.009

[9] Yusuph M, Tester RF, Ansell R, Snape CE. Composition and properties of starches extracted from tubers of different potato varieties grown under the same environmental conditions. Food Chemistry. 2003;**82**:283-289. DOI: 10.1016/S0308-8146(02)00549-6

[10] Noda T, Takigawa S, Matsuura-Endo C, Kim SJ, Hashimoto N, Yamauchi H, Hanashiro I, Takeda Y. Physicochemical properties and amylopectin structures of large, small, and extremely small potato starch granules. Carbohydrate Polymer. 2005;**60**:245-225. DOI: 10.1016/j.carbpol.2005.01.015

[11] Aberle T, Burchard W, Vorwerg W, Radosta S. Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. Starch/Stärke. 1994;**46**:329-335. DOI: 10.1002/star.19940460903

[12] Ratnayake WS, Jackson DS. A new insight into the gelatinization process of native starches. Carbohydrate Polymer. 2007;**67**:511-529. DOI: 10.1016/j.carbpol.2006.06.025

[13] McPherson AE, Jane J. Comparison of waxy potato with other root and tuber starches. Carbohydrate Polymer. 1999;**40**:57-70. DOI: 10.1016/S0144-8617(99)00039-9

[14] Zhu Q, Bertoft E. Composition and structural analysis of alpha-dextrins from potato amylopectin. Carbohydrate Research. 1996;**288**:155-174. DOI: 10.1016/S0008-6215(96)90793-4

[15] Zeeman SC, Kossmann J, Smith AM. Starch: Its metabolism, evolution, and biotechnological modi cation in plants. Annual Review of Plant Biology. 2010;**61**:209-234. DOI: 10.1146/annurev-arplant-042809-112301

[16] Hennen-Bierwagen TA, James MG, Myers AM. Involvement of debranching enzymes in starch biosynthesis. In: Tetlow I, editor. Starch, Origins, Structure and Metabolism. Essential Reviews in Experimental Biology. Vol. 5. London: The Society for Experimental Biology; 2012. p. 179-215

[17] Stark DM, Timmerman KP, Barr GF, Preiss J, Kishore GM. Regulation of the amount of starch in plant-tissues by adp glucose pyrophosphorylase. Science. 1992;**258**:287-292. DOI: 10.1126/science.258.5080.287

[18] Jeon JS, Ryoo N, Hahn TR, Walia H, Nakamura Y. Starch biosynthesis in cereal endosperm. Plant Physiology and Biochemistry. 2010;**48**:383-392. DOI: 10.1016/j.plaphy.2010.03.006

[19] Kossmann J, Abel GJW, Springer F, Lloyd JR, Willmitzer L. Cloning and functional analysis of a cDNA encoding a starch synthase from potato (*Solanum tuberosum* L.) that is predominantly expressed in leaf tissue. Planta. 1999;**208**:503-511. DOI: 10.1007/s004250050587

[20] Commuri PD, Keeling PL. Chain-length specificities of maize starch synthase I enzyme: Studies of glucan affinity and catalytic properties. The Plant Journal. 2001;**25**:475-486. DOI: 10.1046/j.1365-313x.2001.00955.x

[21] Delvallé D, Dumez S, Wattebled F, Roldán I, Planchot V, Berbezy P, Colonna P, Vyas D, Chatterjee M, Ball S. Soluble starch synthase I: A major determinant for the synthesis of amylopectin in *Arabidopsis thaliana* leaves. Plant J. 2005;**43**:398-412. DOI: 10.1111/j.1365-313X.2005.02462.x

[22] McMaugh SJ, Thistleton JL, Anschaw E, Luo J, Konik-Rose C, Wang H, Huang M, Larroque O, Regina A, Jobling SA. Suppression of starch synthase I expression affects the granule morphology and granule size and fine structure of starch in wheat endosperm. Journal of Experimental Botany. 2014;**65**(8):2189-2201. DOI: 10.1093/jxb/eru095

[23] Abel GJW, Springer F, Willmitzer L, Kossmann J. Cloning and functional analysis of a cDNA encoding a novel 139 kDa starch synthase from potato (*Solanum tuberosum* L.). The Plant Journal. 1996;**10**(6):981-991. DOI: 10.1046/j.1365-313X.1996.10060981.x

[24] Marshall J, Sidebottom C, Debet M, Martin C, Smith AM, Edwards A. Identification of the major starch synthase in the soluble fraction of potato tubers. The Plant Cell Online. 1996;**8**:1121-1135. DOI: 10.1105/tpc.8.7.1121

[25] Edwards A, Fulton DC, Hylton CM, Jobling SA, Gidley M, Rössner U, Martin C, Smith AM. A combined reduction in activity of starch synthases II and III of potato has novel effects on the starch of tubers. Plant Journal. 1999;**17**:251-261. DOI: 10.1046/j.1365-313X.1999.00371.x

[26] Flipse E, Suurs LCJM, Keetels CJAM, Kossmann J, Jacobsen E, Visser RGF. Introduction of sense and antisense cDNA for branching enzyme in the amylose-free potato mutant leads to physico-chemical changes in the starch. Planta. 1996;**198**:340-347. DOI: 10.1007/BF00620049

[27] Zhang X, Szydlowski N, Delvallé D, D'Hulst C, James MG, Myers AM. Overlapping functions of the starch synthases SSII and SSIII in amylopectin biosynthesis in Arabidopsis. BMC Plant Biology. 2008;**8**:96. DOI: 10.1186/1471-2229-8-96

[28] Roldan I, Wattebled F, Lucas MM, Delvalle D, Planchot V, Jimenez S. The phenotype of soluble starch synthase IV defective mutants of *Arabidopsis thaliana* suggests a novel function of elongation enzymes in the control of starch granule formation. The Plant Journal. 2007;**49**:492-504. DOI: 10.1111/j.1365-313X.2006.02968.x

[29] Dian W, Jiang H, Wu P. Evolution and expression analysis of starch synthase III and IV in rice. Journal of Experimental Botany. 2005;**56**:623-632. DOI: 10.1093/jxb/eri065

[30] Nazarian-Firouzabadi F, Visser RGF. Potato starch synthases: Functions and relationships. Biochemistry and Biophysics Reports. 2017;**10**:7-16. DOI: 10.1016/j.bbrep.2017.02.004

[31] Lorberth R, Ritte G, Willmitzer L, Kossmann J. Inhibition of a starch-granule-bound protein leads to modified starch and repression of cold sweetening. Nature Biotechnology. 1998;**16**:473-477. DOI: 10.1038/nbt0598-473

[32] Yuan RC, Thompson DB, Boyer CD. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three wx-containing genotypes in two inbred lines. Cereal Chemistry. 1993;**70**:81-89

[33] Srichuwong S, Sunarti TC, Mishima T, Isono N, Hisamatsu M. Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. Carbohydrate Polymers. 2005;**60**:529-538. DOI: 10.1016/j.carbpol.2005.03.004

[34] Safford R, Jobling SA, Sidebottom CM, Westcott RJ, Cooke D, Tober KJ. Consequences of antisense RNA inhibition of starch branching enzyme activity on properties of potato starch. Carbohydrate Polymers. 1998;**35**:155-168. DOI: 10.1016/S0144-8617(97)00249-X

[35] Schwall GP, Safford R, Westcott RJ, Jeffcoat R, Tayal A, Shi YC. Production of very-high-amylose potato starch by inhibition of SBE A and B. Nature Biotechnology. 2000; **18**(5):551-554. DOI: 10.1038/75427

[36] Brummell DA, Watson LM, Zhou J, McKenzie MJ, Hallett IC, Simmons L, Carpenter M, Timmerman-Vaughan GM. Overexpression of starch branching enzyme II increases short-chain branching of amylopectin and alters the physicochemical properties of starch from potato tuber. BMC Biotechnology. 2015;**29**:15-28. DOI: 10.1186/s12896-015-0143-y

[37] Preiss J, Sivak MN. Biochemistry, molecular biology and regulation of starch synthesis. Genetic Engineering. 1998;**20**:177-223. DOI: 10.1007/978-1-4899-1739-3_10

[38] Emes MJ, Neuhaus HE. Metabolism and transport in non-photosynthetic plastids. Journal of Experimental Botany. 1997;**48**:1995-2005. DOI: 10.1093/jxb/48.12.1995

[39] Neuhaus HE, Emes MJ. Nonphotosynthetic metabolism in plastids. Annual Review of Plant Physiology and Plant Molecular Biology. 2000;**51**:111-140. DOI: 10.1146/annurev.arplant.51.1.111

[40] Müller-Röber B, Sonnewald U, Willmitzer L. Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. EMBO Journal. 1992;**11**:1229-1238

[41] Lloyd JR, Springer F, Buleon A, Muller-Rober B, Willmitzer L, Kossmann J. The influence of alterations in ADP-glucose pyrophosphorylase activities on starch structure and composition in potato tubers. Planta. 1999;**209**:230-238. DOI: 10.1007/s004250050627

[42] Wang ZY, Chen XP, Wang JH, Liu TS, Liu Y, Zhao L. Increasing maize seed weight by enhancing the cytoplasmic ADP-glucose pyrophosphorylase activity in transgenic maize plants. Plant Cell, Tissue and Organ Culture. 2007;**88**:83-92. DOI: 10.1007/s11240-006-9173-4

[43] Trethewey RN, Geigenberger P, Riedel K. Combined expression of glucokinase and invertase in potato tubers leads to a dramatic reduction in starch accumulation and a stimulation of glycolysis. Plant Journal. 1998;**15**:109-118. DOI: 10.1046/j.1365-313X.1998.00190.x

[44] Sweetlove M-R, Willmitzer H. The contribution of adenosine 5′-diphosphoglucose pyrophosphorylase to the control of starch synthesis in potato tubers. Planta. 1999;**209**:330-337. DOI: 10.1007/s004250050640

[45] Ji Q, Oomen RJF, Vincken JP, Bolam DN, Gilbert HJ, Suurs L. Reduction of starch granule size by expression of an engineered tandem starch-binding domain in potato plants. Plant Biotechnology Journal. 2004;**2**:251-260. DOI: 10.1111/j.1467-7652.2004.00069.x

[46] Nazarian-Firouzabadi F, Trindade LM, Visser RGF. Production of small starch granules by expression of a tandem-repeat of a family 20 starch-binding domain (SBD3-SBD5) in an amylose-free potato genetic background. Functional Plant Biology. 2012;**39**:146-155. DOI: 10.1071/FP11150

[47] Werij JS, Furrer H, van Eck HJ, Visser RGF, Bachem CWB. A limited set of starch related genes explain several interrelated traits in potato. Euphytica 2012;**186**:501-516. DOI: 10.1007/s10681-012-0651-y

[48] Bustos R, Fahy B, Hylton CM, Seale R, Nebane NM, Edwards A. Starch granule initiation is controlled by a heteromultimericisoamylase in potato tubers. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:2215-2220. DOI: 10.1073/pnas.0305920101

[49] Donath H, Avtomonov EV, Sarraje I, Von Dahlen KH, El-Essawi M, Lorberth J. Organoplatinum compounds VII—Trimethylplatinum fluoride [(CH3)(3)PtF](4), the missing link in organoplatinum cluster chemistry: Its synthesis, crystal structure and a comparison to the crystal structure of [(CH3)(3)PtOH](4). Journal of Organometallic Chemistry. 1998;**559**:191-196. DOI: 10.1016/S0022-328X(98)00481-1

[50] Wang H, Miyazaki S, Kawai K, Deyholos M, Galbraith DW, Bohnert HJ. Temporal progression of gene expression responses to salt shock in maize roots. Plant Molecular Biology. 2003;**52**:873-891. DOI: 10.1023/A:1025093528174

[51] Goddijn OJ, Verwoerd TC, Voogd E, Krutwagen RW, de Graaf PT, van Dun K, Poels J, Ponstein AS, Damm B, Pen J. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiology. 1997;**113**:181-190. DOI: 10.1104/pp.113.1.181

[52] Purcell LC, DeSiva M, King CA, Kim WH. Biomass accumulation and allocation in soybean associated with genotypic differences in tolerance of nitrogen fixation to water deficits. Plant Soil. 1997;**196**:101-113. DOI: 10.1023/A:1004289609466

[53] Zhou L, Jang JC, Jones TL, Sheen J. Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. Proceedings of the National Academy of Sciences of the United States. 1998;**95**:10294-10299. DOI: 10.1073/pnas.95.17.10294

[54] León P, Sheen J. Sugar and hormone connections. Trends in Plant Sciences. 2003;**8**:110-116. DOI: 10.1016/S1360-1385(03)00011-6

[55] Lusser M, Parisi C, Plan D, Rodriguez-Cerezo E. Deployment of new biotechnologies in plant breeding. Nature Biotechnology. 2012;**30**:231-239. DOI: 10.1038/nbt.2142

Chapter 3

Breeding Potato for Quality Improvement

Meenakshi Kumari, Manoj Kumar and
Shashank Shekhar Solankey

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71482

Abstract

Potato is the most important non-cereal food crop in the world, that in general represent a non-fattening, nutritious and wholesome food, which supply important nutrients to the human diet. The potato tubers contain considerable amounts of carbohydrates, vitamin C, essential amino acids and minerals. The potato quality includes biological traits (e.g. proteins, carbohydrates and minerals); sensorial traits (e.g. flavor, texture); and industrial traits (e.g. tuber shape, cold sweetening and starch quality). These traits are deemed very important for fresh consumption, where they are most likely to influence consumer's choice worldwide. Since most quality traits are genetically controlled, breeding work can successfully meet the quality of potato tubers and fulfills the needs of a changing and demanding world. Breeding potato for quality traits requires a continuous flow of new genes and allelic diversity into the *Solanum tuberosum* gene pool. However, recent advances in conventional and non-conventional breeding methods have significantly improved the possibilities of producing novel genetic variability for selection of new genotypes, especially when biotechnologists and plant breeders pool the existing resources. The genetics, biochemical and physiology of several quality traits is to be given equal importance that ultimately makes breeding efforts less empirical and more predictable.

Keywords: *Solanum tuberosum*, quality traits, genotypes, gene, inheritance, conventional breeding, non-conventional breeding

1. Introduction

Potato (*Solanum tuberosum*) is one of the most important tuber crops, is used worldwide for human and animal consumption, and as raw material for starch and alcohol production. It is also one of the world's major staple crops, which produces more dry matter and protein per hectare than the major cereal crops [1]. Potato is mainly grown in Uttar Pradesh, West

Bengal, Punjab, Bihar, Haryana, Madhya Pradesh, Gujarat and Maharashtra. Total potato production is about 320 million tonnes (Mt) globally, of which about 66% is used as food, 12% as feed and 10% as seed [2]. At the highlands of Ethiopia, the potato holds great promise for improving the livelihoods of millions of smallholder farmers. The potential for high yield, early maturity and excellent food value give the potato great potential for improving food security, increasing household income and reducing poverty [3]. Potato is grown in India in almost all the states except Kerala. It is possible to see the crop in field round the year in one part of the country or the other. About 82% of the area under potato crop lies in the plains where the crop is grown during short-days of winters from October to March. About 10% lies in the hills where the crop is grown during long-days of summer from April to September in tropical and sub-tropical parts of the world.

Cultivated potato and its wild relatives belong to the genus *Solanum*, the largest genus with 1500–2000 species [4]. Within the genus *Solanum*, over a 1000 of species have been recognized [5]. The genus *Solanum* comprises 8 cultivated species and 2000 wild relatives out of which 235 *Solanum* species tuberize.

The tuber-bearing *Solanum* species are grouped in the *Petota* section. This section is divided into two sub-sections, *Potatoe* and *Estolonifera* [6]. The sub-section *Potatoe* contains all tuber-bearing potato species, including common potato (*S. tuberosum*, belonging to series *Tuberosa*). Two non-tuber bearing series (*Etuberosa* and *Juglandifolia*) are placed in sub-section *Estolonifera*. However, a number of molecular studies suggest that the series *Etuberosa* and *Juglandifolia* do not belongs to the *Petota* section [7, 8].

The cultivated potato (*S. tuberosum*) is divided into two sub-species: *tuberosum* and *andigena*. The sub-species *tuberosum* (**Table 1**) is the cultivated potato originated from Peru and widely in use as a crop plant in, for example, Asia, North America and Europe. The sub-species *andigena* is also a cultivated species, originated from Andean mountain (South America) and cultivation is restricted to Central and South America [6, 9]. The subsp. *andigena* forms tubers under short day conditions at high altitudes (>2000 msl.) while in the subsp. *tubersoum*, tuber formation is under long days in temperature climate and short days in the tropics at lower altitudes (500–2000). The cultivated potato (*S. tuberosum* subsp. *tuberosum*) is crossable with other cultivated species

Taxonomic rank	**Latin name**
Family	Solanaceae
Genus	*Solanum*
Section	*Petota*
Subsection	*Potatoe*
Series	*Tuberosa*
Species	*Solanum tuberosum*
Sub-species	*tuberosum*

Table 1. Taxonomic position of *S. tuberosum* subsp. *tuberosum*.

(*S. tuberosum* subsp. *andigena*, *Solanum phureja* and *Solanum stenotomum*) and wild diploid species (*Solanum sparsipilum*, *Solanum berthaultii*, *Solanum spegazzinii* and others). For transgression of genes for improvement of quality traits as well as resistance to pathogens and insect pests in potato cultivars, wild species have been found useful. Potato tubers develop from the stolons formed from the lower basal nodes below the soil surface. The potato tuber is a modified stem with a few 'eyes' that are leaf scars with a subtended lateral bud.

Potato production is important due to many reasons but one of the most important aspects of potato production is tuber quality, that includes biological (e.g. proteins, carbohydrates and minerals) and sensorial traits (e.g. flavor, texture) and industrial traits (e.g. tuber shape, cold sweetening and starch quality). The potato is rich source of carbohydrate and has considerable amounts of protein, with a good quality of amino acid balance, vitamins C, vitamin B (B_1 & B_6), folate, minerals (potassium, phosphorus, calcium and magnesium) and in micronutrients (iron and zinc). Dietary fiber found high in the tubers, and potato skin contained higher amount of dietary fibers than flesh. Potato tubers are also rich in antioxidants comprising polyphenols, vitamin C, carotenoids and tocopherols. Freshly harvested potatoes are virtually free of fat and cholesterol as compared to stored potatoes [1, 10]. The nutrient-rich potato can contribute to improved diets, thus reducing mortality rates caused by malnutrition. It can improve food security and health, especially among women and children. To improve the livelihoods and food security of poor farmers, it is very essential to increases in potato yields.

2. Origin of potato

Potato is an auto-tetraploid species (2n = 4× = 48) which was first introduced into Europe, and spreads as a botanical novelty and as fodder crop for livestock. Initially people treated the potato as suspicion relative of the toxic nightshade (*S. nigrum*).Eventually potato was adopted as a human food source and gradually gained popularity and then it introduced to the rest of the world, from the Andes of South America in the late sixteenth century. By the end of the eighteenth century, it was found that it is well adopted under long-day photoperiod then further selection of early tuberization cultivars and high-yielding clones were done from the derived seedlings from naturally occurring berries, the consequence of uncontrolled, largely self-pollination. Potato is an Andean tuber crop that was originally domesticated in South America and started its worldwide dissemination after Columbus voyages brought to Europe in the late sixteenth century some years after the discovery and conquest of Peru. It is believed that cultivated potato originated from its wild ancestors near the lake *Tritica* basin in Peru Bolivian region in high mountains. This plant was selected as article of food by the oldest civilizations of Mayas and Incas. There are strong evidences that potato was widely distributed throughout the Andes, from Colombia to Peru and also in southern Chile. Potatoes are said to have been taken to India and to China by British missionaries in the late seventeenth century and were known in Japan and parts of Africa by about the same period.

Potato was originated from the wild species *Solanum leptophyes* some 10,000–7000 years ago, and the first domesticated species was *Solanum stenotomum.* The evolution of *Solanum stenotomum* was only the beginning of potato evolution. In addition to first wild species *S. leptophyes*

which gave rise to domesticated diploid species, *Solanum stenotomum*, three otherswild species, namely *S. sparsipilum, Solanum acaule* and *Solanum megistacrolobum* were instrumental in evolution of present day cultivated potatoes. Some authors believed that *S. tuberosum* is a straight tetraploid of *S. stenotomum*but there are stronger evidences in support of the allotetraploid origin of *S. tuberosum* by hybridization between *S. stenotomum* and *S. sparsipilum* [11].

3. Nutritional content of potato

Potato tubers contain about 75% water, 21% carbohydrates (of which about 82% is starch), 2.5% protein and less than 1% fat. Often looked upon as primarily a starchy vegetable, potatoes are actually highly nutritious. It is a good source of vitamins C and B_6. About 48% daily values (DV) for vitamin C and 46% for vitamin B_6 are provided by a large (299 g) baked potato. They also have fair amount of fibers (26% DV), proteins and minerals. The DVs are varied with different types of cultivars, various authors have been reported DVs of 46% for potassium, 33% for manganese, 21% for magnesium and 21% for phosphorus in large baked potato. Potatoes are rich source of essential amino acids which required for proper growth and development, hence it is considered as balanced and complete diet for adults. The flesh color varies according to genotypes and climatic conditions of cultivated area. The most common flesh color is white but other than this variety of flesh color cultivars evident in different countries. The carotenoids (yellow and orange colors) and anthocyanins (red and purple colors) are the two most valuable coloring pigments in potato. White-fleshed potatoes are low in carotenoids (<100 μg/100 g fresh weight) whereas, the carotenoid content of yellow-fleshed varieties are higher (about 560 μg/100 g FW) [12]. Intense yellow to near orange flesh color, associated with carotenoid concentrations >2000 μg/100 g FW, have been reported in diploid *Solanum* germplasm [13]. The primary tuber carotenoids in potato tubers are lutein, zeaxanthin and violanxanthin, although some studies have also reported finding neoxanthin and antheraxanthin [13]. In the macula of the human eye, lutein and zeaxanthin are found and it also plays a role in reducing the risk of age-related macular degeneration. In addition, increased intake of zeaxanthin may improve mental acuity in the elderly. The anthocyanin concentration in tubers is 100-fold greater than carotenoids. The red fleshed of tubers is mainly due to the anthocyanin pigment pelogonidin-3-(p-coumaroyltutinoside)-5-glucoside (200–2000 μg/ g FW) and peonidin-3-(pcoumaroyl-rutinoside)-5-glucoside (20–400 μg/g FW) while, petunidin3-(p-coumaroyl-rutinoside)-5-glucoside (1000–2000 μg/g FW) and malvidin-3-(p-coumaroyl-rutinoside)-5-glucoside (2000–5000 μg/g FW) compounds of anthocyanin pigments are responsible for dark purple-fleshed potatoes [14]. There is increasing interest in anthocyanins in potato tubers because of their perceived higher antioxidant content, and ability to combat both prostate cancer and breast cancers. The potatoes with high glycemic index play important role in the diet of persons suffering from diabetes. During digestion, quick breakdown of food with high glycemic index leads to rapid rise of blood sugar level. The two major components of potato starch are amylose (20–25%) and amylopectin (75–80%). While cooking recrystallization of some portion of amylose leads to formation of resistance starch. Resistant starch acts as a dietary fiber. It passes through the small intestines and once in the large intestines microbial fermentation results in the production of small chain fatty acids,

which enhance colon health and lower the risk of colorectal cancer and diverticulosis. The diploid potato species adapted to long-day growing conditions, the concentration of amylose in starch ranged from 25 to 36%. Greater variations for amylose content were present in wild potato species, which ranged from 22 to 43%. There is considerable interest in the potential of potato to lessen micronutrient malnutrition, particularly for iron and zinc. About 40% of the world's population is iron deficient, however, no reliable estimate is available for the number of people with zinc deficiency. The iron content of potato ranges from 15 to 20 μg/g DW. However, iron concentrations ranging from 9 to 158 μg/g DW in Andean potato cultivars and 18–65 μg/g DW in recent American potato cultivars.

4. Genetics and chromosomal variations of potato

Breeding of potato is a cumbersome task due to inherent genetic and biological factors. To understand the genetics, we will first outline some important genetic and reproductive aspects of the potato. Potato has basic chromosome number as 12 and right from diploid to hexaploid species. Majority (about 73%) of the species are diploid followed by tetraploids (about 15%), hexaploids (about 6%), triploids (about 4%) and pentaploids (about 2%). The tuber-bearing *Solanum* species include several diploids (2n = 24), triploids (2n = 36) and a few tetraploids (2n = 48), and hexaploids (2n = 72). Many wild species of the series *Tuberosa* are diploid (*S. berthaultii, Solanum canasense, S. sparsipilum, S. vernei,* etc.) and the cultivated diploid (2n = 2× = 24) species, *S. stenotomum*, *S. phureja and S. ajanhuri*, of which former two are sexually fertile and the later one is less fertile and does not breed true. Triploid potato species are *S. chaucha* and *S. xjuzepczukii* derived from spontaneous crosses between diploid and tetraploid species. There are 3 cultivated diploid species, *S. stenotonomum, S. phureja* and *S.ajanhuri*. The cultivated potato *S. tuberosum and S. demissum* are autotetraploid species with chromosome number, 2n = 4× = 48 are usually fertile except in a number of highly bred clones outside South America. There is only one pentaploid species (2n = 60), that is, *S. curtilobum* which is reasonably fertile in crosses with *S. tuberosum*, but not in selfings. Nearly all the diploid species are self-incompatible while all the tetraploids and hexaploids are self-compatible [11].

4.1. Genetic diversity for quality traits

Genetic diversity is a prerequisite for an effective plant breeding program. It is a useful and essential tool for parent's choice in hybridization to develop high yield potential cultivars and to meet the diversified goals of plant breeding [15]. The cultivated potato have narrow genetic base due to limited introduction of germplasm from their natural range in South America [16]. Most of the potato cultivars are auto-tetraploid (2n = 4× = 48), highly heterozygous and out breeding species, which suffer from inbreeding depression. Cultivated potato species have a base chromosome number of n = 12 and may be diploid (2n = 2× = 24), triploid (2n = 3× = 36), tetraploid (2n = 4× = 48) or pentaploid (2n = 5× = 60). There are 7 cultivated potato species [6], whereas only 9 species and 141 intra-specific taxa have been identified [17]. Recent studies suggested that, genotyping of 742 landraces of all cultivated and wild species have been completed with SSR and chloroplast markers [18]. Based on these studies,

Species	Quality traits
S. medians, S. okadae, S. pinnatisectum, S. raphanifolium, S. sogarandinum	Chip directly from cold storage
S. siparunoides, S. sisymbrifolium, S. stramonifolium, S. tuberosum	Used in medicine
S. phureja, S. stenotomum	High carotenoid content
S. phureja, S. vernei	High starch content
S. phureja, S. estoloniferum	High ascorbic acid content
S. phureja	High protein content, low temperature non-sweetening
S. vernei	High starch and protein content, low reducing sugar content

Table 2. Species for important quality traits.

they reclassified the all cultivated potatoes in following four species: (i) *S. tuberosum*, with two cultivar groups (the *Andigenum* group – diploids, triploids and tetraploids and the *Chilotanum* group – tetraploid); (ii) *S. ajanhuiri* (diploid); (iii) *S. juzepczukii* (triploid) and (iv) *S. curtilobum* (pentaploid) [18].

1. *Solanum ajanhuiri* (2n = 2× = 24): this diploid species was formed by natural hybridization between diploid cultivars of *S. tuberosum* L. *andigenum* group and the tetraploid wild species *S. bolivense* (*S. megistacrolobum*). This landrace possesses frost resistance and is distributed in the high Andean Altiplano between southern Peru and central to North Bolivia, at elevations between 3700 and 4100 m [19, 20].

2. *S. juzepczukii* (triploid) (2n = 3× = 36): formed by hybridization between a diploid cultivar of *S. tuberosum* L. *andigenum* group, and the tetraploid wild species *S. acaule* Bitter [21]. It can be found from central Peru to southern Bolivia and can grow at an altitude of 4000 m (Spooner et al. [19]). This species contains high levels of glycoalkaloids, and local people prepare detoxified processed potato "chuño" by freeze drying [22].

3. *S. curtilobum* (2n = 5× = 60): formed by hybridization between tetraploid forms of *S. tuberosum* L. *andigenum* group (synonym for *S. tuberosum* subsp. *andigenum*) and *S. juzepczukii* [21] is cultivated in the Andean Altiplano at an altitude range of approximately 4000 m [19]. Because the tubers are bitter, owing to high glycoalkaloid content, the species is also used to prepare "chuño" [22].

4. *S. tuberosum:* the most popular cultivated potato is *S. tuberosum*, which is also known as "common potato" in most parts of the world. It has originated from the coastal regions of South Central Chile.

5. *S. chaucha*: *S. chaucha* is a cultivated triploid species that supposedly originated from natural hybridization between *S. tuberosum* subsp. *andigena* and *S. stenotomum* [6] distributed from 2100 to 4100 msl throughout Peru, with lower frequency in Bolivia, and rarely found in Ecuador and Colombia.

6. *S. phureja*: this species was cultivated from central Peru to Ecuador, Colombia, and Venezuela since the pre-Spanish era and is believed to have originated from *S. stenotomum* [6].
7. *S. stenotomum*: the species is diploid and cultivated from Central Peru to Central Bolivia. Most primitive form of cultivated potato. *S. stenotomum* shows the diversity within species, suggesting it to be the first domesticated potato derived from diploid wild species (**Table 2**).

5. Quality traits of potato

In the field of nutrition, there are several topics such as food and nutritional security, dietary adequacy etc. which have been discussed most vigorously at global level. These all are well defined by the quality of the produce. Potato is the fourth most important food crop of the world. Quality of a potato tuber can be defined as the sum of favorable characteristics of the tuber, which is a subjective and dynamic concept that depends on consumer's tradition lifestyles, food habits and the industrial process used.

Potato considered as an important source of nutrition for human diet. Potato tubers are good source of vitamin C and also having significant amount of 12 various essential amino acids along with minerals. In general, different cultivars have different uses. Red-skinned cultivars with low dry matter content, for example, are used for fresh market, whereas long types are suitable for French fries.

There are two broad category groups of tuber quality:

- **External quality:** this is the first category group which comprising the aspects such as skin color, tuber size and shape, eye depth [23]. Besides, dormancy and greening are additional important quality traits [24]. These traits are deemed very important for fresh consumption where external traits are most likely to influence consumer's choice.
- **Internal quality:** this represents the aspects such as nutritional properties, culinary value, after-cooking properties or processing quality. The traits such as dry matter content, flavor, reducing sugar, protein content, starch quality, type and amount of glycoalkaloids, enzymatic discoloration and other nutritional quality, etc. comprising the internal quality of tuber [1].

5.1. Inheritance of quality traits

5.1.1. Fresh market

The traits which are crucial aspects for consumers are tuber shape, eye depth, skin and flesh color, as these are immediately obvious while making the purchase [24]. Tuber shape is a syndrome of many characters that considers the length/width ratio for describing the overall shape, it varies from compressed/round to long [24]. The round shape of tuber mainly governed by a single locus on chromosome X with a dominant allele *Ro* reported that and other reports mention quantitative

trait loci (QTLs) on chromosome II, V and XI, II and XI and VII and XII. Other than this, there are several factors which controlling this trait, most probably it depending on the genetic background of the respective populations used on their researches [24].

Eye depth is an important trait of tuber quality because the appearance of tubers is affected by deep eye which leads to increased cost of peeling in processing factories. As for tuber shape, contradictory hypotheses were formulated to explain the inheritance of eye depth. A single locus *Eyd*/*eyd* controls the appearance of eye depth, this locus is closely linked with *Ro* locus at chromosome X at a distance of 4 centi Morgan (cM) [25], whereas, at least four QTLs on chromosomes III, V and X were identified.

Skin color is one of the most easily notable traits of potato tuber. Potato having lots of variability in skin color as it ranges from white-cream to blackish. The different genetic systems that control the presence and absence of red and blue pigments are responsible for tuber skin color. The potato *R* locus is required for the production of red anthocyanins, which have been shown to be derivatives of pelargonidin, while P is required for the synthesis of purple pigments, which are typically derived from petunidin [14]. Whereas, the synthesis of red or purple anthocyanins in tuber skin is mainly due to I locus. These three loci have been mapped in the potato genome [26, 27].

Several studies [28, 29] reported that three genes *R* (*dfr*), *P* (*f3´5´h*) and *D* (*f3h*) were expressed in the periderm of red and purple skinned clones, while *dfr* and *f3´5´h* were not expressed, and *f3h* was only weakly expressed, in white skinned clones. Moreover, R gene linked to dihydroflavonol 4-reductase (*dfr*), *P* responsible for flavonoid 3′,5′-hydroxylase (*f3′5′h*) and *D* corresponds with *R2R3MYB* transcription factor which is similar to Petunia *an2* [28].The response is similar to expression of flavanone 3-hydroxylase (*f3h*), dihydroflavonol 4-reductase (*dfr*) and flavonoid 3′,5′-hydroxylase (*f3′5′h*) [29].

In potato tuber flesh color also lots of diversity occur, it varies from white to purple. The variation in tuber flesh is mainly due to two naturally occurring pigments, that is, anthocyanin and carotenoids (Lewis et al., [14]). Red, blue and purple color flesh is due to accumulation of anthocyanin pigment [30]. Red and purple fleshed potatoes have acylated glucosides of pelargonidin while purple potatoes have, in addition, acylated glucosides of malvidin, petunidin, peonidin and delphinidin [31]. Natural anthocyanin pigments are the economic constituent of colored potato, since it is a low-cost crop [32], and also are a significant source of potato antioxidant micronutrients [33]. Hence, colored potatoes can serve as novel sources of natural colorants and antioxidants with added value for the food industry and human health. Antioxidant values also depend on color of flesh of tuber. Red fleshed potato genotypes have the high antioxidant value (300%) as compare to white fleshed, while purple fleshed tubers have antioxidant value of 250% than white fleshed [34].

The single dominant allele at the Y locus on potato chromosome III controls the white, yellow or orange flesh of tuber which determines by the carotenoid level. A combination of the dominant β-carotene hydroxilase 2 (*Chy2*) allele and homozygous recessive *Zep* allele controls the yellow flesh (accumulation of high levels of zeaxanthin) of potato tubers [35].

Greening and tuber dormancy also another two important quality parameters. Greening of tubers mainly due to transformation of amyloplasts in the outer layers of the tuber to chloroplasts [36]. Green tubers having high concentration of glycoalkaloids, which is an antinutritional factor (poisonous) for human and animals. Several studies reported that there is no direct metabolic link between chlorophyll biosynthesis and total glycoalkaloids content. There is no single reason which is responsible for greening. Several factors such as light, maturity of tubers, time and temperature of storage, production treatments or tuber size are responsible for greening of potato tubers. Although tuber greening is affected by environmental factors, variability among varieties has been reported by several workers and this character has polygenic inheritance [37].

Tuber dormancy defines as the physiological state of potato tuber after harvest, during which tuber do not sprout under favorable condition for sprouting. Two molecular mapping studies detected a number of QTLs for tuber dormancy and demonstrated the complex character of this trait. There are several categories of genes which involved in breaking dormancy and sprouting action [24]. Among these, the first group represents the genes coding for homeotic proteins and transcription factors [38]. The second class of genes regulates hormone metabolism and hormone response. The dormancy of potato tubers is induced by abscisic acid and ethylene. The abscisic acid maintains dormancy and whereas the cytokinins are involved in loss of dormancy. The third group of genes is involved in metabolism of reserve storage molecules [39]. Recently, it was reported that, in DNA replication fourth gene category were involved [40].

Glycoalkaloid content is another important quality attribute that affects the taste of tubers. These are naturally occurring secondary metabolites which may be toxic against bacteria, fungi, viruses, insects, animals and humans beyond the optimum level and produce in all parts of potato plant. The predominant glycoalkaloids in potato are α-solanine and α-chaconine [41], both trisaccharides of the common aglyconesolanidine. These two compounds comprise about 95% of the glycoalkaloids in potato tubers [42]. The chemical structure of α-solanine consists of branched β-solatriose (α-L-rhamnopyranosyl-β-D-glucopyranosyl-β-galactopyranose) along with the side also attached to the 3-OH group of the same aglycon, while a branched β-chacotriose (bis-α-Lrhamnopyranosyl-β-D-glucopyranose) carbohydrate side chain attached to the 3-OH group of the aglyconsolanidine in case of α-chaconine [43]. Various factors influence the quantity of glycoalkaloid, that is, it increases during storage and transportation and under the influence of light, heat, cutting, slicing, sprouting and exposure to phyto-pathogens. The quantity of these chemicals decides the quality of tubers, that is, when this compound is present in low concentration, it may attribute to flavor of processed potato, but when its level crosses 15 mg/ 100 g fresh weight it cause bitterness of tubers. The maximum amount of glycoalkaloids in the potato tubers occur within the first 1 mm from the outside surface and decrease toward the center of the tuber [43]. The consumption of large amounts of glycoalkaloids by humans could produce toxication symptoms ranging in severity from nausea to, in extreme cases, death [42].

The quality of potato tubers also depends on sugar content of the harvested crop. For the fresh market, sucrose levels above 1% fresh weight (FW) are reported to give an unacceptably sweet taste to the boiled potatoes [1].

5.1.2. Potato for processing

The basic criteria of selection for processing potatoes are tuber shape, eye depth, dry matter and starch content. Selection of varieties is done based on the product to be prepared. For example, French fries need varieties with long tuber whereas for chips varieties with round tubers are preferred [44]. Shallow eyed potato tubers are ideal for processing because these help to minimize waste during peeling. Moreover, dry matter content (DMC) is also a critical component for the processing industry, it represents the internal quality of the produce. Starch is the principal compound of tuber which is having polygenic inheritance and the effects are located on all chromosomes [45]. For French fries preparation, the DMC should not be below 19.5% while for chips it should not be less than 20% [44]. The amount of DMC more than 25% is also not acceptable for French fries preparation. The susceptibility of produce to bruising depends on DMC and its distribution during harvest has an impact on cooking type, for example, a waxy or mealy texture when boiled, organoleptic characteristics and in processed potatoes the final product texture [46]. Several environmental factors such as solar radiation, soil temperatures, soil moisture, fertilizers and haulm killing affect the dry matter content during the growth and development of crop [47]. In general, DMC have negative response to cold climate and short growing period, while positive response, that is, increased in DMC is occurred in warm climate with long growing seasons and an adequate water supply. The accumulation of more DMC is occurred during vegetative phase of the crop. When root system remains active in wet soil there will be sharp decline in tuber DMC after defoliation of the haulm [1]. The quality of potato tubers is also affected by the level of sugars, because at high temperature Maillard reaction occur which leads to interaction between reducing sugars (glucose and fructose) and free amino acid which ultimately affect the color, flavor and it has also been related to acrylamide formation in fried potato products [48]. Amount of sugar in potato tubers depends upon several factors which include genotype, environmental conditions, agronomical practices and several post-harvest factors including storage [48]. Potato has both monosaccharides and disaccharide sugars. Glucose (0.15–1.5%) and fructose (0.15–1.5%) are monosaccharide reducing sugars, while sucrose (0.4–6.6%), is a non-reducing disaccharide [1]. In processing of chips and French fries, the acceptable levels of glucose and fructose is 0.2–0.3% and 0.3–0.5%, respectively. The molecular studies suggested that these sugars are occurred between one and three putative QTL regions per chromosome [49]. There are 24 QTLs which are related to sugar accumulation in potato tubers and were present on all potato chromosome. Among these QTLs, most of the QTL were mapped in the same positions for both glucose and fructose content. Besides, several candidate genes involved in carbohydrate metabolism have been mapped [45].

Enzymatic discoloration (ED) is another important trait which is responsible for loss of quality. Peeling potato tubers and exposing them to air cause the flesh color to change from red to brown. Deterioration of quality is due to the oxidation of phenolics compound to quinones by the enzyme tyrosinase (polyphenol oxidase, PPO) where quinones is transformed to dark pigments [50]. These changes affected the nutritional quality, flavor and color resulting to loss in economic for food processing and marketing industry [51]. The diploid (C × E) population contributes three QTLs for ED, namely, cholorogenic acid and tyrosine levels QTL on C_2 and C_8 parental chromosome, respectively, while chromosome VIII carry a candidate gene

PPO for ED. The absence of genetic correlations between these components represents non-overlapping of QTLs [51]. During cooking, the phenol (mainly chlorogenic acid) and iron combined to formed iron diphenol resulting in post cooking discoloration which is a non-enzymatic reaction. The oxidation process occurs during cooling which results into blackening of potatoes [50]. The dark patches that occur beneath the skin intact were the result of mechanical damage during harvesting, transport, and storage, which are known as black spot bruising. These deteriorate the consumer and processing qualities of potatoes. The damaged cells are oxidized by phenols mediated *PPO* which then results into spot formation like as in post-peeling discoloration [50].

Tuber size has positive correlation with time required for peeling and trimming. Small tuber size possessed more number of tuber eyes and skin ratio per unit weight in comparison with large tubers. For example, the processing cultivar 'Sayaka' used especially for salads required less trimming time due to large tubers and shallow eyes as compared to Irish Cobbler', an old cultivar with deep eyes and relatively small tubers which is one-third of 'Sayaka' [52].

5.1.3. Nutritional quality

Potatoes have gained a special status in world due to cheap availability of nutrients, which play major role to feed the poor population of the world. These are the valuable source of dietary vitamins, minerals and phyto-nutrients because of their per capita consumption. Potato is considered as a good source of antioxidants, such as polyphenols, carotenoids and vitamin C [34] and major contributor of antioxidants in American diet because of the high consumption [53]. Diets rich in antioxidants have been associated with a lower incidence of atherosclerotic heart disease, certain cancers, macular degeneration and severity of cataracts. Furthermore, the considerable high diversity offered by native Andean potato germplasm to contribute to the dietary antioxidant intake and indicates that native potatoes offer a new opportunity to promote the nutritional quality of potato [54]. Polyphenols are most important secondary metabolites obtained from potato, which act as antioxidant. These compounds have wide range of physiological activities, which help to protect against UV radiation, pathogens and herbivores [55]. Phenylpropanoid pathway is responsible for production of polyphenol compounds and contains a wide range of chemical classes, including phenolic acids such as benzoic and hydroxycinnaic acids, flavonoids such as flavonols and anthocyanins, stilbenes and lignans [56].

Food polyphenols have protective role on human however, there is no direct protective role of polyphenol but it gives indirect protection by activating endogenous defense systems and by modulating cellular signaling processes. This compound is important in the human defense system against reactive oxygen species (ROS), which are known to be involved in the pathogenesis of aging and many degenerative diseases such as cardiovascular diseases and cancers [57].

The higher content of lutein, violaxanthin and total carotenoids occur in yellow-fleshed potatoes. Yellow-fleshed varieties obtain their color from xanthophyll carotenoids. Many carotenoids have been identified, including lutein, violaxanthin, neoxanthin, antheraxanthin and β-cryptoxanthin, with deep yellow pigmentation due to the presence of zeaxanthin [34]. The genetic and molecular biology of orange flesh color in potato was studied by several researchers [35]. In orange-fleshed potato, only genotypes combining presence of the dominant beta-carotene hydroxilase

2 (*Chy2*) allele with homozygositiy for the recessive zeaxanthin epoxidase (*Zep*) allele produced orange-fleshed tubers that accumulated large amounts of zeaxanthin [35]. Potatoes are one of the best plant sources of the amino acid lysine. Lysine is one of the amino acids that are a necessary component of complete proteins [58].

5.2. Different quality traits and breeding for quality traits of potato

Breeding for quality traits in potato is a difficult task due to inherent genetic and biological factors. In order to better understand the strategies for quality breeding, first we have to outline some important genetic and reproductive aspects of the potato which help in getting positive results.

First of all, the tetraploid (2n = 4× = 48) cultivated *S. tuberosum*is a polysomic polyploidy with four sets of similar alleles. The genetic components are the primary factor attributing to quality traits.

Groups of genetically controlled traits as follows: (i) biological traits like carbohydrates, proteins, vitamins, minerals and low level of toxic glycoalkaloids. (ii) Sensorial traits such as color, flavor and texture. (iii) Industrial traits, namely, shape and size of tuber, dry matter content, cold sweetening, oil absorption, starch quality and specific gravity.

Biological traits: these traits included under internal quality of tubers. Brief detailed of these traits given below:

- **Proteins:** in potato, several types of proteins were present. A globulin protein has been extracted from potato tubers by using salt extraction method, which is designated as 'tuberin'. The nutritional value of tuberin, is basically a globulin protein of potato as well as it is the true protein present in potato. They also categorized the potato tuber proteins into tuberin, globulin II, albumin, prolamine and glutelin.
- **Potato starch:** starch content in potato is about 11 and 45% of the tuber fresh weight. It varies according to geographical area, climate and cultivar. The tuber starch content of middle European varieties ranges from 10 to 17% for table potatoes, from 14 to 20% for processing potatoes (e.g. chips, French fries) and reaches up to 25% in industrial potatoes. The amylose content of starches ranged between 15.0 and 23.1% and differed significantly among different potato cultivars.

Starch yield (amount of starch obtained per unit of arable land) is one of the most important trait for industrial starch production. Hence, breeding of cultivars with high tuber starch yield (TSY) can be considered as one of the objective for industrial starch production. The total tuber weight per plant, per plot or per unit of arable land, is negatively correlated with tuber starch content [59].

- **Glycoalkaloid:** a bitter chemical compound with a combination of a glycoside and an alkaloid, which is present in potatoes and some other plants. The pasting temperature of starch separated from potato tubers depend on the duration of storage and glycoalkaloid content. Before storage, the pasting temperature ranges from 64.6 to 67.7°C, whereas 90 days after storage the temperature range of 66.9–69.4°C was more suitable. The effect of temperature

was also observed in viscosity of paste. The lower storage temperature (8°C) having the less viscous paste while higher storage temperature (16°C) leads to more viscosity. Storage temperature and processing also influence the viscosity of paste. Processing at high temperature leads to lower viscosity which ultimately leads to breakdown viscosity and set back viscosity after storage while the cold paste viscosity is not affected by temperature variation. For instance, solanine, the storage of raw material leads to increase in pasting temperature but significant reduction in pasting time.

- **Carotenoids:** the carotenoid pattern in four yellow- and four white-fleshed potato cultivars (*S. tuberosum*) was dominated by violaxanthin, antheraxanthin, lutein and zeaxanthin, which were present in different ratios, whereas neoxanthin, β-cryptoxanthin and β-carotene generally were only minor constituents [60].

The genotypes with extremely high levels of total carotenoids had zeaxanthin, an isomer of lutein, also present in the human retina. Total anthocyanins ranged from 1.5 to 48 mg/100 g FW in a solidly pigmented purple skinned, purple-fleshed breeding line.

- **Phenolic compounds (phenolic acids, flavonoids and anthocyanins):** potato tubers are one of the richest sources of polyphenols. The amount of polyphenols is affected by different parameters such as variety, year of cultivation, stress factors (mechanical damage of tubers, attack of pathogens, action of light on tubers or irrigation), storage and cooking treatment. Other than these, upto some extent it is also affected by of geographical location, soil type, potassium fertilization and storage temperature. It is commonly observed that about 50% of the phenolic compounds are located in the potato peel and adjoining tissues, which are often wasted, while the remainder decreases in concentration from outside toward the center of the potato tubers. It is also reported the total phenolic acid content in potato peel was about 3.93 mg/g powder and the major phenolic acids present were predominantly gallic acid, caffeic acid, chlorogenic acid and protocatechuic acid [61].

A number of health-promoting phytonutrients such as phenolics, flavonoids, folates, ketoimines and carotenoids has been found in potato [62]. High concentration of phenolic acids was observed in pigmented potato as compared to white-fleshed potato. Potato pigments are also rich in natural colorants and antioxidants.

- **Sugars:** β-D-fructose; α-glucose, β-D-glucose, myo-inositol and sucrose were the commonly occurring sugars in cold stored Kennebec potato tubers with stearic acid as internal standard [63].

- **Organic acids:** citric acid and malic acid in potato tubers in the ratio of nearly 20:1 together and also having small amount of isocitric acid.

- **Fatty acid:** almost 17 fatty acids were reported in *Solanum phureja* and *S. tuberosum* genotypes [64]. The predominant fatty acid was linoleic followed by α-linolenic and palmitic acids whereas the 15-Methylhexadecanoate was present in minute amount in both species. The amounts of these fatty acids were varied according to storage condition and period. The contents of linoleic acid decreased whereas α-linolenic acid increased in tubers of both species over the whole storage period.

- **Phytohormones and endogenous tuber inducing compounds:** potato plant had been reported to contain phytohormones like jasmonic acid, auxins (IAA), gibberellins (GA), cytokinins, abscisic acid (ABA), ethylene and strigolactones. It was also reported that both ABA and ethylene were required for dormancy induction, but only ABA was needed to maintain bud dormancy [65].
- **Specific gravity:** high specific gravity was found in a South American species *S. commersonii* and this species also possesses tolerance to both frost and heat.
- **Texture:** the texture of the tuber is a direct visible trait and helps to attract the consumers. S. *cardiophyllum* possesses yielding ability and attractive smooth tubers which can be used for breeding purpose.

Cultivars with the table quality demanded by supermarkets: these cultivars should have the following properties.

- Tubers must be resistant to after-cooking-blackening.
- Attractive skin finish paramount.
- Flavor and texture as judged by taste panels.
- Low levels of glycoalkaloids.
- Special purpose cultivars, for example, salad and punnet types.

5.3. Breeding for quality traits

Farmer selection from naturally occurring variation was responsible for evolution of many Andean varieties [66]. The low toxicity of present day cultivated potato tubers is mainly due to selection of tubers with less bitterness during the course of domestication. Large numbers of genotypes with lots of variation in tuber shape and color of skin and flesh has been maintained by Andean farmers. In later stage of development, the selection was made for early maturity, dormancy and resistance to different abiotic and biotic stresses for adaptation in different agro-climatic conditions. During the middle age, the primary objective of plant breeding was to increase yield and resistance to late blight in order to feed the large population of world [66]; followed by quality breeding as second priority of plant breeder. [24].Two aspects of wild potato species utilization: development of breeding methods and incorporation of valuable traits.

Rapid progress in potato breeding requires the correct choice of parents and crosses, and efficient selection procedures. Quality traits improvement can be achieved through two prominent genetic approaches at present. These are: (1) utilization of direct selection, traditional breeding or QTL mapping and subsequent marker assisted selection to exploit natural genetic variation and (2) introduction of transgenic plants or modifying expression of already existing gene which is linked with quality traits by using transgenic approaches.

Conventional potato breeding refers to the development of new varieties from sexual crosses between pairs of parents with complementary features followed by clonal propagation and

selection based on several traits. Before going to breeding, the breeder should know about the parents which are used for crossing. Selection of suitable parents and parental combination depends upon the breeder's experience which helps to get superior offspring [23]. Presence of genetic variation among the parents is the main prerequisite for any breeding program. The large number of variation for different quality traits were reported for Andean potatoes. These variations can play a major role in improvement of quality traits of present cultivated potato, by incorporating the gene linked with particular traits. The exploitation of existing variation into a cultivated species can be done after developing a population having trait of interest. This population can be developed by crossing two parents with contrast characters followed by phenotyping of population and then quantitative trait loci (QTLs) analysis which helps in identification of location for trait of interest [24]. In general, maximum breeding work for improvement of potato have been done by using the diploid populations but rarely by using tetraploid species [49, 51].

Association mapping is a new approach which was used for the first time in human genetics. In crop plants, genetic resources which exhibit the genetic and phenotypic diversity of the species can be utilize for developing the association mapping population. The phenotyping of these population done for quality traits and followed by genotyping. Candidate gene approach and genome wide distribution approach using DNA markers are two ways for genotyping. Then the testing of markers done for identification of trait association. After a large number of meiotic generations, if a specific trait is co-inherited with a specific trait allele due to identity between marker and trait locus or close physical linkage between them, it represents the markers-trait association. The population substructure (result from direct selection for specific trait complexes) is responsible for marker-trait association between unlinked loci.

In an association mapping experiment on potato by using 221 tetraploid genotypes to identify the specific gene associated with quality traits [67]. The DNA markers linked to QTL can be used to more rapidly incorporate the desirable regions into agronomically superior genotypes. Selection of genotype for improving some quality traits such as tuber shape or eye depth is quite easy whereas for some traits (glycoalkaloids and polyphenols), it requires long time and investment. Association mapping of starch and yield related traits also have been performed in potato [68, 69]. The most prominent hot spot comprises the distal segment of approximately five Mbp on the North arm of chromosome V, which harbors among other candidate genes [69], the *StCDF1* locus that controls photoperiod dependent tuberization [70].

Microarray technique helps to identify consistent differences in gene expression profiles between *S. phureja* and *S. tuberosum* cultivars, including genes likely to impact on flavor, texture, carotenoid content and tuber life-cycle. A sesquiterpene synthase gene was identified that consistently expressed at higher levels in *S. phureja* tubers, and also encodes an a-copaene synthase gene [71].

Plant breeding in combination with biotechnology can be proves as boon for breeders in generating the new varieties with superior quality traits such as high starch content, anthocyanin and carotenoid content. In Netherlands, a variety Karnico1 has been developed by genetic modification of starch which leads to production of amylose free potatoes. Amylose

production was completely suppressed by antisense RNA-mediated inhibition of granule-bound starch synthase, an approach made possible by the identification of an amylose-free mutant produced by techniques associated with conventional breeding.

Transgenic approaches have also provided new ways of understanding and manipulating carbohydrate metabolism aimed at developing genetically in-built resistance to low temperature sweetening caused by an accumulation of glucose and fructose.

The introgression of desirable genes from wild species to cultivated species and breeding at diploid and tetraploid level can be possible by using molecular marker-assisted selection (MAS) strategies. These strategies help to overcome the problems which are associated with selection of many economically important quality traits which are influenced by the environmental factors or require a special test for detecting these traits. MAS do not require DNA manipulations but only resides in the analysis of natural DNA variations that occur after intercrossing different genotypes. The MAS has several advantages over conventional breeding. In MAS breeding, 99% of cultivated genome can be recovered with only three backcross generation instead of the six to seven generations required to recover the same percentage of genome without the use of molecular markers. At present many markers are available, but only few markers such as RFLP, RAPD, AFLP and SSR are most widely used for MAS.

More than 350 markers that are uniformly distributed on 12 chromosomes saturates the molecular map of potato. There are more than 25 single dominant genes present on potato map. Among these, most of them show resistance to pest and disease while some of genes related to yield and quality traits together. Interspecific hybridization between wild and cultivated genotype is a valuable approach used to transfer the useful genes and in this case the use of species-specific molecular markers would allow the wild genomic content to be reduced in few backcross generations (negative-assisted selection). Potato map is one of the most highly saturated maps with different molecular markers and there are more than 350 markers which covers approximately 90% of the potato genome which provides an extensive opportunity for optimal use of DNA analysis for MAS and making it valuable tool for fixing the genes that controls the expression of quality traits [72]. The important tuber traits such as skin color, flesh color, tuber shape and leptin content [73] are controlled by single loci. The most of tubers traits are polygenic in nature and a lot of mapping work has been carried out by various researchers to localize the related QTLs on the potato map, using different segregating progenies and marker systems. DNA markers helps in early identification of quality traits such as tuber starch content, yield and starch yield potential in potato breeding populations and helps to facilitate the combination of superior alleles for high starch yield in novel cultivars.

Potatoes are an important source of carbohydrates, ways of reducing the glycemic impact of potatoes is an important research area. Moreover, existing biodiversity of potato varieties and their nutritional composition need to be explored before engaging in transgenics. The nutrient content of tubers needs to be among the criteria in cultivar promotion. As well as the cultivar-specific nutrient analysis and data dissemination should be systematically undertaken.

5.4. Bottlenecks in potato breeding for quality traits

There are several problems which hinder the breeding for quality traits. Among these, one potential problem due to extensive utilization of exotic germplasm is introgression of undesirable traits. For instance, wild species having higher amount of glycoalkaloid which can be introgressed into cultivated species leads to increase its concentration in tubers and made it unfit for human consumption.

6. Conclusions

Tuber quality is one of the most important characteristics of potato, it is probably the most poorly defined and least researched at the genetic level. The potato needs a continued improvement of quality traits to meet the needs of a changing and demanding world. Moreover, breeding objectives related to quality for processed potatoes are normally different from those for fresh use. Exploitation of cultivated and wild species of potato as source of valuable quality traits/allelic diversity, the possibility to manipulate whole chromosome sets make sexual hybridization a powerful strategy to produce new and valuable genotypes with high quality. However, the genetic improvement of potato is hampered by several factors, namely, its tetrasomic inheritance, high level of heterozigosity and incompatibility barriers. Moreover, these days molecular breeding helps the breeders for rapid identification of desirable genes and to produce quality traits like starches with modified amylose to amylopectin ratio, and potatoes with a higher nutritional value. As well as, genetic engineering is an additional tool to produce new genetic variability and to study important metabolic pathways. Equally important is the fact that basic studies have contributed to elucidate our knowledge on the genetics, biochemistry and physiology of several quality traits, making breeding efforts less empirical and more predictable. Since most quality traits are genetically controlled, breeding work can successfully meet the quality of potato tubers and fulfills the needs of a changing and demanding world.

Author details

Meenakshi Kumari[1], Manoj Kumar[2] and Shashank Shekhar Solankey[3]*

*Address all correspondence to: shashank.hort@gmail.com

1 Department of Vegetable Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India

2 Division of Vegetable Crops, Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

3 Department of Horticulture (Vegetable & Floriculture), Bihar Agricultural University, Sabour (Bhagalpur), Bihar, India

References

[1] Storey M. The harvested crop. In: Vreugdenhil D et al., editors. Potato Biology and Biotechnology. Amsterdam: Elsevier Science B.V.; 2007. p. 441-470

[2] FAO. Food Agriculture Organization, 2010. Available from: www.fao.org.in

[3] Devaux A, Kromann P, Ortiz O. Potatoes for sustainable global food security. Potato Research. 2014;**57**:185-199. DOI: 10.1007/s11540-014-9265-1

[4] PBI Solanum Project. Solanaceae, 2014. Available from: http://www.solanaceaesource.org [Accessed: July 10, 2014]

[5] Burton WG. The Potato. 3rd ed. New York: Harlow: Longman Scientific and Technical; 1989. 742 p

[6] Hawkes JG. The Potato: Evolution, Biodiversity and Genetic Resources. London: Belhaven Press; 1990. 259 p

[7] Bohs L, Olmstead RG. Phylogenetic relationships in *Solanum* (Solanaceae) based on ndhF sequences. Systematic Botany. 1997;**22**:5-17

[8] Peralta IE, Spooner DM. Granule-bound starch synthase (GBSSI) gene phylogeny of wild tomatoes (*Solanum* L. section *Lycopersicon* [Mill.] Wettst. subsection *Lycopersicon*). American Journal of Botany. 2001;**88**:1888-1902

[9] Hanneman RE Jr. The testing and release of transgenic potatoes in the North American Center of diversity. In: Krattiger AF, Rosemarin A, editors. Biosafety of Sustainable Agriculture: Sharing Biotechnology Regulatory Experiences of the Western Hemisphere. Stockholm: ISAAA, Ithaca and SEI; 1994. p. 47-67

[10] Camire M, Kubow S, Donnelly D. Potatoes and human health. Critical Reviews in Food Science and Nutrition. 2009;**49**:823-840

[11] Hari RH. Vegetable Breeding. Principles and Practices. Ludhiana-141008: Kalyani Publisher; 1998

[12] Nesterenko S, Sink KC. Carotenoid profiles of potato breeding lines and selected cultivars. Hortscience. 2003;**38**:1173-1177

[13] Lu W, Haynes K, Wiley E, Clevidence B. Carotenoid content and color in diploid potatoes. Journal of the American Society for Horticultural Science. 2001;**126**:722-726

[14] Lewis CE, Walker JRL, Lancaster JE, Sutton KH. Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L. Journal of the Science of Food and Agriculture. 1998;**77**:45-57

[15] Hayder A, Ahmed MB, Hannan MM, Razvy MA, Mandal MA, Salahin M, Karim R, Hossain KM. Analysis of genetic diversity in some potato varieties grown in Bangladesh. Middle-East Journal of Scientific Research. 2007;**2**(3-4):143-145

[16] Rauf S, Silva JAT, Khan AA, Naveed A. Consequences of plant breeding on genetic diversity. International Journal of Plant Breeding. 2010;**4**(1):1-21

[17] Ochoa CM. Las papas de sudamerica: Peru (Parte I). Lima: International Potato Center; 1999. 1036 p

[18] Spooner DM, Núñez J, Trujillo G, Del Rosario Herrera M, Guzmán F, Ghislain M. Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:19398-19403

[19] Spooner DM, Gavrilenko T, Jansky SH, Ovchinnikova A, Krylova E, Knapp S, Simon R. Ecogeography of ploidy variation in cultivated potato (Solanum sect. Petota). American Journal of Botany. 2010;**97**:2049-2060

[20] Ovchinnikova A, Krylova E, Gavrilenko T, Smekalova T, Zhuk M, Knapp S, Spooner DM. Taxonomy of cultivated potatoes (Solanum section Petota: Solanaceae). Botanical Journal of the Linnean Society. 2011;**165**:107-155

[21] Rodríguez F, Ghislain M, Claused AM, Jansky SH, Spooner DM. Hybrid origins of cultivated potatoes. Theoretical and Applied Genetics. 2010;**121**:1187-1198

[22] Irikura Y. Bareisho. In: Matsuo T, editor. Collected Data of Plant Genetic Resources 2. Tokyo: Kodansha Scientific; 1989. p. 498-518

[23] Jansky S. Breeding, genetics, and cultivar development. In: Singh J, Kaur L, editors. Advances in Potato Chemistry and Technology. San Diego: Academic Press; 2009. p. 27-62

[24] Werij J. Genetic Analyisis of Potato Tuber Quality Traits. Wageningen: Laboratory of Plant Breeding, Wageningen University; 2011. 125 p

[25] ŚLiwka J, Wasilewicz-Flis I, Jakuczun H, Gebhardt C. Tagging quantitative trait loci for dormancy, tuber shape, regularity of tuber shape, eye depth and flesh colour in diploid potato originated from six Solanum species. Plant Breeding. 2008;**127**:49-55

[26] van Eck HJ, Jacobs JME, Dijk J, Stiekema WJ, Jacobsen E. Identification and mapping of three flower colour loci of potato (*S. tuberosum*L.) by RFLP analysis. TAG Theoretical and Applied Genetics. 1993;**86**:295-300

[27] van Eck HJ, Jacobs JME, van den Berg PMMM, Stiekema WJ, Jacobsen E. The inheritance of anthocyanin pigmentation in potato (*Solanum tuberosum*L.) and mapping of tuber skin colour loci using RFLPs. Heredity. 1994;**73**:410-421

[28] De Jong WS, Eannetta NT, Jong DM, Bodis M. Candidate gene analysis of anthocyanin pigmentation loci in the *Solanaceae*. Theoretical and Applied Genetics. 2004;**108**:423-432

[29] Jung CS, Griffiths HM, De Jong DM, Cheng S, Bodis M, Kim TS, De Jong WS. The potato developer (D) locus encodes an R2R3 MYB transcription factor that regulates expression of multiple anthocyanin structural genes in tuber skin. Theoretical and Applied Genetics. 2009;**120**(1):45-57

[30] Hung CY, Murray JR, Ohmann SM, Tong CBS. Anthocyanin accumulation during potato tuber development. Journal of the American Society for Horticultural Science. 1997;**122**:20-23

[31] Lachman J, Hamouz K, Sulc M, Orsák M, Pivec V, Hejtmánková A, Dvorák P, Cepl J. Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity. Food Chemistry. 2009;**114**:836-843

[32] Jansen G, Flamme W. Coloured potatoes (*Solanum tuberosum* L.) anthocyanin content and tuber quality. Genetic Resources and Crop Evolution. 2006;**53**:1321-1331

[33] Nayak B, Berrios JDJ, Powers JR, Tang J, Ji Y. Colored potatoes (*Solanum tuberosum* L.) dried for antioxidant-rich value-added foods. Journal of Food Processing and Preservation. 2011;**35**:571-580

[34] Brown C. Antioxidants in potato. American Journal of Potato Research. 2005;**82**:163-172

[35] Wolters AM, Uitdewilligen J, Kloosterman B, Hutten R, Visser R, van Eck H. Identification of alleles of carotenoid pathway genes important for zeaxanthin accumulation in potato tubers. Plant Molecular Biology. 2010;**73**:659-671

[36] Jakuczun H, Zimnoch-Guzowska E. Inheritance of glucose content in tubers of diploid potato families. American Journal of Potato Research. 2004;**81**:359-370

[37] Reeves A. Varietal differences in potato tuber greening. American Journal of Potato Research. 1988;**65**:651-658

[38] Faivre-Rampant O, Bryan GJ, Roberts AG, Milbourne D, Viola R, Taylor MA. Regulated expression of a novel TCP domain transcription factor indicates an involvement in the control of meristem activation processes in *Solanum tuberosum*. Journal of Experimental Botany. 2004;**55**:951-953

[39] Agrimonti C, Visioli G, Bianchi R, Torelli A, Marmiroli N. G1-1 and LeG1-1/LeG1-2 genes are involved in meristem activation during breakage of dormancy and early germination in potato tubers and tomato seeds. Plant Science. 2007;**173**:533-541

[40] Senning M, Sonnewald U, Sonnewald S. Deoxyuridinetriphosphatase expression defines the transition from dormant to sprouting potato tuber buds. Molecular Breeding. 2010;**26**: 525-531

[41] Nema PK, Ramayya N, Duncan E, Niranjan K. Potato glycoalkaloids: Formation and strategies for mitigation. Journal of the Science of Food and Agriculture. 2008;**88**:1869-1881

[42] Friedman M, Levin CE. Analysis and biological activities of potato glycoalkaloids, calystegine alkaloids, phenolic compounds, and anthocyanins. In: Jaspreet S, Lovedeep K, editors. Advances in Potato Chemistry and Technology. San Diego: Academic Press; 2009. p. 127-161

[43] Friedman M. Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. Journal of Agricultural and Food Chemistry. 2006;**54**:8655-8681

[44] Kirkman M. Global markets for processed potato products. In: Vreugdenhil D et al., editors. Potato Biology and Biotechnology. Amsterdam: Elsevier Science B.V; 2007. p. 27-44

[45] Werij J, Furrer H, van Eck H, Visser R, Bachem C. A limited set of starch related genes explain several interrelated traits in potato. Euphytica. 2012;**186**(2):501-516

[46] Taylor MJ, McDougall G, Stewart D. Potato flavour and texture. In: Vreugdenhil D et al., editors. Potato Biology and Biotechnology. Amsterdam: Elsevier Science B.V.; 2007. p. 525-540

[47] Haverkort A. Potato crop response to radiation and daylength. In: Vreugdenhil D et al., editors. Potato Biology and Biotechnology. Amsterdam: Elsevier Science B.V.; 2007. p. 353-365

[48] Kumar D, Singh BP, Kumar P. An overview of the factors affecting sugar content of potatoes. Annals of Applied Biology. 2004;**145**:247-256

[49] Menendez C, Ritter E, Schafer-Pregl R, Walkemeier B, Kalde A, Salamini F, Gebhardt C. Cold sweetening in diploid potato: Mapping quantitative trait loci and candidate genes. Genetics. 2002;**162**:1423-1434

[50] Friedman M, Mc Donald GM, Filadelfi-Keszi M. Potato glycoalkaloids: Chemistry, analysis, safety, and plant physiology. Critical Reviews in Plant Sciences. 1997;**16**:55-132

[51] Werij J, Kloosterman B, Celis-Gamboa C, de Vos C, America T, Visser R, Bachem C. Unravelling enzymatic discoloration in potato through a combined approach of candidate genes, QTL, and expression analysis. Theoretical and Applied Genetics. 2007;**115**:245-252

[52] Mori M. Breeding new potato cultivars to meet processing needs and increase the contents of functional ingredients. Gamma Field Symposia. 2008;**47**:49-57

[53] Chun OK, Kim DO, Smith N, Schroeder D, Han JT, Lee CY. Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. Journal of the Science of Food and Agriculture. 2005;**85**:1715-1724

[54] Andre CM, Oufir M, Guignard C, Hoffmann L, Hausman JF, Evers D, Larondelle Y. Antioxidant profiling of native andean potato tubers (*Solanum tuberosum* L.) reveals cultivars with high levels of β-carotene, α-tocopherol, chlorogenic acid, and petanin. Journal of Agricultural and Food Chemistry. 2007;**55**:10839-10849

[55] Andre CM, Oufir M, Hoffmann L, Hausman JF, Rogez H, Larondelle Y, Evers D. Influence of environment and genotype on polyphenol compounds and in vitro antioxidant capacity of native Andean potatoes (*Solanum tuberosum* L.). Journal of Food Composition and Analysis. 2009;**22**:517-524

[56] Andre CM, Schafleitner R, Legay S, Lefèvre I, Aliaga CAA, Nomberto G, Hoffmann L, Hausman JF, Larondelle Y, Evers D. Gene expression changes related to the production of phenolic compounds in potato tubers grown under drought stress. Phytochemistry. 2009;**70**:1107-1116

[57] Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden M, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A. Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theoretical and Applied Genetics. 2006;**113**:1409-1420

[58] Peksa A, Kita A, Kułakowska K, Aniołowska M, Hamouz K, Nemś A. The quality of protein of coloured fleshed potatoes. Food Chemistry. 2013;**141**:2960-2966

[59] Li L, Tacke E, Hofferbert H-R, Lübeck J, Strahwald J, Draffehn AM, Walkemeier B, Gebhardt C. Validation of candidate gene markers for marker-assisted selection of potato cultivars with improved tuber quality. Theoretical and Applied Genetics. 2013;**126**: 1039-1052

[60] Breithaupt DE, Bamedi A. Carotenoids and carotenoid esters in potatoes (*Solanum tuberosum* L.): New insights into an ancient vegetable. Journal of Agricultural and Food Chemistry. 2002;**50**(24):7175-7181

[61] Singh N, Rajini PS. Antioxidant-mediated protective effect of potato peel extract in erythrocytes against oxidative damage. Chemico-Biological Interactions. 2008;**173**(2):97-104

[62] Ezekiel R, Singh N, Sharma S, Kaur A. Beneficial phytochemicals in potato – A review. Food Research International. 2013;**50**(2):487-496

[63] Varns JL, Shaw R. An internal standard for rapid analysis of potato sugars by gas chromatography. Potato Research. 1973;**16**(3):183-187

[64] Dobson G, Griffiths DW, Davies HV, McNicol JW. Comparison of fatty acid and polar lipid contents of tubers from two potato species, *Solanum tuberosum* and *Solanum phureja*. Journal of Agricultural and Food Chemistry. 2004;**52**:6306-6314

[65] Suttle J. Physiological regulation of potato tuber dormancy. American Journal of Potato Research. 2004;**81**:253-262

[66] Bradshaw JE. Potato-breeding strategy. Potato Biology and Biotechnology: Advances and Perspectives. Chapter 8; 2007:157-177

[67] D'hoop B, Paulo MJ, Kowitwanich K, Sengers M, Visser RF, van Eck H, van Eeuwijk F. Population structure and linkage disequilibrium unravelled in tetraploid potato. Theoretical and Applied Genetics. 2010;**121**:1151-1170

[68] D'hoop B, Keizer PC, Paulo MJ, Visser RF, Eeuwijk F, Eck H. Identification of agronomically important QTL in tetraploid potato cultivars using a marker – trait association analysis. Theoretical and Applied Genetics. 2014;**127**:731-748

[69] Schreiber L, Nader-Nieto AC, Schönhals EM, Walkemeier B, Gebhardt C. SNPs in genes functional in starch-sugar interconversion associate with natural variation of tuber starch- and sugar content of potato (*Solanum tuberosum* L.). G3: Genes, Genomes, Genetics. 2014;**4**: 1797-1811

[70] Kloosterman B, Abelenda JA, Gomez MMC, Oortwijn M, de Boer JM, Kowitwanich K, Horvath BM, van Eck HJ, Smaczniak C, Prat S, Visser RGF, Bachem CWB. Naturally occurring allele diversity allows potato cultivation in northern latitudes. Nature. 2013;**495**:246-250

[71] Ducreux LJ, Morris WL, Prosser IM, Morris JA, Beale MH, Wright F, Taylor MA. Expression profiling of potato germplasm differentiated in quality traits leads to the identification of candidate flavour and texture genes. Journal of Experimental Botany. 2008;**59**(15):4219-4231

[72] Gebhardt C, Ritter E, Salamini F. RFLP map of the potato. In: Phih'pps RL, Vasfl IK, editors. DNA-Based Markers in Plants. Vol. 6. Dordrecht: Kluwer Academic Publishers; 2001. p. 319-336

[73] Hutvagner G, Banfalvi Z, Milankovics I, Silhavy D, Polagr Z, Horvath S, Wolters P, Nap JP. Molecular markers associated with leptinine production are located on chromosome 1 in *Solanumchacoense*. Theoretical and Applied Genetics. 2001;**102**:1065-1071

Section 2

Control of Pests and Diseases

Chapter 4

Simulations of Colorado Potato Beetle Development in Poland Based on Four Climate Change Scenarios

Andrzej Wójtowicz, Marek Wójtowicz,
Maciej Zacharczuk, Henryk Ratajkiewicz and
Maria Pasternak

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70777

Abstract

The simulations were conducted using actual data and virtual data. The actual data were recorded in the period of 1986–2005 at 16 localities representing 16 regions of Poland. The virtual data were obtained after transformation of the recorded data to reflect a temperature changes under RCP2.6, RCP4.5, RCP6.0 and RCP8.5 scenarios according to giss_e2_r climate model. The model used in the study was based on scientific reports describing the influence of temperature on acceleration of the onset of egg laying and on successive stages of Colorado potato beetle as well as publications on the effects of photoperiod on the pest diapause. The study showed a growing threat to potato from Colorado potato beetles as a result of the temperature rise. The fastest development of the pest appeared in simulations under RCP8.5 scenario. Of 16 regions surveyed in the study, the south-western part of Poland was found to be most threatened by Colorado potato beetle as a result of anticipated climate change.

Keywords: Colorado potato beetle, number of generations, model, climate change, RCP scenarios

1. Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most destructive pest of the potato in many countries all over the world [1]. The pest consumes about 40 cm^2 foliage at the larval stage and almost 10 cm^2 per day as an adult [2]. The distribution of the Colorado potato beetle covers about 8 million km^2 in North America [3] and about 6 million km^2 in Europe and Asia [4]. It has recently appeared in western China [1] and Iran [5]. According to Vlasova [6], Worner [7] and Jolivet [4], the expected climate change may promote the pest expansion into

Korea, Japan, certain areas of the Indian subcontinent, parts of North Africa and the temperate Southern Hemisphere. In Poland, the Colorado potato beetle appeared in 1944 [8]. In 1950, the first great invasion of this species was noticed [9]. Despite the systematic reduction of potato land, the Colorado potato beetle is still a major pest affecting potato crops in Poland [10–12]. Yield losses caused by the feeding of the pest, in the absence of chemical protection, are estimated at 35–40% [13], and in extreme cases, losses can reach 70% of yield [14]. Potato crop losses caused by the Colorado potato beetle are highly dependent on the growth rate of the pest population, which is heavily dependent on meteorological conditions, among which temperature plays a leading role. According to the data from a number of studies, temperature is also the main environmental factor which determines the number of pest generations. The close connection between these two factors indicates the opportunity of using mathematical models expressing relationships between temperature and the rate of Colorado potato beetle development for predicting the influence of climate change on the number of pest generations.

This has already been studied in Poland [15], but only for the Wielkopolska region and without considering new emission scenarios termed representative concentration pathways (RCPs) recommended by the international climate modeling community through the Fifth Assessment Report (AR5) of the Intergovernmental Panel on Climate Change (IPCC) to be used in climate modeling and research [16].

RCP2.6, RCP4.5, RCP6 and RCP8.5 are four pathways named according to their 2100 radiative forcing level expressed in Watts per meter square. RCP2.6 is a "peak-and-decline" scenario. By mid-century, its radiative forcing level reaches a value of around 3.1 W/m^2 and then decreases to 2.6 W/m^2 by 2100 [17]. RCP4.5 [18–20] and RCP6.0 [21, 22] are stabilization scenarios in which total radiative forcing is stabilized shortly after 2100, following the reduction of greenhouse gas emissions. RCP8.5 is characterized by increasing greenhouse gas emissions over time. This is a representation of scenarios in the literature that lead to high greenhouse gas concentration levels [23].

The aim of this study was to determine the impact of climate change on the development of the Colorado potato beetle and to identify the region most at risk of increase in the number of pest generations.

2. Material and methods

2.1. Climate change model selection

In order to select the climate model, out of the 16 models presented on the Climate Change Knowledge Portal created by the World Bank (http://sdwebx.worldbank.org/climateportal/), we used the Taylor diagram technique. This diagram enables to assess how closely a pattern matches observations on the basis of three measures of model quality presented on one chart. These measures are: the correlation (R), the centered rootmean-square-error (RMSE) and the amplitude of the standard deviations (Std) [24]. We compared monthly temperature registered at 16 localities in the period 1986–2005 and the temperatures generated for this period and these locations by the climate models.

http://dx.doi.org/10.5772/intechopen.70777

2.2. Meteorological data

Two kinds of meteorological data were used in the study: first, data were registered in the years 1986–2005 at 16 localities representing the 16 regions of Poland; and second, data obtained after transformation of the recorded data to reflect temperature changes under RCP2.6, RCP4.5, RCP6.0 and RCP8.5 scenarios according to the giss_e2_r climate model. Latitudes and longitudes of the analyzed localities are presented in **Table 1**.

2.3. Simulation of the impact of climate change on Colorado potato beetle development

The study was performed using the NumoGen 2 model, which was developed for the present study based on the earlier version called NumoGen 1 [15]. The main difference between these two models is that NumoGen 2 enables the calculation of differences in the dates of egg laying between regions and years, while NumoGen 1 was only able to consider the changes in temperature triggered by climate changes. But, for all these purposes, the same equation were used, presented by Wójtowicz et al. in [15], describing the relationship between the onset of the egg-laying period and temperature increase.

From experiments conducted in the Wielkopolska region at WinnaGóra in the years 2003–2005 when Colorado potato beetle egg laying was noticed in the first decade of June, it was decided to perform simulations of the pest development with the use of meteorological data collected in Poznan in 2005 starting from 27 May to 15 June. This covers the period from 5 days before the start to 5 days after the first decade of June. The start of simulations performed with the use of data collected in Poznań in 1986–2004, as well as those registered at the other 15 localities in the period 1986–2005 and virtual data generated by the giss_e2_r model were obtained with

Locality	longitude	latitude
Białystok	53°07'N	23°10'E
Gdańsk	54°22'N	18°38'E
Katowice	50°15'N	19°00'E
Kielce	50°53'N	20°37'E
Kraków	50°03'N	19°55'E
Lublin	51°15'N	22°34'E
Łódź	51°49'N	19°28'E
Olsztyn	53°47'N	20°30'E
Opole	50°40'N	17°56'E
Poznań	52°25'N	16°53'E
Rzeszów	50°02'N	22°00'E
Szczecin	53°25'N	14°32'E
Toruń	53°02'N	18°37'E
Warszawa	52°35'N	21°05'E
Wrocław	51°05'N	17°00'E
Zielona Góra	51°56'N	15°30'E

Table 1. Latitude and longitude of localities analyzed in the study.

the use of an equation describing the relationship between onset of the egg-laying period and temperature increase [15].

NumoGen 2 simulates the development of colorado Potato Beetle from the occurrence of egg clusters until meteorological conditions or photoperiods prevent further development of the pest. The model was developed from information presented in scientific reports. The development of the pest from egg to adult was based on information presented by Łarczenko [25]. The dates of egg laying by female beetles of succeeding generations were estimated according to data presented by Alyokhin and Ferro [26]. The beginning of the winter diapause was determined from data reported by Tauber et al. [27], who found that all females reared at a photoperiod between 10:14 and 14:10 (L:D) entered diapause. This information was used to determine the dates of diapause based on day length at the 16 localities analyzed in the study. Information about day length was found on the internet at: http://www.timebie.com/sun/.

For each locality, 20 simulations were performed. Each simulation generated information about CPB development based on meteorological data registered in the 20 years, 1986–2005, and data obtained after transformation of the recorded data to reflect temperature changes under four scenarios (RCP2.6, RCP4.5, RCP6.0 and RCP8.5) and four periods (2020–2039, 2040–2059, 2060–2079 and 2080–2099) according to the giss_e2_r climate model.

Additionally for each locality, a model was developed to estimate the minimum temperature increase, in relation to 1986–2005, that ensured the emergence of the second generation of CPB.

The models were developed based on meteorological data (registered and obtained after transformation of the recorded data to reflect the temperature changes under four RCP scenarios) and simulation results describing the probability of the occurrence of CPB second generation. The models were developed with the use of the exponential function:

$$SGCPBP = a + \exp(b + c \times T) \quad (1)$$

where SGCPBP is the probability of the occurrence of CPB second generation; T the temperature increase for the temperature registered in 1986–2005; a, b and c are the equation coefficients.

3. Results

3.1. Climate change model selection

The results from the giss_e2_r model appeared on the Taylor diagram at the shortest distance from the observation point (**Figure 1**). On that basis, the giss_e2_r model was selected out of the 16 analyzed climate models for further analysis (**Figure 2**).

3.2. Simulation of climate change on Colorado potato beetle development

Simulations performed on data registered at 16 localities showed that the best meteorological conditions for the earliest egg laying in 2005 occurred in the west and south-west, while the

http://dx.doi.org/10.5772/intechopen.70777

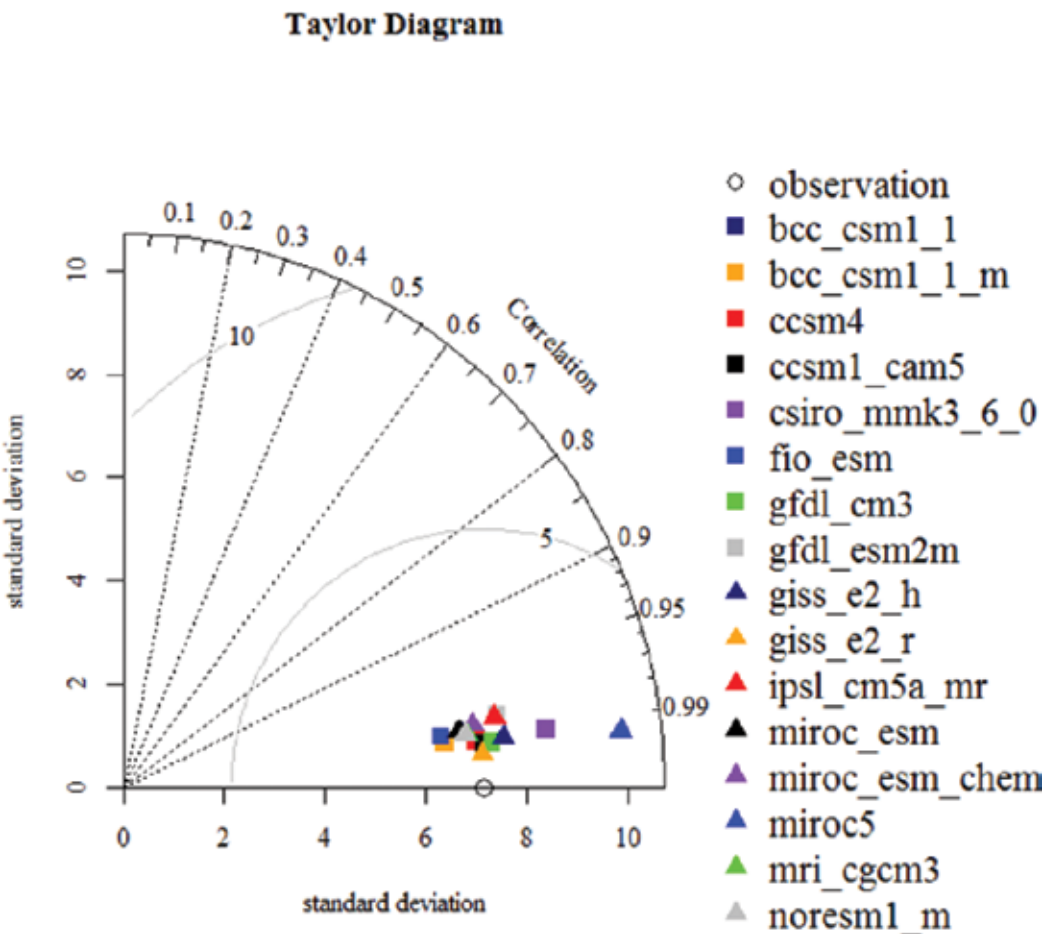

Figure 1. Taylor diagram illustrating the statistics of the comparison between observed and 16 model estimates of air temperature at 16 localities in 1986–2005. Bcc_csm1_1, bcc_csm1_1_m, ccsm4, cesm1_cam5, csiro_mk3_6_0, fio_esm, gfdl_cm3, gfdl_esm2m, giss_e2_h, giss_e2_r, ipsl_cm5a_mr, miroc_esm, miroc_esm_chem, miroc5, mri_cgcm3, noresm1_m: Model names used in the study.

worst conditions were noted in the northern and north-eastern parts of Poland (**Table 3**). Out of the four RCP scenarios, three (RCP4.5, RCP6.0 and RCP8.5) generated the greatest acceleration of egg laying in the period 2080–2099. But, according to the RCP2.6 scenario, the earliest egg laying is expected in the period 2020–2039 (**Table 2**). Comparison of meteorological data registered in 1986–2005 revealed that the differences between the earliest and the latest day of egg laying ranges from 12 at Białystok to 16 at Opole (**Table 3**).

Simulations performed on real data, except for Białystok (95.8%), Gdańsk (85%) and Olsztyn (99.3%), as well as on transformed data showed a 100% probability of the appearance of the first generation of CPB (**Figure 3**). Simulations performed on real data revealed that the average number of days needed for the development of the first generation of CPB was 56. Use of transformed data resulted in a shortening of the first generation development of the

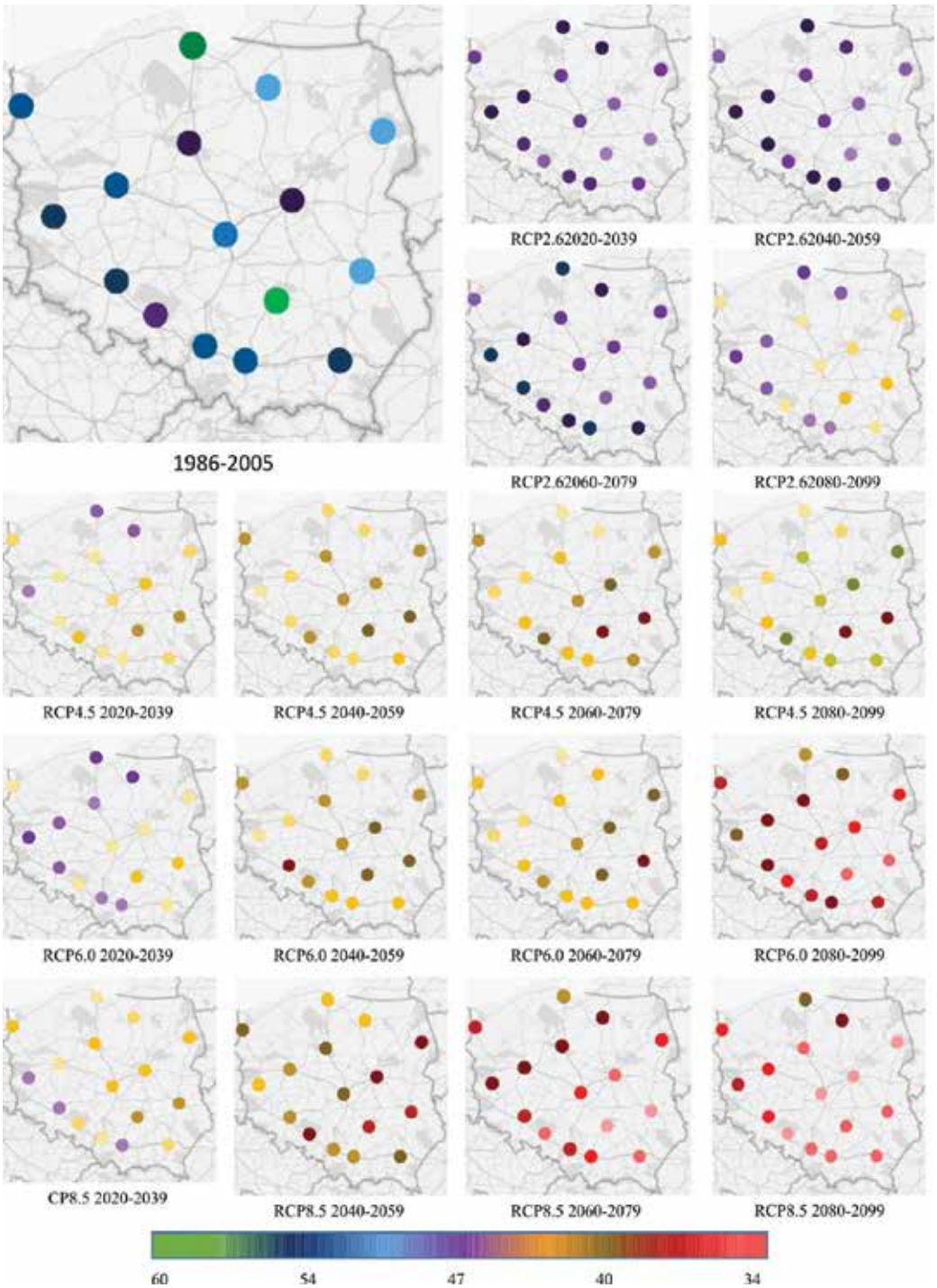

Figure 2. Effects of climate change on number of days needed to complete the first generation of CPB at 16 localities in Poland.

http://dx.doi.org/10.5772/intechopen.70777

Locality	Registerd temperature	RCP2.6				RCP4.5				RCP6.0				RCP8.5			
		20-39	40-59	60-79	80-99	20-39	40-59	60-79	80-99	20-39	40-59	60-79	80-99	20-39	40-59	60-79	80-99
Białystok	0	-2	-1	-2	1	-1	-1	-4	-3	-1	-2	-1	-5	0	-6	-7	-9
Gdańsk	0	-1	0	0	3	0	0	-2	-2	0	-1	0	-4	1	-5	-6	-8
Katowice	0	-2	-1	-1	2	0	-1	-3	-3	-1	-2	-1	-5	0	-4	-6	-10
Kielce	0	-4	-3	-3	-1	-2	-3	-5	-5	-3	-4	-3	-7	-2	-7	-8	-12
Kraków	0	-1	0	-1	2	0	0	-3	-2	-1	-1	0	-4	0	-4	-6	-9
Lublin	0	-3	-2	-3	0	-2	-2	-5	-4	-2	-3	-2	-6	-1	-7	-8	-11
Łódź	0	-1	0	-1	2	0	0	-3	-2	-1	-2	0	-4	0	-5	-6	-9
Olsztyn	0	1	2	2	5	2	2	0	0	2	1	2	-2	2	-3	-5	-6
Opole	0	0	1	0	3	1	1	-2	-2	0	-1	1	-4	1	-3	-5	-8
Poznań	0	-1	1	0	3	1	1	-2	-2	0	-1	1	-4	1	-4	-5	-8
Rzeszów	0	-1	-1	-1	1	-1	-1	-4	-3	-1	-2	0	-5	0	-5	-6	-10
Szczecin	0	-2	0	-1	2	0	0	-3	-3	-1	-2	0	-5	0	-5	-6	-8
Toruń	0	0	1	0	4	1	1	-2	-1	1	0	1	-3	2	-4	-5	-7
Warszawa	0	-1	0	0	3	1	0	-2	-2	0	-1	0	-4	1	-5	-5	-8
Wrocław	0	-1	0	0	2	0	0	-3	-2	-1	9	0	-4	1	-4	-6	-9
Zielona Góra	0	-1	1	0	3	1	1	-2	-2	0	-1	1	-4	1	-3	-5	-8
average	0	-1,3	-0,1	-0,7	2,2	0,1	-0,1	-2,8	-2,4	-0,6	-0,8	-0,1	-4,4	0,4	-4,6	-5,9	-8,8

Table 2. Effects of RCP scenario on the offset of the egg-laying period in relation to temperature registered in 1986–2005.

pest to 46–51 days for RCP2.6, 44 days for RCP4.5, 41–48 days for RCP4.5 and 39–46 days for RCP8.5. The greatest decreases were obtained for Gdańsk (9–14 days for RCP2.6, 15–16 days for RCP4.5, 11–19 days for RCP6.0 and 14–21 days for RCP8.5), Białystok (10–14 days for RCP2.6, 14–17 days for RCP4.5, 13–19 days for RCP6.0 and 16–22 days for RCP8.5), Kielce (9–15 days for RCP2.6, 16–17 days for RCP4.5, 13–19 days for RCP6.0 and 14–21 days for RCP8.5), Lublin (9–14 days for RCP2.6, 15–16 days for RCP4.5, 12–18 days for RCP6.0 and 14–20 days for RCP8.5) and Olsztyn (8–12 days for RCP2.6, 13–14 days for RCP4.5, 10–17 days for RCP6.0 and 14–20 days for RCP8.5). The smallest decreases were noted in simulations performed for Opole (1–6 days for RCP2.6, 7–8 days for RCP4.5, 4–11 days for RCP6.0 and 5–12 days for RCP8.5), Wrocław (0–6 days for RCP2.6, 8–9 days for RCP4.5, 4–11 days for RCP6.0 and 6–13 days for RCP8.5) and ZielonaGóra (1–6 days for RCP2.6, 8–9 days for RCP4.5, 4–12 days for RCP6.0 and 6–14 days for RCP8.5).

Simulations performed on meteorological data registered in the period 1986–2005 showed that the average probability of the appearance of the Colorado potato beetle second generation was 26% (**Figure 4**). The highest probabilities were obtained for Opole 62%, Wrocław 46% and ZielonaGóra 45%, whereas the smallest probabilities were achieved for Gdańsk 0%, Olsztyn 3.2% and Białystok 6%.

The use of data obtained after transformation of the recorded data to reflect temperature changes under four scenarios (RCP2.6, RCP4.5, RCP6.0 and RCP8.5) and four periods (2020–2039, 2040–2059, 2060–2079 and 2080–2099), according to the giss_e2_r climate model, did not lead to much alteration in the list of the localities least threatened by the occurrence of CPB second generation. Out of 16 combinations of scenarios and periods, only four resulted in the replacement of Białystak by another locality in third position on the list. But, changes in the list

year	Białystok	Gdańsk	Katowice	Kielce	Kraków	Lublin	Łódź	Olsztyn	Opole	Poznań	Rzeszów	Szczecin	Toruń	Warszawa	Wrocław	Zielona Góra
Differences in the onset of the egg lying period between localities in 2005in relation to Poznań																
2005	5	8	2	3	-2	2	1	5	-1	0	0	4	3	1	-1	-1
Differences in the onset of the egg lying period between years for each locality in relation to 2005																
2005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2004	3	0	2	2	2	2	2	2	1	1	3	0	1	2	2	2
2003	-1	-1	-1	-1	-1	-1	0	0	0	-2	-1	-1	-2	-1	0	0
2002	-6	-6	-5	-4	-4	-4	-4	-7	-4	-4	-3	-3	-7	-5	-4	-2
2001	-2	0	-1	0	1	-1	2	0	1	0	0	-1	-1	0	1	1
2000	-8	-8	-8	-8	-6	-8	-9	-8	-6	-8	-6	-8	-10	-8	-7	-7
1999	1	0	-2	0	0	1	1	0	0	0	1	-1	-1	0	-1	0
1998	-3	-3	-4	-4	-2	-3	-2	-4	-4	-4	-2	-5	-4	-3	-2	-2
1997	4	5	3	4	5	4	5	5	4	5	5	3	3	5	5	6
1996	-2	0	0	-1	2	-2	0	-1	2	2	-1	1	-2	-1	3	4
1995	2	2	2	3	4	3	3	2	2	2	4	0	1	3	3	3
1994	1	1	0	1	2	1	1	2	2	2	1	-2	0	1	2	2
1993	-4	-6	-5	-3	-1	-3	-4	-6	-10	-6	-1	-9	-7	-4	-3	-5
1992	2	1	1	2	3	3	2	2	-4	1	3	-3	0	2	1	1
1991	4	4	5	5	7	5	5	5	6	5	7	3	4	5	6	7
1990	0	-1	0	1	3	1	0	-1	0	1	2	-3	-2	0	1	1
1989	-2	-1	-2	0	2	-1	0	-2	0	0	0	-2	-2	-1	1	1
1988	0	2	-1	1	4	1	0	0	0	0	2	-3	-1	0	1	1
1987	4	4	4	4	6	4	5	4	4	4	5	3	3	4	5	5
1986	-1	0	-5	-3	-1	-2	-1	-2	-3	0	-4	0	-1	-2	0	2
max	4	5	5	5	7	5	5	5	6	5	7	3	4	5	6	7
min	-8	-8	-8	-8	-6	-8	-9	-8	-10	-8	-6	-9	-10	-8	-7	-7

Table 3. Effects of locality and year on the offset of the egg-laying period.

http://dx.doi.org/10.5772/intechopen.70777

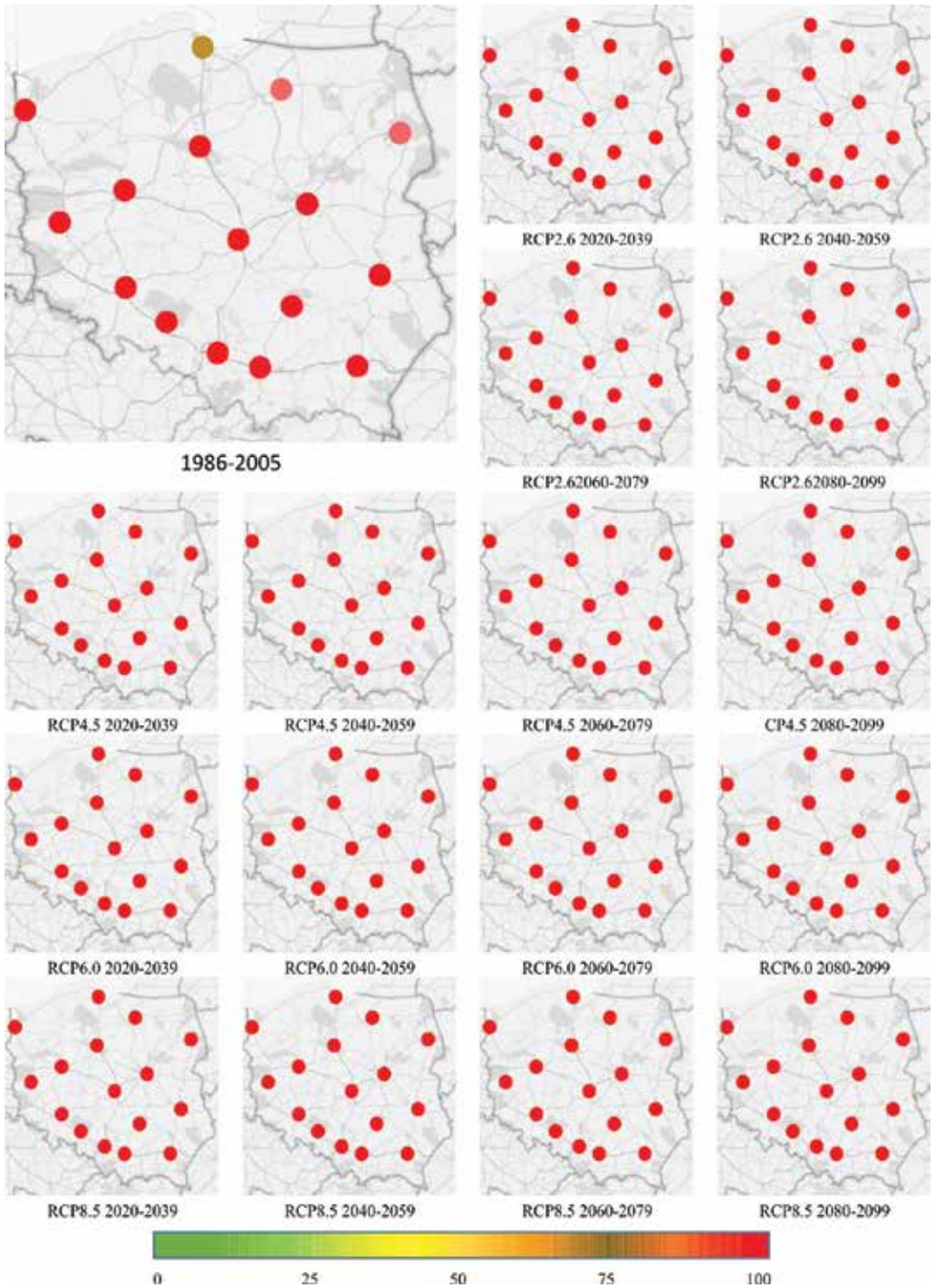

Figure 3. Effects of climate change on probability of the appearance of CPB first generation.

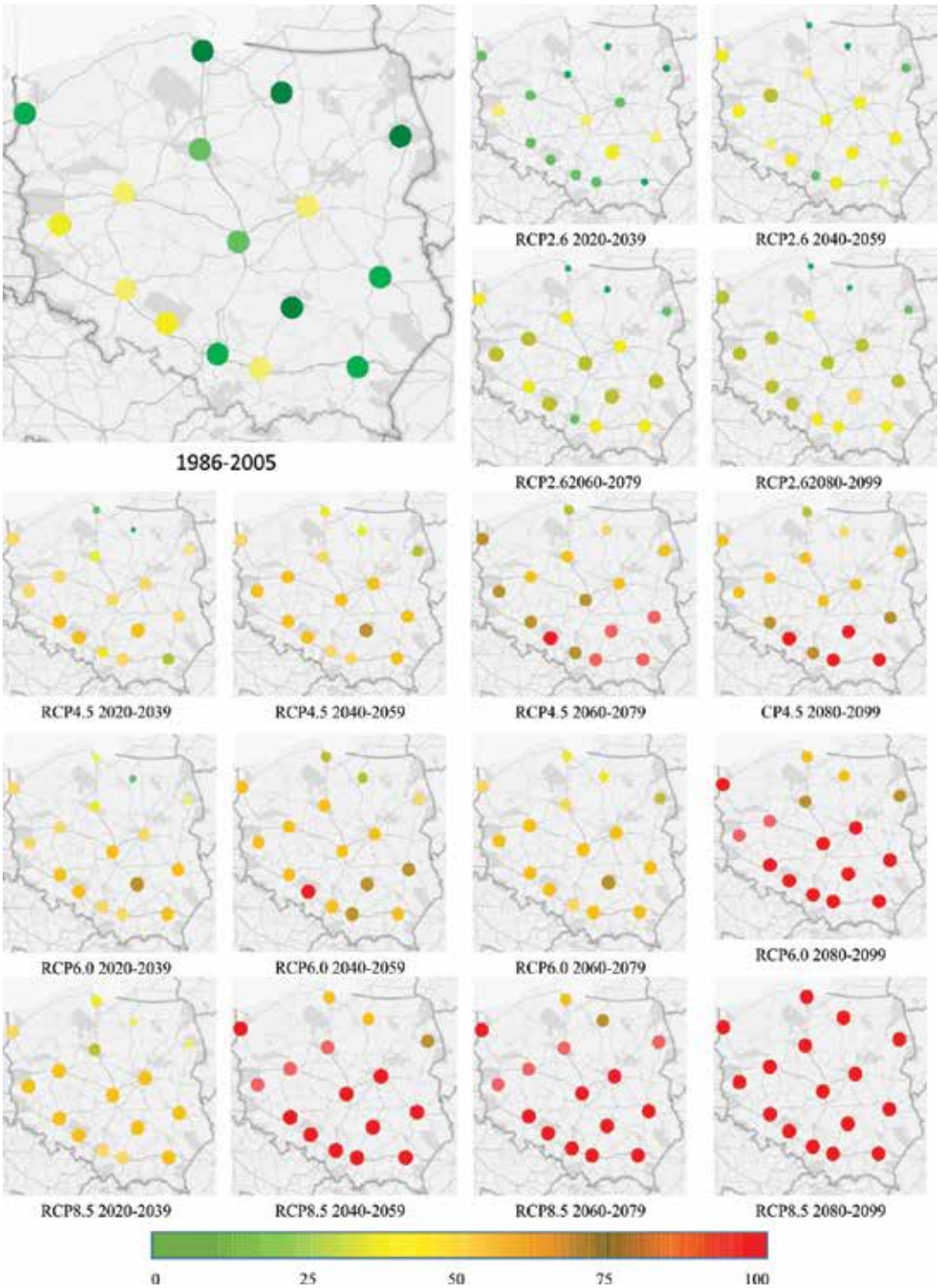

Figure 4. Effects of climate change on probability of the appearance of CPB second generation.

of the highest threatened localities were noticed. ZielonaGóra and Wrocław were replaced mostly by Lublin and Kielce.

Of the four scenarios (RCP2.6, RCP4.5, RCP6.0 and RCP8.5), three (RCP4.5, RCP6.0 and RCP8.5) generated a significant increase in the probability of CPB second generation appearance at the end of the century. But, in the periods 2020–2039, 2040–2059 and 2060–2079, increases in the probability of the second generation pest occurrence were generated under all four scenarios.

For the end of the century, the highest probability of the appearance of the second generation of CPB (99.5–100%) was obtained under scenario RCP8.5, whereas the smallest (5.5–50.3) was under RCP2.6. Scenarios RCP4.5 and RCP6.0 generated the following results, respectively: 60.3–97–3% and 79–100%.

Simulation under scenarios RCP2.6, RCP4.5 and RCP6.0 revealed that for the end of the century, the highest increase in probability (SGCPBP) were generated respectively for Kielce (42.3, 88.5, 92), Lublin (34, 84.8, 92) and Białystok (12.5, 70.3, 86.3), whereas under scenario RCP8.5, these were for Gdańsk (99.5) Olsztyn (96.8) and Białystok (94). The smallest increase in SGCPBP obtained in simulations under scenarios RCP4.5, RCP6.0 and RCP8.5 were, respectively, revealed for Opole (35.3, 37.3, 38), Wrocław (41.5, 52, 53.8) and ZielonaGóra (34.3, 52, 55.3). In simulations under scenario RCP2.6, besides an increase in SGCPBP, a decrease was also achieved. The highest decrease was generated for Opole (−27.5), Wrocław (−17.8) and ZielonaGóra (−12).

Simulations under scenario RCP2.6 produced 29 results with SGCPBP lower than 50%, 34 with SGCPBP ranging from 50 to 75% and one higher than 75%. The use of scenarios RCP4.5 and RCP6.0 resulted, respectively, in two and three results with SGCPBP lower than 50%, 18 and 17 results with SGCPBP ranged from 50 to 75% and 43 results higher than 75%. Simulations under RCP8.5 produced 10 results with SGCPBP ranging from 50 to 75% and 54 results higher than 75%.

Simulations also showed that the average number of days needed for completion of the second generation was 51–54 for RCP2.6, 47–52 for RCP4.5, 44–51 for RCP6.0 and 39–51 for RCP8.5.

Simulations on real data sets revealed no possibility of the third generation of CPB appearance at all analyzed localities, except for Opole (**Figure 5**). Introduction of transformed data did not change the results very much, except for scenarios RCP6.0 (period 2080–2099) and RCP8.5 (periods 2060–2079, 2080–2099).

The results of the simulations were also used to estimate the minimum temperature increase for 1986–2005 that ensure the emergence of the second generation of CPB. To achieve that aim, exponential models for 16 localities were developed (**Table 4**).

With a 95% probability, 1°C temperature rise at Opole guaranteed the appearance of CPB second generation (**Figure 6**). At Katowice, Rzeszów, Szczecin and Wrocław, temperatures ought to increase by 1.6°C. At Kraków and Toruń, 1.7 and 1.8°C temperature rises, respectively, led to the appearance of the pest second generation. At Łódź, Poznan, Warszawa and ZielonaGóra, temperatures ought to rise by 1.9°C. A temperature increase of 2.1 and 2.3°C

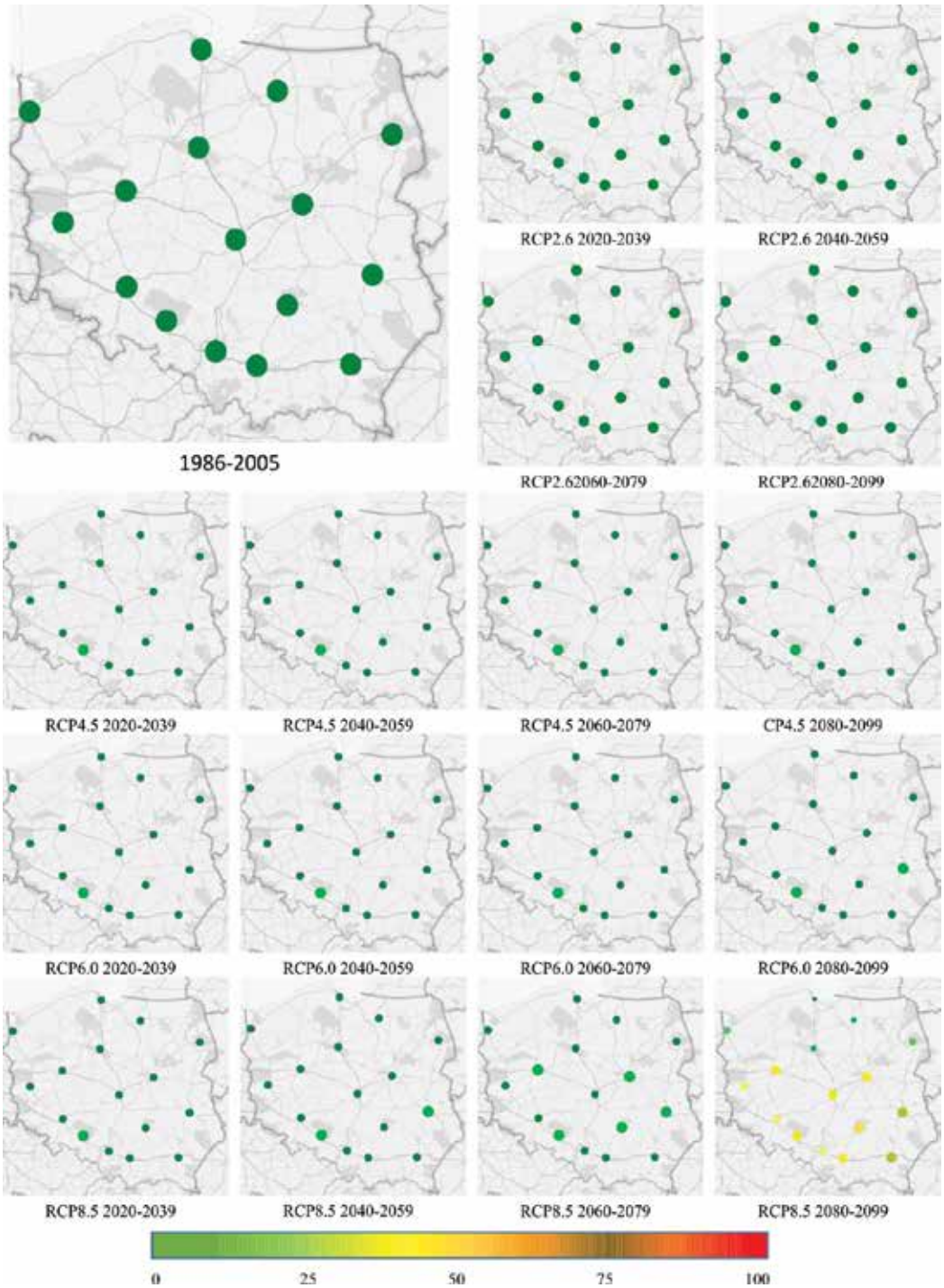

Figure 5. Effects of climate change on probability of the appearance of CPB third generation.

http://dx.doi.org/10.5772/intechopen.70777

Locality	a	b	c	R^2
Białystok	1,014298	2,992209	1,923994	0,96
Gdańsk	0,928751	3,029688	2,179541	0,97
Katowice	1,015548	1,612499	2,540181	0,98
Kielce	1,029395	2,297799	1,934486	0,99
Kraków	1,033059	0,646070	1,892346	0,98
Lublin	1,028426	1,560441	1,933356	0,97
Łódź	1,051250	0,956814	1,696449	0,97
Olsztyn	0,968657	2,817530	2,298582	0,93
Opole	1,021316	-0,340691	2,289298	0,97
Poznań	1,043817	0,412160	1,468694	0,98
Rzeszów	1,033329	1,023897	2,295942	0,98
Szczecin	1,031734	0,880774	2,092321	0,95
Toruń	1,010716	0,341909	1,814728	0,96
Warszawa	0,997326	1,310552	2,273030	0,95
Wrocław	1,032427	0,079441	1,675169	0,89
Zielona Góra	1,058929	0,475526	1,437996	0,97

Table 4. Parameters of the exponential models [SGCPBP = a + exp.(b + c × T)] expressing the influence of temperature increase (Ti) on probability of the appearance of the second generation of CPB (SGCPBP).

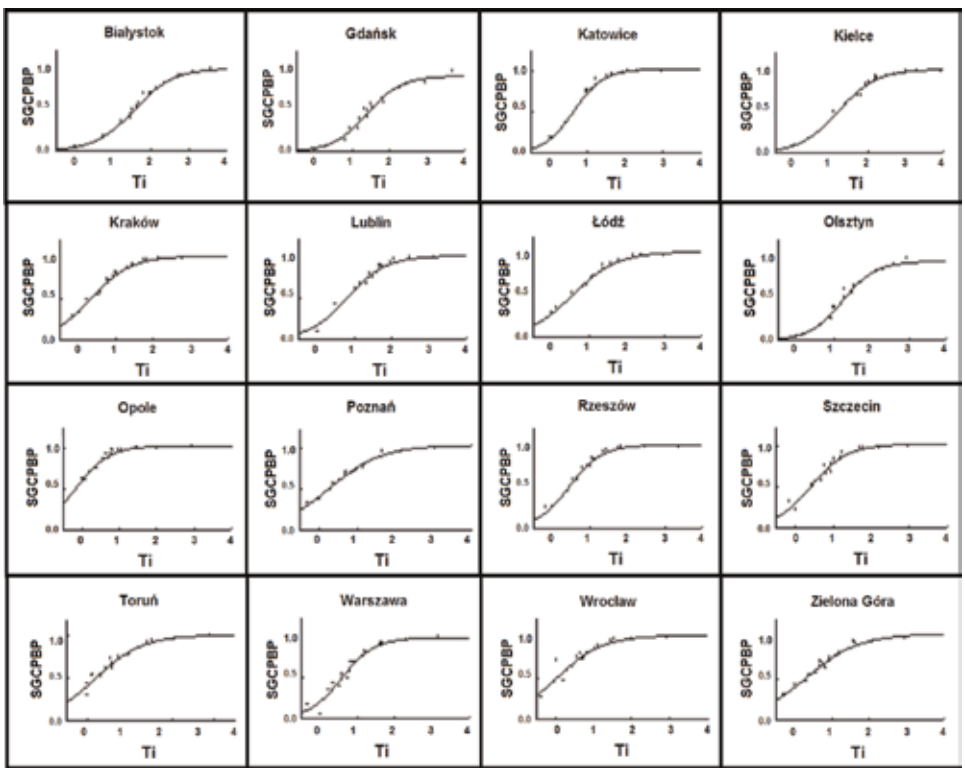

Figure 6. Effects of temperature increase on probability of the appearance of the second generation of CPB.

generated the appearance of the second pest generation at Lublin and Białystok, respectively. At Kielce and Olsztyn, temperature rises of 2.5 and 3°C were needed to trigger the appearance of the second generation, whereas at Gdańsk, the probability of the appearance of the second generation did not exceed 93%.

With a probability of 99%, a temperature rise of 1.4°C generated the second generation of CPB at Opole. At Katowice and Rzeszów, temperature had to increase by 1.9°C. At Kraków, Szczecin and Wrocław, a 2°C temperature rise triggered the appearance of the pest second generation. At ZielonaGóra and Łódź temperature had to rise by 2.2°C. A temperature increase of 2.3, 2.4 and 2.5°C generated the appearance of the second pest generation at Poznań, Toruń and Lublin. At Kielce and Białystok, temperature rises of 2.9 and 3.2°C were needed to trigger the appearance of the second generation, whereas for Gdańsk and Olsztyn, the models did not generate the appearance of the second generation with a probability of 99%.

4. Discussion

Results obtained in the present study are in line with our earlier prediction of CPB development under climate change in the Wielkopolska region, located in the western part of Poland [15]. In that study, two CPB generations were generated following a temperature increase of around 2°C for the Wielkopolska region by a model developed on simulation results obtained using meteorological data registered at WinnaGóra, located 60 km south of Poznań, and data obtained after transformation of the recorded data to reflect a temperature increase of 1–6°C. Similar results are presented in the present study for Poznań, where two generations of CPB are expected with a probability of 0.95 and 0.99 following a temperature increase of 1.9 and 2.3°C, respectively. Comparison of the results of 16 models describing the influence of temperature rises on SGCPBP indicated that thermal conditions in south-western Poland are most similar to those that guarantee the appearance of two generations. On the other hand, an increase in the number of CPB generations in north-eastern Poland required a much higher temperature rise. The main advantage of the current study over the study published in 2013 is not only the increase in the number of localities but also the inclusion of four RCP scenarios, which enable an assessment of regional variations in climate change.

Simulation results produced with the use of RCP scenarios in the present study show that an increase in the number of CPB generations is very likely to appear in Poland. Under three (RCP4.5, RCP6 and RCP8.5) out of the four analyzed scenarios, average SGCPBP calculated for 16 localities is going to exceed 75% after 2039. The only scenario which shows that, at the end of the century, the situation will not differ very much from that observed in 1986–2005 is RCP2.6. However, even under that scenario, simulations showed a shortening of the CPB first generation development time, especially in the northern (Gdańsk), north-eastern (Olsztyn, Białystok), eastern (Lublin) and southern parts of central Poland (Kielce). The same regions were indicated as being the most vulnerable to shortening of the CPB first generation development from simulation results under scenarios RCP4.5, RCP6 and RCP8.5. Moreover, analysis

http://dx.doi.org/10.5772/intechopen.70777

of the pest development under scenarios RCP4.5, RCP6 and RCP8.5 showed that the second generation usually developed faster than the first generation. We decided not to compare the development time of the generations obtained in simulations on real data and under scenario RCP2.6 because of the excessive number of SGCPBP results lower than 50%.

As expected, the lowest values for SGCPBP were generated under scenario RCP2.6, whereas the highest were noted under RCP8.5. The SGCPBP values produced by the former are usually lower than 75%. That is why we did not use it to specify the regions to be threatened by CPB in the future. The values for SGCPBP produced by RCP8.5 are usually higher than 75%, but there are almost no differences in this analyzed parameter between localities, so this scenario was also not used for specification of the regions at most risk from CPB in the future. On the other hand, scenarios RCP4.5 and RCP6 can be helpful in identifying regions at risk from CPB in the future. Both produced quite differential results, usually higher than 75%. Comparison of simulation results obtained under scenario RCP4.5 enables identification of the south-western region (Opole, Wrocław), the south-eastern (Rzeszów), eastern (Lublin) and southern parts of central Poland (Kielce), as being the most threatened by CPB in the future. Simulations under scenario RCP6.0 additionally included the south of Poland (Katowice) as one of the region most at risk of CPB.

Comparison of SGCPBP obtained in simulations on real and transformed data also enables identification of the regions vulnerable to higher changes in SGCPBP. Based on scenarios RCP2.6, RCP4.5 and RCP6.0, the south of central Poland (Kielce) and the eastern part of Poland (Lublin) should be included into that category. The predicted increase in SGCPBP obtained in simulations under these scenarios for these two localities distinctly differs from the rest. According to simulation results from scenario RCP8.5, it appears that besides these two localities, another three (Białystok, Gdańsk and Olsztyn) are more vulnerable to increase in SGCPBP than other localities.

Considering the results of the study dealing with the risk of CPB third generation appearance, it seems that the south-western region (Opole), eastern region (Lublin) and southern part of central Poland (Kielce) may face the most problems caused by increased numbers of CPB generations.

Results obtained in the present study are also in line with predictions of CPB development under expected climate change in the Czech Republic presented by Kocmankowa et al. [28], who used a simulation performed with the use of the CLIMEX model to show a growing danger of an increase in the number of CPB generations. This is in line with the predictions by Menéndez [29], Das et al. [30] and Sangle et al. [31], who expected greater numbers of generation of so-called "stop and go" insects following climate change. Kocmankowa et al. [28] also predicted a widening of the area of CPB occurrence and a shifting of the pest to higher altitudes. The significant increase in SGCPBP in the Małopolska upland located in the southern part of central Poland (Kielce) showed in our study confirms the findings of Kocmankowa et al. [28]. Similar results were also presented by Pulatov et al. [32], who analyzed the effect of climate change on the potential spread of the Colorado potato beetle in Scandinavia. They showed a substantial increase in the frequency of years in which the temperature requirement for development of one generation was fulfilled. Additionally, they indicated regions where two generations per year may occur more often.

The results of the simulations performed by Žalud et al. [33] for middle Europe, including Poland, also show an increase in the number of CPB generations per year based on the temperature increase predicted by various scenarios. Possible increases in the number of CPB generations are also expected in some areas of Russia by Popova [34], who used cartographic modeling to show that this phenomenon is caused by an increase in the sum of effective air temperatures in those territories.

The differences in the effect of climate change on CPB development between regions shown in the present study are also in consistent with the findings of Wittchen and Freyer [35], who analyzed the impact of temperature increases at two localities in Germany (Potsdam and Ulm) on the appearance of subsequent developmental stages of CPB. Simulations performed under real temperatures generated faster development of CPB first generation at Potsdam (30 m a. s. l.) than at Ulm (470 m a. s. l.) (by 5 days). But, under increased temperatures, CPB developed faster at Ulm than at Potsdam (by 3 days). A similar situation was noticed while comparing the development of CPB in Poland at Toruń (46 m. a. s. l.) and Kielce (270 m. a. s. l.). Using real data, development of CPB first generation at Toruń was 5 days shorter than at Kielce, while under transformed data and depending on the RCP scenario at Toruń development was 2–3 days longer than at Kielce.

The results of this study clearly indicate not only increased rates of CPB development following expected climate change across Poland, but also the regions exposed to the most rapid changes in the number of pest generations. But, one has to be aware that the interaction among the environmental factors is very complex and changeable. Most insect pests can adapt to a wide range of environments through selection and evolution. Therefore, prediction based on factor-limited simulations produce results with limited accuracy. On the other hand, only simulations can aid a rapid investigation of the effects of a change in a real life situation that will take place in the future over several years. From simulation results, a problem expected in the future can be mitigated now. So, systematic monitoring of potato crops in the regions indicated in the present study as the most threatened by the appearance of additional generations of CPB should be our first priority. Knowledge about pest trends gained in simulations coupled with results of field monitoring allows the determination of the feasibility of using certain pest management strategies.

Author details

Andrzej Wójtowicz[1]*, Marek Wójtowicz[2], Maciej Zacharczuk[3], Henryk Ratajkiewicz[4] and Maria Pasternak[1]

*Address all correspondence to: a.wojtowicz@iorpib.poznan.pl

1 Institute of Plant Protection – National Research Institute, Poznań, Poland

2 Plant Breeding and Acclimatization Institute, Research Division in Poznań, Poznań, Poland

3 Regional Agricultural Advisory Center, Poznań, Poland

4 Poznań University of Life Sciences, Poznań, Poland

References

[1] Li C, Liu H, Huang F, Cheng DF, Wang JJ, Zhang YH, Sun JR, Guo WC. Effect of temperature on the occurrence and distribution of colorado potato beetle (Coleoptera: Chrysomelidae) in China. Environmental Entomology. 2014;**43**:511-519. DOI: 10.1603/EN13317

[2] Ferro DN, Logan JA, Voss RH, Elkinton JS. Colorado potato beetle (Coleoptera: Chrysomelidae) temperature-dependent growth and feeding rates. Environmental Entomology. 1985;**14**:343-348

[3] Hsiao, T. H. 1985. Ecophysiological and genetic aspects of geographic variations of the Colorado potato beetle, p. 63-77. In D. N. Ferro and R. H. Voss (ed.), Proceedings of Symposium on the Colorado Potato Beetle, XVIIth International Congress of Entomology. Agricultural Experiment Station Research Bulletin 704. University of Massachusetts, Amhers

[4] Jolivet P. The Colorado beetle menaces Asia (*Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae). L'Entomologiste. 1991;**47**:29-48

[5] Malekmohammadi M., Galehdari H. Target site insensitivity mutations in the AChE 15 enzyme confer resistance to organophosphorous insecticidesin Leptinotarsa decemlineata (SAY), Pesticide Bio16 chemistry and Physiology. 2015; **126**:85-91. DOI: http://dx.doi.org/10.1016/j.pestbp.2015.08.002

[6] Vlasova VA. A prediction of the distribution of Colorado beetle in the Asiatic territory of the USSR. ZaschitaRastenii. 1978;**6**:44-45

[7] Worner SP. Ecoclimatic assessment of potential establishment of exotic pests. Journal of Economic Entomology. 1988;**81**:973-983

[8] Węgorek W. The Colorado potato beetle (*Leptinotarsa decemlineata* Say). Prace Naukowe Instytutu Ochrony Roślin. 1959;**1**:1-105

[9] Kałmuk J., Pawłowski J: Księga gatunków obcych inwazyjnych w faunie Polski, 2008. Available from: http://www.iop.krakow.pl/gatunkiobce/default6db7.html?nazwa=opis&id=60&je=pl [Accessed: June 15, 2017]

[10] Mrówczyński M. Występowanie i zwalczanie stonki ziemniaczanej (*Leptinotarsa decemlineata* Say) w latach 1978–1999. Progress in Plant Protection/Postępy w Ochronie Roślin. 2000;**40** (1):292-299

[11] Pawińska M, Mrówczyński M. Występowanie i zwalczanie stonkiziemniaczanej (*Leptinotarsa decemlineata* Say) w latach 1978–1999. Progress in Plant Protection/Postępy w Ochronie Roślin. 2000;**40**(1):292-299

[12] Węgorek P, Stachecki S. Ryzyko wystąpienia odporności agrofagów na środki ochrony roślin. In: Proceedings of the Conference: Integrowana ochrona ziemniaka przed chorobami, szkodnikami i chwastami; Poland Poznań. September 1999. p. 83-92

[13] Wójtowicz A, Jörg E. Prognozowanie rozwoju stonki ziemniaczanej za pomocą program ukomputerowego SIMLEP. Progress in Plant Protection/Postępy w Ochronie Roślin. 2004;**44**(1):538-541

[14] Gugała M, Zarzecka K. Porównanie skuteczności insektycydów w zwalczaniu stonki ziemniaczanej. Biuletyn instytutu hodowli i aklimatyzacji roślin. 2007;**246**:151-157

[15] Wójtowicz A, Wójtowicz M, Sigvald R: Forecasting the influence of temperature increase on the development of the Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] in the Wielkopolska region of Poland. Acta Agriculturae Scandinavica Section B Soil and Plant Science, 2013;**2**:136-146. DOI: http://dx.doi.org/10.1080/09064710.2012.733021

[16] Cubasch U, Wuebbles D, Chen D, et al. In: Climate Change 2013: The Physical Science Basis. Introduction. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013:119-58

[17] Van Vuuren DP, Den Elzen MGJ, Lucas PL, Eickhout B, Strengers BJ van Ruijven B Wonink S van Houdt R: Stabilizing greenhouse gas concentrations at low levels: An assessment of reduction strategies and costs. Climatic Change. 2007;**81**:119-159

[18] Clarke LE, Edmonds JA, Jacoby HD, Pitcher H, Reilly JM, Richels R. Scenarios of Greenhouse Gas Emissions and Atmospheric Concentrations Sub-Report 2.1a of Synthesis and Assessment Product 2.1. Washington DC: Climate Change Science Program and the Subcommittee on Global Change Research; 2007

[19] Smith SJ, TML W. MultiGas forcing stabilization with minicam. The Energy Journal. 2006;**3**:373-392

[20] Wise M, Calvin K, Thomson A, Clarke L, Bond-Lamberty B, Sands R, Smith SJ, Janetos A, Edmonds J. Implications of limiting CO_2 concentrations for land use and energy. Science. 2009;**324**:1183-1186

[21] Fujino J, Nair R, Kainuma M, Masui T, Matsuoka Y. Multigas mitigation analysis on stabilization scenarios using aim global model. The Energy Journal. 2006;**3**:343-354

[22] Hijioka Y, Matsuoka Y, Nishimoto H, Masui T, Kainuma M. Global GHG emission scenarios under GHG concentration stabilization targets. Journal of Global Environment Engineering. 2008;**13**:97-108

[23] Riahi K, Krey V, Rao S, Chirkov V, Fischer G, Kolp P, Kindermann G, Nakicenovic N, Rafai P. RCP-8.5: Exploring the consequence of high emission trajectories. Climatic Change. 2011;**109**:33-57. DOI: 10.1007/s10584-011-0149-y

[24] Taylor KE. Summarizing multiple aspects of model performance in a single diagram. Journal of Geophysical Research. 2001;**106**:7183-7192

[25] Łarczenko K. Długość rozwoju stonki ziemniaczanej w zależności od temperatury. Roczniki Nauk Rolniczych. 1958;**78**(1):27-42

[26] Alyokhin AV, Ferro DN. Modifications in dispersal and oviposition of Bt-resistant and Bt-susceptible Colorado potato beetles as a result of exposure to *Bacillus thuringiensis* subsp. tenebrionis Cry3A toxin. Entomologia Experimentalis et Applicata. 1999;**90**:93-101

[27] Tauber M, Tauber C, Obrycki J, Gollands B, Wright R. Voltinism and the induction of aestival diapause in the colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America. 1988;**81**:748-754

[28] Kocmankova E, Trnka M, Eitzinger J, Formayer H, Dubrovsk M, Semeradova D, Žalud Z, Juroch J, Mozny M. Estimating the impact of climate change on the occurrence of selected pests in the central European region. Climate Research. 2010;**44**:95-105

[29] Menéndez R. How are insects responding to global warming. Tijdschrift voor Entomologie. 2007;**150**:355-365

[30] Das DK, Singh J, Vennila S. Emerging crop pest scenario under the impact of climate change – A brief review. Journal of Agricultural Physics. 2011;**11**:13-20

[31] Sangle PM, Satpute SB, Khan FS, Rode NS. Impact of climate change on insects, Trends in Biosciences. 2015;**8**(14):3579-3582. Print:ISSN 0974-8

[32] Pulatov B, Hall K, Linderson ML, Jönsson AM. Effect of climate change on the potential spread of the Colorado potato beetle in Scandinavia: An ensemble approach. Climate Research. 2014;**62**:15-24. DOI: 10.3354/cr01259

[33] Zdeněk Žalud, Miroslav Trnka, Petr Hlavinka, Martin Dubrovský, Eva Svobodová, Daniela Semerádová, Lenka Bartošová, Jan Balek, Josef Eitzinger and Martin Možny (2011). Climate Change Impacts on Czech Agriculture, Climate Change - Socioeconomic Effects, Dr Houshan Kheradmand (Ed.), InTech, Rijeka, Croatia, DOI: 10.5772/23966. Available from: https://www.intechopen.com/books/climate-change-socioeconomic-effects/climate-change-impacts-on-czech-agriculture

[34] Popova. The influence of climatic changes on range expansion and phenology of the Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera, Chrysomelidae) in the Territory of Russia. Entomological Review. 2014;**94**:643-653

[35] Wittchen U, Freier B. Nutzung von Datenreihen und Simulationsmodellen zur Abschätzung des Einflusses des Klimawandels auf die Befallsentwicklung von Schadorganismen in landwirtschaftlichen Pflanzenbeständen. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes. 2008;**60**(7):157-162

Chapter 5

Management of Late Blight of Potato

Mehi Lal, Sanjeev Sharma, Saurabh Yadav and Santosh Kumar

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72472

Abstract

Potato (*Solanum tuberosum* L.) is the most important crop and *Phytophthora infestans* (Mont.) de Bary is the oomycete, which was responsible for infamous Irish potato famine during 1843–45 and it continues to cause worldwide devastation of the potato. Moreover, this disease is re-emerging in the forms of different genotypes and causes huge yield loss in the potato crop. The factors which are responsible for huge yield loss of potato are applied improper management strategies and pathogen behavior. Management strategies includes; forecasting, cultural, biological, varietal and chemical management. Forecasting is the better option for management of late blight, if accurately forecasted and promptly information reaches to the end users. As infected potato tubers cause the primary sources of infection in next season. The cultural practices will also helpful in reducing inoculum load and managing the disease. The host resistance is best option for management of this disease. However, due to very divers' virulence nature of *P. infestans;* the resistance of the varieties is wiped out within a decade. Several fungicides including contact, systemic and translaminar have been evaluated from time to time; however, the pathogen has shown a remarkable capacity for change with respect to host genotype and fungicides. Nowadays biological control is gaining importance due to its eco-friendly in nature.

Keywords: potato, late blight, disease, management, fungicides, *Phytophthora*

1. Introduction

The Potato was originated in the hills of Andes and Bolivia in South America, subsequently it was introduced into Europe by Spaniards in the second half of the 16th century, from there it spread throughout Europe and rest of the world in the mid 17th to mid of 18th century. In Asia, particularly in India, it was introduced by Portuguese in 17th century [1]. The late blight fungus co-evolved with potato in Central and South America and subsequently spread

to other parts of the world mainly through infected seed tubers. The late blight disease caused by oomycete, which was initially reported as *Botrytis infestans* in 1845 by C. Montagne, later on German scientist Anton de Bary renamed as *Phytophthora infestans* (Mont.) de Bary [2]. The entire potato crop across Europe, especially in Ireland, was killed prematurely during 1844–45; leading to worst ever famine the 'Irish Potato Famine' [3]. One million people died of starvation due to that famine and another million migrated to the USA and other parts of the world.

The late blight disease was recorded in India for the first time between 1870 and 1880 in the Nilgiri hills [4]. Under subtropical plains particularly in eastern part of the India, it was first observed in 1898–1900 in Hooghly district of West Bengal [5]. In the northern part, it appeared for the first time in 1883 in Darjeeling and subsequently spread rapidly to other adjoining hills [6]. The late blight disease was observed in Khasi hills (North-eastern Region) in 1885, Kumaon hills in 1897 and in Shimla hills (North-western Region) in 1902 [5, 7]. During 1913, it appeared at several places in Assam and Bihar [6, 8–11]. In plains of Uttar Pradesh, it was reported for the first time in 1943 in Dehradun and Meerut [10]. Severe attack of the late blight was observed in Meerut district in 1949, 1950 and 1951 and subsequently in many other districts of Uttar Pradesh [12]. In Punjab, the disease was occurred annually from 1958 to 1963 except during 1961 [13]. Potatoes had been grown in Mahabaleshwar hills and other parts of Maharashtra but late blight was observed there only in 1973 [14]. In Gujarat and Madhya Pradesh, the disease was observed in traces in 1968 and in Rajasthan in 1958 [12]. Afterwards, appearance of late blight disease is regular feature with high disease severity in hill areas while in plains disease severity is moderate to high level.

2. Crop losses

Phytophthora infestans causes late blight diseases in potato and tomato crops worldwide. It is not cause only economic losses of yield but also the quality and quantity of the crop. It is a highly researchable pathogen in plant diseases. The worldwide late blight disease is re-emerging, therefore this disease is constantly observed by the late blight researchers [15]. The late blight disease is considered emerging disease, it is not only having important in global crop production, but also pose severe risks on a local level, especially on small farms in developing countries [16]. The losses caused by late blight disease, it varied countries to countries, as per their adopted plant protections measures and grown cultivars. The yield losses due to late blight of potato were reported up to 50–70% during the 2007 under favorable environmental condition in Pakistan [17]; however recently Ahmed et al. [18] reported that late blight can induce 100% yield loss under epidemic condition in Pakistan. As far as Indian scenario is concerned, reduction in potato production due to late blight ranged between 5 and 90% depending upon climatic conditions, with an average of 15% across the country [19]. However, recently yield loss was reported, overall basis a range of 10–20% due to late blight in the year 2013–2014 major potato growing sites of the India viz., Uttar Pradesh, West Bengal, Punjab, Karnataka and Uttarakhand [20]. Whenever, disease appeared in epiphytotic form at early stage of the crop yield loss would be more. Tuber yield decline was significantly higher in unmanaged crop, which could go as high as 90% of total productivity in hilly regions. The changing climate pattern is being influenced appearance of late blight as it is occurring every

year in plain region with moderate to high disease severity. Variations in disease severity are mainly due to climatic factors i.e. rainfall, relative humidity, temperature and pathogen virulence. In Punjab (main potato growing belt), severe epidemics of late blight disease have appeared during 1985–1986, 1989–1990, 1992–1993 and 2006–2007 [21]. In 2006–07, average crop loss of 22% in productivity resulting in a net loss of around 0.16 mt of potato in the state of Punjab alone. The increase in disease severity could be due to a change in the pathogen population [22]. The varying degree of crop losses was also reported due to late blight from Punjab, Haryana, UP, Maharashtra Karnataka, Bihar and West Bengal [23]. The decline in productivity and yield of potato was in between 25 and 85% due to late blight, depending mainly on degree of susceptibility of the host plant [24]. The economic costs associated with late blight to be somewhere around US $3–5 billion per year was estimated by several authors [25, 26]. A method had been used to conservative estimate costs of late blight and it was observed that lowest yields mainly in developing countries and previous eastern block countries which suffered over €10 billion per annum at least, whereas in developed countries with high yields (7.5% of global potato production) suffered damage of about €1 billion per year [27].

3. Symptomatology

The late blight disease affects all plant parts especially leaves, stem and tubers.

3.1. Leaves

Pale green water soaked spots (2–10 mm) appear mostly on the margin and tips. In moist weather, spots may appear anywhere on the leaves, enlarge rapidly and turn necrotic and black killing the entire leaf instantly. On the corresponding lower side, whitish cottony growth containing millions of sporangia forms around the dead area in a ring pattern (**Figure 1**).

3.2. Stem and petiole

Light brown lesions develop which elongates and encircles the stem and petioles breaking them and killing the plant/leaves instantly. Stem infection is more severe under high temperature and relative humidity conditions (**Figure 2**). Symptoms of stem blight are observed more in last ten years.

3.3. Tubers

Rusty brown discoloration of the flesh is the typical symptom of late blight (**Figure 3**). On outside tuber surface, hard depressions with purplish tinge on the sides are a common feature. Normally, late blight infected tubers are hard but associated secondary pathogens may set in soft rot symptoms.

3.4. Field infection

Generally, late blight appears on lower most leaves of the plant which goes unnoticed from a distance. Slowly, the disease spreads to the middle and then upper leaves. Subsequently it

Figure 1. Whitish cottony growth on the lower surface of leaf.

Figure 2. Late blight symptom on potato stem.

http://dx.doi.org/10.5772/intechopen.72472

Figure 3. Late blight symptom on potato tubers.

Figure 4. Late blight affected potato field.

spreads whole plants and near of the plants. The disease spreads faster and the entire crop gets killed as if burnt by fire (**Figure 4**). The heavily infected field gives fetid odor which can be felt from a distance.

4. Disease epidemiology

The late blight infected tubers are the major sources to cause the infection. Moreover, refuse piles and volunteer plants also serve as primary source of disease particularly in the hilly

region. Wherever, both mating type is existed oospore formation take place and oospore also has the potential to cause and initiate the disease. The spores germinate and infect the exposed tubers. Although, some of the infected tubers get completely rotted by the time, crop is harvested but, still lot of tubers carry incipient infection, and escape in the cold store/country store where they remain dormant but alive. These tubers if used as seed, becomes the source of infection of the disease in the next crop season [28]. Sporangia are formed wide range of temperature (3 to 26°C) and optimum is 18–22°C. The sporangia are germinated by two ways process i.e. indirect and direct germination. It depends mainly on temperature. Indirect germination generally occurs at temperatures of 6 to 15°C (optimum 12°C) by means of sporangia produces zoospores. Direct germination takes place under warm temperature and a range of 4 to 30°C (optimum 25°C). High relative humidity (>90%) is required for spore formation, germination and infection; whereas >80% is essential for lesions expansion. Extreme light is harmful for *P. infestans* and sometimes sporangia may be killed due to extreme light. Cloudy weather is favorable for late blight. The cool (12–15°C) and high humidity (>90%) weather with heavy dews or rains alternating with warm (18–20°C) moist period favor for rapid development of disease. Infection and disease development is observed a range of 7.2–26.6°C [29].

5. Management

Several management strategies have been developed for late blight of potato and adopted by the farmers/potato growers as per availability of the resources. Amongst them chemicals, host resistant, biological control, cultural control are discussed below:

5.1. Chemical management

Chemical management is very popular strategy for the management of late blight. Since the discovery of Bordeaux mixture in 1885 and it was first important landmark in the history of chemical disease control. Bordeaux mixture belongs to first generation of fungicides along with other inorganic chemicals. After more than 130 years, the introduction of Bordeaux mixture (Copper sulfate, hydrated lime and water), large numbers of fungicides (first generation Bordeaux mixture to fourth generation Mandipropamid & Azoxystrobin) were evaluated at worldwide against late blight of potato/tomato. In practice, the traditional management of late blight depends highly on preventative fungicides, application on a regular calendar basis (e.g. weekly) during the growing season [30]. The population diversity and disease incidence of *P. infestans* has been increased through the development of systemic fungicide resistance (insensitivity) and the transcontinental shipment of the late blight infected potato tubers and tomato plantlets [31]. Metalaxyl fungicide which comes under Phenylamide group with FARC 4, was introduced against oomycetes, very effective for late blight management and highly adopted worldwide. However, after introduction within three years metalaxyl resistant isolates were detected on field grown potatoes in Ireland, The Netherlands and Switzerland [32]. The site-specific systemic fungicide, mefenoxam (the active isomer in metalaxyl), inhibits sporulation and mycelial growth inside host tissues by specifically inhibiting RNA polymerase-1, a mutation that changes the affinity of target sites could easily lead to fungicide resistance [33]. In Indian scenario, metalaxyl based fungicides were

introduced on experimental basis for management of late blight during late 1980's however, their commercial use started only during 1994–1995 [34]. In India, 200–400 ppm tolerance level was observed with metalaxyl. After 12 years, its introduction during 2006, the metalaxyl based fungicides failed to protect the potato crop from the late blight in temperate highlands leading to 40–70% crop losses. Systemic fungicide metalaxyl is cause of concern for management of late blight disease due to quickly developed resistance. Pathogen had developed.

Tolerance up to 400 ppm and genetic studies crosses indicated that a semi dominant major locus determines resistance to metalaxyl, since insensitive and sensitive parents usually yielded progeny with those phenotypes at a 1:1 ratio [35, 36]. The heterothallic single mating type isolates of *P. infestans* was exposed to 9 of the 11 commercial fungicide formulations for assess their effect on formation of oospores. The highest numbers of oospores were observed on media amended with Ridomil 2E (metalaxyl) and Ridomil Gold EC (mefenoxam) at 0.1 to 10 μg a.i./ml, when averaging it was found that 471 and 450 oospores/petri dish, respectively. The remaining fungicides viz., Maneb, Manzate (Mancozeb), Curzate (cymoxanil +mancozeb), and Acrobat MZ (dimethomorph + mancozeb) also induced oospore formation, which ranged from 0 to 200 oospores/petri at fungicide concentrations from 0.1 to 10 μg a.i./ml. No oospores were formed on media amended with Bravo (chlorothalonil) or Tattoo C (chlorothalonil + propamocarb HCl), moreover both the compounds completely suppressed growth of the isolates at 0.1 and 1 μg a.i./ml. The metalaxyl resistant isolates formed oospores in response to the fungicides more often than the metalaxyl sensitive isolates [37]. Metalaxyl + mancozeb (Ridomil MZ) and ofurace (Orafce 50WP) were reported to provide highly effective control of late blight [38]. The fenamidone is a novel fungicide, which acts on cytochrome bc1 in mitochondrial complex III of *P. infestans* at a number of points in its life cycle [39]. Cymoxanil based fungicides possess a novel mode of action by preventing electron transfer between cytochrome b and c1 in mitochondrial complex III and provide good scope for the control of late blight of potato and tomato [40, 41]. Efficacy of seven fungicides was tested under *in vitro* conditions and the fungicides, which showed promising results, were further evaluated under field conditions and fenamidone based fungicide was found most effective in controlling late blight followed by cymoxanil based while mancozeb was found least effective; similarly the systemic fungicides viz., fenamidone and dimethomorph were reported most effective *in vitro* for management of late blight [42, 43]. Various studies showed that a reduced use of fungicides lowers the selection pressure for mefenaxam-resistant strains and mixture with a contact fungicide improves efficacy and may slow the development of resistance to mefenoxam [44, 45]. The systemic fungicides have better persistence on the host surface and are being used as mixture with contact fungicides against late blight so as to avoid development of resistance in pathogen [46]. The fungicide mixtures, containing two or more fungicides with different modes of action, have been developed with the twin objectives of broadening the activity spectrum against diverse plant diseases and to check the development of resistance in the target pathogens [47]. In commercial production of potato is not viable without fungicides for management of late blight. Fungicide mixtures and targeted application based on late blight forecasting model are very important for managing late blight. However, due to delisting of many fungicides products under the EU Pesticide Directive and environmental concerns, provides impetus for potato breeding and more effective fungicide application [48]. It has been reported from European country that the same fungicide should not applied more than two sequential

applications [49]. The severe late blight can be effectively managed with prophylactic spray of mancozeb at 0.25% followed by cymoxanil+mancozeb or dimethomorph+mancozeb at 0.3% at the onset of disease and one more spray of mancozeb at 0.25% seven days after application of systemic fungicides in West Bengal [50]. Similarly, one spray of mancozeb followed by three spray of cymoxanil + mancozeb was effective on cv. Kufri Bahar under western UP [34]. Due to development of resistance to fungicides, a new fungicide, Victory 72 WP was first used in controlling late blight of potato and tomato in West Shoa of Ethiopia [51]. The late blight specific spray scheduling method and a method of scheduling sprays for both diseases (early and late blight) suppressed early and late blight as well as did weekly sprays (conventional methods) and with the same average number of applications as with weekly sprays [52]. The customarily, spray schedules were one prophylactic spray using contact fungicides followed by systemic fungicides and one more spray of either same contact or same systemic fungicides. A unique combination of treatments was developed keeping in view the sensitivity of *P. infestans* to develop fungicide resistance. The post spray (curative spray) of same mode of action fungicide was not taken. Prophylactic sprays of chlorothalonil/mancozeb followed by systemic/trans laminar fungicides were found effective than post symptom sprays. This will be useful to minimize the yield losses due to late blight and assist in reducing development of resistance against fungicides in pathogen [53]. The spray schedule of mancozeb 75% WP (0.2%- before appearance) followed by two more spray with mancozeb 75% WP (0.2%) + dimethomorph 50% WP (0.2%) at 7–10 days intervals showed less terminal disease severity (24.55%) with highest disease controlled (74.45%), which was at statistically par with treatment mancozeb 75% WP (0.2%, before appearance) followed by cymoxanil 8% + mancozeb 64% WP (0.3%) with two more spray at 7–10 days intervals, with 27.56% terminal disease severity along with disease controlled 71.29%. One spray of mancozeb (contact fungicides: before appearance) and latter two more sprays of translaminar/systemic + contact fungicides at 7–10 days interval give better results for managing late blight of potato [54]. The highest marginal benefit was achieved by applying first Ridomil then Dithane M-45 at 14–21 days interval. The lowest marginal benefit was with alone application of Ridomil at 21 day spray interval. At 7 days sprays was more economical to apply Dithane M-45 than Ridomil first followed by Dithane M-45 subsequently [55]. Twelve fungicides were evaluated on isolates of three identified clonal lineages (US-22, US-23, and US-24) of *P. infestans* using a detached tomato leaf assay in preventative and post-infection methods. The results revealed that these fungicides were suitable in conventional and organic systems, which can effectively control late blight caused by new clonal lineages of *P. infestans* when applied preventatively and late blight caused by the US-24 clonal lineage may require less fungicide than US-22 or US-23 to manage the disease [56]. The efficacy of Ametoctradin 27% + dimethomorph 20.27% (w/w) as a new molecule for management of late blight of potato was reported in India [57]. Initium (ametoctradin) is a new fungicide for management of *Phytophthora infestans*. It affects mitochondrial respiration inhibitor interfering with the complex III (complex bc1) in the electron transport chain of the pathogen, thus ATP synthesis in the fungal cells is inhibited. It is a non-systemic fungicide that remains primarily on the leaf surface where it is adsorbed with high affinity to the epicuticular wax layer of the epidermis [58]. Many oomycete-specific fungicides such as QoI compounds, dimethomorph, propamocarb, etc. [59] were commercialized, but currently, we are unaware of any fungicide that could effectively halt epidemics caused by metalaxyl-resistant strains under conditions favorable to *P. infestans* growth and development [60]. Isolates of *Phytophthora*

infestans showed 10-fold or more variation in baseline sensitivity to many fungicides including cymoxanil, dithiocarbamates, mandipropamid, and strobilurins [61–63]. Various substances other than fungicides also were tested for management of late blight of potato. Ammonium molybdate, cupric sulfate and potassium metabisulfate at 1 mM partially inhibited the growth and spore germination of *P. infestans*, whereas ferric chloride, ferrous ammonium sulfate and $ZnSO_4$ at 10 mM completely inhibited growth and spore germination [64]. The foliar spray of $ZnSO_4$ and $CuSO_4$ (0.2%) micronutrients, 12 days delayed the onset of late blight when used with host resistance, subsequently reduced disease severity with higher yield [65]. Sub-phytotoixc dose of boron with reduced rate of propineb + iprovalidicarb has been found more effective than treated with fungicides alone [66]. β-aminobutyric acid (BABA) has been known as an inducer of disease-resistance. However, only the R but not the S enantiomer of BABA primes for resistance. Unfortunately, BABA can also impose growth stress in some treated plants therefore BABA analogs with reduced stress effects are highly desirable for agricultural field [67]. Plant activator viz., BABA and phosphoric acid was evaluated against late blight by various researchers with combination of fungicides or alone [68–71]. A 20–25% reduction of the fungicide dose in combination with BABA gave on average the same result on late blight development as full dose Shirlan alone in field condition, while reduced dose of Shirlan alone sometimes resulted in less effective protection. However, *in vitro* results indicated that the efficacy was lasted for only 4–5 days after BABA treatment and subsequently efficacy was lowered. The partially resistant cultivars Ovatio and Superb reacted to lower concentrations of BABA where no effect was found in susceptible cv. Bintje [72]. Two SAR activators (BABA and phosphorous acid) were found effective against late blight of potato with significantly reduced disease severity (40–60%). The expression of the defense related genes and *P. infestans* effecter proteins β-1,3 glucanase, PR-1 protein, *phytophthora* inhibitor, protease inhibitor, xyloglucanase, thaumatin protein, steroid binding proteins, proline, endochitinase and cyclophilin genes were up regulated with the SAR activator treatment compared to unsprayed [73]. Since last one and half decades, various fungicides have been developed for management of late blight. Isolates of *P. infestans* might develop resistant over the period. Fungicides resistance with currently used fungicides, including dimethomorph, has been reported [74, 75]. There are three key phases in the development of fungicide resistance (i) emergence, (ii) selection, and (iii) adjustment. In emergence, the resistant strain has to arise through mutation and invasion whereas in selection, the resistant strain is present in the pathogen population and a small portion of the pathogen population carrying the resistance increases due to the selective pressure imposed by the fungicides. In case of adjustment phase, the resistant fraction of the pathogen population has become large, crop managers have to adjust fungicide programs, by changing the dose or active substance(s) used, in order to maintain control [76].

5.2. Biological control/eco-friendly management

Generally, management of late blight by eco-friendly means is a difficult task particularly when the level of disease pressure is high along with prevailing congenial environmental condition. However, due to negative impact of chemicals on environment as well as human health, nowaday's eco-friendly management is gaining more importance. Management of late blight through eco-friendly way, using botanicals has been initiated in European and American countries during the last years of 20th century [77, 78]. Out of 100 species in 54 plant families tested against

P. infestans, the leaf extracts from onions, garlic, *Malustoringo*, *Reynoutria japonica* and *Rheum coreanum* revealed positive inhibition of mycelial growth of *P. infestans*. *M. toringo* extracts strongly inhibited *P. infestans* and was effective in managing late blight also [79]. The effectiveness of some antifungal compound was reported against late blight from botanicals [80]. The antagonist *Bacillus subtilis* B5 was found effective in inhibiting the growth of *P. infestans* [81]. The efficacy of bacterial and fungal antagonist found effective as lowest average disease severity (27.89%) was recorded in treatment when *Bacillus subtilis* (B5–0.25%) + *Trichoderma viride* (TV-0.7%) was applied before disease appearance followed by cymoxanil 8% + mancozeb 64%WP (0.3%) at onset of late blight and one more spray of B5 + TV after 7 days [82]. The different isolates of *Trichoderma* were evaluated against *P. infestans* and found that *Trichoderma* isolates HNA 14 was most effective under both laboratory and field conditions and showed mycoparasitism against *P. infestans* when observed under scanning electron microscope [83], whereas *T. koningiopsis* and *T. asperellum* were effective against *P. infestans* under both laboratory and field conditions [84]. Rhamnolipid bacterial based formulation (0.25%) was tested under field trials at three different locations for managing late blight of potato. It was observed that the terminal disease severity in rhamnolipid formulation sprayed plot was 45% (against control plot 100%), 47.5% (against control plot 92.5%) and 59.2% (against control plot 76.64%) at Modipuram, Lawar (Meerut) and Jalandhar, respectively [85]. The some phyllospheric microorganisms *viz.*, yeasts *Sporobolomyces* spp., *Acetenobacter* spp., isolates of *Pseudomonas* spp. and *Bacillus* spp. were reported antagonistic to *P. infestans* [86, 87]. The *Bacillus* sp. inhibited mycelial growth of 7 plant pathogenic fungi *in vitro* and *in vivo* and the same bacterium protected tomato plants against *P. infestans* [88]. A bacterium (*Serratia* sp.), and 4 fungi (*Trichoderma* sp., *Fusarium* sp. and 2 *Penicillium* spp.) were tested against *P. infestans* on tomatoes under field conditions and found that *Penicillium* reduced the lesion area/plant between 8 and 40% [89]. One hundred twenty two microorganisms isolated from the phyllosphere of potatoes on the development of *P. infestans,* 23 effective microorganisms (spore-forming and non-spore-forming bacteria, yeasts and fungi) were tested in dual cultures and different patterns of inhibition of *P. infestans* were observed [90]. Various naturally occurring microorganisms, i.e., *Trichoderma viride*, *Penicillium virdicatum*, *P. aurantiogiseum, Chetomium brasilense* [91], *Acremonium strictum* [92], *Myrothecium varrucaria* and *Penicillium aurantiogriseum* [93] showed antagonistic effect against *P. infestans*. The antagonistic activities of *Pseudomonas fluorescens, Pseudomonas* sp., *Aspergillus flavus, A. niger, Penicillium* sp., *T. virens* and *T. harzianum* were tested *in vitro* conditions against *P. infestans, Fusarium sp.* and *Rhizoctonia solani.* All bio-agents inhibited the mycelial growth of the pathogens in comparison to control [94]. The defense enzymes viz., chitinase and β.1, 3-glucanase activities of *B. subtilis* and *T. harzianum* were well reported against late blight of potato and early and late blight of tomato [95, 96]. Forty-three bacteria were isolated from the phylloplane and rhizosphere of potato and canola plants, evaluated against *P. infestans* causing late blight on potato. It was reported that more than one system (*in vitro* culture media, detached leaves, and whole plants) should be used for selecting and identifying potential of bioagents [97]. A well-known group of microorganism used is the fluorescent *Pseudomonas* which excretes secondary metabolites including antibiotics and biosurfactants that are inhibitory to plant pathogens [98]. Naturally occurring surface active compounds derived from micro-organisms are called biosurfactants. These are amphiphilic biological compounds produced extra-cellularly as part of the cell membrane by a variety of bacteria, yeast and fungi [99]. Biosurfactants can be used as alternatives to chemical surfactants as their capability of reducing surface and interfacial tension with low toxicity,

high specificity and biodegradability make them important for inhibiting pathogens. The best antagonistic activity against *P. infestans* is observed in the genera of *Pseudomonas* and *Bacillus* as they produce wide range of antibiotics and biosurfactants and can be used as alternatives to chemical surfactants [100]. The metabolite of biosurfactant producing bacterium, *P. aeruginosa* has shown high efficacy against *P. infestans* under *in vitro* conditions [101]. Ninety five isolates of bacteria were evaluated for their biosurfactant as well as biocontrol activity against *P. infestans.* It is observed that only 15.8% isolates showed biosurfactant activity and only five isolates were found effective against *P. infestans* for biocontrol properties [102].

5.3. Cultural practices

The cultural practices, includes inoculum free seeds and planting materials, crop and field sanitation and adjustment of crop cultures. Cultural practices classified into three categories: i. Practices, which are usually applied for agriculture purposes not directly connected with crop protection, such as fertilization and irrigation. They may or may not have a positive or a negative side effect on disease incidence or severity, ii. Practices that are used completely for disease control, such as sanitation and flooding and iii. Practices, which are used for both agricultural purposes and for disease control, such as crop rotation, grafting and composting [103]. Late blight of potato can be managed up to some extent using cultural practices. The infected potato tubers are the primary source of inoculums for causing initial infection of late blight. Besides, areas wherever both mating type (A1 & A2) are co-existed, oospore formation takes place and a possibility to survive longer period in the soil and cause the infection from soil sources also. The oospores as soil-borne inoculums and its significant are determined by formation of oospore in plant tissue and their survival in soil. There is a clear cut correlation between crop rotation and early infections of late blight disease. Generally, infection starts early in fields which are not used for crop rotations. The decline in early infection was most pronounced in fields subjected to crop rotations for three or more years between the potato crops [104, 105]. It might be a reason that inoculums are less survived in non-crop rotation field than the crop rotated fields. It is clearly indicated that practices of crop rotation is an important aspects for reducing the risk of soil-borne infections of *P. infestans*. The date of potato planting is also useful to avoid the late blight of potato, especially by changing in planting dates. On average, planting in the last 10 days of September resulted in less severe late blight epidemics [106]. Mixed cropping, barrier crops and strip cropping are also helpful for reducing disease severity of potato late blight. Concept of mixed cropping and barrier crops were investigated for managing/delaying the spread and build up of late blight in western Uttar Pradesh at Meerut. Results revealed that spreads of the disease were delayed by 7 days by planting resistant cultivars in alternation with susceptible one whereas barrier crop (oat) delayed the spread of disease by 4 days [107]. Strip cropping of potatoes significantly reduced late blight severity in organic production when the crop was planted perpendicular to the wind neighbored by grass clover [108]. Control of contaminated sources such as infected tubers, volunteer plants, waste heaps, disease in neighboring fields and re-growth after haulms destruction can help in management of the disease [109]. It has been assessed that onset of epidemic can be delayed by 3 to 6 weeks if all primary infection from early potato can be eliminated. It has been shown that during most years late blight epidemics start from infected plants on dumps [110]. Covering of dumps with black plastic sheet throughout the season and preventing seed tubers from

becoming infected is an important step to reduce the primary inoculum [111]. Avoiding use of excess nitrogen and use of moderate nitrogen fertilization is often recommended as cultural practices to delay the development of late blight [112]. Higher dose of phosphorus and potassium has been found to give a higher yield in a late blight year [113]. The selection of suitable cultivars with late blight resistant, well aerated fields, pre-sprouting of tubers and early planting are some of the measures for foliar blight while planting potatoes on large steep ridges, right time of mechanical weeding and harvesting, avoiding rapid shift of harvested tubers or long transports could minimize tuber blight [114].

5.4. Host resistance

Host resistance is the best option for management of late blight of potato and it is eco-friendly in nature. Generally, after a decade, resistant level of the cultivars is being defeated, due to matching of new virulence genes. To find out the source(s) of resistance to late blight in potato was serious concern after Irish famine, during late 19th century. The fact that *P. infestans* originated in Mexico where lots of wild *Solanum* species also grow and co-exist with late blight led to the belief that wild *Solanum* species would possess a fair degree of resistance to balance the *Phytophthora* attack. In India, selection of late blight resistant genotypes dates back to 1936 when potato germplasm was screened in the field. In subsequent selections, clones of *S. demissum* and *S. antipoveizii* were found immune and later used as parents for late blight resistance breeding. Development of resistant cultivars and exploitation of screening methodology has played an important role in the management of late blight [115–120]. CPRI has released varieties having moderate to high degree of resistance to late blight for cultivation both for plains and hills. Some of them are Kufri Giriraj, K. Shailja, K. Himalini and K. Himsona (for hills) and K. Pukhraj, K. Anand, K. Sutlej, K. Badshah, K. Arun, K. Jawahar, K. Garima, K. Chipsona-1, K. Chipsona-2, K. Chipsona-3 and K. Frysona (for plains). Advanced hybrid MS/99–1871 derived from cross PH/F-1045 X MS/82–638 has been released for commercial cultivation under the name Kufri Garima. Foliage resistance of advanced hybrids tested under laboratory and field conditions did not establish close relationship. The expression of late blight resistance in foliage and tuber were not related [121]. K. Mohan is a new variety with field resistance to late blight reported [122]. Recently, Payette Russet: a dual-purpose potato cultivar with late blight resistance (both tuber and foliage) and high resistance to potato virus Y released in USA [123]. Somatic hybrids having high degree of resistance to late blight can be used as one of the parent for potato breeding [124]. The somatic hybrids P4, P8 and P10 reported for the introgression of important characters such as high tuber dry matter concentration, resistance to late blight into the cultivated potato via conventional breeding methods for cultivar development in the sub-tropical plains of India [125].

5.5. Forecasting models

The late blight pathogen is highly dependent on the environmental factors like temperature, relative humidity and leaf wetness etc. for causing late blight disease. Therefore, various forecasting model had been developed for forecasting late blight disease. Initially, Van Everdingen [126] evolved 'Dutch rules' for predicting the initial occurrence of late blight and for scheduling fungicide applications under Holland condition. Subsequently, Beaumont's period [127];

Irish rules, moving day concept [128]; severity value accumulation [129]; negative prognosis [130] and mathematical based models were developed worldwide. Large number of forecasting systems like BLITECAST, SIMCAST, ProPhy, PROGEB, PhytoPre, NegFry, Web-Blight, Plant Plus, PhytoPRE + 2000, China Blight, Bio-PhytoPre etc. have been developed for different regions of the world [112]. International Potato Centre has linked two disease forecasting models, Blitecast and Simcast to climate database in a Geographical Information System (GIS) to estimate global severity of potato late blight. Using GIS database, they suggested that an increased access to host resistance and fungicides in developing countries could have a strong economic impact on potato production [131]. A web-based Decision Support System (DSS) was developed for management of potato and tomato late blight [132] which links various models into a system that enables prediction of disease dynamics based on weather conditions, crop information, and management strategies. Growers identify the location of their production unit of interest and the system automatically obtains observed weather data from the nearest available weather station, and location-specific forecast weather data from the National Weather Service – National Digital Forecast Database [133]. Recently a new forecasting model BLITE-SVR developed for prediction of first appearance of late blight of potato. A total of 13 kinds of weather data had been utilized for development of this model and performance of BLITE-SVR compared with the conventional moving-average method as well as through pace regression and linear regression. The accuracy of prediction was 64.3% by BLITE-SVR, with 42.9% by the conventional moving-average method, 42.9% by pace regression and 35.7% by linear regression for first appearance of late blight of potato [134].

In Indian scenarios, a forecasting model has been developed for Darjeeling hills utilizing 12 years rainfall data on the concept of Cook's moving graph and Hyre's [135]. Another forecasting model had been developed using daily weather data (temperature, rainfall and RH) for actual appearance of late blight for Shimla, Shillong and Ootacamund [136]. The computerized forecasting model 'JHULSACAST' developed for western UP for both the rainy and non-rainy conditions and it is being utilized for forecasting of first appearance of late blight in the regions and large scale of farmers are benefited by timely adopting control measures [137]. The wireless sensor network was used for validation of 'JHULSACAST' with other forecasting late blight models in western Uttar Pradesh using human participatory sensing approach. It was observed that the 'JHULSACAST' has been found to be significantly accurate than the Ullrich, Fry, Winsteland Wallin models for the Hapur region of Uttar Pradesh, India [138]. JHULSACAST model template was used for calibration for development of forecasting models for Punjab [139], Tarai region of Uttarakhand [140] and plains of West Bengal [141]. A decision support system also developed for assisting in management of late blight by ICAR-CPRI, which includes three modules i.e. i) decision rules for forecasting first appearance of late blight in plains during rainy and non-rainy years based on temperature, relative humidity, and rainfall data, ii) decision rules for need based application of fungicides, and iii) regression models for yield loss assessment. All these modules have been combined and a web based decision support system for western Uttar Pradesh has been developed and hosted on ICAR-CPRI server. The yield loss assessment model was developed using two parameters *i.e.* per cent yield loss as a dependent variable and AUDPC as an independent variable. Twenty five linear and non-linear regression lines were fitted with three years data and amongst best non-linear reciprocal hyperbola regression line, which has $R^2 = 0.84$ was selected. Further, this model was validated and results revealed that the

deviation from 0.5 to 13.70% in 2010–2011, 1.16 to 9.69% in 2011–2012 and −3.01 to 9.23% in 2012–13 between actual and predicted yield loss [142]. Recently, INDO-BLIGHTCAST- a web based Pan-India model for forecasting potato late blight which is an improvement over JHULSACAST has been developed. It predicts late blight appearance using daily mean temperature and relative humidity data available with meteorological stations and does not require hourly weather data, not region/location specific and can be used across the country without any calibration [143]. An algorithm to determine the severity of potato late blight was developed using image processing techniques and neural network. The proposed system takes images of a group of potato leaves with complex background as input which are captured under uncontrolled environment [144]. It could further modified for spray of fungicides based on disease severity. Thus, the disease forecasting model is not only forecast for initial appearance of late blight but also assist in managing the late blight with proper spray schedules.

6. Conclusion

Late blight disease could be managed by taking in account all available resources i.e. chemical, host resistant, cultural or biological in the form of integrated disease management. Although the chemical and varietal management are being used widely all over the world, biological control could be used especially in organic potato cultivation or reducing the number of fungicides sprays/objectives to less use of fungicides. It is cause of concern wherever, oospores are survived and emergence of new strain/re-emerging the late blight. It will in future line of action that how disease is re-emerging and how to manage at short span after its re-emerging.

Author details

Mehi Lal[1]*, Sanjeev Sharma[2], Saurabh Yadav[1] and Santosh Kumar[3]

*Address all correspondence to: mehilalonline@gmail.com

1 ICAR-Central Potato Research Institute, Meerut, Uttar Pradesh, India

2 ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India

3 Department of Plant Pathology, Bihar Agricultural University, Bihar, India

References

[1] Mehi L, Yadav S, Singh V, Nagesh M. The use of bio-agents for management of potato diseases. In: Rigobelo EC, editor. Plant Growth. InTech Publisher; 2016. http://dx.doi.org/10.5772/62601

[2] Alexopoulos CJ, Mims CW, Blackwell M. Introductory Mycology. Fourth ed. USA: John Wiley & Sons. Inc; 1996

[3] Large EC. The Advance of the Fungi. London: Jonathan Cape; 1940. p. 488

[4] Butler EJ. Fungi and Disease in Plants. Calcutta: Thacker Spink and Co; 1918

[5] Butler K. Potato disease of India. Agriculture Ledger Crop Disease Pest Series. 1903; **8**:87-124

[6] Dastur JF. Conditions influencing the distribution of potato blight in India. Indian Journal of Agricultural Research (Special Indian Congress). 1917;**12**:90-95

[7] Dastur JF. Potato blight in India. Member Department Agriculture India Botany Series. 1915;**7**:163-176

[8] Basu SK. The late blight of potato. Agricultural Journal of Bihar and Orissa. 1913;**1**:142-149

[9] Dey NR. Cultivation and storage of potatoes in Bihar with special reference to the disease prevalent in the stores and the field. Allahabad Fmg. 1947;**21**:177-204

[10] Lal TB. Occurrence of late blight in the plains of India. Indian Phytopathological Society. 1949;**1**:164-181

[11] Woodhouse EJ, Dutt NL. Potato disease in Bihar. Indian Journal of Agricultural Sciences. 1913;**1**:15-38

[12] Dutt BL. Bacterial and Fungal Disease of Potato, ICAR, New Delhi.1979; pp. 169

[13] Srikantaiaya M. Late blight of potato in Mysore state. Potato Journal. 1962;**4**:49-50

[14] Kadam VC, Sarode MS, Bendre NJ, Shingte VV, Khot SB, Lokhande SB. Late blight of potato *Phytophthora infestans* (Mont.) de Bary. Current Science. 1974;**43**:260

[15] Fry WE, Birch PRJ, Judelson HS, Grünwald NJ, Danies G, Everts KL, Gevens AJ, Gugino BK, Johnson DA, Johnson SB, McGrath MT, Myers KL, Ristaino JB, Roberts PD, Secor G, Smart CD. Five reasons to consider *Phytophthora infestans* a re-emerging pathogen. Phytopathology. 2015;**105**:966-981

[16] Subbarao KV, George SW, Klosterman SJ. Focus issue articles on emerging and re-emerging plant diseases. Phytopathology. 2015;**105**:852-854

[17] Haq I, Rashid A, Khan SA. Relative efficacy of various fungicides, chemicals and biochemicals against late blight of potato. Pakistan. Journal of Phytopathology. 2008;**21**:129-133

[18] Ahmed N, Khan MA, Khan NA, Ali MA. Prediction of potato late blight disease based upon environmental factors in Faisalabad, Pakistan. Journal of Plant Pathology and Microbiology. 2015;**S3**:008

[19] Collins WW. The global initiative on late blight- alliance for the future. In: Khurana SMP, Shekhawat GS, Singh BP, Pandey SK, editors. Potato Global Research and Development, Vol I, Indian Potato Association, CPRI, Shimla, India. 2000. pp. 513-524

[20] Lal M, Arora RK. Maheshwari Uma, Rawal S and Yadav S. Impact of late blight occurrence on potato productivity during 2013-14. Int. J. Agricult. Statistical Science. 2016;**12**(1):187-192

[21] Thind TS, Bala A, Mukerjee J, Jolly RK, Kumar P, Singh A, Sekhon PS. Development of a web-based decision support system for late blight of potato. Plant Disease Research. 2013;**28**:67-70

[22] Arora RK. Late blight: an increasing threat to seed potato production in the north-western plains of India. Acta Horticulture (ISHS). 2009;**834**:201-204

[23] Bhat MN, Singh BP, Arora RK, Rai RP, Garg ID, Trehan SP. Assessment of crop losses in potato due to late blight disease during 2006-2007. Potato Journal. 2010;**37**:37-43

[24] Kumar S, Singh PH, Garg ID, Paul SMP. Integrated management of potato diseases. Indian Horticulture. 2003;**48**:25-27

[25] Judelson HS, Blanco FA. The spores of *Phytophthora* weapons of the plant destroyer. Nature Reviews. Microbiology. 2005;**3**:47-57

[26] Haldar K, Kamoun S, Hiller NL, Bhattacharje S, van Ooij C. Common infection strategies of pathogenic eukaryotes. Nature Reviews. Microbiology. 2006;**4**:922-931

[27] Haverkort AJ, Struik PC, Visser RGF, Jacobsen E. Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. Potato Research. 2009;**52**:249-264

[28] Sharma S, Sundaresh S and Singh BP. Late blight of potato. In: BP singh, M Nagesh, Sanjeev Sharma, Vinay Sagar, A Jeevvlatha and J Sridhar, editors. A manual on diseases and pest of potato-Technical Bulletin No. 101 ICAR-central potato research institute, Shimla, HP, India. 2015. pp. 1-6

[29] Lal M. Fungal foliar diseases of potato and their management (Course on disease and pest management in potato complied and produced by Sanjeev Sharma, Vinay Sagar, Ravinder Kumar, Sridhar Jandrajupalli, Dhiraj Kumar Singh) published by ICAR-CPRI Shimla. 2016; pp. 22-27

[30] Liu Y, Langemeier M, Small I, Joseph L, Fry W. Risk management strategies using precision agriculture technology to manage potato late blight. Agronomy Journal. 2017;**109**:562-575

[31] Hwang YT, Wijekoon C, Kalischuk M, Johnson D, Howard R, Prüfer D, Kawchuk L. Evolution and management of the Irish potato famine pathogen *Phytophthora infestans* in Canada and the United States. American Journal of Potato Research. 2014;**91**:579-593

[32] Gisi U, Cohen Y. Resistance to phenylyamide fungicides: A case study with *Phyophthora infestans* involving mating type and race structure. Annual Review of Phytopathology. 1996;**34**:549-572

[33] Davidse LC, Hofman AE, Velthuis GCM. Specific interference of metalaxyl with endogenous RNA polymerase activity in isolated nuclei from *Phytophthora megasperma* f. Sp.medicaginis. Experimental Mycology. 1983;**7**:344-361

[34] Bhat MN, Tyagi P, Singh BP. Effect of translaminar fungicides against late blight of potato in subtropical plains. Journal of Mycology and Plant Pathology. 2009;**139**:109

[35] Fabritius AL, Judelson HS. Mating type loci segregation aberrantly in *Phytophthora infestans* but normal in *Phytophthora parasitica*: Implication for models of mating type determination. Current Genetics. 1997;**32**:60-65

[36] Judelson HS, Robert S. Multiple loci determining insensitivity to phenlaymide fungicides in *Phytophthora infestans*. Phytopathology. 1999;**89**:754-760

[37] Groves CT, Ristaino JB. Commercial fungicide formulations induce in vitro oospore formation and phenotypic change in mating type in *Phytophthora infestans*. Phytopathology. 2000;**90**:1201-1208

[38] Thind TS. Singh Lakhvir, Mohan C, Paul J. Monitoring for metalxayl resistance in populations of *Phytophthora infestans* and their characteristics in Punjab. Indian Phytopathological Society. 2001;**54**:91-97

[39] Bardsley ES, Seitz A, Mercer RT. Control of potato late blight (*Phytophthora infestans*) with a fenamidone based product in the UK. The-BCPC-Conf. Pests and Diseases, 2002; Vol. 1 & 2. Proc. Inte Conf. Brighton Hilton Metropole Hotel, Brighton, UK. pp. 595-604

[40] Thind TS, Mohan C, Raj P. Competitive fitness of metalxayl resistant population of *Phytophthora infestans* and its cross resistance to strobiturins and other fungicides. Journal of Mycology and Plant Pathology. 2002;**32**:122-124

[41] Rodriguez F, Perez L, Almandoz J. Proc 24 Int Plant Protect Cong (IPPC), Jerusalem, Israel, July 25-30, 1999; p 15 (Abs)

[42] Lal M, Lal C, Yadav S. Gunjan, Singh BP, Kaushik SK, Sharma S. Biological characterization, mt haplotyping, and chemical management of *Phytophthora infestans* causing late blight of potato. Int. J. Agricult. Statistical Science. 2015;**11**:259-266

[43] Rani A, Bhat MN, Singh BP. Effect of fungicides on growth and germination of zoosporangia and zoospore of *Phytophthora infestans*. Journal of Mycology and Plant Pathology. 2007;**37**:527-529

[44] Samoucha Y, Cohen Y. Efficacy of systemic and contact fungicide mixtures in controlling late blight in potatoes. Phytopathology. 1986;**76**:855-859

[45] Grünwald NJ, Flier WG, Sturbaum AK, Garay-Serrano E, van den Bosch TBM, Smart CD, Matuszak JM, Lozoya-Saldana H, Turkensteen LJ, Fry WE. Population structure of *Phytophthora infestans* in the Toluca valley region of central Mexico. Phytopathology. 2001; **91**:882-890

[46] Davidse LC, Henken J, VanDalen A, Jaspers APK, Mantel BC. Nine year of practical experience with phenylamide resistance in (*Phytophthora infestans* (Mont.) de Bary). *Boletin-Tecnio*-INTA. 1989;**96**:11

[47] Thind TS. Fungicides in crop health security–the road ahead. Indian Phytopathological Society. 2012;**65**:109-115

[48] Fry WE. Phytophthora: Late blight on Ireland. Microbiology Today. 2007:13-14

[49] http://vegetables.wisc.edu

[50] Chakraborty A, Mazumdar D. Development of effective spray schedule for the management of late blight of potato in plains of West Bengal. Potato Journal. 2012;**39**:92-94

[51] Amin M, Mulugeta N, Selvaraj T. Field evaluation of new fungicide, victory 72 WP for management of potato and tomato late blight (*Phytophthora infestans* (Mont) de Bary) in west Shewa highland, Oromia, Ethiopia. Journal of Plant Pathology and Microbiology. 2013;**4**:192

[52] Shtienberg D, Fry WE. Field and computer simulation evaluation of spray-scheduling methods for control of early and late blight of potato. Phytopathology. 1990;**80**:772-777

[53] Lal M, Yadav S, Chand S, Kaushik SK, Singh BP, Sharma S. Evaluation of fungicides against late blight (*Phytophthora infestans*) on susceptible and moderately resistant potato cultivars. Indian Phytopathological Society. 2015;**68**:345-347

[54] Lal M, Yadav S, Singh B. P. Efficacy of new fungicides against late blight of potato in subtropical plains of India. Journal of Pure and Applied Microbiology. 2017;**11**:599-603

[55] Kankwatsa P, Hakiza JJ, Olanya M, Kidenamariamand HM, Adipala E. Efficacy of different fungicide spray schedules for control of potato late blight in southwestern Uganda. Crop Protection. 2003;**22**:545-552

[56] Seidl Johnson AC, Jordan SA, Gevens AJ. Efficacy of organic and conventional fungicides and impact of application timing on control of tomato late blight caused by US-22, US-23, and US-24 isolates of *Phytophthora infestans*. Plant Disease. 2015;**99**:641-647

[57] Lal M, Singh BP, Yadav S, Sharma S. Ametoctradin 27% + dimethomorph 20.27% (w/w) SC: A new molecule for management of late blight of potato in India. Journal of Experimental Zoology, India. 2017;**20**(2):1119-1123

[58] Merk M, Gold RE, Schiffer H, Levy T, Frechen T, Saramago J. Initium®: A new innovative fungicide of a new chemical class for the control of late blight and downy mildew diseases. Acta Horticulturae. 2011 No 917

[59] Köller W. Chemical approaches to managing plant pathogens. In: Ruberson JR, editor. Handbook of pest management. New York: Marcel Dekker, Inc.; 1998. pp. 337-376

[60] Mizubuti ESG, Fry WE. Late blight. In: Cooke BM, Gareth Jones D, Kaye B, editors. The Epidemiology of Plant Diseases. 2nd ed. Netherlands: Springer; 2006. pp. 445-471

[61] Daayf F, Platt HW. Variability in responses of US-8 and US-11 genotypes of potato and tomato isolates of *Phytophthora infestans* to commercial fungicides in vitro. American Journal of Potato Research. 2002;**79**:433-441

[62] Samoucha Y, Cohen Y. Synergistic interactions of cymoxanil mixtures in the control of metalaxyl resistant *Phytophthora infestans* of potato. Phytopathology. 1988;**78**:636-640

[63] Saville A, Graham K, Grunwald NJ, Myers K, Fry WE, Ristaino JB. Fungicide sensitivity of U.S. genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. Plant Disease. 2015;**99**:659-666

[64] Bhat MN, Rani A, Singh BP. Efficacy of inorganic salt against potato late blight. Potato Journal. 2006;**34**:83-84

[65] Basu A, Md. Hossain M, Konar A, Chettri M. Integrated management of late blight disease of potato in West Bengal. Annals of Plant Protection Sciences. 2003;**1**:64-66

[66] Frenkel O, Yermiyahu U, Forbes GA, Fry WE, Shtienberg D. Restriction of potato and tomato late blight development by sub-phytotoxic concentrations of boron. Plant Pathology. 2010;**59**:626-633

[67] Cohen Y, Vaknin M, Mauch-Mani B. BABA-induced resistance: Milestones along a 55-year journey. Phytoparasitica. 2016;**44**:513-538. DOI: 10.1007/s12600-016-0546-x

[68] Baider A, Cohen Y. Synergistic interaction between BABA and mancozeb in controlling *Phytophthora infestans* in potato and tomato and *Pseudoperonospora cubensis* in cucumber. Phytoparasitica. 2003;**31**:399

[69] Cook LR, Little G. The effect of foliar application of phosphonate formulations on the susceptibility of potato tubers to late blight. Pest Management Science. 2002;**58**:17-25

[70] Johnson SB. Post-harvest applications of phosphorous acid materials for control of *Phytophthora infestans* and *Phytophthora erythroseptica* on potatoes. Plant Pathology Journal Faisalabad. 2008;**7**:50-53

[71] JyhNong T, PaoJen A, Wang IT, Wang SY, Hu CY. Control of *Phytophthora* late blight of potato and tomato with neutralized phosphorous acid. Journal of Taiwan Agricultural Research. 2009;**58**:185-195

[72] Liljeroth E, Bengtsson T, Wiik L, Anderson E. Induced resistance in potato to *Phytophthora infestans* effects of BABA in greenhouse and field tests with different potato varieties. European Journal of Plant Pathology. 2010;**127**:171-183. DOI: 10.1007/s10658-010-9582-4

[73] CPRI. 2014-15. Annual Scientific Report. Central Potato Research Institute, Shimla, India

[74] Stein JM, Kirk WW. Variations in the sensitivity of *Phytophthora infestans* isolates from different genetic backgrounds to dimethomorph. Plant Disease. 2003;**87**:1283-1289

[75] Stein JM, Kirk WW. The generation and quantification of resistance to dimethomorph in *Phytophthora infestans*. Plant Disease. 2004;**88**:930-934

[76] Van den Bosch F, Paveley N, Shaw M, Hobbelen P, Oliver R. The dose rate debate: Does the risk of fungicide resistance increase or decrease with dose? Plant Pathology. 2011;**60**:597-606

[77] Sas-Piotrowska B, Piotrowski W, Misiak M. The growth and development of potato pathogens on media with extracts from *Polygonaceae* plants. I. Pathogens causing dry leaf-spot disease. Phytopathologia Polonica. 1996;**11**:103-109

[78] Blaser P, Steiner U, Lyr H, Russel PE, Dehne HW, Sisler HD. Antifungal activity of plant extracts against potato late blight (*Phytophthora infestans*). Modern fungicides and antifungal compounds II Frie drichroda, Thuringia, Germany. 24th–29th May 1998-1999; 491-499pp

[79] Paik SB. Screening for antagonistic plants for control of *Phytophthora* spp in soil. Korean Journal of Mycology. 1989;**17**:39-47

[80] Khan MA, Singh BP, Kaushik SK, Lal M. Evaluation of antifungal potential extracts against potato pathogens. National Seminar on emerging problems of potato, 1-2 November 2014, CPRI Shimla. 143p

[81] Ajay S, Sunaina V. Direct inhibition of *Phytophthora infestans*, the causal organism of late blight of potato by *Bacillus* antagonist. Potato Journal. 2005;**32**:179-180

[82] Lal M, Yadav S, Sharma S, Singh BP, Kaushik SK. Integrated management of late blight of potato. Journal of Applied and Natural Science. 2017;**9**(3):1821-1824

[83] Yao Y, Yan L, Chen Z, Zheng B, Zhang L, Niu B, Meng J, Li A, Zhang J, Qi W. Biological control of potato late blight using isolates of Trichoderma. American Journal of Potato Research. 2016;**93**:33-42

[84] Yuan-Hang QU, Qi W, Yan-Po Y, Zhen-Lin H, Yan L. Antagonistic *Trichoderma* isolates against potato late blight caused by *Phytophthora infestans*. Mycosystema. 2014;**33**:1231-1241

[85] Indian Institute of Spices Research. PhytoFuRa Annual Report 2013-14. Kozhikode: IISR; 2014. pp. 20-26

[86] Ramos L, Ciampi L, Gonzales S. Biological control of *Phytophthora infestans* in potato plants. *Simiente.* 1993;**63**:53-54

[87] Sanchez V, Bustamante E, Shattock R. Selection of antagonists for biological control of *Phytophthora infestans* in tomato. Manejo Integrado de Plagas. 1998;**48**:25-34

[88] Sadlers HM. Use of bacteria in controlling fungal diseases. Gemuse Munchen. 1996, 1996;**32**:180-181

[89] Garita VS, Bustamante E, Shattock R. Microbiological control of *Phytophthora infestans* on tomato. Manejo Integrado de Plagas. 1999;**51**:47-58

[90] Stephan D, Koch E, Elad Y, Kohl J, Shtienberg D. Screening of plant extracts, micro-organisms and commercial preparations for biocontrol of *Phytophthora infestans* on detached potato leaves. Bulletin OILB-SROP. 2002;**25**:391-394

[91] Gupta H, Singh BP, Mohan J. Biocontrol of late blight of potato. Potato Journal. 2004; **31**:39-42

[92] CPRI. 1999. Annual Progress Report. Central Potato Research Institute, Shimla, India

[93] Roy S, Singh BP, Bhattacharyya SK. Biocontrol of late blight of potato. Phytophthora News Letter. 1991;**17**:18

[94] Lal M, Singh AP, Tomar S, Hussain T, Sharma S, Kaushik SK, Singh BP. Antagonistic effect of bioagents against three potato fungal diseases and their fungicidal sensitivity. *Vegetos*. 2013;**26**:362-367

[95] El-Naggar MA, Abouleid HZ, El-Deeb HM, Abd-El-Kareem F, Elshahawy IE. Biological control of potato late blight by means of induction systemic resistance and antagonism. RJPBCS. 2016;**13**:1338-1348

[96] Chowdappa P, Mohan Kumar SP, Jyothi Lakshmi M, Upreti KK. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biological Control. 2013;**65**:109-117

[97] Daayf F, Adam L, Fernando WGD. Comparative screening of bacteria for biological control of potato late blight (strain US-8), using in-vitro, detached-leaves, and whole-plant testing systems. Canadian Journal of Plant Pathology. 2003;**25**:276-284

[98] Haas D, Defago G. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews. Microbiology. 2005;**3**:307-319

[99] Chen SY, Wei YH, Chang JS. Repeated pH-satisfied batch fermentation for rhamnolipid production with indigenous *Pseudomonas aeruginosa* S2. Applied Microbiology and Biotechnology. 2007;**76**:67-74

[100] Banat IM, Makkar RS, Cameotra SS. Potential commercial application of microbial surfactants. Applied Microbiology and Biotechnology. 2000;**53**:495-508

[101] Tomar S, Singh BP, Khan MA, Kumar S, Sharma S, Lal M. Identification of *Pseudomonas aeruginosa* strain producing biosurfactant with antifungal activity against *Phytophthora infestans*. Potato Journal. 2013;**40**:155-163

[102] Tomar S, Singh BP, Lal M, Khan MA, Hussain T, Sharma S, Kaushik SK, Kumar S. Screening of novel microorganisms for biosurfactant and biocontrol activity against *Phytophthora infestans*. Journal of Environmental Biology. 2014;**35**:893-899

[103] Katan J. Cultural practices and soil borne disease management. In: Utkhde RS, Gupta VK, editors. Management of Soil Borne Diseases. India: Kalyani Publishers; 1996. p. 100

[104] Bodker L, Pedersen H, Kristensen K, Moller L, Lehtinen A, Hannukkala A. Influence of crop history of potato on early occurrence and disease severity of potato late blight caused by *Phytophthora infestans*. In: Westerdijk CE, Schepers HTAM, editors. Proceedings of the 9th workshop of an European network for development of an integrated control strategy of potato late blight. 2006; PPO Special Report No. 11. 53-56p

[105] Hannukkala AO, Kaukoranta T, Lehtinen A, Rahkonen A. Late blight epidemics on potato in Finland 1933-2002; increased and earlier occurrence of epidemics associated with climate change and lack of rotation. Plant Pathology. 2007;**56**:167-176

[106] Sekhon PS, Sokhi SS. Effect of date of planting on late blight development in Punjab. Indian Phytopathological Society. 1999;**52**:267-269

[107] CPRI. 2004-2005. Annual Progress Report. Central Potato Research Institute, Shimla, India

[108] Bounes H, Finckh MR. Effects of strip intercropping of potatoes with non-hosts on late blight severity and tuber yield in organic production. Plant Pathology. 2008;**57**:916-927

[109] Turkensteen LJ, Mulder A. The potato disease *Phytophthora infestans*. Gewasbescherming. 1999;**30**:106-112

[110] Zwankhuizen MJ, Govers F, Zadoks JC. Inoculum sources and genotypic diversity of *Phytophthora infestans* in southern Flevoland, the Netherlands. European Journal of Plant Pathology. 2000;**106**:667-680

[111] Cooke LR, Schepers HTAM, Hermansen A, Bain RA, Bradshaw NJ, Ritchie F, Shaw DS, Evenhuis A, Kessel GJT, Wander JGN, Anderson B, Hansen JG, Hannukkala A, Naerstad R, Nielsen BJ. Epidemiology and integrated control of potato late blight in Europe. Potato Research. 2011;**54**:183-122

[112] Arora RK, Shrama S, Singh BP. Late blight disease of potato and its management. Potato Journal. 2014;**41**:16-40

[113] Roy SK, Sharma RC, Trehan SP. Integrated nutrient management by using farmyard manure and fertilizers in potato-sunflower-paddy rice rotation in the Punjab. The Journal of Agricultural Science. 2001;**137**:271-278

[114] Meinck S, Kolbe H. Control of leaf and tuber blight in ecological potato cultivation. Kartoffelbau. 1999;**50**:172-175

[115] Bhardwaj V, Kaushik SK, Singh PH, Singh BP. Tuber and foliage resistance to late blight in advanced potato hybrids. Potato Journal. 2005;**32**:131-132

[116] Bhardwaj V, Kaushik SK, Chakrabarti SK, Pandey SK, Singh PH, Manivel P, Singh BP. Combining resistance to late blight and PVY in potato. Potato Journal. 2007;**34**:41-42

[117] Kaushik SK, Bhardwaj V, Singh PH, Singh BP. Evaluation of potato germplasm for adaptability and resistance to late blight. Potato Journal. 2007;**34**:43-44

[118] Joseph TA, Kaushik SK, Singh BP, Bhardwaj V, Pandey SK, Singh SV, Singh PH, Gupta VK. Kufri Himalini: A high yielding, late blight resistant potato variety suitable for cultivation in Indian hills. Potato Journal. 2007;**34**:168-173

[119] Joseph TA, Singh BP, Kaushik SK, Bhardwaj V, Pandey SK, Singh PH, Singh SV, Gopal J, Bhat MN, Gupta VK. 2011. Kufri Girdhari: A medium maturing, late blight resistant potato variety for cultivation in Indian hills. Potato Journal. 2011;**38**:26-31

[120] Bhardwaj V, Srivastava AK, Sharma S, Kumar V, Kaushik SK, Singh BP. Efficiency of different potato (*Solanum tuberosum* L) cross combinations in late blight resistance breeding. International journal of Horticulture and agriculture. 2013;**2**:63-69

[121] Lal M, Luthra SK, Singh BP, Yadav S. 2013. Screening of potato genotypes for late blight. Potato Journal. 2013;**40**:80-83

[122] Luthra SK, Gupta VK, Lal M, Rawal S, Kumar V, Singh BP. Kufri Mohan-a new high yielding table potato variety. Potato Journal. 2017;**44**:65-73

[123] Novy RG, Whitworth JL, Stark JC, Schneder BL, Knowles NR, Pavek MJ, Knowles LO, Charlton BA, Sathuvalli V, Yilma S, Brown CR, Thornton M, Brandt TL, Olsen N. Payette russet: A dual-purpose potato cultivar with cold sweetening resistance, low acrylamide formation and resistance to late blight and potato virus Y. American Journal of Potato Research. 2017;**94**:38-53

[124] Tiwari JK. Poonam, Kumar V, Singh BP, Sharma S, Luthra SK and Bhardwaj V. Evaluation of potato somatic hybrids of dihaploid *S. tuberosum* (+) *S. pinnatisectum* for late blight resistance. Potato Journal. 2013;**40**:176-179

[125] Luthra SK, Tiwari JK, Lal M, Chandel P, Kumar V. Breeding potential of potato somatic hybrids: Evaluations for adaptability, tuber traits, late blight resistance, keeping quality and backcross (BC1) progenies. Potato Research. 2016;**59**:375-391

[126] Van Everdingen E. The relation between weather conditions and potato blight, *Phytophthora infestans* (in Dutch). *Tijdschr.* Pfantenziekten. 1962;**32**:129-140

[127] Beaumont A. The dependence of weather on the dates of outbreak of potato blight epidemics. Transactions of the British Mycological Society. 1947;**31**:45-53

[128] Hyre BA. Progress of forecasting late blight of potato and tomato. Plant Disease Report. 1954;**38**:245-253

[129] Wallins JR. Summary of recent progress in predicting late blight epidemics in United States and Canada. American Journal of Potato Research. 1962;**39**:306-312

[130] Ullrich J, Schrodter H. Das problem der vorhersage des aufretens der kartoffelkrautfaule (*Phytophthora infestans*) und die moglichkeit seiner losung durcheins negaturprognose. Nachrichtenblatt Dt. Pflanzenschnezdienst. 1966;**18**:33-40

[131] Hijmans RJ, Forbes GA, Walker TS. Estimating the global severity of potato late blight with GIS-linked disease forecast models. Plant Pathology. 2000;**49**:697-705

[132] Small IM, Joseph L, Fry WE. Evaluation of the blight decision support system for the integrated management of potato and tomato late blight. Phytopathology. 2013;**103**:134-135

[133] Small IM, Joseph L, Fry WE. Development and implementation of the BlightPro decision support system for potato and tomato late blight management. Computers and Electronics in Agriculture. 2015;**115**:57-65

[134] YH G, Yoo SJ, Park CJ, Kim YH, Park SK, Kim JS, Lim JH. BLITE-SVR: New forecasting model for late blight on potato using support-vector regression. Computers and Electronics in Agriculture. 2016;**130**:169-176

[135] Chaudhury SD, Pal SC. Forecasting late blight of potatoes in the hills of West Bengal. American Journal of Potato Research. 1959;**36**:284-287

[136] Bhattacharyya SK, Raj S, Singh DS, Khanna RN, Ram S. Forecasting late blight of potato in Indian hills. In: Nagaich BB et al., editors. Potato in Developing Countries. CPRI, Shimla: Indian Potato Association; 1982. pp. 414-424

[137] Singh BP, Ahmed I, Sharma VC, Shekhawat GS. JHULSACAST: A computerized forecast of potato late blight in western Uttar Pradesh. Potato Journal. 2000;**27**:25-34

[138] Jagyasi B, Kumar V, Pande A, Singh BP, Lal M, Ahmad I, Lohia P. Validation of Jhulsacast model using human participatory sensing and wireless sensor networks. Potato Journal. 2015;**42**:44-49

[139] Arora RK, Ahmed I, Singh BP. Forecasting late blight of potato in Punjab using JHUSLACAST model. Potato Journal. 2012;**39**:173-176

[140] Pundhir VS, Singh BP, Ahmad I, Sharma S, Kushwaha HS, Singh VK, Joshi V. Forecasting late blight of potato in Tarai region of Uttarakhand using JHULSACAST model. Potato Journal. 2015;**41**:95-104

[141] Chakraborty A, Singh BP, Ahmad I, Sharma S. Forecasting late blight of potato in the plains of West Bengal using JHULSACAST model. Potato Journal. 2015;**42**:50-57

[142] Lal M, Sharma S, Ahmad I, Singh BP, Yadav S. Development of yield loss assessment model for potato late blight disease in indo-gangetic plains. Potato Journal. 2014;**41**:130-136

[143] Singh BP, Govindakrishnan PM, Ahmad I, Rawat S, Sharma S, Sreekumar J. INDO-BLIGHTCAST–a model for forecasting late blight across agroecologies. International Journal of Pest Management. 2016;**62**(4):360-367. DOI: 10.1080/09670874.2016.1210839

[144] Biswas S, Bhushan J, Singh BP, Lal M. Severity identification of potato late blight disease from crop images captured under uncontrolled environment. IEEE Canada International Humanitarian Technology Conference (IHTC) 2014, Montreal QC, 1-4 June, 2014. DOI:10.1109/IHTC.2014.7147519

Chapter 6

Nematodes Affecting Potato and Sustainable Practices for Their Management

Fábia S.O. Lima, Vanessa S. Mattos, Edvar S. Silva,
Maria A.S. Carvalho, Renato A. Teixeira,
Janaína C. Silva and Valdir R. Correa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73056

Abstract

Plant-parasitic nematodes are a significant factor limiting potato production and tuber quality in several regions where potato is produced. Overall, parasitic nematodes alone cause an estimated annual crop loss of $ 78 billion worldwide and an average crop yield loss of 10–15%. As a result, sustainable food production and food security are directly impacted by pests and diseases. Degrading land use with monocultures and unsustainable cropping practices have intensified problems associated with plant pathogens. Proper identification of nematode species and isolates is crucial to choose effective and sustainable management strategies for nematode infection. Several nematode species have been reported associated with potato. Among those, the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*, the root-knot nematode *Meloidogyne* spp., the root lesion nematode *Pratylenchus* spp., the potato rot nematode *Ditylenchus destructor* and the false root-knot nematode *Nacobbus aberrans* are major species limiting potato yield and leading to poor tuber quality. Here, we report a literature review on the biology, symptoms, damage and control methods used for these nematode species.

Keywords: control, disease, lesion nematodes, pest, potato cyst nematodes, root-knot nematodes, *Solanum tuberosum*, yield loss

1. Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important staple food worldwide after maize, rice and wheat and the first vegetable and non-grain economically important food crop. It is cultivated in several regions worldwide, especially in Europe, America and Asia. Europe and Asia are major

producers and account for about 80% of world potato's production, and the main consumers are Europe, North America and Asia. Potato is cultivated in an area of 20 million hectares and produces close to 400 million tons annually that are consumed freshly or processed [1–3].

There has been increasing demand for food supply and food security. Unsustainable cropping production systems with monocultures, intensive planting and expansion of crops to newly opened areas have increased problems associated with new pests and diseases [4].

Nematodes are diverse, microscopic multicellular animals comprising free living to plant parasitic species. They parasitize a wide range of plant species, including monocots and dicots, and are one of the most limiting factors for major crops, causing substantial annual crop loss worldwide. Plant parasitic nematodes are a limiting factor for potato production and lead to decreased yield, physical and chemical changes in potato tubers, poor tuber quality and malformations, which overall make them unmarketable [3, 5–6]. Nematodes alone can cause average yield losses in potato up to 12% [7]. Nonetheless, potato yield losses due to nematode parasitism also depend on a combination of factors, including cultivar, favorable environment, soil structure, population density and time of planting, and could lead to a more severe decline in yield at particular cropping systems [3, 5, 8–10].

Several nematode species are found associated with potato; some of which cause significant yield losses, while others may cause minor injuries and are of local importance. The main nematode species associated with potato includes the yellow potato cyst nematode *Globodera rostochiensis* (Woll.) and the white potato cyst nematode *G. pallida* (Stone), the two worldwide most significant nematode species found in temperate regions where potato is cultivated. The false root-knot nematode *Nacobbus aberrans* (Thorne), the potato rot nematode *Ditylenchus destructor* (Thorne), the root lesion nematode *Pratylenchus* spp. and the root-knot nematode *Meloidogyne* spp. can also cause significant yield losses in potato [3, 5]. Other minor nematode species can be a problem to potato field depending on conditions that favor nematode growth, including the stubby-root nematodes *Trichodorus* spp. and *Paratrichodorus* spp., the lance nematode *Hoplolaimus galeatus* (Cobb) and the dagger nematode *Xiphinema* spp., among others.

Since pathogens such as plant parasitic nematodes represent major losses in agricultural systems, especially when the crops are not managed sustainably, the searches for information on the occurrence of nematodes in the production system, population density, species, level of damage and monitoring and management of these populations are essential in regions where crops will be set [3–4]. In addition, reliable, fast and proper nematode diagnosis and specimen identification are mandatory for choosing adequate management control strategies and for avoiding spreading of exotic nematodes in quarantine materials.

The objective of this chapter is to report a literature review on major nematode species that affect potato growth, yield and quality worldwide and to point out methods normally used for their sustainable management in the field. We will focus on the main nematode species that cause mostly damage to potato, including (i) the potato cyst nematodes *G. rostochiensis* and *G. pallida*; (ii) the root-knot nematode *Meloidogyne* spp.; (iii) the false root-knot nematode *Nacobbus aberrans*; (iv) the root lesion nematode *Pratylenchus* spp.; (v) the potato root rot nematode *Ditylenchus destructor* and (vi) the stubby-root nematodes *Trichodorus* and *Paratrichodoros* spp. A list of most common nematode species associated with potato is summarized in **Table 1**.

http://dx.doi.org/10.5772/intechopen.73056

Common name	Species name
Potato cyst nematodes	*Globodera pallida*
	G. rostochiensis
Root-knot nematodes	*Meloidogyne acronea*
	M. arenaria
	M. chitwoodi
	M. incognita
	M. fallax
	M. hapla
	M. javanica
Root lesion nematodes	*Pratylenchus andinus*
	P. brachyurus
	P. coffeae
	P. crenatus
	P. mediterraneus
	P. minyus
	P. neglectus
	P. penetrans
	P. scribneri
	P. thornei
	P. vulnus
	P. zeae
Potato rot nematode	*Ditylenchus destructor*
The false root-knot nematode	*Nacobbus aberrans*
	N. dorsalis
Bulb & stem nematode	*D. dipsaci*
Stubby root nematode	*Paratrichodorus* spp.
	Trichodorus spp.
Sting nematode	*Belonolaimus longicaudatus*
Spiral nematode	*Helicotylenchus pseudorobustus*
Lance nematode	*Hoplolaimus galeatus*
Stunt nematode	*Tylenchorhynchus claytoni*
Dagger nematode	*Xiphinema* spp
The reniform nematode	*Rotylenchulus reniform*
Burrowing nematode	*Radopholus similis*

Table 1. List of some nematode species associated with potato.

2. Major nematode species affecting potato

2.1. Potato cyst nematodes (*Globodera* spp.)

Potato cyst nematodes (PCN)—the golden nematode *G. rostochiensis* and the pale nematode *G. pallida*, are the two major yield-limiting nematode species that affect potato in several subtropical regions where this crop is cultivated. Within the genera *Globodera*, there are about 15 minor species [11] and are taxonomically positioned alongside the genus *Heterodera*. They belong to the Order Rhabditida, Suborder Tylenchina and Family Heteroderidae, and due to their similar morphological characteristics, *Globodera* spp. was initially classified within the genus *Heterodera* [12].

Mature females of PCN, *Globodera* spp., form cysts, which are dead females that become darker in color and store their eggs inside their body when conditions are not proper for their survival [11]. In the species *G. rostochiensis*, the cysts turn from white to yellow and then become brownish, whereas in *G. pallida*, the cysts do not become yellow, it turns from white to brownish directly [11]. This dormant stage of the eggs inside the cysts can last for up to 20 years, even in the absence of host or other adverse environmental conditions [12]. Due to these non-ideal conditions, the death of eggs that are internally within the cysts occurs gradually. Annual mortality varies from 50% for temperate regions and over 75% in warmer climates [13]. This higher egg mortality in warmer climates occurs due to *Globodera* spp. is better adapted to subtropical regions and its cycle is interrupted at temperatures above 28°C [12]. The species *G. pallida* develops best between 10 and 18°C, while *G. rostochiensis* is better adapted to a warmer temperatures, between 15 and 25°C [14].

The nematodes, *Globodera* spp., have a worldwide distribution and are present in every continent. In the Oceania, the species occur in countries such as Australia and New Zealand. In Asia, this species can be found in the Philippines, India, Israel, Japan, Lebanon, Malaysia, Oman, Pakistan, Sri Lanka and Turkey. The species is more widespread in Europe, occurring in most countries, such as Austria, Belgium, Croatia, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Sweden, Switzerland and United Kingdom. They are present in Algeria, Egypt, Libya, Morocco and South Africa, as well as in North America—e.g. Canada, USA and Mexico (*G. rostochiensis*). In Central America, they have been detected in El Salvador (*G. rostochiensis*), Costa Rica, Guatemala and Panama (*G. pallida*), while in South America, they have been detected in Argentina (*G. pallida*), Bolivia, Chile, Colombia (*G. pallida*) and Ecuador (*G. pallida*) [15]. In Brazil, the potato cyst nematodes have not been detected and are considered an A1 quarantine pest [16].

Field dissemination of PCN occurs through several ways, including irrigation water, rainfall runoff, infested soil particles, infested commercial seed potato tubers, contaminated packing of seed potato tubers, footwear, animal hooves, as well as with infested implements and machineries, among others [17, 18].

The host range of PCN includes potato, tomato (*S. lycopersicum*), African eggplant (*S. aethiopicum*), eggplant (*S. melongena*) and other solanaceous plants, including *Physalis* spp., *Datura* spp., *Hyoscyamus* spp., *Physoclaina* spp., *Salpiglossis* spp. and *Saracha* spp. [15]. Species

such as *D. ferox*, *Nicotiana acuminata*, *S. ligustrinum* and *S. pinnatum* are also reported as hosts of *G. rostochiensis* patotype Ro1 in Chile [19].

PCN are considered major pest to potato production in which yield losses can vary from slight losses, reach up to 70% or to a complete loss [20]. The level of damages and losses, however, depend on a combination of factors, including nematode population buildup, number of generations per year, length of potato growing season, soil temperature and host factors [20, 21]. In addition, due to non-specific symptoms in potato, especially above ground, losses are often not taken into account or attributed to adverse factors, such as other pathogens, inadequate plant nutrition and lack of soil moisture [18]. Typical symptoms of nematode infestations occur in patches of poor growth in the field, modifying the genetic characteristics of the crop, causing smaller, curled and abnormally colored leaves, tending to show brown spots on the margins and reduction of the numbers and sizes of leaflets, which overall affect the photosynthesis [6].

Potato plants infected with *Globodera* spp. show yellowish symptoms similar to water and nutrients deficiency, reduced size and number of tubers, with small lesions, making them unmarketable. Plants with damaged roots become wilted, especially during warmer temperatures within the day and may remain wilted even with irrigation. The root system becomes less developed and plants produce a greater amount of lateral roots, leading to overall decreased plant growth, premature death and do not respond properly to fertilization input [14, 22].

Globodera spp. are quarantine A1 pests in Brazil and some other countries. Due to the difficulties in eradicating this nematode once they have been introduced into potato fields, preventive containment measures to avoid their introduction, including non-importation of potato from countries where the nematodes are reported, regardless whether potato is for consumption or for planting, are some of the regulations to avoid spread of the nematode [23].

Once the nematodes are reported into potato fields, other management measures should be used in order to avoid their dissemination or to decrease their population level and thus improve yield. The success in decreasing their population level is variable and depends on the initial population density, soil type and plant genotype, among others. Generally, long-cycle potatoes planted in the fall and harvested in spring have more pronounced yield losses than short-cycle cultivars [21].

Control methods for PCN include the use of quarantine regulations, crop rotation and crop succession [15, 21] and the use of resistant varieties and nematicides. For instance, crop rotation with barley has showed reduction in *G. rostochiensis* up to 87% [15]. Crop rotation with the nematode main hosts, e.g. potato, tomato and egg plants or other solanaceous species, should be avoided as well.

The use of resistant varieties against nematode infection is one of the most effective and environmentally safe methods to control their infection. Resistant varieties against *Globodera* spp. have been successfully used with control rate up to 95% [19]. In addition, there are several breeding programs worldwide aiming to find resistance genes to these nematodes [19].

Trap plants can also be used to control these nematodes. These plants will trigger hatching of nematode eggs with posterior prevention from completing their cycle by destroying the host. The length of time is critical and plants should be destroyed at a proper time after planting in

order to stop the nematode cycle [24]. If plants are not destroyed or occur too late, the nematode population will build up. Some examples of the use of trap plants include the plant species *S. sisymbriifolium* (Lam.), which have been shown significant reduction of PCN population density in the field up to 80% [25]. Other includes *S. tuberosum, S. nigrum*, *S. dulcamara* and *D. stramonium*, which have been shown promising results as well [20].

Alternatively, the use of antagonist plants can be used to control these nematodes. Antagonistic plants will initially stand nematode infection; however, later in their cycle, plant factors will stop their further development. The following plant species *Crotalaria spectabilis*, *C. juncea*, *Tagetes patula*, *T. minuta*, *T. erecta* and *Estizolobium* spp. are being used to manage root-knot nematode problems in potato fields in Brazil [23] and could be used to control *Globodera* spp. as well.

Other control methods include soil solarization, especially in regions with warmer temperatures. Chemical control with nematicides has also being used in several regions with satisfactory rate of control. Products, such as carbamates, aldicarb and carbofuran, have been used successfully. However, soil solarization and the use of nematocides are costly, and nematicides may cause side effects to human and to the environment [21].

2.2. Root-knot nematodes (*Meloidogyne* spp.)

Root-knot nematodes (RKNs), *Meloidogyne* spp., are the most aggressive, damaging and economically important group of plant parasitic nematodes significantly impacting major crops worldwide [9, 26]. Currently, there are more than 90 described species [26], of which *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* represent up to 95% of RKN in cultivated soils, some of them having several races which parasitize more than 2000 susceptible plant species [27], and overall represent a real threat to the agriculture worldwide [26, 28].

This group of nematodes is highly diverse, mainly due to their variations in cytogenetics (aneuploidy and polyploidy states), types of reproduction (amphimixis to parthenogenesis), complex mode of parasitism (advanced interactions with their hosts), interspecific hybridization, cryptic species and wide host ranges [27, 29–32].

Root-knot nematodes are endo-sedentary parasitic nematodes. The second-stage juvenile (J2) is the infective stage. After RKN hatch from eggs, the J2 migrates through the soil towards suitable root and uses special enzymes and the stylets to force penetration into the vascular cylinder where RKN establishes their feeding site by inducing hypertrophy and hyperplasia of a group of cells leading to swelling and formation of giant cells. On this site, nematode goes through three more ecdysis (molting) to become a swollen young female. Mature females begin laying eggs in the root, forming mass eggs wrapped in a gelatinous matrix. Each egg mass contains 400–500 eggs on average, and it is formed in the midst of cortical parenchyma or on the surface of the roots. The embryonic development of the nematode results in the first stage (J1), passing through an ecdysis (molting) in the egg, followed by the second stage (J2). Adult males do not feed on infected plants; they leave the roots and move freely in the soil until they die [4, 27].

Symptoms in the field include yellowing, stunting, wilting, brown spots and rotting of tubers. RKNs induce hypertrophy and hyperplasia of infected cells leading to swelling of tissues commonly known as galls. Affected tubers also develop galls, known as 'popcorn', which overall leads to low quality of tubers (**Figure 1**) [5, 33]. The number and sizes of galls vary depending on

http://dx.doi.org/10.5772/intechopen.73056

the susceptibility of the cultivars, population density and favorable temperatures [26]. RKN-infected roots change their nutrient and water uptake, leading to pronounced poor growth and tuber quality and decreased yield. Commonly, there are high levels of intraspecific variation within *Meloidogyne* genome, and this variability may play an important role in changes in morphology and cytogenetics and ultimately their ability to reproduce in certain hosts [27].

Often, the invasion of potato root system is non-damaging, but as soon as tubers begin to initiate, tubers are invaded by the infective second-stage juvenile (J2) and a rapid development and spread occur. Thus, if potato roots and tubers become infected early, several generations of the pathogen will occur before harvest, which typically is about 110 days after planting [34]. However, this depends on each cultivar and the management systems. Potato production in warmer regions or in sandy soils with irrigation system will result in a mix of favorable temperatures, soil structure and moisture status, which may lead to a significant increase in the severity of RKN infections [34].

Losses caused by RKN infection may in extreme cases reach up to 100% in potato fields. Also, variable losses occur as a result of the planting season and the level of soil infestation [33].

RKN species have been increasingly found in association with potato crops in the tropics and subtropics, causing substantial economic impact due to crop losses depending mainly on the cultivars, favorable climate and nematode density present during planting [5, 8–9, 27, 35, 36]. A few RKN species have been reported as increasing problems for potato cultivation in several

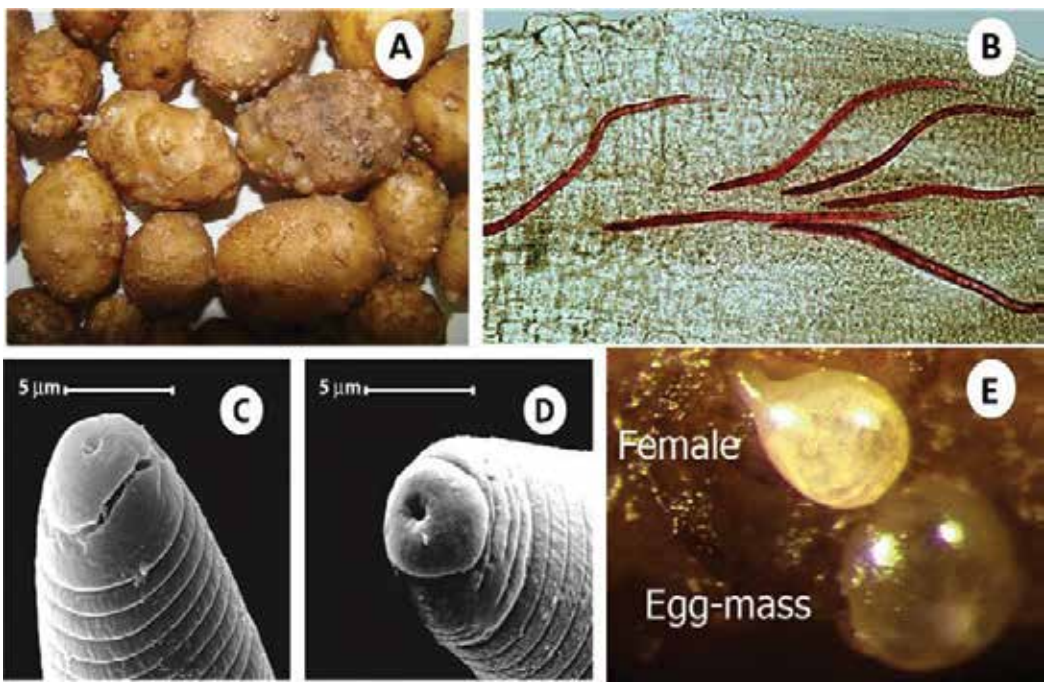

Figure 1. Nematode induced symptoms and nematode characteristics. (A) Potato tuber showing RKN galling (swellings) symptoms, (B) RKN second-stage juveniles (J2) inside root tissues, (C, D) scanning electron micrographs showing an overview of the RKN labial region and (E) RKN female and their egg-mass protruding from plant tissue. Courtesy of (A) Israel Medina (Univ. Nac. Del Altiplano Puno, Peru), (B, E) Jonathan Isenback (Viginia Tech, USA) and (C, D) Regina Carneiro (Embrapa Recursos Genéticos e Biotecnologia, Brazil).

regions worldwide, and the most important ones are *M. chitwoodi* (Golden), *M. fallax* (Karssen), *M. incognita* (Kofoid and White), *M. javanica* (Treub), *M. arenaria* (Neal) and *M. hapla* (Chitwood) [5, 35–37].

In temperate regions, i.e. North America, Europe and Australia, *M. chitwoodi* is a widespread and the most important RKN species affecting potato, in which severe damage, poor tuber quality and economic losses have been reported [34]. This RKN species tolerates much lower temperatures than *M. incognita* and *M. javanica* and is reported to cause damage to tubers at temperatures below 6°C [34]. In addition, the approved rates of some nematicides in the USA for controlling *M. chitwoodi* are higher than for other *Meloidogyne* spp. Thus, any spread of this nematode will further complicate its control. *Meloidogyne fallax* is also recognized as a serious pest of potato in Europe, New Zealand and Australia, where it is also a cold-tolerant species [34]. *Meloidogyne acronea* (Coetzee) has also been reported as infecting potato but are rare in commercial plantings and little is known about its distribution [34].

In a survey for RKN species in potato fields in southern Brazil [3], it was found that *M. javanica* was the most prevalent species (90%), followed by *M. incognita* (6.4%), *M. arenaria* (4.3%) and *M. ethiopica* (2.1%). The authors also found that *M. javanica* isolates showed differences in aggressiveness towards two susceptible potato cultivars tested, an information important for screening promising progenies to develop resistant materials.

In a similar survey, [36], using multiple loci sequencing approaches such as the intergenic region (IGS), D2-D3 expansion segments within 28S rDNA and *cytochrome oxidase subunit II* gene (COII) of mitochondrial DNA, it was reported several *Meloidogyne* species parasitizing potatoes in South Africa, including *M. javanica* (23%), *M. incognita* (23%), *M. arenaria* (17%), *M. enterolobii* (14%), *M. chitwoodi* (3%), *M. hapla* (1%) and unidentified *Meloidogyne* spp. (19%). Thus, these surveys show a trend of increased problems associated with RKN infection to potato fields related to climate change and to the breeding of potato cultivars suitable for planting in warmer regions worldwide. The extent of yield losses in potato field associated with RKN is to be better quantified.

The most effective, low cost, environmentally and healthy sound way to control RKN is to use resistant cultivars that stand good yield performance and have been tested for a particular region where potato is produced. However, currently there is no potato cultivar resistant to *Meloidogyne* spp., even though there are studies reporting the identification of resistant genes in wild potato genotypes and posterior introgression into breeding lines [38, 39].

The best characterized resistant gene against RKN in wild potato (*Solanum* sect. *Petota*, solanaceae) is R*Mc1(blb)* from *S. bulbocastanum*, a gene that is effective against some races of *M. chitwoodi* [38]. The resistance mechanism of R*Mc1(blb)* is based on a hypersensitive response and involves calcium signaling [40]. Recent studies suggested that *M. chitwoodi* resistance in different species of *Solanum* is based on the same gene, thereby limiting the diversity of available resistant genes [41]. Resistance to *M. incognita, M. javanica* and *M. arenaria* have also been reported in the wild potato species *S. sparsipilum* and are being used as breeding lines to develop resistant potato cultivars (International Center for Potato—CIP).

Alternatively, chemical nematicides have been used for the control of RKN in potato, especially in large cropping areas [6]. Other control methods, include crop rotation or succession with non-host and poor hosts, even though they should be used carefully since RKN have a

very wide host range and it could host other non RKN species, i.e. *Pratylenchus* spp. and be a further problem [42]. To be effective, rotation should last for at least 4 years, together with effective weed control. Due to this requirement, crop rotation may not be economically the best way to manage these nematodes for certain cropping system and regions. Some oat cultivars (*Avena sativa*), cotton (*Gossypium hirsutum*) and grasses that exhibit resistance to RKN may be used in rotation with potato in order to minimize the damage. Other crop rotation schemes that have been employed for *M. javanica* include the use of sorghum, maize and castor bean resistant to this species. Overall, brassica crops, including cabbage, cauliflower, mustard and Chinese cabbage, are used to rotate with RKN nematodes [42]. Other recommended management practices are the regular and timely cultivation and drying of soil, immediate destruction of volunteer potato plants and tubers, planting certified potato tuber, selecting planting dates to avoid high RKN population during tuber growth, shortening of potato cycle by using early maturing cultivars and, finally, just before planting, at planting and post-planting, the rational use of registered nematicides [34].

2.3. The false root-knot nematode (*Nacobbus aberrans*)

The false root-knot nematode *Nacobbus aberrans* (Thorne & Allen) (Nematoda, Pratylenchidae) is a plant parasitic nematode found mainly in some regions of North (Mexico and the USA) and South America (Argentina, Peru, Ecuador, Chile and Bolivia) [43–45]. In the USA, *N. aberrans* parasitizes sugar beet and other vegetable crops, but do not infect potato, while in Mexico and some South America countries, this nematode species is a serious problem on potato fields [43, 45].

This species is a quarantine regulated pest to several regions and it is considered a serious pest to potato in which yield losses up to 55–90% have been reported [43–45]. This nematode species has been detected in greenhouses in England, Netherlands, Finland, Russia, India and China [43, 45]; however, it has not been found in the field of any other region outside North and South America [43] and may have been eradicated following its detection in Europe and Asia countries. In the Andean region of Peru and Bolivia, for instance, *N. aberrans*, along with the potato cyst nematodes, *G. rostochiensis* and *G. pallida* and the RKN *Meloidogyne* spp. are considered major nematode species significantly affecting potato production when heavily infested soils are commonly reported [43, 44].

Potato is the most significant host of *N. aberrans*; however, it has a broad host range and parasitizes several other economically important plant species. Among these species includes solanaceous plants, i.e. *Solanum* and *Capsicum*; carrots, lettuce, cabbage, pea, sugar beets, cucumbers, *Opuntia* spp., Cactaceae, Poaceae and some weeds as well [43, 44].

Symptoms of *N. aberrans* infection in potato are similar to those caused by *Meloidogyne* spp., including the formation of galls, which are more discrete and rounded, whereas galls from RKN are elongated forming swellings along the roots. Affected potato roots also negatively impact tuber formation [43–45].

The life cycle of *N. aberrans* is similar to those of RKN species. The second-stage juveniles (J2) hatch from eggs, migrate through the soil towards suitable roots and use its stylet and enzymes to force entry. They penetrate the vascular system and modify a group of cells that lead to the development in galls. Different from RKN species, the third, fourth stage and immature females of *N. aberrans* are migratory. Eggs are produced in a gelatinous matrix that

protrudes from cortical tissue. The nematode can complete 2–3 generations during the crop season depending on ideal temperature which range from 14 to 25°C [43, 44].

Control methods for *N. aberrans* include the use of nematicides, crop rotations (4–6 years), biological control with antagonist fungi and bacteria, the use of resistant or tolerant potato cultivars and quarantine regulations for regions free from this nematode species [43, 44].

2.4. Root lesion nematodes (*Pratylenchus* spp.)

Root lesion nematodes *Pratylenchus* spp. are important plant parasites in the tropics and subtropics [46]. They have a broad host range and are widely distributed in tropical and subtropical regions, especially in Brazil, Southern United States and Africa [46, 47]. At least 15 species have been reported parasitizing potato worldwide, including *P. andinus*, *P. brachyurus*, *P. coffeae*, *P. crenatus*, *P. minyus*, *P. penetrans*, *P. scribneri*, *P. thornei*, *P. vulnus*, *P. neglectus* and *P. zeae* [35, 48]. In Brazil, at least 10 species have been reported in several crops, in which *P. brachyurus*, *P. coffeae*, *P. zeae* and *P. penetrans* are reported as the most frequent in potato [46], with the predominance of *P. brachyurus* in most potato fields throughout the country [6, 46]. *Pratylenchus penetrans* is considered the most important species of *Pratylenchus* spp. for potato fields, especially in Canada and other countries in North America, where substantial yield losses have been reported [49].

Pratylenchus species is commonly referred to as the root lesion nematodes due to the typical symptoms of necrosis they cause in the roots. The species is considered a migratory endoparasitic nematode, normally found within the roots and between the roots and soil particles [46]. *Pratylenchus* species are smaller than 1 mm in length. Males and females are wormlike, differing only in the sexual characters. Females have one ovary (monovarial) and reproduce by sexual reproduction called amphimixis or by mitotic and meiotic parthenogenesis. They are easily recognized by the sclerotized labial region and ventral overlapping esophageal glands and usually by dark intestinal contents. The stylet is well developed with broad basal bulbs. Most species are polyphagous, showing the ability to parasitize cultivated plants-perennials, semi-perennials, annuals as well as weeds [46].

Pratylenchus spp. of varying life stages penetrate the sub epidermal layers of potato tubers and move through the cortex where they feed on parenchyma cells and cause lesions that become dark spots on the tubers. They migrate continuously in the tissues, intra and inter cells causing such lesions. Depending on favorable temperature and soil conditions, they may have up to 3 generations during the potato cycle and may reach up to 10,000 individuals per 10 g of potato samples [46]. Besides that, these lesions in the root and tubers facilitate invasion of secondary infection by bacterial and fungal pathogens, which results in further necrosis of tubers [46, 50]. Infected tubers may rot and have a shorter shelf life as compared to healthy tubers. On the field, infected potato plants have poor growth as evidenced by scattered patches of stunted plants and show late flowering and intense necrosis in the roots [46]. Overall, infection of *Pratylenchus* spp. to potato may reduce yield by up to 50% in some reported cases, depending on several factors such as population level, temperature, soil conditions and potato cultivars [46, 50].

Management practices for *Pratylenchus* spp. include a successful integration of rotation and succession of crops, the use of resistant cultivars and genotypes, proper physical and chemical management of soil and elimination of weeds in the harvest and off season. Potato planting should be avoided during high temperatures and excessive rainfall [42].

All *Pratylenchus* ssp. have a wide host range, which include several cultivated crops and weed species as well. Successive planting of soybean, potato, pasture and corn favors the buildup of nematode population [6]. The authors [51] evaluated the effects of crop rotation and soil management practices such as reduced planting, cover crops and organic fertilizer applications on *P. neglectus* population densities in potato in a sandy soil in the south of Alberta, Canada. Rotated crops from 3 to 6 years included potato, dry beans, wheat, sugar beet and oats. Conservation practices included autumn cover crops and incorporation of compost as a substitute for inorganic fertilizer. *Pratylenchus neglectus* populations were affected by rotation length, but not by soil management practices. Population densities at 3-year rotation were higher as well as potato yields in the conventional 3-year rotation was consistently lower than at longer rotation periods.

Some other plant species such as *Crotalaria* and *Tagetes* are excellent options for rotation/succession with potato. The authors [52] carried out a study to monitor root lesion nematodes, mainly *P. penetrans* on two marigolds (*Tagetes tenuifolia* cv. Nemakill and cv. Nemanon), annual ryegrass (*Lolium multiflorum* cv. Lemtal), red clover (*Trifolium pratense* cv. Florex) and soybean (*Glycine max* cv. Proteus) and potato (cv. Superior) for three subsequent plantings. The population levels of the nematode were consistently lower with *Tagetes* spp. compared to other cover crops tested. As a result, the mean yield of potato was significantly higher (8–14%) when planting potato after *Tagetes* spp. Red clover and soybean allowed the highest nematode build up with mean potato yield lower than other cover crops tested.

The lack of crop rotation or succession with crops that are good hosts, such as soybeans, beans, corn, sorghum and several forage grasses, no-tillage system; the use of sandy to medium texture soils and poor nutrition of plants are some of the facts that may increase root lesion nematode-associated problems in potato. Favorable temperatures and humidity (ca. 20–25°C and 60–80% humidity) and excess of nitrogen fertilization also intensify problems associated with these nematodes [42].

2.5. The root rot nematode (*Ditylenchus* destructor)

The genera *Ditylenchus* (Nematoda, Tylenchida) has a complex systematic position and has been renamed several times, with several synonyms [53]. Currently, there are 81 described species within the genera, and *D. angustus, D. dipsaci* and *D. destructor* are plant parasitic nematodes of economic importance, with the last two species being important pathogens of potato, especially when associated with fungal pathogens. In addition, these nematodes are listed as quarantine pests to several countries.

Ditylenchus spp. have been reported in several regions worldwide causing direct and indirect damage to cultivated crops and overall have a wide host range [48]. The species *D. destructor* and *D. dipsaci* are important pathogens of potato, especially in temperate climates. *Ditylenchus destructor* is a major pathogen of potatoes in regions such as Europe, specially Russia, Asia, North America, Oceania and some isolated regions of South America and South Africa [48].

The symptoms caused by *D. destructor* are not commonly seen in the above ground of the affected plant. The heavily infected tubers give rise to a compromised plant due to physiological and morphological disorders caused by the nematode infection which may eventually lead to plant death. Early infections can be detected by peeling off the tuber and may reveal small and whitish spots [48].

Ditylenchus dipsaci, also known as the stem and bulb nematode, is more common in garlic, but it also damages other plant species as well, including potato, and affects stalks, stolons and tubers. Affected potato tubers show gray to brownish lesions with an overall poor plant growth. Diseased stems become swollen and curved. Galls may also form on the leaves, which cause significant leaf distortion [54].

The use of crop rotation for controlling *D. destructor* and *D. dipsaci* is not feasible due to their wide host ranges. The use of resistant genotypes is the most effective, economically and environmentally safe control method for nematode infection. Currently, there is limited information on the availability of resistant and tolerant potato cultivars against these nematodes. The authors [55] evaluated 25 potato varieties for resistance and tolerance against infection of *D. destructor* and *D. dipsaci*, and based on the nematode reproduction factor (RF), 16 varieties were rated as susceptible (S), while 5 other as resistant (R) to *D. destructor*; potato varieties 'Innovator', 'Aveka' and 'Spunta' were rated as resistant to *D. dipsaci* as well. In their study, the potato cultivar 'Désirée' was rated as highly susceptible to both *D. destructor* and *D. dipsaci*.

Other control measures for these nematodes include the use of nematode free field, nematode free seed potato tuber, and ultimately the use of chemical nematicides may be recommended.

2.6. The stubby-root nematodes (*Trichodorus* and *Paratrichodorus* spp.)

The stubby-root nematodes, *Trichodorus* and *Paratrichodorus* spp. (Trichodorids), belong to the family Trichodoridae (Thorne). Nematodes of this family are a group of important plant parasitic pathogens and include nearly 100 described species within five genera [56]. The stubby-root nematodes are ectoparasites that usually aggregate at the root tips and have a long, solid and curved stylet called onchiostyle that they use to pierce plant cells during feeding, preferably meristem cells of root tips. The damage caused by their direct feeding may be considerable, with thickened root and atrophy, early senescence and interruption of plant growth (stunting), a condition known as 'stubby root' [56].

Trichodorids have a wide distribution around the world, although some species are restricted to a particular region. Studies on the distribution and ecology of trichodorids, as well as the mechanisms involved in the transmission of certain viruses, may eventually result in new strategies for their control [56]. *Trichodorus* spp. are found in sandy soils in several regions worldwide. Although they are more specialized to monocots, they parasitize dicots as well and are considered an important nematode to potato in the tropics and subtropics. However, the level of damage in the field, their overall distribution and economic losses associated with their infection are not well studied [57].

Even though there is a considerable amount of data reporting the impact of these nematodes as a plant parasite, their ability to vector certain viruses has increased their importance to the agriculture, when several species of Trichodoridae were identified as a viral vector [56]. Thus, they are considered economically important for potato production, both because of their direct damage and due to the viruses they transmit. For instance, *Trichodorus* spp. have the ability to disseminate viruses to potato varieties, especially the Tobacco Rattle Virus—TRV, a virus of the genus Tobravirus, which cause the potato disease known as corky-ring spot [6, 57]. The juvenile and adult stages of *Trichodorus* spp. can vector these viruses after feeding on diseased plants. The viruses are stuck in the stylet region and do not circulate inside the nematode

body; however, the nematodes may remain with the virus for up to 4 months or until ecdysis occur, in which the nematode loses its viral load [56].

TRV-infected potato plants exhibit systemic and local symptoms, such as necrosis, chlorosis and overall stunting. Affected potato tubers may exhibit necrotic lesions with brittle tissues and low quality commercial potato tubers may occur even when mild symptoms are seen [58].

Other symptoms associated with TRV infection include delayed emergence of plant and subsequent poor growth, reduced tuber weight and potato yield, high incidence of poor commercial potato tubers, with low dry matter content and oxidation of tuber post cooking [56].

On the whole, control methods for stubby-root nematodes include those listed for other species as well, i.e. prevention (the use of certified planting material, cleaning soil from machineries and equipments, preventing the movement of animals within infested fields), crop rotation, cultural practices and the use of nematicides [6].

3. Other minor nematode species

Other nematode species, including the sting nematode (*B. longicaudatus*), the spiral nematode (*H. pseudorobustus*), the lance nematode (*H. galeatus*), the stunt nematode (*T. claytoni*) and the dagger nematode (*Xiphinema* spp), among others (**Table 1**), may be of economic importance to potato fields at some regions, for instance, some regions in the USA. Among these ectoparasitic nematode species, *B. longicaudatus* seems to be the most impacting one [59]. Other minor nematode species might be associated with potato, however, with isolated importance

4. Overall strategies for managing nematodes in potato fields

The effective control of potato nematodes are overall difficult and complex due to the particular biology of these plant parasites—they inhabit soil, have a short life cycle, multiply fast and have a large population build up; there are just few plant genotypes resistant to them, and chemical nematicides have limited effect due to their interaction with soil components or are being avoided due to their side effects to human and to the environment [42]. Therefore, control strategies for nematodes affecting potato should be planned carefully in order to succeed. The use of more than one control strategy (integrated management) is advised in order to optimize the control efficiency. Information required for proper nematode management, include: (i) proper diagnosis of nematode species and isolate; (ii) relationship between population density and yield losses; (iii) nematode biology (life cycle, environmental requirements, parasitism); (iv) host range; (v) population dynamics; (vi) efficiency of control methods and (vii) economic feasibility of control methods [21].

Generally used control strategies for potato nematodes are (i) planting in fields free of nematode pests, (ii) the use of certified nematode-free seed potato tubers, (iii) crop rotation and succession with non-host or poor host, (iv) fallow (including elimination of weeds), (v) antagonist plants, (vi) trap plants, (vii) resistant cultivars, (viii) avoidance to disseminate the nematodes, i.e. cleaning of tools and machineries, clean irrigation water and cleaning of

footwear, (ix) planting potato at season that is less favored to nematode reproduction, i.e. dry and cold season, (x) quarantine regulations for exotic nematode species, i.e. potato cyst nematodes *G. pallida* and *G. rostochiensis*, (xi) removal of infected plants, (xii) isolation of infested areas, (xiii) the use of biological control, (xiv) cultural and tillage practices and (xv) the use of chemical nematicides [10, 21, 42, 48].

5. Concluding remarks

Several nematode species are associated with potato and few of them negatively impact yield and tuber quality. Severe yield losses and poor tuber quality have been reported in most regions where potatoes are grown. The importance of these nematode species depends on their adaptation to each geographical region (local climate), plant host factors and management practices of potato crop. Other minor species may be a problem to local regions as well. Nematode species have unique biology, behavior and are usually difficulty to be managed or eradicated once they are introduced in a field. In addition, their morphological similarities make them difficult to be diagnosed. Nonetheless, proper nematode identification to species and isolate level are mandatory to choose the proper control method. Overall, these nematode problems in potato are better managed when integrated management practices are used, i.e. exclusion (quarantine regulations, certified plant material, use of clean equipment and machineries), cultural practices (crop rotation, succession, cover crops), genetic control, and ultimately by the use of nematicides. Therefore, for a sustainable cropping of potato cultivars, growers, extension services and researchers must consider these nematodes holistically, the impact they cause and whether these management practices are economically, environmentally and technically sound.

Author details

Fábia S.O. Lima[1], Vanessa S. Mattos[2], Edvar S. Silva[3], Maria A.S. Carvalho[1], Renato A. Teixeira[4], Janaína C. Silva[1] and Valdir R. Correa[1]*

*Address all correspondence to: valdir.correia@ifto.edu.br

1 Instituto Federal de Educação, Ciência e Tecnologia do Tocantins, Dianópolis, TO, Brazil

2 Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, Brazil

3 Instituto Federal de Educação, Ciência e Tecnologia do Acre, Brazil

4 Instituto Federal de Educação, Ciência e Tecnologia do Mato Grosso, Brazil

References

[1] FAO. Food and Agriculture Organization of the United Nations [Internet]. 2014. Available from: http://www.fao.org/faostat/en/#data/QC [Accessed: September 16, 2017]

[2] Suinaga FA, Pereira AS. Introdução e importância econômica. In: Silva GO, Lopes CA, editors. Sistema de Produção da Batata. 1st ed. Brasilia, Brazil: Embrapa-CNPTIA; 2010. pp. 1-20

[3] Medina IL, Gomes CB, Correa VR, Mattos VS, Castagnone-Sereno P, Carneiro RMDG. Genetic diversity of *Meloidogyne* spp. parasitizing potato in Brazil and aggressiveness of *M. javanica* populations on susceptible cultivars. Nematology. 2016;**1**:1-12. DOI: 10.1163-15,685,411/00003032

[4] Lima FSO, Correa VR, Nogueira SR, Santos PRR. Nematodes affecting soybean and sustainable practices for their management. In: Kasai M, editor. Soybean – The Basis of Yield, Biomass and Productivity. 1st ed. Rijeka, Croatia: InTech Open; 2016. pp. 1-16. DOI: 10.5772/67030

[5] Vovlas N, Mifsud D, Landa BB, Castillo P. Pathogenicity of the root knot nematode *Meloidogyne javanica* on potato. Plant Pathology. 2005;**54**:657-664

[6] Pinheiro JB, Silva FO, Pereira RB. Nematoides na cultura da batata. Circular Técnica. 2015;**143**:1-12

[7] Barker KR, Koenning R. Developing sustainable systems for nematodes management. Annual Review of Phytopathology. 1998;**36**:165-205

[8] Wesemael WML, Viaene N, Moens M. Root knot nematodes (*Meloidogyne* spp) in Europe. Nematology. 2011;**13**:3-16

[9] Jones JT, Haegeman A, Danchin EG, Gauer HS, Helder J, Jones MG, Kikuch T, Manzanilla-Lopes R, Palomares-Rius JE, Wesemael WM, et al. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology. 2013;**14**:946-961

[10] Noling JW. Nematode management in potatoes (Irish or White). University of Florida Agricultural Service – Extension – ENY 029. 2016;**1**:1-10

[11] Ferraz LCCB, Brown DJF. Nematologia de Plantas: Fundamentos e importância. Manaus: Manaus Norma Editora; 2016. 251 p

[12] Caixeta LB, Furlaneto C, Cares JE. Gênero Heterodera. In: Oliveira CMG, Santos MA, Castro LHS, editors. Diagnose de Fitonematoides. 1st ed. Campinas: Millennium; 2016. pp. 149-162

[13] Marks RJ, Brodie BB. Potato Cyst Nematodes: Biology, Distribution and Control. 1st ed. Wallingford: CAB International; 1998. 408 p

[14] EPPO. Globodera rostochiensis and G. Pallida PM 7-40. EPPO Bulletin. 2013;**49**:354-368

[15] Senasica. Nematodo dorado de la papa- Globodera rostochiensis – Mexico – Segarpa. Ficha Técnica. 2013;**19**:1-24

[16] MAPA. Instrução Normativa n. 41. Ministério da Agricultura Pecuária e Abastecimento. 2008;**1**:1-40

[17] Jatala P, Bridge J. Nematode parasites of root and tuber crops. In: Luc M, Sikora RA, Bridge J, Editors. Plant Parasitic Nematodes in Tropical and Subtropical Agriculture. 1st ed. Wallingford: CAB International; 1990. pp. 137-180

[18] Henrique. Nematodos de quisto de batateira Globodera rostochiensis and *G. pallida*. Boletim Técnico – INIA. 2012;**12**:1-2

[19] Sullivan MJ, Inserra RN, Franco J, Moreno-Lereude I, Greco N. Potato cyst nematodes: Plant host status and their regulatory impact. Nematropica. 2007;**37**:193-201

[20] Sparkes J. Potential trap crops for the control of potato cyst nematodes (PCN). Agriculture and Horticulture Development Board. 2013;**1**:1-19

[21] Nematode GN. Problems affecting potato production in subtropical climates. Nematropica. 1993;**23**(2):213-220

[22] DGAV. Organismos de quarentena- ações realizadas ano de 2014. Direção de Serviços de Sanidade Vegetal. 2015;**1**:41-48

[23] Embrapa. Nematoides na cultura da batata. Circular Técnica. 2015;**143**:1-12

[24] Halford PD, Russel MD, Evan K. Use of resistant and susceptible potato cultivars in the trap cropping of potato cyst nematodes G. Pallida and G. Rostochiensis. Annals of Applied Biology. 1999;**134**:321-327

[25] Timmermans BGH, Vos J, Stomph TJ, Van Nieuwburg J, Van der Putten PEL. Field Performance of Solanum Sisymbriifolium, a trap crop for potato cyst nematodes. Annals of Applied Biology. 2007;**150**:99-106

[26] Hunt D, Handoo Z. Taxonomy, identification and principal species. In: Perry RN, Moens M, Star JL, editors. Root Knot Nematodes. 1st ed. London: CAB International; 2009. pp. 55-88

[27] Moens M, Perry RN, Starr JL. Meloidogyne species – A diverse group of novel and important plant parasites. In: Perry RN, Moens M, Starr JL, editors. Root Knot Nematodes. Wallingford, UK: CAB International 2009. pp. 1-18

[28] Carneiro RMDG, Dos Santos MFA, Almeida MRA, Mota FC, Gomes ACMM, Tigano MS. Diversity of *Meloidogyne arenaria* using morphological, cytological and molecular approaches. Nematology. 2008;**10**:819-834

[29] Blok VC. Achievements in and future prospects for molecular diagnosis of plant parasitic nematodes. Canadian Journal of Plant Pathology. 2005;**27**:176-185

[30] Blok VC, Powers TO. Biochemical and molecular identification. In: Perry RN, Moens M, Star J, editors. Root Knot Nematodes. 1st ed. London: CAB International; 2009. pp. 98-112

[31] Randig O, Bongiovanni M, Carneiro RMDG, Castagnone-Sereno P. Genetic diversity of root knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. Genome. 2002;**45**:862-870

[32] Carneiro RMDG, Lima FSO, Correa VR. Methods and tools currently used for the identification of plant parasitic nematodes. In: Shah MM, Mohamood M, editors. Nematology: Concepts, Diagnosis and Control. 1st ed. Rijeka, Croatia: InTech Open; 2017. pp. 19-35. DOI: 10.5772/intechopen.69403

[33] Gomes CB, Souza RM. Doenças causadas por nematoides. In: Pereira AS, Daniels J, editors. O cultivo da batata na região sul do Brasil. 1st ed. Brasilia, Brazil: Embrapa; 2003. pp. 321-349

[34] Jones RK, Storey SG, Knoetze R, Fourie H. Nematode Pests of Potato and Other Vegetables – Nematology in South Africa. A View from the 21st Century. Vol. 1. Cham, Switzerland: Springer International Publishing; 2017. pp. 231-260

[35] Brodie BB, Evans K, Franco J. Nematode parasites of potato. In: Evans K, Trudgill DL, Webster JM, editors. Plant Parasitic Nematodes in Temperate Agriculture. 1st ed. Wallingford, UK: CAB International; 1993. pp. 87-132

[36] Okendi EM, Moleleki LN. Distribution and genetic diversity of root-knot nematodes (*Meloidogyne* spp) in potatoes in South Africa. Plant Pathology. 2013;**62**:1184-1192

[37] Molendijk LPG, Mulder A. The Netherlands, nematodes and potatoes: Old problems are here again. Potato Research. 1996;**39**:471-477

[38] Brown CR, Mojtahedi H, Zhang LH, Riga E. Independent resistant reactions expressed in root and tuber of potato breeding lines with introgressed resistance to *Meloidogyne chitwoodi*. Phytopathology. 2009;**99**(9):1085-1089

[39] Williamson VM, Kumar A. Nematode resistance in plants: The battle underground. Trends in Genetics. 2006;**22**:396-403

[40] Davies LJ, Brown CR, Elling AA. Calcium is involved in the RMc1 (blb)- mediated hypersensitive response against *Meloidogyne chitwoodi* in potato. Plant Cell Reports. 2015; **34**:167-177

[41] Brown CR, Zhang L, Mojtahedi H. Tracking the RMc1 gene for resistance to race 1 of Columbia root knot nematode (*Meloidogyne chitwoodi*) in three Mexican wild potato species with different ploidies. American Journal of Potato Research. 2014;**91**:180-185

[42] Pinheiro JB, Lopes CA, Henz GP. Medidas gerais de controle de nematoides da batata. Circular Técnica. 2009;**76**:1-9

[43] EPPO. Data sheets on quarantine pests – *Nacobbus aberrans*. EPPO/CABI Bulletin. 1997;**144**:1-4

[44] Manzila-Lopes RH, Costilla MA, Doucet M, Franco J, Inserra RN, Lehman PS, Cid del Prado-Vera I, Souza RM, Evans K. The genus Nacobbus (Thorne & Allenn, 1944 Nematoda: Pratylenchidae): Systematics, distribution, biology and management. Nematropica. 2002; **32**:149-227

[45] Vovlas N, Nico AI, De Luca F, De Giorgi C, Castillo P. Diagnosis and molecular variability of an Argentinean population of *Nacobbus aberrans* with some observations on histopathology in tomato. Journal of Nematology. 2007;**39**(1):17-26

[46] Ferraz LCCB. Gênero Pratylenchus – Os nematoides das lesões radiculares. In: Luz WC, editor. Revisão Anual de Patologia de Plantas. 1st ed. Passo Fundo: RAPP; 1999. pp. 158-195

[47] De Waele D, Elser A. Migratory endoparasites: Pratylenchus and Radopholus species. In: Star JJ, Cook R, Bridge J, editors. Plant Resistance to Parasitic Nematodes. Wallingford, UK: CAB International; 2002. pp. 175-206

[48] Mai WF, Brodie BB, Harisson MB, Jatala P. Nematodes. In: Hooker WJ, editor. Compendium of Potato Diseases. 1st ed. Saint Paul, USA: The American Phytopathological Society; 1990. pp. 93-101

[49] EPPO. Data sheet on quarantine pests: *Meloidogyne chitwoodi* and *M. fallax*. EPPO Bulletin. 2004;**34**(2):315-320

[50] Castillo P, Vovlas N. Pratylenchus (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management. Leiden: Brill; 2007. 529 p

[51] Forge TA, Larney FJ, Kawchuk LM, Pearson DC, Kock C, Blackshaw RE. Crop rotation effects on *Pratylenchus neglectus* populations in the root zone of irrigated potato in Southern Alberta. Canadian Journal of Plant Pathology. 2015;**37**(3):1-12

[52] Kimpinski J, Arsenaut WJ, Gallant CE, Sanderson JB. The effects of marigolds (*Tagetts* spp) and other cover crops on *Pratylenchus penetrans* and on following potato crops. Journal of Nematology. 2000;**32**:531-536

[53] Sturhan D, Brzeski MW. Stem and bulb nematodes *Dytilenchus* spp. In: Nickle WR, editor. Manual of Agricultural Nematodes. 1st ed. New York: Marcel Dekker; 1991. pp. 423-464

[54] Asscheman IE, Brinkman H, Bus CB, van Delft M, Hotsma PH, Mejers CP, Mulder A, Turkensteen LJ, Wustman R, editors. Potato Diseases (Diseases, Pests, Defects). 1st ed. Holland: NIVAA; 1996. 179 p

[55] Mlwara P, Niere B, Vidal S. Resistance and tolerance of potato varieties to potato rot nematode (*Ditylenchus destructor*) and stem nematode (*D. dipsaci*). Annals of Applied Biology. 2015;**16**(2):257-270

[56] Decraemer W, editor. The Family Trichodoridae: Stubby Root and Virus Vector Nematodes. 1st ed. Rotterdam: Springer Netherlands; 1995. 360 p. DOI: 10.1007/978-94-015-8482-1

[57] Scurrah MI, Niere B, Bridge J. Nematodes parasite of Solanum and sweet potatoes. In: Luc M, Sikora RA, Bridge J, editors. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. 2nd ed. London: CAB Publishing; 2205. pp. 193-220

[58] Taylor CE, Brown DJF, editors. Nematode Vectors of Plant Viruses. 1st ed. Wallingford, UK: CAB International; 1998. 286 p

[59] Bird GW, Wernette LG. Potato Nematodes: A Farm Guide to Nematode Diagnostic and Management [Internet]. December 2011. Available from: https://www.canr.msu.edu/ent/…Bird_bio…/Potato_nematodes.pdf [Accessed: November 1, 2017]

Chapter 7

Ralstonia solanacearum: A Bacterial Disease and Its Biological Control by Essential Oils on *Solanum tuberosum* L.

Cristina Ruiz Alvarado,
Ramón Jaime Holguin Peña and
Edgar Omar Rueda Puente

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.70744

Abstract

Worldwide, potato is considered the fourth most important crop for human consumption. In recent years, in some regions of the USA and Canada, the bacterium *Ralstonia solanacearum* (*Rs*), called bacterial wilt (Mb), has caused serious damage. Given the proximity of these countries, with Mexico as a tuber importer, the odds of an eventual introduction of these diseases are significant, especially in areas with large tracts of potato. Therefore, this research was performed to detect the presence of *Rs* in tuber and vegetative material of *Solanum tuberosum* and evaluated the bactericidal effect of essential oils. The results indicated that the presence of the bacterium *Rs* was negative in tuber from abroad. Nevertheless, we detected the presence of the causal agent of bacterial wilt in potatoes for domestic consumption that producers could use these tubers as production material. Oils of oregano and thyme showed inhibitory effects on the growth of *Rs*. Essential oils are considered as an alternative for the control of *Rs*.

Keywords: diagnosis, detection, phytopathogen, control, essential oils

1. Introduction

Global agriculture has been affected in recent years by phytosanitary problems caused mainly by fungi, bacteria, nematodes, weeds, and insects. With a radical change in international trade, and with the movement of vegetative material and sowing of these plant products, pests are dispersing throughout the world, becoming a more complex problem. Even when efforts are made, in the first instance to measure the introduction of diseases from other countries and secondly to

prevent their dispersion from primary inoculum sources, the negative aspects mentioned above have, to a certain degree, caused losses and major disruptions in some regions to national agriculture, so it is advisable to have the necessary measures to prevent the entry and secondly the control and dispersion of these biological agents harmful to plants [1]. In the Mexican Republic, specifically in northwestern Mexico, the area targeted for the cultivation of vegetables (mainly tomato, potato, chili, and watermelon) has increased considerably in recent years [2]. Mexico does not produce quality seed, it obliges producers to acquire tuber of foreign origin, mainly from the United States, since the varieties acquired produce fruits that meet the characteristics preferred by the consumer and also cause the tuber volumes to enter the country which is a gateway for microorganisms of quarantine importance such as *Pseudomonas solanacearum* = (currently) *Ralstonia solanacearum* to potato, *Xanthomonas campestris* pv. *vesicatoria* to chili and tomato, *Clavibacter michiganensis* ssp. *sepedonicus* to potato, *C. michiganensis* spp. *michiganensis* to tomato, and *Acidovorax avenae* pv. *citrulli* to watermelon, among others [1]. Also, the controversy is currently being generated among producers about their presence in agricultural fields in the state of Sonora, Mexico. Therefore, it is of great importance to know the current situation of *R. solanacearum* in potato (*Solanum tuberosum* L.), on material and during the different phenological stages of the crop in the agricultural zone of the state of Sonora, Mexico.

This research aims to expand knowledge of the situation that occurs in the detection of bacteria of quarantine importance, in addition to updating and reaffirming its null presence in the agricultural areas of the state, and later extend to the interior of the country. In addition, this screening study is aimed at involving producers with a new production scheme under phytosanitary conditions in order to safeguard our national agriculture.

On the other hand, in recent years, there has been a growing interest in the use of biologically active compounds extracted from plant species that have the ability to eliminate pathogenic microorganisms by themselves, mainly due to the resistance that microorganisms have developed to antibiotics [3]. In addition, agriculture in the new millennium must establish new control alternatives that produce a lower environmental impact, as day-to-day increases in the percentage of consumers who demand healthy and chemical-free food [4]. Therefore, the importance of knowing new control strategies arises, especially those that have a sustainable aspect. Based on the above, the need to evaluate bactericidal products to perform tests of antimicrobial activity against *R. solanacearum* is presented.

According to the abovementioned details, the principal goals were to detect the presence of *R. solanacearum* in tuber and vegetative material of *S. tuberosum* L. and to evaluate the bactericidal effect of essential oils.

2. Material and methods

2.1. Detection of brown rot (*Ralstonia solanacearum*) of potato crop (*Solanum tuberosum* L.) of the state of Sonora, Mexico

The research was carried out in two stages: the first one consisted in the increase of *R. solanacearum* (*Rs*) with specific culture media, tuber pathogenicity tests, seedling and fruit for familiarization

purposes in *Rs*, and their identification by ELISA technique. The second stage included a field sampled of tuber, seedling, flowering, and tubers produced in physiological maturity plants in Sonora state, to detect *Rs* by specific culture media and ELISA. Likewise, pathogenicity tests were carried out on those samples that were positive for the presence of *Rs*. Fit in mention that Detection of *Rs* in potato tuber was carried out in two types of tuber: (a) those from Canada and the United States of America (USA) and (b) tuber which it is to consumption human and used as a seed, it was sampled in commercial stores in Sonora state.

2.1.1. First stage

It was initiated with the increase of *Rs* and was developed according to the protocol established by Rueda [1], using means of specific cultures. According to the technique described by the same author, ten test tubes were obtained in 0.85% NaCl saline solution with a concentration of 108 colony-forming units (CFU)/ml, verified with the aid of a hematometer. Bacterial suspensions in tubes were stored in refrigeration at 4°C to stabilize the bacterium and avoid a shock in the immunization.

Pathogenicity tests (PPs) were developed in order to familiarize themselves with the symptomatology of *Rs* which were carried out in tuber and potato seedlings. In the case of tuber, 20 tubers were split in half and inoculated into the bundle by 200 ml with a bacterial suspension of 108 CFU/ml, and were placed in humid chambers under favorable conditions of the disease; likewise, another 20 tubers were considered as negative control when going through the same process but making use of sterile distilled water in the incision. Regarding PP in seedlings, 40 tubers were previously disinfected and were germinated in germination plates with sterile substrate, the conditions in which the seedling produced was at 25°C using sterile tap water for irrigation; at the end of 30 days after the emergency, 20 seedlings were inoculated with the bacterial solution of *Rs* at a concentration of 10^8 CFU/ml with the aid of a cotton swab on the cotyledons of the seedlings, and the remaining 20 seedlings were considered as negative control when passing through the same process with swab plus sterile distilled water. The tubers, seedlings used for PP, after inoculation, were covered with polyethylene bags and placed in an incubation chamber with a relative humidity between 80 and 90% and a temperature of 35–41°C in a period of 4–7 days [1]; these conditions are appropriate to induce the signs of the disease.

For the process of identification of *Rs*, the serological ELISA technique was developed, following the general protocol of identification of bacteria AGDIA. The *Rs* kit was donated by the project to which this research belongs.

2.1.2. Second stage

In the second stage, farmers donated tubers from Canada and the USA; the tuber, which is consumed by human and used as a seed, was sampled in commercial stores in Sonora state. Also, fields of potato crop were sampled in three stages: seedling, flowering stage, and physiological maturity when the plants produced tubers considered to be cut and later for sale. It should be noted that seedlings, leaves, or fruits of plants showing a symptomatology similar to that of *Rs* were also collected. Batch sampling was according to the National Potato Sampling in

Mexico [8]. Sampling was carried out in 10% of the total cultivated area of nine municipalities of Sonora state (Agua Prieta, Caborca, Cajeme, Hermosillo, Moctezuma, Navojoa, Sahuaripa, Santa Ana, and Ures). Located in the geographical coordinates, Agua Prieta 31° 17′ north latitude and 109° 33′ west longitude, Caborca 30° 42′ north latitude and 112° 09′ longitude west, Cajeme 27° 29′ north latitude and 109° 56′ longitude west, Hermosillo 29° 05′ north latitude and 110° 57′ longitude west, Moctezuma 29° 47′ north latitude and 109° 40′ west longitude, Navojoa 27° 03′ north latitude and 109° 25′ west longitude, Sahuaripa 29° 03′ north latitude and 109° 14′ west longitude, Santa Ana 30° 33′ north latitude and 111° 07′ west longitude, and Ures 29° 25′ north latitude and 110° 23′ west longitude. In the 10% of the surface of each municipality, the following was done: each batch of 5 ha was considered as a sampling area. Each hectare of that surface was a must-see. At each point an imaginary diagonal line was drawn from corner to corner, and on that straight line, ten samples were collected. Donations from producers were obtained. Each of the collected samples, previously identified, was wrapped with wet paper and placed in a cooler to be transferred to the laboratory for analysis.

Detection of Rs in tuber. According to Rueda [1], each tuber sample, consisting of 10 tubers from each batch of cooperating producers, weighed separately, washed in running water for 30 min, and placed in plastic trays with a capacity of 2 L. Each tray, with its respective sample of tuber, is left with an amount of 100 ml of distilled water, and each of these trays was added 2 ml of buffer solution phosphatase with a pH = 7. The water-phosphatase mixture containing each tuber sample is called a "mother suspension." The trays were incubated for 12 hours in cooling at 4°C in order to release the bacteria to the stock suspension. After the incubation, 10 ml was taken from suspension of each of the trays, four dilutions were made to such suspension (10:1, 10:2, 10:3, 10:4), and the last dilution of 0.1 ml was taken and sowed in specific culture medium in Petri dishes by the rod dispersion method The media were incubated for 7 days at 34°C. The inoculated media were then incubated at a temperature of 35°C for 7 days [1].

Detection of Rs in vegatitive material sampled (tuber, sedling, leaf and fruit), by ELISA technique. For the detection of *Rs*, with respect to the serological ELISA technique, the protocol described in the detection of the microorganism in the PP was considered.

Detection of *Rs* in tuber, seedling, leaf developed and fruit by PCR technique. Commercial primers were obtained from the 16Sr intergenic region, and screening tests were performed to reaffirm the null or positive presence.

Pathogenicity tests to positive bacteria with the different methods of detection. For the reaffirmation of *Rs* bacteria that proved to be positive in previous detection methods, they were carrying out pathogenicity tests [5]. The PPs were applied to seedlings 25–30 days after emergence, as described above in the PP of the first stage. The diseased tissue bacteria were reinoculated using the Randhawa technique, and the pathogen was confirmed by ELISA.

2.2. Evaluation of the in vitro antibacterial activity of essential oils of oregano and thyme against *Ralstonia solanacearum*

This stage which consisted of the evaluation of the antibacterial activity of two essential oils was carried out in the laboratory microbiology and mycotoxins of the Department of

http://dx.doi.org/10.5772/intechopen.70744

Research and Postgraduate in Food of the University of Sonora, in the city of Hermosillo, Sonora, Mexico. The experiment was carried out in vitro under controlled conditions at 30 °C and 90% humedity.

The bacterial strain used in the study was *R. solanacearum*, was isolated, and characterized from pathogenicity tests of potatoes from commercial houses in the state of Sonora.

The bacterial strain was grown in a culture of 24 hours at 30°C in Nutrient Broth (Difco, Sparks, MD) (extract of 3.0 g and peptone of 5.0 g) and adjusted to a concentration of 10^8 CFU/ml with phosphate-buffered saline (PBS). The bacterial inoculum was massively planted on dextrose and potato agar plates using a sterile cotton swab to achieve uniform microbial growth [6].

Once the plates were inoculated with the bacteria, filter paper disks of approximately 10 mm in diameter were placed in the center of the dish, in which different amounts of the essential oils were applied.

Essential oils were prepared at different concentrations using 70% ethyl alcohol as diluent. The concentrations used were 1:1, 1:5, and 1:10. Aseptically, 7.5, 10, and 15 μl of each of the concentrations of the essential oils were placed on the filter paper disks. Seventy percent alcohol was used in one of the filter paper disks as a negative control to discard the antimicrobial activity of the same. In addition, a disk of streptomycin (10 μg/disk) and one ampicillin (10 μg/disk) were used as the reference control. After impregnating the disks with the respective treatment, the plates were incubated at 30°C for 24 hours. After the incubation period, bacterial growth inhibition halos were measured in millimeters using a ruler. Analyses were carried out in triplicate.

The experimental design was trifactorial A × B × C where factor A has two oils [oregano (*Lippia graveolens*) and thyme (*Thymus vulgaris*)], factor B has three dilutions (1:1, 1:5, and 1:10), and factor C has three amounts applied 7.5, 10, and 15 μl. The data were analyzed in Statistix 8.0 program (2003).

3. Results

3.1. Detection of brown rot (*Ralstonia solanacearum*) of potato crop (*Solanum tuberosum* L.) of the state of Sonora (Mexico)

3.1.1. First stage

When testing for pathogenicity for *R. solanacearum* (*Rs*) symptoms in potato seedlings, the results indicate that between the tubers embedded in the bacterial suspension of 108 CFU/ml and between 7 and 15 days under favorable conditions of the disease, the vascular bundle of the tuber was darkened, and, when making a cross section, a grayish bacterial mucilage was exuded by the eyes and by the end of the stolon in the tubers. There were grayish-white outcrops that exudate from the darkened vascular ring of the cut tubers. On the other hand, in the inoculated seedlings when making a transverse cut at the stem level, the exudation of a gray-brown mucilage was noted. This could be verified by makng a transverse cut at the base of the

stem of the seedling and observing a milky-white filamentous fluid emanating from the vascular bundles and submerging a piece of the stem in clean, sterile water. In the same way, in the first true leaves for the seedling case, irregular spots of whitish appearance were observed in relation to the healthy area, and finally the death of the seedling occurred. For this, first appeared irregular spots at first clear and then obscure, and after 7–14 days death occurred. An opposite result was for those PPs that were directed as negative control by the use of sterile distilled water, in tuber-tubercle and seedling, being observed that the organs used were shown healthy, except in tuber where the punctured area showed a light brown oxidation (2 mm) which, when pressed, showed the same solid consistency as the healthy area [7].

Regarding the sowing of "bacterial suspensions" obtained from the pathogenicity test on media Mannitol salt agar (SMSA), and tetrazolium chloride (TZC) cultures, the results indicate that on the TZC medium, developed colonies showed a pinkish-white color, while on the SMSA medium, colonies of developed bacteria showed an irregular shape, of white color and with centers of pink color after 72 hours of incubation at 30°C. A similar result was obtained for those PPs that were inoculated with the positive control (*Rs*), whereas for those samples generated from the PPs that served as negative control (sterile distilled water), the results on both media used were non-mucoid dry colonies [7].

By ELISA confirmation, a positive result was obtained for the control strain (*Rs*), as well as for the bacterial suspensions isolated from the aforementioned pathogenicity tests and from those grown on the culture media SMSA and TZC. The opposite happened with the negative control [7].

3.1.2. Second stage

Sampling of potatoes was carried out in the municipalities of Agua Prieta, Caborca, Cajeme, Hermosillo, Moctezuma, Navojoa, Sahuaripa, Santa Ana, and Ures according to the sampling method [8] and detection of *Rs* (**Table 1**). It indicates the surface that is directed to the production of potato in the state of Sonora. The sampled area (889.8) can be identified, with the municipality of Navojoa and Cajeme with 394 and 240.6 ha, respectively [7].

The results of the phytopathological diagnosis show positive *Rs* for the municipalities of Navojoa, Hermosillo, and Agua Prieta in a consumption tuber that can be used as seed (**Table 2**). When the tests were carried out on the SMSA, TZC, and PPO media, the results were positive since the visible colonies on SMSA medium were after 36–48 hours of growth at 30°C, white with pink to colored centers of cream and irregularly round; on the TZC medium, the colonies appeared white with pink centers. It is possible to indicate that those bacterial colonies grown on SMSA and TZC medium obtained from samples of potatoes for consumption indicated that the bacterial cells were Gram-negative, in a bar form, strictly aerobic, and with measurement of 0.5–0.7 × 1.5–2.0 μm. These colonies were tested positive by ELISA and PCR [7].

In import tuber, the results were negative. Nonetheless, it is important to note that in response from abroad, there was variability of response for the municipalities of Agua Prieta, Sahuaripa, Moctezuma, Hermosillo, and Ures, as they were positive with the specific medium TZC but negative to the PPO and ELISA test [7].

Regarding the analyses carried out in seedlings, a leaf developed during the flowering stage and tubercle of fruit sampled at physiological maturity, the results indicate that for samples

District	Area sowed (ha)				
	Total	Irrigation	Raining area	Surface sampled from irrigation area 10%	Surface sampled from raining area
Navojoa	3940	3940	0	394	0
Cajeme	2406	2406	0	240.6	0
Hermosillo	371	371	0	37.1	0
Ures	115	115	0	11.5	0
Caborca	2000	2000	0	200	0
Agua Prieta	35	35	0	3.5	0
Sahuaripa	5	0	5	0	0.5
Santa Ana	30	30	0	3	0
Moctezuma	1	1	0	0.1	0
Total	8903	8898	5	889.8	0.5

Table 1. Surface sowed by potato crop in different districts of Sonora state (surface sampled to detect *Ralstonia solanacearum*).

obtained from the municipalities of Agua Prieta, Navojoa, Hermosillo, and Caborca, they were negative when using specific media (SMSA and TZC) and serological tests, except in the PPO test that was positive for leaf and fruit (**Table 3**) [7].

On the other hand, when analyzing the vegetative samples from Sahuaripa, Cajeme, and Ures, the analyses showed negative results in specific medium SMSA and serological tests, the opposite occurred in culture medium TZC and PPO test. An additional test was developed to the isolated colonies of the different sampling points, resulting being Gram-negative, in a bar form, strictly aerobic, and with measurement of 0.3–0.5 × 1.0–1.5 μm. For the municipality of Moctezuma, the results indicate the negative presence of *Rs*. A favorable result was obtained for the positive control in the tests SMSA, TZC, PPO, and ELISA [7]. Concerning the PP of the positive samples of consuming tuber, tubers embedded in the bacterial suspension of 10^8 CFU/ml, between 5 and 15 days under favorable conditions of the disease, presented a wateriness in the vascular bundle of the tuber. It was also detected that in tubers inoculated with *Rs*, whitish exudates of paste consistency appeared. As the infection evolved, there was a darkening of the entire vascular ring, and the adjacent tissues began to decompose, presenting a yellowish, creamy, or brownish coloration, eventually ending up rotting. When corroborating these symptoms by the ELISA technique, the result was positive for *Rs*. In the case of seedlings, the pathogenicity tests showed, in the first true leaves, irregular spots of whitish appearance relative to the healthy area and finally the death of the seedling. For this, irregular, initially clear and subsequently obscure patches were first presented, slight yellowing of the bacterial wilt disease of the plant, which is observed first on a single side of the leaf or on a branch, and after 7–14 days, death occurred. A positive result for *Rs* was obtained by ELISA, when analyzing the organs of PP in the seedling stage. The opposite occurred for those PP organs inoculated with sterile distilled water [7].

	Technical of diagnosis			
	Test SMSA	**Test TZC**	**Test PPO**	**Serology technique**
Potato from Canada and the USA				
Navojoa	−	−	−	−
Cajeme	−	−	−	−
Hermosillo	−	+	−	−
Ures	−	+	−	−
Ures	−	+	−	−
Caborca	−	−	−	−
Agua Prieta	−	+	−	−
Sahuaripa	−	+	−	−
Santa Ana	−	−	−	−
Moctezuma	−	+	−	−
Potato tuber's sampled in commercial stores in Sonora state, which is for human consumption and used as a seed				
Navojoa	+	+	+	+
Cajeme	−	−	−	−
Hermosillo	+	+	+	+
Ures	−	−	−	−
Caborca	−	−	−	−
Agua Prieta	+	+	+	+
Sahuaripa	−	−	−	−
Santa Ana	−	−	−	−
Moctezuma	−	−	−	−
Control positivo *Rs*	+	+	+	+
Control negativo (distilled water)	−	−	−	−

+, Test positive; −, test negative; specific media, SMSA and TZC. *Rs*, *Ralstonia solanacearum*; PPO, oxidase test.

Table 2. Detection of *Ralstonia solanacearum* in potato tuber from Canada and the United States of America (USA) and tuber of potato sampled in commercial stores in Sonora state, which is for human consumption and used as a seed.

3.2. Evaluation of the in vitro antibacterial activity of essential oils of oregano and thyme against *Ralstonia solanacearum*

Both oregano and thyme essential oils presented inhibition halos, such as antimicrobial activity against *R. solanacearum*, at all concentrations and amounts applied.

Districts sampled	Techniques of diagnosis			
	Test SMSA	Test TZC	Test PPO	Serology technique
Navojoa				
Seedling	–	–	–	–
Leaf	–	–	+	–
Fruit	–	–	+	–
Cajeme	–	+	+	–
Seedling	–	+	+	–
Leaf	–	+	+	–
Fruit				
Hermosillo	–	–	–	–
Seedling	–	–	+	–
Leaf	–	–	+	–
Fruit				
Ures	–	+	+	–
Seedling	–	+	+	–
Leaf	–	+	+	–
Fruit				
Caborca	–	–	–	–
Seedling	–	–	+	–
Leaf	–	–	+	–
Fruit				
Agua Prieta	–	–	–	–
Seedling	–	–	+	–
Leaf	–	–	+	–
Fruit				
Sahuaripa	–	+	+	–
Seedling	–	+	+	–
Leaf	–	+	+	–
Fruit				
Santa Ana	–	–	–	–
Seedling	–	–	–	–
Leaf	–	–	–	–
Fruit				
Moctezuma				
Seedling	–	–	–	–
Leaf	–	–	–	–
Fruit	–	–	–	–
Control positive *Rs*	+	+	+	+
Control negative *Rs*	–	–	–	–

+, Test positive; –, test negative; specific medium, SMSA and TZC. PPO, oxidase test; control negative = sterile distilled water.

Table 3. Detection of *Ralstonia solanacearum* in seedling, leaf, and fruit sampled from crop potato areas in Sonora (Mexico).

The average antibacterial activity obtained from all concentrations and different amounts of oregano extract applied was 25.8 mm. The mean inhibition values found were 38.3, 20.5, and 20.2 mm in diameter, for concentrations 1:1, 1:5, and 1:10, respectively. In a similar study in which the antibacterial activity of the oils of four different varieties was evaluated, oregano on *C. michiganensis* subsp. *michiganensis* (Cmm) bacteria average values of 47.5, 35.6, and 30.8 mm in diameter for the same concentrations and in the amount used applied 15 μl [6] were found. These inhibition results are greater than those found in the present experiment; this may be due not only to the different sensitivity of the bacteria under study but also to the different composition of the oregano oils used. The concentration of essential oil of oregano showed that the greater antibacterial activity against *R. solanacearum* was 1:1 in different amounts applied with 7.5 μl with 35.16, 10 μl with 39.15 mm, and 15 μl with 40.83 mm in diameter. On the other hand, the results of inhibition of *R. solanacearum* were very similar when they were applied with 7.5 and 10 μl of the extract in concentrations 1:5 (average value, 19 mm) and 1:5 (average value, 19.5 mm). However, the amount applied had the greatest effect when 15 μl of the extract was applied, especially in the 1:5 concentration (23.8 mm).

The average antibacterial activity obtained from all concentrations and different amounts of thyme extract evaluated was 26.2 mm. The mean inhibition values found were 36.3, 28.0, and 14.1 mm in diameter, for the concentrations 1.1, 1:5, and 1.10, respectively. Our results were lower compared with [6], which obtained 50.3, 33.0, and 21.0 mm for the same concentrations to *C. michiganensis* subsp. *michiganensis*.

The concentration of thyme oil that showed the greatest halo of growth inhibition of the bacterium *R. solanacearum* was the 1:1 concentration in the different amounts applied, with 33.5, 35.2, and 40.5 mm, when 7.5, 10, and 15 μl, respectively, as shown in **Figure 1(a)**.

When comparing the essential oils of oregano (*L. graveolens*) and thyme (*T. vulgaris* L.) with respect to mean halo values of inhibition in the growth of *R. solanacearum* ($P > 0.05$), there is no significant difference in oregano (25.907) and thyme (27,204). The two oils are considered to have an inhibitory effect on the growth of *R. solanacearum*. Other studies also showed inhibitory capacity in bacterias such as *Agrobacterium tumefaciens*, *C. michiganensis* subsp. *michiganensis*, *Erwinia amylovora*, *E. carotovorum*, *E. Xanthomonas vesicatoria* [9–11]. Regarding the comparison of the average values of the dilutions (1:1, 1:5, 1:10) of the essential oils of oregano and thyme in halo of inhibition of growth of *R. solanacearum* (**Figure 1(a, b)**), the resoults showed a significant difference (p0.05) in the 1:1 dilution. In addition a similar result with the 1:1 dilution was obtained in the growth inhibition study of *C. michiganensis* subsp. *michiganensis* with oregano and thyme oils [6].

In the comparison of the mean values of applied amounts (7.5, 10.15 μl) of oregano and thyme essential oils in growth inhibition halo of *R. solanacearum* ($P < 0.05$), there was a significant difference in applied amount of 15 μl:30, 7.5 μl:22.7, and 10 μl:26.05 (**Figure 1(a, b)**). The growth inhibition halo values of the bacterium *R. solanacearum* by the effect of oregano and thyme oils

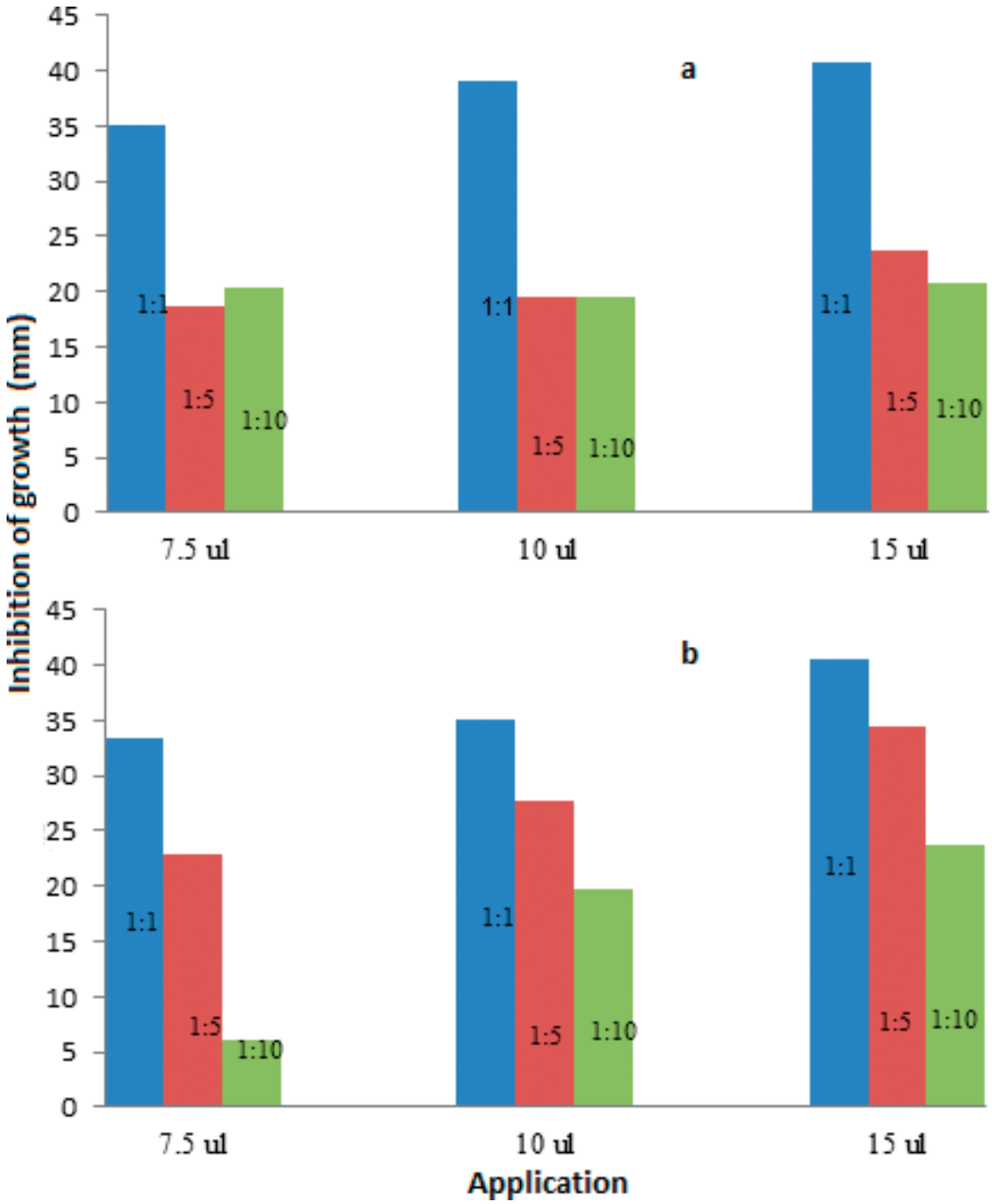

Figure 1. Inhibition of growth of *Ralstonia solanacearum* by oils of oregano (a) and thyme (b) in dilutions of 1:1, 1:5, and 1:10 at 7.5, 10, and 15 μl applied at 24 hours.

are shown in **Table 4** in which it is indicated that ($P > 0.05$) there is no significant difference between oregano stockings 40,833 and thyme 40,500 ha.

Essential oil	Dilutions	Applications	Values
Oregano	1:1	15 μl	40.833 a
Thyme	1:1	15 μl	40.500 a
Oregano	1:1	10 μl	39.000 ab
Thyme	1:1	10 μl	35.167 abc
Oregano	1:1	7.5 μl	35.167 abc
Thyme	1:5	15 μl	34.500 abcd
Thyme	1:1	7.5 μl	33.500 abcde
Thyme	1:5	10 μl	27.833 abcdef
Thyme	1:10	15 μl	24.500 bcdef
Oregano	1:5	15 μl	23.833 cdef
Thyme	1:5	7.5 μl	23.000 cdef
Oregano	1:10	15 μl	20.833 def
Oregano	1:10	7.5 μl	20.333 ef
Thyme	1:10	10 μl	19.833 efg
Oregano	1:5	10 μl	19.500 efg
Oregano	1:5	7.5 μl	18.667 fg
Oregano	1:10	10 μl	15.000 fg
Thyme	1:10	7.5 μl	6.000 g

Values with the same letter does not exist significance ($P > 0.05$).

Table 4. Inhibition of growth of *Ralstonia solanacearum* by essential oils (trifactorial A × B × C).

4. Conclusion

The presence of *Rs* bacterium was proven to be negative in tuber from abroad. Nevertheless, the presence of the causative agent of bacterial wilt in potatoes of national consumption was detected that some producers could use tuber. The presence of *R. solanacearum* was verified through the use of specific culture media called SMSA and TZC under controlled conditions, PPO test, and pathogenicity tests. It is concluded that separate screening tests should not be used as a single detection method. *R. solanacearum* was found to be positive in the tubercle of consumption, in the different vegetative stages (seedling, leaf developed, and fruit tuber) in which samples were taken for detection of bacterial disease; these were negative in all cases, being corroborated under the same detection techniques implemented. However, since the presence of *Rs* in consumption tubers is positive, it represents a risk of a possible manifestation of the disease; it is necessary that the producing areas carry out activities to prevent the disease from developing, such as certified tuber verification, cleaning, and disinfection of machinery, among others, and, even more, to test on imported potatoes to prevent the entry of tuber-containing bacteria.

According to the essential oils of oregano and thyme, they showed inhibitory effects on the growth of the bacterium *R. solanacearum* at the 1:1 dilution result ($P < 0.05$) to be more effective than the rest of the dilutions evaluated, and the most effective applied amount was 15 μl of oregano and thyme essential oil.

The essential oils of oregano and thyme showed ($P < 0.05$) better inhibitory effect than the antibiotics used streptomycin (10 μg) and ampicillin. Therefore, essential oils are excellent alternatives to antibiotics in the control of the bacterium *R. solanacearum.* However, it is very important to consider others studies to evaluate the phytotoxic activity of essential oils studied in this research (*L. graveolens* and *T. vulgaris*) on *Rs* in different phenological stages of potato crop under production system.

Author details

Cristina Ruiz Alvarado[1], Ramón Jaime Holguin Peña[2] and Edgar Omar Rueda Puente[3]*

*Address all correspondence to: erueda2@hotmail.com

1 Universidad Autonoma de Baja California, Instituto de Ciencias Agrícolas, Méxicali, Baja California, México

2 Center for Biological Research of the Northwest, Instituto Politecnico Nacional 195, Colonia Playa Palo de Santa Rita, La Paz, BCS, Mexico

3 Sonora University, Agriculture Department, Boulevard Luis Encinas y Rosales, Colonia Centro, Hermosillo, Son, Mexico

References

[1] Rueda PEO. Producción de antisuero contra *Acidovorax avenae* pv. *citrulli* y su detección en la Comarca Lagunera. [thesis]. Saltillo, Coahuila, México: Universidad Autónoma Agraria Antonio Narro; 1998

[2] SIAP (Servicio de Información Agroalimentaria y Pesquera). 2012. Available from: http://www.siap.go.mx/index.php?option=comwrapper&view=wrapper&itemid=351. [Accessed: Sep 21, 2012]

[3] Daferera DJ, Ziogas BN, Polissiou MG. The effectiveness of plant essential oils in the growth of *Botrytis cinerea*, Fusarium sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protection. 2003;**22**:39-44

[4] Ponce AG, del Valle C, Roura SI. Evaluation of plant essential oil as natural posharvest disease control of tomato (*Lycoperciom esculentum* L.). International Society Horticultural Science. 2004;**628**:737-745

[5] Randhawa P. Fruit Blotch Testing Protocol. Roseville, California, USA: Plant Lab; 1996. p. 110

[6] Borboa FJ, Rueda PE, Acedo EF, Ponce FJ, Cruz VM, García LJH, Ortega NMM. Evaluación de la actividad antibacteriana in vitro de aceites esenciales contra *Clavibacter michiganensis* subespecie *michiganensis*. Tropical and Subtropical Agroecosystems. 2010;**12**:539-547

[7] Alvarado-Martínez Ana G, Rueda-Puente EO, Ponce-Medina JF, Avendaño-Reyes L, Santillano-Cazares J, Borboa-Flores J, Hernández-Montiel L, Holguín-Peña R. Detection of *Ralstonia solanacearum* in *Solanum tuberosum* L. in Sonora, Mexico. Revista de laFaculatad de Ciencias Agrarias Universidad Nacional de Cuyo. 2013;**45**:2

[8] SARH (Secretaría de Agricultura y Recursos Hidráulicos). Manual de muestreo y procesamiento para la identificación de los principales patógenos de la papa. México, D.F.: Dirección General de Sanidad Vegetal; 1994. p. 16

[9] Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M. Antimicrobial and cytotoxic activities of *Origanum essential* oils. Journal of Agricultural and Food Chemistry. 1996;**44**:1202-1205

[10] Smith MD, Navilliat PL. A new protocol for antimicrobial testing of oils. Journal of Microbiology Methods. 1997;**28**:21-24

[11] Soylu S, Soylu EM, Baysal O, Zeller W. Antibacterial activities of the essential oils from medicinal plants against the growth of Clavibacter michiganensis subsp. michiganensis // Biocontrol of Bacterial Plant Diseases, 1st Symposium, 23-26 October, 2005, Darmstadt, Germany. 2005;**408**:82-85

Edited by Mustafa Yildiz

Potato is the world's fourth food crop after maize, wheat, and rice and is a staple crop in many diets throughout the world with a high source of proteins, carbohydrates, minerals, and vitamins. Biotic and abiotic stress factors give rise to decrease in yield. That is why improvement of new cultivars resistant to stress factors by conventional and biotechnological methods is extremely important. The most important factor in production increase is the use of healthy seed tubers along with using drought-, heat-, and salt-tolerant cultivars. On the other hand, protection and storage of surplus crops, which are the most important stage in its marketability, are the main problems in potato. In this book, all these issues are discussed, and it is hoped that the book *Potato* will help growers and researchers in solving problems in potato cultivation.

Published in London, UK

IntechOpen

ISBN 978-1-83881-344-4