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Physical Methods for Stimulation of Plant and Mushroom Development

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Meet the editor

Dr. Mohamed Ahmed El-Esawi is currently a visiting research fellow at the University of Cambridge in the United Kingdom and an associate professor of Molecular Genetics at the Botany Department of Tanta University in Egypt. He received his BSc and MSc degrees from the Tanta University and his PhD degree in Plant Genetics and Molecular Biology from the Dublin Institute of Technology in Ireland. Afterwards, he joined the University of Warwick in the UK, University of Sorbonne (Paris VI) in France and University of Leuven (KU Leuven) in Belgium as a visiting research fellow. His research focuses on plant genetics, genomics, molecular biology, molecular physiology, developmental biology, plant-microbe interaction and bioinformatics. He has authored several international journal articles and book chapters and participated at more than 60 conferences and workshops worldwide. He is currently involved in several research projects in biological sciences.

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Preface

Chemical additives used for increasing plant productivity can contaminate the raw materials used in food production. Physical methods represent alternative promising sources for stimulating plant development and increasing vegetable production. Many physical factors are currently used for plant treatment, including electromagnetic waves, optical emission, laser, magnetic field, gamma rays and ultrasound and ionizing radiation. The sensitivity of plants to the effect of these physical factors has been demonstrated.

This book discusses such physical methods for stimulation of plant development and seed invigoration. Current research trends, future research directions and challenges are also discussed. This book will be of interest to many readers, researchers and scientists who can find this information useful for the advancement of their research works towards a better understanding of physical methods in plant development.

This book includes seven chapters. The first introductory chapter "Physical Methods for Stimulating Plant Growth and Development" presents an introduction to the physical methods and their important applications in plant growth and development. The second chapter "The Effect of Leaf Removal-Based Physical Injury on High Seed and Crude Oil Yields in Sunflower (*Helianthus annuus* L.)" aims to increase the photosynthetic activity in the sunflower via leaf defoliation and consequently to enhance seed and crude oil yields. The third chapter "A Bayesian Multiple-Trait and Multiple-Environment Model Using the Matrix Normal Distribution" provides an improved version of the Bayesian multiple-trait and multiple-environment (BMTME) model that takes into account the correlation between traits (genetic and residual) and environments. The fourth chapter "Branch Formation and Yield by Flower Bud or Shoot Removal in Tomato" investigates the effects of flower bud or shoot removal on plant growth, flowering and yield. The fifth chapter "Using Abrasive Grit for Weed Management in Field Crops" evaluates a fertilizer grit and a non-fertilizer grit for abrasive in-row weed management in maize and soybean. The sixth chapter "Use of Some Bacteria and Mycorrhizae as Biofertilizers in Vegetable Growing and Beneficial Effects in Abiotic Stress Conditions" describes the effects of bacteria and mycorrhiza on vegetable growth and their responses to abiotic stresses. The seventh chapter "High-Voltage Methods for Stimulation of Mushroom Fruit Body Developments" describes the role of high-voltage methods in stimulating the development of the mushroom body.

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Chapter 1

Introductory Chapter: Physical Methods for Stimulating Plant Growth and Development

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Additional information is available at the end of the chapter

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1. Introduction

Various physiological, biochemical, and molecular genetic markers have been applied to enhance plant performance and crop yield [1–19]. The required increase of agricultural production has imposed the essentiality for probing incipient and secured decisions due to the incremented requisite of environmental agricultural products and raw materials, which are both used in food and industrial purposes [20]. The substantial alterations of the atmosphere, soil, or even water which all happen due to the excess utilization of divergent chemical supplements used to increment the yield level are some of the most recent results of anthropogenic adjustments that consequently have led to probing these new alternative methods [20]. Such ways for incrementing the products contain the plausible utilization of supersessions or chemicals through using congruous or applicable physical influences or factors [20]. These influences when used on some biologically controlled comportment are considered as a contemporary trend in amalgamating the consolidation of plant technology with the environmental requisites [20]. Physical methods represent alternative promising sources for stimulating plant development and increasing vegetable production. Many physical factors are currently used for plant treatment, including electromagnetic waves, optical emission, laser, magnetic field, gamma rays, and the ultrasound and ionizing radiation [20]. The sensitivity of plants to the effect of these physical factors has been demonstrated.

Various studies demonstrated that the effect of the magnetic field on the seeds enhances their expeditious growth, root growth, and activated protein formation [20–25]. The results of those studies revealed that the treatment of seeds with the magnetic fields incremented nonstandard seed germination and quality. The rationale behind these reactions can be detected in some of the characteristics of green plastids, namely chloroplasts, which represent the photosynthesis apparatus of higher plants.

Several studies recently showed that the treatment by utilizing the ultrasound radiations can transform the conditions of some substances and hence expedite the interactions between them [20]. Such facts have incentivized their implementation to stimulate the development of various cultures [26, 27]. Effects of 22 kHz frequency and 150 W power ultrasound treatments on germination energy and the seed of carrot (*Daucus carota* L.) showed that the superior influences were verified to be 5 minutes only [20]. Seeds of *Robinia pseudoacacia*, *Caragana arborescens, Laburnum anagyroides*, and *Gleditsia triacanthos* treated with ultrasound radiation have revealed increases in the germinations of the seeds, shoot length, and fresh weights [20]. It can be inferred that ultrasound treatment has played the vital role of the factors stimulating plant growth. Ionizing radiation effect on plant growth has also studied [28].

2. Importance of application of physical methods on plant growth

Chemical additives used for increasing plant productivity cause the contamination of raw materials required for food production [20]. Physical methods are applied for enhancing crop yield and plant growth and development. These methods include the plant treatment with electromagnetic waves, particularly optical emission, ultrasound and ionizing radiation, and magnetic field [20]. Using physical methods for stimulating plant growth has recently increased [21, 22, 29–32]. Additionally, further studies demonstrated that the development of the living organisms is recognized by the effect on physical factors, such as magnetic field, electromagnetic spectrum, and gamma rays [20, 27]. Those factors define the environment for plant growth. Upon physical treatment, the energy in cells is involved in facilitating molecular transformations; therefore, the cells are provided with the required substances [20]. This work discusses the physical methods and properties for stimulation of plant development and seed invigoration. Current research trends, future research directions, and challenges are also discussed.

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Chapter 2

The Effect of Leaf Removal–Based Physical Injury on High Seed and Crude Oil Yields in Sunflower (*Helianthus annuus* L.)

Mustafa Yildiz, Mehdi Taher, Marieh Javani, Ramazan Beyaz and Mehtap Gursoy

Additional information is available at the end of the chapter

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Abstract

Yield in agricultural production decreases due to biotic (diseases and pests) and abiotic (salinity, drought, high temperature, etc.) stress factors. Chemical methods have been widely used to fight against biotic stress factors. However, the use of chemicals in agriculture causes extra financial cost and environmental pollution. Improvement of high yielded cultivars via plant breeding methods does not seem to be adequate for meeting food demand of increasing population. That is why, the improvement of environmentally friendly new methods for high yield is obligatory. Leaves in plants form an active surface for photosynthesis. High photosynthetic activity affects yield directly by increasing matter production. The aim of this study was to increase seed and oil yields in sunflower via leaf defoliation. Oil-type sunflower cultivars used in the study, "08-TR-003," "TR-3080," and "TARSAN-1018," were obtained from the "Trakya Agricultural Research Institute." When plants reached to "star-shaped head stage," which is the beginning of the reproductive period, four different defoliation treatments were performed. They were control (no leaves removed), two leaves removed, four leaves removed, and six leaves removed. Half of the leaves were removed from just below the head, while the other half was removed from the middle part of the plant. After harvest, seed yield per plant, seed yield per decare, crude protein percentage, crude oil percentage, crude protein yield per decare, and crude oil yield per decare were determined. At the end of the study, it was observed that the application of defoliation, compared to the control, affected all characteristics positively.

Keywords: Sunflower, defoliation, seed yield, crude protein yield, crude oil yield

1. Introduction

Plants comprise the source of life on earth. In total, 90% of the energy and 80% of the protein consumed by humans are of plant origin. The remaining energy and protein requirements are met by animal products. 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.

It is estimated that world population will increase by 1.5% per year to 8 billion in 2020 and 11 billion in 2050 [1]. The area of land covering the Earth is 14 billion hectares. Currently, 10% of this land area is cultivated. About 20% of the world's land is covered with pastures, 20% with mountains, 20% with glaciers, and 20% with deserts. The remaining 10% of the area has a very shallow soil cover. Given the impossibility of agricultural activities in mountains and glacier-covered areas, there are areas of potential agriculture, such as marshlands, deserts, or areas with insufficient land cover. It is largely impossible to use pastures that cover rugged and very sloping areas as cultivating fields. The conversion of deserts and inadequate land cover into agricultural land requires great investment.

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. It is estimated that agricultural land per capita, which is now 0.26 hectares, will decrease to 0.15 hectares by 2050. In addition, the availability of water resources for modern agriculture will become difficult due to increased water consumption and increasing water pollution [1]. It is expected that food requirements in the most populous parts of the world will double by 2025 [1].

The yield in agricultural production declines due to biotic and abiotic stress factors. Developing a resistant or tolerant cultivar against these stress factors is the main goal of plant breeding. Chemical methods are commonly used to combat biotic stressors (diseases and pests) that reduce crop production. However, the use of chemicals in agriculture causes an extra financial burden and pollutes the environment. In Turkey, 2.3 million tons of chemical fertilizer and 25,000 tons of pesticides (insecticides, fungicides, and herbicides) were used according to the data from 2013. In the last 25 years, it has emerged that the unconscious use of fertilizers and chemicals applied in plant production has negatively affected long-term ecological balance. For example, it has been determined that overused nitrogen fertilizers are washed from the soil and pollute drinking water and the seas, while the nitrogen components that are escaping from the gaseous state are adversely affecting the ozone layer, which protects the earth from harmful rays of the sun. In addition, herbicides and insecticides applied to combat weeds and pests have been shown to destroy the natural equilibrium in agricultural areas, causing the emergences of new diseases and pesticides. It has also been understood that certain chemicals, which have permanent effects, accumulate in plants, and this negatively affects the health of people and animals fed on those plants. As a result, it is

not possible to increase the crop production by using more chemical fertilizers and pesticides in the future.

On the one hand, the world population is increasing day by day, and on the other hand, the limits of agricultural land have been reached; it is clear that yield increases still need to continue into the future [2].

Two types of sunflower are grown in Turkey and the rest of the world for oil production and for producing snacks. The production of sunflower oil in Turkey is mostly concentrated in the Trakya-Marmara region, while the production of sunflowers for snacks is mostly carried out in the Central and Eastern Anatolia regions. Oil-type sunflowers are generally black colored, thin-crusted, with 38 to 50% oil and 20% protein in their seeds. Sunflower oil has one of the highest nutritional values among vegetable oils because it contains a high percentage of poly-unsaturated fatty acids and a low proportion of saturated fatty acids.

Highly efficient genotypes are used to increase the yield in a unit area of land. Chemical fertilization is carried out, and chemical treatments are applied to combat the diseases and pests that cause yield losses in large quantities. However, it is possible to increase the production to a certain degree by using high-yielding cultivars, fertilizing and applying chemicals where necessary. Development of new plant cultivars resistant to biotic and abiotic stress factors by using plant breeding (classical and modern) methods is a difficult task, because the resistance to these stress factors is caused by more than one gene (additive gene effect). Therefore, the development of environmentally friendly new methods to enhance crop production is extremely important.

In plants, the leaves form the active surface for photosynthesis. The high level of photosynthetic activity also increases the production of substances [3, 4]. In our greenhouse trials, it was observed that defoliation, to a certain level, increased metabolic activity and photosynthetic activity. In sunflower, "star-shaped head stage" is the beginning of the flowering and fertilization period (the reproductive period) followed by the formation of seeds. After this cycle, substances formed as a result of photosynthesis are stored in the seeds. High levels of photosynthetic activity in this stage will increase the production of the material in the leaves and will increase important agricultural characteristics such as seed yield, crude protein yield, and crude oil yield.

In the study conducted by Taher et al. [5], seed yield and crude oil yield have been increased significantly by defoliation of the leaves forming the surface for photosynthesis. By the use of the production method described in this study, the amount of crude oil needed in Turkey has been reduced and the large amount of money currently paid for imports was decreased significantly.

2. Materials and method

The study conducted by Taher et al. [5] was carried out in the research fields of the Faculty of Agriculture, Ankara University in the years of 2013 and 2014. Oil-type sunflower cultivars

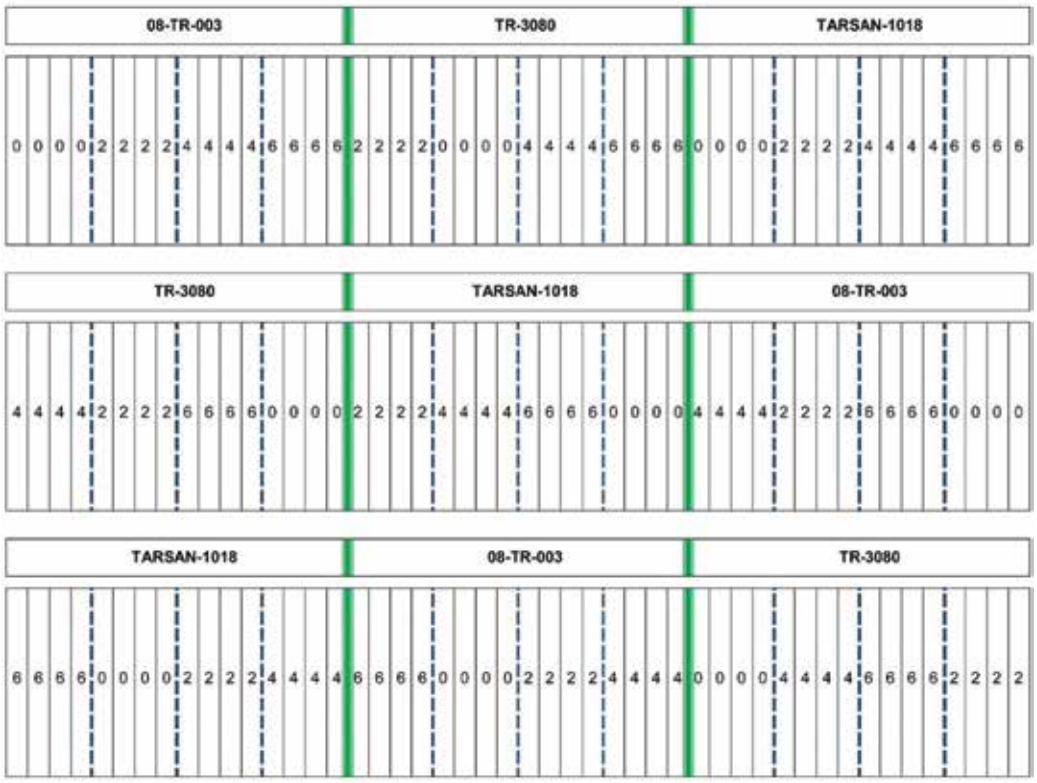

Figure 1. Sowing plan for sunflower cultivars according to the "randomized complete block, split-plots" design with three replications.

"08-TR-003," "TR-3080," and "TARSAN-1018" obtained from "Trakya Agricultural Research Institute" were used in the study. Soil of trial field was plowed 30 cm in depth in fall before winter. In spring, it was plowed again for 10–15 cm in depth to make soil ready for sowing. Sowing was performed in the first week of April with spaces of 70 cm inter-row and 25 cm on-row. Three seeds were put in each dibbling to guarantee the emergence. Two weeks after emergence, two of the plants were eliminated and only one plant left in each dibbling. For all defoliation treatments, plots were fertilized with 14 kg/da diammonium phosphate (DAP) before sowing. During growing, weed control was achieved by hand in experimental field. The study sowing plan is given in **Figure 1**.

When plants reached to "star-shaped head stage," which is the beginning of reproductive period, defoliation was carried out and the plants were labeled. Half of the leaves were removed from just below the head, while the other half was removed from the middle part of the plant for each defoliation treatment (**Figure 2**). Four different defoliation treatments were performed. They were:

- First treatment (Control): Defoliation was not carried out in this case.
- Second treatment: A total of two leaves were removed from the plant. One of these leaves was selected from the below of the head, and the other from the middle of the plant.

- Third treatment: A total of four leaves were removed from the plant. Two of these leaves were taken from the below of the head, and the other two were taken from the middle of the plant.
- Fourth treatment: A total of six leaves were removed from the plant. Three of these leaves were selected from the below of the head, and the remaining three from the middle of the plant.

Plants were irrigated during development according to water need of the plants. During the application of irrigation, the most attention was given to watering each parcel equally. After the flowering and fertilization has been completed, the heads of the plants from which the measurements were taken were covered with paper bags to protect the seeds from damage by birds (**Figure 3**). Plants were harvested when 80% of sunflower heads were brown.

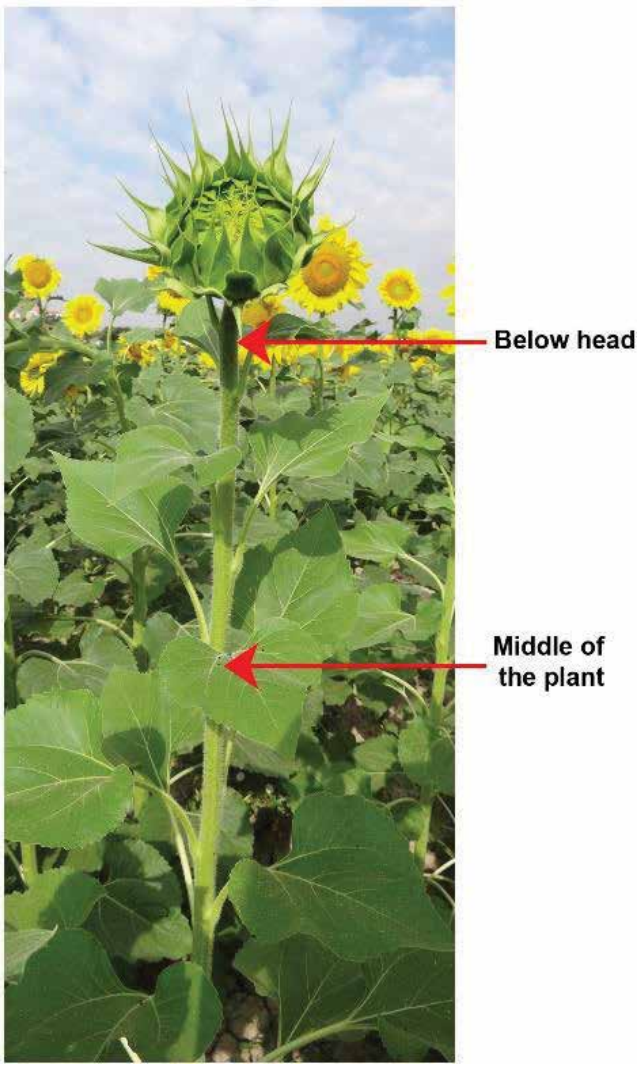

Figure 2. The places from where leaves were removed in "star-shaped head stage" (from below the head and from the middle of the plant).

Figure 3. Flowering in the head (on the left) and covering the head with paper bags to protect seeds from bird's damage (on the right).

Measurements were performed in totally 30 plants (10 plants per replication) in each defoliation treatments in all cultivars. Seed yield per plant (g/plant), seed yield per decare (kg/decare), protein and oil percentages, crude protein, and crude oil yields (kg/decare) were recorded.

Experiments were arranged at "randomized complete block, split-plot" design with three replications. In the experiment, oil-type sunflower cultivars were main plots and four defoliation applications were subplots. Data were statistically analyzed by Duncan's multiple range test using "IBM SPSS Statistics 22." Data given in percentages were subjected to arcsine ($\sqrt{X}$) transformation before statistical analysis [6].

3. Results and discussion

There are research studies examining the effects of defoliation on seed and crude oil yields in sunflower. However, in all these studies, it was reported that leaf removal from plant gave rise to decreases in seed yield and crude oil yield. It was thought that these negative results were caused by the incorrect and incomplete application of the methods used in those researches. In some studies, all leaves in plant were removed [7–9], while 1/3 or 2/3 of leaves were removed in some other ones [9, 10]. Or defoliation was carried out in the lower, middle, and upper leaves of the plants [11, 12]. It was reported that effective leaves on yield were in top and middle of the plant [13]. In our study, a certain amount of defoliation (0 = control,

two, four, and six leaves removed) was carried out in the middle of the plant and from the below of the head in "star-shaped head stage," which is the beginning of the reproductive period in the plant, and the results of photosynthesis are assimilated and transported to the seeds. When the amount of assimilation produced by photosynthesis is increased, the seed yield will also directly increase.

In this study, on the effects of different defoliation treatments on seed, crude protein, and crude oil yields per decare in "star-shaped head stage," which is the beginning of the reproductive period in sunflower, it was determined that different defoliation treatments, according to cultivars, significantly increased seed, crude protein, and crude oil yields compared to the control group with no defoliation treatment (**Table 1**).

For cv. "08-TR-003," the seed yield per decare was 385.4 kg in the control treatment in which no leaf was removed, while it was 431.2 kg in the four-leaf defoliation treatment in the plant. This means that the yield increased by 11.87%. An increase of 1.90% was observed in the crude protein yield obtained from a decare. When the oil yield per decare value was examined, it was 175.0 kg for the control treatment, while it was 207.7 kg — an increase of 18.67% — in the four-leaf defoliation group. The highest values for seed, crude protein, and crude oil yields in the cv. "08-TR-003" were obtained when four leaves per plant were removed (**Table 1**).

In cv. "TR-3080," the seed yield per decare in control treatment was measured as 398.3 kg, whereas there was an 8.64% increase to 432.7 kg when two leaves were removed at the beginning of the reproductive period. The protein yield was 66.2 kg in the control, whereas it increased by 6.40% up to 70.4 kg when two leaves were removed. Examining the crude oil yield per decare values, it was 184.8 kg for the control treatment, whereas it increased by 13.36% to 209.5 kg when two leaves were removed from the plant. In cv. "TR-3080," the highest values for seed, crude protein, and crude oil yields were obtained from the two-leaf defoliation treatment (**Table 1**).

In cv. "TARSAN-1018," 407.3 kg/da seed yield determined for the control treatment was 451.6 kg/da when six leaves were removed from the plant. This indicates that in the six-leaf defoliation treatment, seed yield increased by 10.87% compared to the control. Crude protein yield per decare was found to be 75.6 kg for control treatment, while it was 85.1 kg for the six-leaf defoliation, an increase of 12.61%. The crude oil yield per decare value in the control group was 190.7 kg, while it was 215.3 kg for the six-leaf defoliation treatment, with an increase of 12.92%. In cv. "TARSAN-1018," the highest values for seed, crude protein, and crude oil yields were obtained from the six-leaf defoliation treatment (**Table 1**).

Leaves forming the surface for active photosynthesis in plants can be damaged due to environmental factors (such as storms and hail) and mechanical factors (tools and machines used in maintenance operations such as drilling and spraying). The extent of this damage is directly proportional to the amount of defoliation. In other words, as the number of defoliations increases in the plant, the agricultural characteristics decrease proportionally, based on the cultivar. This is confirmed by the lowest values for the seed yield per decare values obtained in our study for the six-leaf defoliation treatments for cvs. "08-TR-003" and "TR-3080."

Cultivars	Defoliation treatment	Seed yield (g/plant)	Seed yield (kg/da)	The increase in the seed yield compared to control (%)	Crude protein percentage (%)	Crude protein yield (kg/da)	The increase in the crude protein yield compared to control (%)	Crude oil percentage (%)	Crude oil yield (kg/da)	The increase in the crude oil yield compared to control (%)
"08-TR-003"	0 (Control)	70.1 ± 0.68 c	385.4 ± 3.74 c	11.87	18.2 ± 0.41 a	70.4 ± 1.12 a	1.90	45.4 ± 1.49**a**	175.0 ± 4.16 c	18.67
	2	67.6 ± 0.85 d	371.9 ± 4.68 d		16.7 ± 0.38 a	62.8 ± 1.10 b		46.7 ± 0.63**a**	173.1 ± 1.08 c	
	4	78.4 ± 0.28 a	431.2 ± 1.52 a		16.6 ± 0.56 a	71.7 ± 1.25 a		48.3 ± 0.45**a**	207.7 ± 1.18 a	
	6	72.6 ± 0.72 b	399.0 ± 3.97 b		16.9 ± 0.49 a	68.2 ± 0.53 ab		46.5 ± 0.50**a**	185.1 ± 1.84 b	
"TR-3080"	0 (Control)	72.4 ± 0.10 b	398.3 ± 0.57 ab	8.64	16.7 ± 0.76 a	66.2 ± 0.75 b	6.40	46.3 ± 1.32**a**	184.8 ± 1.47 b	13.36
	2	78.7 ± 0.82 a	432.7 ± 4.50 a		16.3 ± 0.41 a	70.4 ± 0.67 a		48.4 ± 0.44**a**	209.5 ± 1.60 a	
	4	70.6 ± 0.69 b	388.1 ± 3.77 b		17.5 ± 0.17 a	67.7 ± 0.81 b		47.6 ± 1.34**a**	184.7 ± 2.06 b	
	6	68.5 ± 1.00 c	376.6 ± 5.51 b		15.7 ± 0.03 a	59.2 ± 1.13 c		46.3 ± 1.79**a**	173.8 ± 1.29 c	
"TARSAN-1018"	0 (Control)	74.1 ± 0.80 b	407.3 ± 4.41 b	10.87	18.6 ± 0.72 a	75.6 ± 0.86 c	12.61	46.8 ± 0.46**a**	190.7 ± 1.32 c	12.92
	2	77.3 ± 0.42 b	425.3 ± 2.30 ab		18.1 ± 0.34 a	77.1 ± 0.90 c		46.9 ± 0.26**a**	199.7 ± 1.67 bc	
	4	80.6 ± 0.24 ab	443.4 ± 1.30 a		20.3 ± 0.09 a	81.0 ± 1.02 b		46.4 ± 0.25**a**	206.0 ± 5.96 b	
	6	82.1 ± 0.10 a	451.6 ± 1.20 a		18.9 ± 1.31 a	85.1 ± 0.77 a		47.8 ± 1.29**a**	215.3 ± 1.46 a	
Mean				10.46			6.97			14.98

The difference between the averages indicated by different letters in the same column is statistically significant at the 0.01 level.

Table 1. The effect of different defoliation treatments on seed yield per plant, seed yield per decare, crude protein percentage, crude protein yield, crude oil percentage, and crude oil yield in sunflower (*Helianthus annuus L.*).

Considering the general average values of the three cultivars used in this study, seed yield increased by 10.46%, crude protein yield increased by 6.97%, and crude oil yield increased by 14.98% compared to the control group when defoliation treatment was applied in "star-shaped head stage," which is the beginning of the reproductive period.

4. Economic analysis

In 2014, we estimated that the Turkish population was 77,695,904 [14] and that annual consumption of vegetable oil per capita should be 21 kg, corresponding to 1,630,000 tons of vegetable oil needs. Accordingly, 800,000 tons of this was met with domestic production, and a shortage of 835,000 tons of crude oil was identified [15]. In 2014, 795,000 tons of crude oil was obtained from sunflower.

Turkey paid 1194 US dollars for importing 1 ton of vegetable crude oil in 2014, and 835,000 tons of crude oil met by imports would be 996, 990,000 US dollars [15]. In 2014, oil-type sunflower seeds were sown over an area of 5,524,651 decares in Turkey and 1,480,000 tons of sunflower seeds were produced; the seed yield was 269.00 kg/da [16]. The crude oil yield in the sunflower was 143.90 kg/da.

According to the results obtained in this study, when leaves are reduced in sunflower cultivation, the seed yield per decare will increase from 269.00 to 297.13 kg, a 10.46% increase, and the crude oil yield will increase from 143.90 to 165.95 kg, a 14.98% increase. This means that crude oil production in Turkey from sunflower will be 914,054 tons (165.45 × 5,524,651). In other words, when the method described in the current study based on defoliation is applied to the production of sunflower, the production of crude oil in Turkey will increase to 119,054 tons (914,054–795,000). Considering that 1194 US dollars was paid for 1 ton of crude oil import, it is seen that 142,150,476 dollars (119,054 × 1194) will be retained domestically by applying the method developed in our research.

5. Conclusion

Due to the increasing world population and the rapid consumption of natural resources, there is a need to increase crop production. Aside from increasing the agricultural lands to increase crop production, existing agricultural lands are decreasing day by day. In this case, it is necessary to develop new high-yielding cultivars and to apply agricultural techniques (fertilization, irrigation, and agricultural pest control) as the best way to increase crop production. However, with the rapidly increasing world population and ever-narrowing areas available for agriculture, the development of new cultivars resistant to biotic and abiotic stress factors (extreme heat, extreme cold, salinity, new pest culprits, and pest breeds) is extremely difficult due to time limitations and to the resistance characteristics being under the control of more than one gene. Therefore, it is necessary to develop new methods in order to increase the yield per unit area for plants that play an important part in human nutrition (wheat, corn, rice, sunflower, etc.).

With this research, it has been shown that crop production can be increased by physiological stimulation of plants. In greenhouse researches, it has been determined that reduction in the photosynthetic surface, through a certain number of defoliations in the plant, results in an increase in photosynthetic activity in the remaining leaves of the plant, which causes significant increases in the agricultural characteristics.

Using the method developed in this research, decreasing the number of leaves at the beginning of the reproductive stage in sunflower plant has resulted in significant increase in agricultural characteristics such as seed yield, crude protein yield, and crude oil yield. Thanks to this developed environmentally friendly production method, an increase of about 120,000 tons of crude oil production has been achieved in sunflower. The developed method can be successfully used in other plants to increase the crop production.

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Chapter 3

A Bayesian Multiple-Trait and Multiple-Environment Model Using the Matrix Normal Distribution

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Abstract

Genomic selection (GS) is playing a major role in plant breeding for the selection of candidate individuals (animal or plants) early in time. However, for improving GS better statistical models are required. For this reason, in this chapter book we provide an improved version of the Bayesian multiple-trait and multiple-environment (BMTME) model of Montesinos-López et al. that takes into account the correlation between traits (genetic and residual) and between environments since allows general covariance's matrices. This improved version of the BMTME model was derived using the matrix normal distribution that allows a more easy derivation of all full conditional distributions required, allows a more efficient model in terms of time of implementation. We tested the proposed model using simulated and real data sets. According to our results we have elements to conclude that this model improved considerably in terms of time of implementation and it is better than a Bayesian multiple-trait, multiple-environment model that not take into account general covariance structure for covariance's of the traits and environments.

Keywords: genomic selection, multiple-trait and multiple-environment, Bayesian, general covariance's matrices

1. Introduction

Genomic selection is revolutionizing plant breeding, since allows the selection of candidate individuals (animal or plants) early in time. However, the success of genomic selection is

linked directly to the use of statistical models, since the process of selection of candidate individuals is done using statistical models. However, most of the models currently used in genomic selection are univariate models mostly for continuous phenotypes, which not exploit the existing correlation between traits when the selection of individuals (genotypes or animals) is done with the purpose to improve simultaneously multiple-traits. The advantage of jointly modeling multiple-traits compared to analyzing each trait separately, is that the inference process appropriately accounts for the correlation among the traits, which helps to increase prediction accuracy, statistical power, parameter estimation accuracy, and reduce trait selection bias [1, 2]. For this reason, there is a great interest of plant and animal scientist to develop appropriate genomic selection models for multiple-traits and multiple-environments to take advantage of this correlation and to improve the prediction accuracy in the selection of candidate individuals.

For this reason, in this chapter we propose an improved version of the Bayesian multiple-trait, multiple-environment (BMTME) model proposed by Montesinos-López et al. [3] that is appropriate for correlated multiple-traits and multiple-environments but instead of building this model using the multivariate normal distribution we propose to build it using the matrix normal distribution which should avoid that the number of rows of the datasets grows proportional to the number of traits under study.

Also, the BMTME model was improved adding a general covariance structure for the genetic covariance of environments in place of assuming a diagonal matrix as the original BMTME model. Additionally, in this chapter we compare the improved model in terms of prediction accuracy and time of implementation with the original BMTME model of Montesinos-López et al. [3] and with a multiple-trait and multiple-environment model where it is ignored the correlation between traits and between environments. Our hypothesis is that the improved model should be similar in terms of prediction accuracy, but considerably faster in terms of time of implementation with regard to the original BMTME of Montesinos-López et al. [3] and a little better in terms of prediction accuracy that a multiple-trait and multiple-environment model that ignore the correlation between traits and environments. Also, we propose to implement the proposed model with simulated and real data sets. Our results suggest that the construction and implementation of the proposed model should be of great help for breeding scientist and programs since will help to select candidate genotypes early in time with more accuracy.

2. Material and methods

2.1. Matrix normal distribution

The matrix normal distribution is a probability distribution that is a generalization of the multivariate normal distribution to matrix-valued random variables. According with Rowe [4] the $n \times p$ matrix normal distribution can be derived as a special case of the np-variate Multivariate Normal distribution when the covariance matrix is separable. A np-dimensional vector $\boldsymbol{x}$ is distributed according to multivariate normal distribution with np-dimensional mean $\boldsymbol{\mu}$ and $np \times np$ covariance matrix $\boldsymbol{\Omega}$ if its probability density function is given by

$$P(\boldsymbol{x}|\boldsymbol{\mu}, \boldsymbol{\Omega}) = (2\pi)^{-\frac{np}{2}}|\boldsymbol{\Omega}|^{-\frac{1}{2}}e^{-\frac{1}{2}(\boldsymbol{x}-\boldsymbol{\mu})^T\boldsymbol{\Omega}^{-1}(\boldsymbol{x}-\boldsymbol{\mu})} \tag{1}$$

When the covariance matrix $\boldsymbol{\Omega}$ is separable, that is, is one of the form $\boldsymbol{\Omega}=\boldsymbol{\Sigma}\otimes\boldsymbol{\Phi}$, where $\otimes$ is the Kronecker product which multiplies every entry of its first matrix argument by its entire second matrix argument, Eq. (1) becomes

$$p(\boldsymbol{X}|\boldsymbol{M}, \boldsymbol{\Sigma}, \boldsymbol{\Phi}) = (2\pi)^{-\frac{np}{2}}|\boldsymbol{\Sigma}|^{-\frac{n}{2}}|\boldsymbol{\Phi}|^{-\frac{p}{2}}e^{-\frac{1}{2}tr\left[\boldsymbol{\Sigma}^{-1}(\boldsymbol{X}-\boldsymbol{M})^T\boldsymbol{\Phi}^{-1}(\boldsymbol{X}-\boldsymbol{M})\right]} \tag{2}$$

upon using the following matrix identities

$$|\boldsymbol{\Omega}| = |\boldsymbol{\Sigma}\otimes\boldsymbol{\Phi}| = |\boldsymbol{\Sigma}||\boldsymbol{\Phi}| \tag{3}$$

and

$$(\boldsymbol{x}-\boldsymbol{\mu})^T(\boldsymbol{\Sigma}\otimes\boldsymbol{\Phi})^{-1}(\boldsymbol{x}-\boldsymbol{\mu}) = tr\left[\boldsymbol{\Sigma}^{-1}(\boldsymbol{X}-\boldsymbol{M})^T\boldsymbol{\Phi}^{-1}(\boldsymbol{X}-\boldsymbol{M})\right] \tag{4}$$

where $\boldsymbol{X}$ and $\boldsymbol{M}$ are matrix of dimension $n\times p$ such that $\boldsymbol{x}=vec(\boldsymbol{X})$ and $\boldsymbol{\mu}=vec(\boldsymbol{M})$, with *vec* is the *vec* operator that stacks the columns of its matrix argument from left to right into a single vector, *tr*(.) is the trace operator which gives the sum of the diagonal elements of a square matrix argument.

Then, according with Rowe [4] the density function given in Eq. (2) correspond to a random variable that follows a $n\times p$ matrix normal distribution and it is denoted as

$$\boldsymbol{X}|\boldsymbol{M}, \boldsymbol{\Sigma}, \boldsymbol{\Phi} \sim MN_{n\times p}(\boldsymbol{M}, \boldsymbol{\Phi}, \boldsymbol{\Sigma}) \tag{5}$$

where $(\boldsymbol{M}, \boldsymbol{\Sigma}, \boldsymbol{\Phi})$ parametrize the above distribution with $\boldsymbol{M}\in\mathbb{R}^{n\times p}$, and $\boldsymbol{\Sigma}$ and $\boldsymbol{\Phi}$ are positive defined matrix of dimension $n\times n$ and $p\times p$, respectively. The matrices $\boldsymbol{\Sigma}$ and $\boldsymbol{\Phi}$ are commonly referred to as the within and between covariance matrices. Sometimes they are referred to as the right and left covariance matrices [4].

Some useful properties of the matrix normal distribution are: the mean and model is equal to $E(\boldsymbol{X}|\,\boldsymbol{M}, \boldsymbol{\Sigma}, \boldsymbol{\Phi})=\boldsymbol{M}$ and the variance $\text{var}(vec(\boldsymbol{X})|\,\boldsymbol{M}, \boldsymbol{\Sigma}, \boldsymbol{\Phi})=\boldsymbol{\Sigma}\otimes\boldsymbol{\Phi}$, which can be found by integration and differentiation. Since $\boldsymbol{X}$ follows a Matrix Normal distribution, the conditional and marginal distributions of any row or column subset are Multivariate Normal distributions [4].

2.2. Univariate model with genotype by environment interaction (M1)

First, for each trait we considered the following univariate linear mixed model:

$$y_{ij} = E_i + g_j + gE_{ij} + e_{ij} \tag{6}$$

were y_{ij} represents the normal response from the jth line in the ith environment ($i=1, 2, \ldots, I$, $j=1, 2, \ldots, J$). For illustration purposes, we will use $I=3$. E_i represents the fixed effect of the ith environment and is assumed as a fixed effect, g_j represents the random effect of the genomic

effect of jth line, with $\boldsymbol{g} = \left(g_{j'} \ldots, g_J\right)^T \sim N\left(\mathbf{0}, \sigma_1^2 \boldsymbol{G}_g\right)$, $\boldsymbol{G}_g$ is of order $J \times J$ and represents the Genomic Relationship Matrix (GRM) and is calculated using the VanRaden method [5] as $\boldsymbol{G}_g = \frac{\boldsymbol{W}\boldsymbol{W}^T}{p}$, with $\boldsymbol{W}$ as the matrix of marker of order $J \times p$. gE_{ij} is the random interaction term between the genomic effect of the jth line and the ith environment where $\boldsymbol{gE} = \left(gE_{11}, \ldots, gE_{IJ}\right)^T \sim N\left(\mathbf{0}, \sigma_2^2 \mathbf{I}_I \otimes \boldsymbol{G}\right)$ and e_{ij} is a random error term associated with the jth line in the ith environment distributed as $N(0, \sigma^2)$. As previously mentioned, this model was used for each of the $l = 1, \ldots, L$ traits, where L denotes the number of traits under study.

2.3. Multivariate correlated model with multiple-trait and multiple-environment (M2)

To account for the correlation between traits, all of the L traits given in Eq. (6) should be jointly modeled in a whole multiple-trait, multiple-environment mixed model as the following:

$$\boldsymbol{Y} = \boldsymbol{X}\boldsymbol{\beta} + \boldsymbol{Z}_1\boldsymbol{b}_1 + \boldsymbol{Z}_2\boldsymbol{b}_2 + \boldsymbol{e} \tag{7}$$

where $\boldsymbol{Y}$ is of order $n \times L$, with $n = I \times J$, $\boldsymbol{X}$ is of order $n \times I$, $\boldsymbol{\beta}$ is of order $I \times L$ and contains the beta coefficients of the environment by trait combinations, $\boldsymbol{Z}_1$ is of order $n \times J$, $\boldsymbol{Z}_2$ is of order $n \times IJ$, $\boldsymbol{b}_1$ is of order $J \times L$ and follows a normal matrix distribution $MN_{J \times L}(\mathbf{0}, \boldsymbol{G}_g, \boldsymbol{\Sigma}_t)$, $\boldsymbol{b}_2$ is of order $IJ \times L$ with a normal matrix distribution $\boldsymbol{b}_2 \sim MN_{IJ \times L}(\mathbf{0}, \boldsymbol{\Sigma}_E \otimes \boldsymbol{G}_g, \boldsymbol{\Sigma}_t)$ and $\boldsymbol{e}$ is of order $n \times L$ with a normal matrix distribution $\boldsymbol{e} \sim MN_{n \times L}(\mathbf{0}, \boldsymbol{I}_n, \boldsymbol{R}_e)$, where $\boldsymbol{\Sigma}_t$ is the genetic covariance matrix between traits and it is assumed unstructured (or general), $\otimes$ denotes a Kronecker product, $\boldsymbol{\Sigma}_E$ is assumed as a general matrix of order $I \times I$, $\mathbf{R}_e$ is the residual general covariance matrix between traits. It is important to point out that the trait × environment (T × E) interaction term is included in the fixed effects, while the trait × genotype (T × G) interaction term is included in the random effect $\boldsymbol{b}_1$ and the three-way (T × G × E) interaction term is included in $\boldsymbol{b}_2$.

2.4. Joint posterior density and prior specification

In this section, we provide the joint posterior density and prior specification for the improved BMTME model. Assuming independent prior distributions for $\boldsymbol{\beta}$, $\boldsymbol{\Sigma}_t$, $\boldsymbol{\Sigma}_E$, and $\boldsymbol{R}_e$, the joint posterior density of the parameter vector becomes:

$$P\left(\boldsymbol{\beta}, \boldsymbol{b}_1, \boldsymbol{b}_2, \boldsymbol{\Sigma}_t, \boldsymbol{\Sigma}_E, \mathbf{R}_e\right) \propto P\left(\boldsymbol{Y}\middle|\boldsymbol{\beta}, \boldsymbol{b}_1, \boldsymbol{b}_2, \boldsymbol{R}_e\right) P\left(\boldsymbol{\beta}\right) P(\boldsymbol{b}_1|\boldsymbol{\Sigma}_t)\boldsymbol{P}(\boldsymbol{b}_2|\boldsymbol{\Sigma}_t, \boldsymbol{\Sigma}_E)P(\boldsymbol{\Sigma}_t)\boldsymbol{P}(\boldsymbol{\Sigma}_E)\boldsymbol{P}(\boldsymbol{R}_e) \tag{8}$$

where $P(\boldsymbol{\beta})$, $P(\boldsymbol{\Sigma}_t)$, $P(\boldsymbol{\Sigma}_E)$ and $P(\mathbf{R}_e)$ denote the density prior distributions of $\boldsymbol{\beta}$, $\boldsymbol{\Sigma}_t$, $\boldsymbol{\Sigma}_E$, and $\boldsymbol{R}_e$, respectively. Specifically, we are assuming an Inverse-Wishart (IW) for $\boldsymbol{\Omega}_v$ with shape parameter κ and scale matrix parameter $\boldsymbol{B}$, and is denoted by $\boldsymbol{\Omega}_v \sim IW(\kappa, \boldsymbol{B})$, with density function given by $\mathrm{P}(\boldsymbol{\Omega}_v) \propto |\boldsymbol{B}|^{\frac{\kappa}{2}} |\boldsymbol{\Omega}_v|^{-\frac{\kappa+p+1}{2}} \exp\left[-\frac{1}{2} tr\left(\boldsymbol{B}\boldsymbol{\Omega}_v^{-1}\right)\right]$, $\kappa > 0$, $\boldsymbol{B}$, $\boldsymbol{\Omega}_v$ both are positive definite matrices. For the remaining parameters we are assuming the following prior distributions: $\sim MN_{n \times p}(\boldsymbol{\beta}_0, \boldsymbol{I}_I, \boldsymbol{I}_L)$, $\boldsymbol{b}_1 | \boldsymbol{\Sigma}_t \sim MN_{J \times L}(\mathbf{0}, \boldsymbol{G}_g, \boldsymbol{\Sigma}_t)$, $\boldsymbol{\Sigma}_t \sim IW(\nu_t + L - 1, \boldsymbol{S}_t)$, $\boldsymbol{b}_2 | \boldsymbol{\Sigma}_t, \boldsymbol{\Sigma}_E \sim MN_{IJ \times L}(\mathbf{0}, \boldsymbol{\Sigma}_E \otimes \boldsymbol{G}_g, \boldsymbol{\Sigma}_t)$, $\boldsymbol{\Sigma}_E \sim IW(\nu_E + I - 1, \boldsymbol{S}_E)$, and $\mathbf{R}_e \sim IW(\nu_e + L - 1, \boldsymbol{S}_e)$. Next we combine the joint posterior density of the parameter vector with the priors to obtain the full conditional distribution for parameters $\boldsymbol{\beta}$, $\boldsymbol{b}_1$, $\boldsymbol{b}_2$, $\boldsymbol{\Sigma}_t$, $\mathbf{R}_e$. All full conditionals, as well as details of their derivations, are given in Appendix A.

2.5. Gibbs sampler

In order to produce posterior means for all relevant model parameters, below we outline the exact Gibbs sampler procedure that we proposed for estimating the parameters of interest. The ordering of draws is somewhat arbitrary; however, we suggest the following order:

Step 1. Simulate $\boldsymbol{\beta}$ according to the normal distribution given in Appendix A (A.1).

Step 2. Simulate $\boldsymbol{b}_h$ for $h = 1, 2$, according to the normal distribution given in Appendix A (A.2 and A.3).

Step 3. Simulate $\boldsymbol{\Sigma}_t$ according to the IW distribution given in Appendix A (A.4).

Step 4. Simulate $\boldsymbol{\Sigma}_E$ according to the IW distribution given in Appendix A (A.5).

Step 5. Simulate $\mathbf{R}_e$ according to the IW distribution given in Appendix A (A.6).

Step 6. Return to step 1 or terminate when chain length is adequate to meet convergence diagnostics.

2.6. Multivariate uncorrelated model with multiple-trait and multiple-environment (M3)

To compare the model given in Eq. (7) we considered also model **M3** (Eq. 6) that consists of using the following multi-trait, multi-environment model that ignore the correlation between traits and between environments:

$$y_{ijl} = E_i + g_j + T_l + gE_{ij} + TE_{il} + gT_{jl} + gET_{ijl} + e_{ijl} \tag{9}$$

where y_{ijl} represents the normal response from the jth line in the ith environment for trait l ($i = 1, 2, \ldots, I$, $j = 1, 2, \ldots, J$, $l = 1, \ldots, L$). T_l represents the fixed effect of the lth trait, TE_{il} is the fixed interaction term between the lth trait and the ith environment, gT_{jl} represents the random effect of the interaction of genotype j and the lth trait, with $\boldsymbol{gT} = \left(gT_{11}, \ldots, gT_{JL}\right)^T \sim N\left(\mathbf{0}, \sigma^2_{11}\boldsymbol{G} \otimes \boldsymbol{I}_L\right)$, gET_{ijl} is the three-way interaction of genotype j, the ith environment and the lth trait, with $\boldsymbol{gET} = \left(gET_{111}, \ldots, gET_{IJL}\right)^T \sim N\left(\mathbf{0}, \sigma^2_{22}\mathbf{I}_I \otimes \boldsymbol{G} \otimes \boldsymbol{I}_L\right)$ and e_{ijl} is a random error term associated with the jth line in the ith environment distributed as $N(0, \sigma^2)$.

2.7. Experimental data sets

2.7.1. Simulate data sets

For testing the proposed models and methods we simulated multiple-trait and multiple-environment data using model in Eq. (7). We studied six scenarios depending of the parameters used. For the first scenario (S1) we used the following parameters: three environments, three traits, 80 genotypes, 1 replication for environment-trait-genotype combination. We assumed that $\boldsymbol{\beta}^T = [15, 12, 7, 14, 10, 9, 13, 11, 8]$, where the first three beta coefficients belong to traits 1, 2 and 3 in environment 1, the second three values for the three traits in environment 2 and the last three for environment 3. We assumed that the genomic relationship matrix is

known and is equal to $\boldsymbol{G}_g = 0.3\boldsymbol{I}_{80} + 0.7\boldsymbol{J}_{80}$, where $\boldsymbol{I}_{80}$ is an identity matrix of order 80 and $\boldsymbol{J}_{80}$ is a matrix of order 80×80 of ones. Therefore, the total number of observations is $3 \times 80 \times 3 \times 1 = 720$, that is, 240 for each trait. Since a covariance matrix can be expressed in terms of a correlation matrix ($\boldsymbol{R}_r$) and a standard deviation matrix ($\boldsymbol{D}_r^{1/2}$) as: $\boldsymbol{\Sigma}_r = \boldsymbol{D}_r^{1/2}\boldsymbol{R}_r\boldsymbol{D}_r^{1/2}$, with $r = t, E, e$, where $r = t$ represent the genetic covariance between traits, $r = E$ represents the genetic covariance matrix between environments and $r = e$, represents the residual covariance matrix between traits. For the three covariance matrices ($r = t, E, e$) in this scenario we used $\boldsymbol{R}_r = 0.15\boldsymbol{I}_3 + 0.85\boldsymbol{J}_3$, where $\boldsymbol{J}_3$ is a matrix of order 3x3 of ones, and $\boldsymbol{D}_t^{1/2} = \text{diag}(0.9, 0.8, 0.9)$, $\boldsymbol{D}_E^{1/2} = \text{diag}(0.5, 0.65, 0.75)$ and $\boldsymbol{D}_e^{1/2} = \text{diag}(6, 0.43, 0.33)$. For the second scenario (S2) we used exactly the same set of parameters defined in S1 except that for the correlation matrix now we assumed that the pair of correlations between traits and between environments is equal to 0.5, that is, $\boldsymbol{R}_r = 0.5\boldsymbol{I}_3 + 0.5\boldsymbol{J}_3$, while the third scenario (S3) also is exactly as S1 with the exception that $\boldsymbol{R}_r = 0.75\boldsymbol{I}_3 + 0.25\boldsymbol{J}_3$, that is, the pair of correlations between traits and between environments is equal to 0.25. These three set of correlation matrices given in S1, S2 and S3 were proposed in order to study the performance of the methods proposed in the context of high correlation (S1), medium (S2) and low correlation (S3) between traits (genetic and residual) and between environments. Other 3 scenarios were studied: scenario 4 (S4) is exactly as scenario S1 but in place of 80 lines were used 100 lines, scenario 5 (S5) was exactly as scenario S2 but with 100 lines and the last scenario (S6) was exactly as scenario S3 but using 100 lines in place of 80.

2.7.2. Real wheat data set

Here, we present the information on the first real data set used for implementing the proposed models. This real data set composed of 250 wheat lines that were extracted from a large set of 39 yield trials grown during the 2013–2014 crop season in Ciudad Obregon, Sonora, Mexico [6]. The trials under study were days to heading (DTHD), grain yield (GRYLD), plant height (PTHT) and the green normalized difference vegetation index (GNDVI), each of these traits were evaluated in three environments (Bed2IR, Bed5IR and Drip). The marker information used after editing was 12,083 markers. This data set was also used by Montesinos-López et al. [3] for this reason those interested in more details of this data set see this publication.

2.7.3. Real maize data set

The second real data set used for implementing the proposed models is composed of 309 double-haploid maize lines. Traits available in this data set include grain yield (Yield), anthesis-silking interval (ASI), and plant height (PH); each of these traits were evaluated in three optimum rainfed environments (EBU, KAT, and KTI). The marker information used after editing was 12,083 markers. Also, this data set was also used by Montesinos-López et al. [3] for this reason those interested in more details of this data set see this publication.

2.8. Assessing prediction accuracy

For assessing prediction accuracy for the simulated and real data sets a 20 training (trn)-testing (tst) random partitions were implemented under a cross-validation that mimicked a situation

where lines were evaluated in some environments for the traits of interest; however, some lines were missing in all traits in the other environments, this cross-validation scheme is called CV1. Under this cross-validation, we assigned 80% of the lines to the trn set and the remaining 20% to the tst set. We used the Pearson correlation and mean square error of prediction (MSEP) to compare the predictive performance of the proposed models. Models with Pearson correlation closet to one indicated better predictions, while under the MSEP values closed to zero are better in terms of prediction accuracy. It is important to point out that model **M2** was implemented with R code done for the authors implementing the Gibbs sampler given above for this model, while model **M3** was implemented in the R package BGLR [7].

3. Results

The results are presented in two sections. The first section presents the results of the simulated data set, while the second the results with the real data sets.

3.1. Simulated data sets

In **Table 1**, under scenario S1 we can observe that the proposed model **M2** was the best in terms of prediction accuracy (with Pearson correlation and MSEP) since in the 9 trait-environment combinations model **M2** (improved BMTME model) was better than model **M3** (uncorrelated multiple-trait multiple-environment). In average in terms of Pearson correlation the model **M2** was better than the model **M3** by 8.72%, while in terms of MSEP model **M2** was better than model **M3** in average by 6.24%. Under scenario S2, in terms of Pearson correlation model **M2** was better in 7 out of 9 trait-environment combinations and in 6 out of 9 trait-environment combination in terms of MSEP. In terms of Pearson correlation model **M2** was better than **M3** in average by 7.76%, while in terms of MSEP was better by 2.27% in average (**Table 1**). While under scenario S3 also model **M2** was better than model **M3**, since in 7 out of 9 trait-environment was the best, while under MSEP model **M2** was better than **M3** in 5 out of 9 trait-environment combination, however, in average model **M2** was better than model **M3** by 3.98 and 1.028% in terms of Pearson correlation and MSEP, respectively (**Table 1**).

In **Table 2**, under scenario S4 model **M2** was the best in terms of prediction accuracy (with Pearson correlation and MSEP) since in the 9 trait-environment combinations was better than model **M3**. In average in terms of Pearson correlation and MSEP model **M2** was better than model **M3** by 4.4 and 4.1%, respectively. Also, under scenario S5, in terms of Pearson correlation and MSEP, model **M2** was better than model **M3** in 7 out of 9 and in 6 out of 9 trait-environment combinations, respectively**.** Model **M2** was better than **M3** in average by 1.6% in terms of Pearson correlation and by 1.2% in average in terms of MSEP (**Table 2**). While under scenario S6 also model **M2** was better than model **M3** in terms of Pearson correlation, since in 7 out of 9 trait-environment was the best, while under MSEP model **M2** was better than **M3** in 5 out of 9 trait-environment combination, however, in average model **M2** was better than model **M3** by 1.6 and 1.02% in terms of Pearson correlation and MSEP, respectively (**Table 2**).

Scenario	Trait_Env	M2				M3			
		CorP	**SE**	**MSEP**	**SE**	**CorP**	**SE**	**MSEP**	**SE**
	11	**0.401**	0.052	**0.693**	0.050	0.375	0.048	0.723	0.050
	21	**0.481**	0.044	**0.561**	0.033	0.434	0.044	0.605	0.035
	31	**0.563**	0.042	**0.494**	0.033	0.530	0.043	0.522	0.033
	12	**0.408**	0.037	**0.658**	0.045	0.343	0.041	0.715	0.046
S1	22	**0.485**	0.049	**0.648**	0.049	0.393	0.053	0.728	0.056
	32	**0.506**	0.042	**0.580**	0.049	0.420	0.049	0.642	0.049
	13	**0.595**	0.030	**0.528**	0.033	0.570	0.034	0.535	0.034
	23	**0.473**	0.043	**0.565**	0.036	0.461	0.039	0.582	0.039
	33	**0.629**	0.031	**0.424**	0.027	0.619	0.036	0.441	0.033
	Average	**0.505**	0.041	**0.572**	0.040	0.461	0.043	0.610	0.042
	11	**0.349**	0.054	**0.748**	0.057	0.302	0.052	0.750	0.055
	21	**0.486**	0.044	**0.571**	0.030	0.447	0.042	0.603	0.031
	31	**0.503**	0.044	0.588	0.033	0.508	0.045	**0.579**	0.031
S2	12	**0.384**	0.037	**0.590**	0.045	0.335	0.038	0.602	0.040
	22	**0.476**	0.049	**0.664**	0.047	0.407	0.053	0.726	0.057
	32	**0.415**	0.044	**0.626**	0.059	0.368	0.048	0.651	0.061
	13	**0.599**	0.028	0.548	0.043	0.566	0.030	**0.537**	0.037
	23	0.373	0.051	**0.719**	0.058	**0.374**	0.048	0.723	0.057
	33	0.565	0.034	0.530	0.043	**0.584**	0.037	**0.513**	0.047
	Average	**0.448**	0.044	**0.632**	0.046	0.413	0.045	0.646	0.046
	11	**0.326**	0.054	**0.764**	0.055	0.297	0.053	0.777	0.055
	21	**0.480**	0.043	**0.588**	0.030	0.443	0.041	0.616	0.030
	31	0.446	0.045	0.657	0.035	**0.465**	0.047	**0.629**	0.030
S3	12	**0.404**	0.038	**0.545**	0.045	0.391	0.038	0.553	0.039
	22	**0.470**	0.047	**0.661**	0.045	0.402	0.050	0.721	0.055
	32	**0.343**	0.045	**0.630**	0.062	0.311	0.048	0.648	0.064
	13	**0.567**	0.035	0.598	0.048	0.552	0.030	**0.592**	0.042
	23	**0.327**	0.054	0.832	0.067	0.324	0.052	**0.831**	0.067
	33	0.498	0.034	0.615	0.055	**0.522**	0.036	**0.584**	0.056
	Average	**0.429**	0.044	**0.654**	0.049	0.412	0.044	0.661	0.049

CorP: average of Pearson correlation; SE: standard error, MSEP: mean square error of prediction. S1: scenario with high correlation (0.85); S2: scenario with medium correlation (0.5); S3: scenario with low correlation (0.25). The values of this table correspond to the simulations done with 80 lines in each environment. In bold are the best predictions of each row (Trait-Env).

Table 1. Comparison in terms of prediction accuracy of models **M2** and **M3** under scenarios S1, S2 and S3.

Scenario	Trait_Env	M2				M3			
		CorP	SE	MSEP	SE	CorP	SE	MSEP	SE
	11	**0.495**	0.042	**0.782**	0.052	0.483	0.043	0.800	0.056
	21	**0.569**	0.028	**0.693**	0.050	0.534	0.035	0.731	0.055
	31	**0.621**	0.028	**0.589**	0.038	0.596	0.033	0.619	0.044
	12	**0.467**	0.043	**0.814**	0.044	0.449	0.044	0.850	0.043
S4	22	**0.471**	0.040	**0.689**	0.040	0.440	0.041	0.740	0.046
	32	**0.572**	0.034	**0.548**	0.035	0.534	0.035	0.597	0.034
	13	**0.498**	0.040	**0.975**	0.060	0.486	0.035	0.984	0.060
	23	**0.535**	0.035	**0.812**	0.051	0.520	0.032	0.824	0.054
	33	**0.631**	0.034	**0.638**	0.043	0.604	0.029	0.674	0.044
	Average	**0.540**	**0.036**	**0.727**	**0.046**	**0.516**	**0.036**	**0.758**	**0.049**
	11	0.403	0.052	0.805	0.055	**0.405**	0.050	0.807	0.056
	21	**0.537**	0.029	**0.666**	0.047	0.510	0.035	0.688	0.049
	31	**0.567**	0.031	**0.595**	0.040	0.555	0.032	0.608	0.042
S5	12	**0.399**	0.051	**0.899**	0.051	0.397	0.053	0.907	0.053
	22	**0.432**	0.041	**0.722**	0.043	0.406	0.043	0.749	0.048
	32	**0.509**	0.034	**0.554**	0.037	0.503	0.035	0.564	0.035
	13	**0.416**	0.043	1.025	0.056	0.413	0.040	**1.024**	0.055
	23	0.487	0.033	0.791	0.042	**0.488**	0.034	**0.784**	0.045
	33	**0.588**	0.037	0.625	0.040	0.589	0.032	**0.630**	0.038
	Average	**0.482**	**0.039**	**0.742**	**0.046**	**0.474**	**0.039**	**0.751**	**0.047**
	11	**0.370**	0.054	0.798	0.057	0.369	0.052	0.802	0.057
	21	**0.512**	0.028	0.635	0.043	0.485	0.033	0.654	0.043
	31	**0.521**	0.034	0.587	0.040	0.511	0.034	0.596	0.041
S6	12	**0.367**	0.052	0.945	0.057	0.364	0.055	0.948	0.060
	22	**0.412**	0.040	0.759	0.045	0.382	0.042	0.776	0.047
	32	0.449	0.034	0.576	0.036	**0.466**	0.034	0.568	0.034
	13	**0.379**	0.045	1.013	0.053	0.374	0.042	1.016	0.051
	23	**0.462**	0.032	0.759	0.038	0.463	0.033	0.751	0.039
	33	0.542	0.039	0.618	0.040	**0.558**	0.035	0.610	0.036
	Average	**0.446**	**0.040**	**0.743**	**0.045**	**0.441**	**0.040**	**0.747**	**0.045**

CorP: average of Pearson correlation obtained across all trait-environment combination; SE: standard error; MSEP: mean square error of prediction. S4: scenario with high correlation (0.85); S5 the scenario with medium correlation (0.5); S6: scenario with low correlation (0.25). The values of this table correspond to the simulations done with 100 lines in each environment. In bold are the best predictions of each row (Trait-Env).

Table 2. Comparison in terms of prediction accuracy of models **M2** and **M3** under scenarios S4, S5 and S6.

3.2. Real data sets

In **Table 3** we can observe that in the wheat data set the best predictions were observed under the proposed improved BMTME model (**M2**), since in all trait-environment combinations was better model **M2** in terms of Pearson correlation and in 10 out of 12 was the better in terms of MSEP than model **M3** (that ignore the correlation between traits and between environments). However, in the maize data set the best predictions were observed under

Data set	Trait_Env	M2				M3			
		CorP	**SE**	**MSEP**	**SE**	**CorP**	**SE**	**MSEP**	**SE**
	DTHD_Bed2IR	**0.876**	0.008	**8.117**	0.692	0.875	0.009	10.636	0.882
	GNDVI_Bed2IR	**0.848**	0.008	**0.000**	0.000	0.009	0.022	0.103	0.006
	GRYLD_Bed2IR	**0.639**	0.014	**0.055**	0.002	0.463	0.015	0.161	0.007
	PTHT_Bed2IR	**0.658**	0.014	**22.527**	0.841	0.566	0.020	25.798	0.895
	DTHD_Bed5IR	**0.873**	0.007	**13.074**	0.733	0.845	0.010	15.312	0.508
Wheat	GNDVI_Bed5IR	**0.758**	0.019	**0.000**	0.000	0.496	0.023	0.219	0.011
	GRYLD_Bed5IR	**0.178**	0.023	**0.251**	0.008	0.175	0.020	0.336	0.014
	PTHT_Bed5IR	**0.076**	0.016	24.064	0.620	0.245	0.023	**20.831**	0.721
	DTHD_Drip	**0.915**	0.005	4.514	0.201	0.895	0.006	**3.321**	0.224
	GNDVI_Drip	**0.681**	0.012	**0.000**	0.000	−0.262	0.022	0.123	0.008
	GRYLD_Drip	**0.653**	0.011	**0.126**	0.005	0.638	0.011	0.144	0.005
	PTHT_Drip	**0.658**	0.019	21.565	0.531	0.602	0.012	**21.306**	0.728
	Average	**0.651**	0.013	**7.858**	0.303	0.462	0.016	8.191	0.334
	Yield_EBU	0.320	0.019	0.789	0.018	**0.365**	0.018	**0.731**	0.017
	ASI_EBU	0.501	0.016	0.396	0.012	**0.510**	0.015	**0.391**	0.012
	PH_EBU	**0.308**	0.025	0.015	0.003	0.305	0.011	**0.010**	0.000
	Yield_KAK	0.402	0.022	0.446	0.020	**0.416**	0.020	**0.438**	0.019
Maize	ASI_KAK	0.389	0.015	0.936	0.043	**0.423**	0.018	**0.822**	0.029
	PH_KAK	**0.462**	0.025	**0.011**	0.001	0.369	0.022	0.013	0.001
	Yield_KTI	0.276	0.015	0.848	0.022	**0.318**	0.018	**0.825**	0.024
	ASI_KTI	**0.290**	0.018	**0.607**	0.018	0.280	0.020	0.614	0.019
	PH_KTI	**0.460**	0.017	**0.019**	0.001	0.443	0.017	0.020	0.001
	Average	0.379	0.019	0.452	0.015	**0.381**	0.018	**0.42**9	0.014

CorP: average of Pearson correlation obtained across all trait-environment combination; SE: standard error; MSEP: mean square error of prediction. Trait_Env means trait-environment combination. In bold are the best predictions of each row (Trait-Env).

Table 3. Comparison in terms of prediction accuracy of models **M2** and **M3** using the two real data sets.

model **M3**, since in 5 out of 9 trait-environment combinations this model was superior to model **M2**, however there is not a great superiority of the results under model **M3** regarded to model **M2**. This results obtained in the maize data set are in agreement with the correlation study performed since this data set has a very low genetic correlation between traits and between environments.

According to the results observed with the simulated data sets (**Tables 1** and **2**) and real data sets (**Table 3**) there is evidence that the larger the correlation between traits (genetic and residual) and environments (genetic) the better the performance of the proposed improved BMTME (**M2**) model with regard to the uncorrelated multiple-trait and multiple-environment model (**M3**), which means that when the there is considerable correlation between traits and between environments this help to increase prediction accuracy.

4. Conclusions

In this paper we proposed an improved version of the Bayesian multiple-trait multiple-environment (BMTME) model of Montesinos-López et al. [3] that was derived using the matrix normal distribution. The advantage of the proposed model (**M2**) is that it is more efficient in terms of time of implementation since this improved version works using as rows the genotypes by environment combinations in place of using as rows the combination of traits, genotypes and environments which allows a more practical implementation of the Gibbs sampler in terms of time of implementation. Another, improvement of the BMTME model is that now allows unstructured covariance matrix for modeling environments in place of only a diagonal matrix as the original BMTME model. We compared the extended model (**M2**) with an uncorrelated multiple-trait and multiple-environment model (**M3**) that ignores the general correlation between traits (genetic and residual) and between environments and we found that the proposed improved BMTME model (**M2**) outperforms model (**M3**) in all the scenarios under study with simulation, however the larger the correlation between traits and between environments the better the performance in terms of prediction accuracy of the improved BMTME model. Additionally, we provided all full conditionals required for the implementation of the improved BMTME model (see Gibbs sampler section and Appendix A). However, we are aware that more empirical evidence with real and simulated data is needed to support our findings, and for this reason, we encourage researcher to implement our proposed improved model and compare with models that ignore the correlation between traits and between environments like the model **M3** given in Eq. (8).

A. Derivation of full conditionals of the improved BMTME model under the matrix normal distribution

Full conditional distribution for $vec(\boldsymbol{\beta})$

$$
\begin{aligned}
&P\left(vec(\boldsymbol{\beta})|ELSE\right)\\
&\quad\propto\exp\left\{-\frac{1}{2}tr\left[\boldsymbol{R}_e^{-1}\left(\boldsymbol{Y}-\boldsymbol{X\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)^T\boldsymbol{I}_n\left(\boldsymbol{Y}-\boldsymbol{X\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)\right]\right.\\
&\qquad\left.-\frac{1}{2}tr\left[\boldsymbol{I}_L^{-1}\left(\boldsymbol{\beta}-\boldsymbol{\beta}_0\right)^T\boldsymbol{I}_I^{-1}\left(\boldsymbol{\beta}-\boldsymbol{\beta}_0\right)\right]\right\}\\
&\quad\propto\exp\left\{-\frac{1}{2}vec\left(\boldsymbol{Y}-\boldsymbol{X\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)^T\left(\boldsymbol{R}_e^{-1}\otimes\boldsymbol{I}_n\right)vec\left(\boldsymbol{Y}-\boldsymbol{X\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)\right.\\
&\qquad\left.-\frac{1}{2}vec\left(\boldsymbol{\beta}-\boldsymbol{\beta}_0\right)^T\left[\boldsymbol{I}_L^{-1}\otimes\boldsymbol{I}_I^{-1}\right]\left(\boldsymbol{\beta}-\boldsymbol{\beta}_0\right)\right\}\\
&\quad\propto\exp\left\{-\frac{1}{2}\left[vec(\boldsymbol{Y})-(\boldsymbol{I}_L\otimes\boldsymbol{X})vec\left(\boldsymbol{\beta}\right)-vec(\boldsymbol{Z}_1\boldsymbol{b}_1)\right.\right.\\
&\qquad-vec(\boldsymbol{Z}_2\boldsymbol{b}_2)\left]^T\left[\boldsymbol{R}_e^{-1}\otimes\boldsymbol{I}_n\right]\left[vec(\boldsymbol{Y})-(\boldsymbol{I}_L\otimes\boldsymbol{X})vec\left(\boldsymbol{\beta}\right)-vec(\boldsymbol{Z}_1\boldsymbol{b}_1)-vec(\boldsymbol{Z}_2\boldsymbol{b}_2\right]\right.\\
&\qquad\left.\left.-\frac{1}{2}\left[vec\left(\boldsymbol{\beta}\right)-vec\left(\boldsymbol{\beta}_0\right)\right]^T\left[\boldsymbol{I}_L^{-1}\otimes\boldsymbol{I}_I^{-1}\right]\left[vec\left(\boldsymbol{\beta}\right)-vec\left(\boldsymbol{\beta}_0\right)\right]\right\}\right.\\
&\quad\propto\exp\left\{-\frac{1}{2}\left[vec\left(\boldsymbol{\beta}\right)-vec(\widetilde{\boldsymbol{\beta}})\right]^T\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{\beta}}^{-1}\left[vec\left(\boldsymbol{\beta}\right)-vec(\widetilde{\boldsymbol{\beta}})\right]\right\}\propto N(vec(\widetilde{\boldsymbol{\beta}}),\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{\beta}})
\end{aligned}
\tag{A1}
$$

where $\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{\beta}}=\left[\boldsymbol{I}_L^{-1}\otimes\boldsymbol{I}_I^{-1}+\boldsymbol{R}_e^{-1}\otimes\boldsymbol{X}^T\boldsymbol{X}\right]^{-1}$, $vec\left(\widetilde{\boldsymbol{\beta}}\right)=\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{\beta}}\left\{\left(\boldsymbol{I}_L^{-1}\otimes\boldsymbol{I}_I^{-1}\right)vec\left(\boldsymbol{\beta}_0\right)+\left(\boldsymbol{R}_e^{-1}\otimes\boldsymbol{X}^T\right)\right.$ $\left.[vec(\boldsymbol{Y})-vec(\boldsymbol{Z}_1\boldsymbol{b}_1)-vec(\boldsymbol{Z}_2\boldsymbol{b}_2)]\right\}$.

In the simplification of some calculations the following properties were involved: $tr(\boldsymbol{AB})=vec(\boldsymbol{A}^T)^Tvec(\boldsymbol{B})=vec(\boldsymbol{B})^Tvec(\boldsymbol{A}^T)$, and $vec\,(\boldsymbol{AXB})=(\boldsymbol{B}^T\otimes\boldsymbol{A})vec(\boldsymbol{X})$.

Full conditional for $vec(\boldsymbol{b}_1)$

$$
\begin{aligned}
&P(vec(\boldsymbol{b}_1)|ELSE)\\
&\quad\propto\exp\left\{-\frac{1}{2}\left[vec(\boldsymbol{Y})-vec\left(\boldsymbol{X\beta}\right)-(\boldsymbol{I}_L\otimes\boldsymbol{Z}_1)vec(\boldsymbol{b}_1)\right.\right.\\
&\qquad-vec(\boldsymbol{Z}_2\boldsymbol{b}_2)\left]^T\left[\boldsymbol{R}_e^{-1}\otimes\boldsymbol{I}_n\right]\left[vec(\boldsymbol{Y})-vec\left(\boldsymbol{X\beta}\right)-(\boldsymbol{I}_L\otimes\boldsymbol{Z}_1)vec(\boldsymbol{b}_1)-vec(\boldsymbol{Z}_2\boldsymbol{b}_2)\right]\right.\\
&\qquad\left.\left.-\frac{1}{2}\left[vec(\boldsymbol{b}_1)^T\left[\boldsymbol{\Sigma}_{\boldsymbol{t}}^{-1}\otimes\boldsymbol{G}_{\boldsymbol{g}}^{-1}\right]\right]vec(\boldsymbol{b}_1)\right\}\right.\\
&\quad\propto\exp\left\{-\frac{1}{2}\left[vec(\boldsymbol{b}_1)-vec\left(\widetilde{\boldsymbol{b}}_1\right)\right]^T\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_1}^{-1}\left[vec(\boldsymbol{b}_1)-vec\left(\widetilde{\boldsymbol{b}}_1\right)\right]\right\}\\
&\quad\propto N\left(vec\left(\widetilde{\boldsymbol{b}}_1\right),\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_1}\right)
\end{aligned}
\tag{A2}
$$

where $\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_1} = \left(\boldsymbol{\Sigma}_t^{-1} \otimes \boldsymbol{G}_g^{-1} + \boldsymbol{R}_e^{-1} \otimes \boldsymbol{Z}_1^T \boldsymbol{Z}_1\right)^{-1}$ and $vec\left(\widetilde{\boldsymbol{b}}_1\right) = \widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_1}\left(\boldsymbol{R}_e^{-1} \otimes \boldsymbol{Z}_1^{\boldsymbol{T}}\right)\left[vec(\boldsymbol{Y}) - vec\left(\boldsymbol{X}\boldsymbol{\beta}\right) - vec(\boldsymbol{Z}_2\boldsymbol{b}_2)\right]$.

Full conditional for $vec(\boldsymbol{b}_2)$

$$
\begin{aligned}
&P(vec(\boldsymbol{b}_2)|ELSE)\\
&\propto \exp\left\{-\frac{1}{2}\left[vec(\boldsymbol{Y}) - vec\left(\boldsymbol{X}\boldsymbol{\beta}\right) - vec(\boldsymbol{Z}_1\boldsymbol{b}_1)\right.\right.\\
&\left.-(\boldsymbol{I}_L \otimes \boldsymbol{Z}_2)vec(\boldsymbol{b}_2)\right]^T\left(\boldsymbol{R}_e^{-1} \otimes \boldsymbol{I}_n\right)\left[vec(\boldsymbol{Y}) - vec\left(\boldsymbol{X}\boldsymbol{\beta}\right) - vec(\boldsymbol{Z}_1\boldsymbol{b}_1) - (\boldsymbol{I}_L \otimes \boldsymbol{Z}_2)vec(\boldsymbol{b}_2)\right]\\
&\left.-\frac{1}{2}vec(\boldsymbol{b}_2)^T\left(\boldsymbol{\Sigma}_{\boldsymbol{t}}^{-1} \otimes \boldsymbol{\Sigma}_E^{-1} \otimes \boldsymbol{G}_{\boldsymbol{g}}^{-1}\right)vec(\boldsymbol{b}_2)\right\}\\
&\propto \exp\left\{-\frac{1}{2}\left[vec(\boldsymbol{b}_2) - vec\left(\widetilde{\boldsymbol{b}}_2\right)\right]^T\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_2}^{-1}\left[vec(\boldsymbol{b}_2) - vec\left(\widetilde{\boldsymbol{b}}_2\right)\right]\right\}\\
&\propto N\left(vec\left(\widetilde{\boldsymbol{b}}_2\right), \widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_2}\right)
\end{aligned}
\tag{A3}
$$

where $\widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_2} = \left(\boldsymbol{\Sigma}_t^{-1} \otimes \boldsymbol{\Sigma}_E^{-1} \otimes \boldsymbol{G}_g^{-1} + \boldsymbol{R}_e^{-1} \otimes \boldsymbol{Z}_2^T\boldsymbol{Z}_2\right)^{-1}$ and $vec\left(\widetilde{\boldsymbol{b}}_2\right) = \widetilde{\boldsymbol{\Sigma}}_{\boldsymbol{b}_2}\left(\boldsymbol{R}_e^{-1} \otimes \boldsymbol{Z}_2^T\right)\{vec(\boldsymbol{Y}) - vec\left(\boldsymbol{X}\boldsymbol{\beta}\right) - vec(\boldsymbol{Z}_1\boldsymbol{b}_1)\}$.

Full conditional for $\boldsymbol{\Sigma}_t$

$$
\begin{aligned}
&P(\boldsymbol{\Sigma}_t|ELSE) \propto P(\boldsymbol{b}_1|\boldsymbol{\Sigma}_t)P(\boldsymbol{b}_2|\boldsymbol{\Sigma}_t)P(\boldsymbol{\Sigma}_t)\\
&\propto |\boldsymbol{\Sigma}_t|^{-\frac{J}{2}}\left|\boldsymbol{G}_g\right|^{-\frac{L}{2}}\exp\left\{-\frac{1}{2}tr\left[\boldsymbol{b}_1^{*T}\boldsymbol{G}_g^{-1}\boldsymbol{b}_1^*\boldsymbol{\Sigma}_t^{-1}\right]\right\}\\
&\times|\boldsymbol{\Sigma}_t|^{-\frac{IJ}{2}}\left|\boldsymbol{\Sigma}_E \otimes \boldsymbol{G}_g\right|^{-\frac{L}{2}}\exp\left\{-\frac{1}{2}tr\left[\boldsymbol{b}_2^{*T}\left(\boldsymbol{\Sigma}_E^{-1} \otimes \boldsymbol{G}_g^{-1}\right)\boldsymbol{b}_2^*\boldsymbol{\Sigma}_t^{-1}\right]\right\}P(\boldsymbol{\Sigma}_t)\\
&\propto \boldsymbol{\Sigma}_t^{-\frac{J+IJ}{2}}\exp\left\{-\frac{1}{2}tr\left[\boldsymbol{b}_1^{*T}\boldsymbol{G}_g^{-1}\boldsymbol{b}_1^* + \boldsymbol{b}_2^{*T}\left(\boldsymbol{\Sigma}_E^{-1} \otimes \boldsymbol{G}_g^{-1}\right)\boldsymbol{b}_2^*\right]\boldsymbol{\Sigma}_t^{-1}\right\}|\boldsymbol{S}_t|^{\frac{\upsilon_t+L-1}{2}}\\
&\times|\boldsymbol{\Sigma}_t|^{-\frac{\upsilon_t+L}{2}}\exp\left\{-\frac{1}{2}tr\left(\boldsymbol{S}_t\boldsymbol{\Sigma}_t^{-1}\right)\right\}\\
&\propto \boldsymbol{\Sigma}_t^{-\frac{\upsilon_t+J+IJ+L-1+1}{2}}\exp\left\{-\frac{1}{2}tr\left(\boldsymbol{b}_1^{*T}\boldsymbol{G}_g^{-1}\boldsymbol{b}_1^* + \boldsymbol{b}_2^{*T}\left(\boldsymbol{\Sigma}_E^{-1} \otimes \boldsymbol{G}_g^{-1}\right)\boldsymbol{b}_2^* + \boldsymbol{S}_t\right)\boldsymbol{\Sigma}_t^{-1}\right\}\\
&\propto IW\left(\upsilon_t + J + L + IJ - 1, \boldsymbol{b}_1^{*T}\boldsymbol{G}_g^{-1}\boldsymbol{b}_1^* + \boldsymbol{b}_2^{*T}\left(\boldsymbol{\Sigma}_E^{-1} \otimes \boldsymbol{G}_g^{-1}\right)\boldsymbol{b}_2^* + \boldsymbol{S}_t\right)
\end{aligned}
\tag{A4}
$$

Full conditional for $\boldsymbol{\Sigma}_E$

$$
\begin{aligned}
P(\boldsymbol{\Sigma}_E|ELSE) &\propto P(\boldsymbol{b}_2|\boldsymbol{\Sigma}_E)P(\boldsymbol{\Sigma}_E) \\
&\propto |\boldsymbol{\Sigma}_E|^{-\frac{JL}{2}}|\boldsymbol{\Sigma}_T \otimes \boldsymbol{G}_g|^{-\frac{I}{2}}\exp\left\{-\frac{1}{2}tr\left[\boldsymbol{b}_2^{*T}\left(\boldsymbol{\Sigma}_t^{-1}\otimes \boldsymbol{G}_g^{-1}\right)\boldsymbol{b}_2^{*}\boldsymbol{\Sigma}_E^{-1}\right]\right\}|\boldsymbol{S}_E|^{\frac{v_E+I-1}{2}} \\
&\times|\boldsymbol{\Sigma}_E|^{-\frac{v_E+I}{2}}\exp\left\{-\frac{1}{2}tr\left(\boldsymbol{S}_E\boldsymbol{\Sigma}_E^{-1}\right)\right\} \\
&\propto |\boldsymbol{\Sigma}_E|^{-\frac{v_E+I+JL}{2}}\exp\left\{-\frac{1}{2}tr\left[\left(\boldsymbol{b}_2^{*T}\left(\boldsymbol{\Sigma}_t^{-1}\otimes \boldsymbol{G}_g^{-1}\right)\boldsymbol{b}_2^{*}+\boldsymbol{S}_E\right)\right]\boldsymbol{\Sigma}_E^{-1}\right\}|\boldsymbol{S}_E|^{\frac{v_E+I-1}{2}} \\
&\propto IW\left(v_E+JL+I-1, \boldsymbol{b}_2^{*T}\left(\boldsymbol{\Sigma}_t^{-1}\otimes \boldsymbol{G}_g^{-1}\right)\boldsymbol{b}_2^{*}+\boldsymbol{S}_E\right)
\end{aligned}
\tag{A5}
$$

Full conditional for $\mathbf{R}_e$

$$
\begin{aligned}
P(\boldsymbol{R}_e|ELSE) &\propto P\left(\boldsymbol{Y}|\boldsymbol{\beta}, \boldsymbol{b}_1, \boldsymbol{b}_2, \boldsymbol{R}_e\right)P(\boldsymbol{R}_e) \\
&\propto |\boldsymbol{R}_e|^{-\frac{n}{2}}\exp\left\{-\frac{1}{2}tr\left[\left(\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)^T\boldsymbol{I}_n\left(\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)\boldsymbol{R}_e^{-1}\right]\right\} \\
&\times|\boldsymbol{S}_e|^{\frac{v_e+L-1}{2}}|\boldsymbol{R}_e|^{-\frac{v_e+L}{2}}\exp\left\{-\frac{1}{2}tr\left(\boldsymbol{S}_e\boldsymbol{R}_e^{-1}\right)\right\} \\
&\propto |\boldsymbol{R}_e|^{-\frac{v_e+n+L-1+1}{2}}\exp\left\{-\frac{1}{2}tr\left[\left(\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)^T\left(\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)+\boldsymbol{S}_e\right]\boldsymbol{R}_e^{-1}\right\} \\
&\propto IW\left(v_e+n+L-1, \left\|\left(\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2\right)\right\|+\boldsymbol{S}_e\right)
\end{aligned}
\tag{A6}
$$

where $\|(\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2)\| = (\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2)^T(\boldsymbol{Y}-\boldsymbol{X}\boldsymbol{\beta}-\boldsymbol{Z}_1\boldsymbol{b}_1-\boldsymbol{Z}_2\boldsymbol{b}_2)$.

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Chapter 4

Branch Formation and Yield by Flower Bud or Shoot Removal in Tomato

Katsumi Ohta

Additional information is available at the end of the chapter

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Abstract

Branch formation might be used as indices for improving productivity in tomatoes. However, there has been little research to elucidate the relationship between the emergence of terminal flower bud (TFB) and the elongation of lateral shoots. Therefore, the effects of flower bud or shoot removal on plant growth, flowering, and yield were investigated. In indeterminate cultivar, the lateral shoot of the second node below TFB was suppressed by flower bud removal but not by shoot removal compared with untreated plants. In determinate cultivar, the opposite results were observed. TFB emergence was affected and not affected during lateral shoot elongation of both type cultivars, respectively. In determinate-type tomato, growth, dry weight, and the distribution of nitrogen and calcium in the lateral shoots in the pinching treatments (shoot removal) were greater than those in the control. The flowering periods and number of flowers per lateral shoot in the pinching treatments were shorter and greater, respectively, than those in the control. Initial weekly yields in the pinching treatments were increased compared with those in the control. From these results, since the branch formation and productivity by flower bud or shoot removal was clarified, it would be useful information for future tomato production.

Keywords: flower bud, lateral shoot, morphogenesis, *Solanum lycopersicum*, yields

1. Introduction

Tomatoes are an important fruit vegetable in many countries. Tomato plants differentiate terminal flower buds (TFB) on the apex of the main stem and formed flower truss, known as the determinate pattern with branching characteristics [1, 2]. The axillary bud (AB) adjacent to TFB differentiates and forms a lateral shoot as a sympodial branching. As mentioned above, the lateral shoot that grows as a main branch is a characteristic of indeterminate-type

tomatoes that are cultivated mainly for the fresh market. On the contrary, determinate-type tomatoes with a self-pruning growth habit with only short sympodial branches form a few flower trusses [3]. These cultivars are mainly grown for processing and cooking tomatoes [4].

In general, the lateral shoots of indeterminate tomato cultivars are periodically removed to prevent nutrient competition between vegetative and reproductive organs during cultivation period. Several lateral shoots extends greatly unless all the lateral shoots are removed [5]. Since the sink strength of lateral shoots with flower buds and trusses is stronger than that of the main stem or lateral shoot without flower buds and trusses [6], strong growth of some lateral shoots may cause uneven distribution of photosynthetic products, resulting in undesirable effects on fruit production. As an example of using lateral shoots, during tomato cultivation during winter in the Netherlands, lateral shoots generated from the first or second nodes below TFB are used to increase stem numbers per area in indeterminate cultivars and increase tomato yield [7]. The utilization of lateral shoots can both promote high-quality fruit production [8–10] and also increase crop yield [11]. In contrast, for determinate tomato cultivars, lateral shoots are generally not removed to save labor and ensure yield [12–15]. However, lack of fruit set on the first flower truss due to low or high temperatures or rainfall or due to pinching at the seedling stage could affect the lateral shoot lengths and flowering periods of determinate processing tomatoes.

Differentiation of AB occurs at every node during the growth of most commercial cultivars. Although AB at lower nodes extends during the vegetative stage, AB at the upper nodes below TFB does not extend much due to apical dominance [1, 16]. When TFB at the shoot apex emerges and grows, the entire AB in general begins to elongate. Branch formation in indeterminate cultivars differs from that in determinate ones because of generally remaining the lateral shoots. Also, to investigate the growth properties of lateral shoots generated from each node could be used to increase productivity in tomato cultivation.

The growth of lateral shoots in the indeterminate cultivars can be extended by pinching (shoot removal) from the results of the previous reports [17–20]. In some tomato cultivars, the numbers and weights of fruits that grew on double-stemmed plants created by pinching treatments were greater than those that grew on single-stemmed plants [21–23]. Pinching at the seedling stage can increase the number of double clusters and flowers on lateral shoots of cherry tomatoes [24, 25]. Pinching is often performed to increase initial tomato yield, but there are differences among cultivars as to the effects of pinching [26, 27]. In addition, the lengths of the lateral shoots at each node do differ depending on the pinching position [14]. As the number of remaining true leaves is increased by pinching, there is a difference among the lateral shoot lengths. Since a relationship among the lengths of lateral shoots, the number of flowers per plant, and per lateral shoot is expected to be changed by pinching in determinate processing tomatoes, growth of the lateral shoot would be influenced by the uptake and distribution of mineral nutrients in each organ. Furthermore, because pinching can enhance the uniformity of fruit maturity [14], pinching could shorten the harvest term while also, due to this shorter flowering period, leading to harvest periods with more than 80% total fruit yield.

However, there has been little research to elucidate the relationships between the TFB and the elongation of lateral shoots in indeterminate and determinate-type tomatoes. Furthermore,

there has been little information about the effects of pinching treatments on the harvest term, yield, growth of lateral shoots, flowering, and number of flowers in determinate processing tomatoes, and about the relationship between the growth of lateral shoots and the uptake of mineral nutrients. Therefore, the objective of this study was to clarify and summarize the effects of flower bud or shoot removal on these parameters based on the previous research [28, 29].

2. Materials and methods

2.1. Lateral shoot elongation after terminal flower buds (TFB) and shoot (including TFB and axillary bud (AB) at the first node below TFB) removal

2.1.1. Plant materials, cultivation, and treatments

Indeterminate-type "Mini Carol" (*Solanum lycopersicum* L.) (Sakata Seed Co. Ltd., Japan) and determinate-type "Suzukoma" (Tohoku Agricultural Research Center, National Agriculture and Food Research Organization and ZEN-NOH, Japan) were used for this experiment. Seeds were sown in plastic containers (34.5 × 27.0 × 7.5 cm). One plant was potted black plastic pots at a ratio of sandy loam:bark compost of 1:1 (v/v). Tomato plants were transplanted into Wagner pots (1/5000 a) in the same potting substrate described above. All pots were placed in a greenhouse at Shimane University, Matsue, Japan. TFB (maximum bud length of about 1 mm) were removed by pinching them off, and the stems were decapitated at the upper portions of shoots of the second node below TFB (**Figure 1**). Ten plants per treatment were evaluated.

2.1.2. Measurements

The lateral shoot length of the second node below TFB was measured at 0, 3, 6, and 9 days after the treatments.

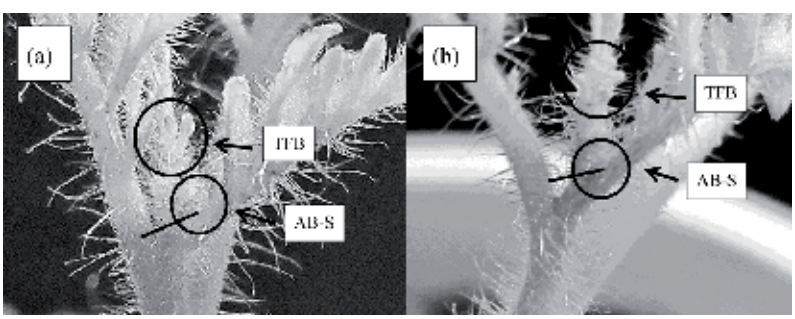

Figure 1. Axillary bud of the second node (AB-S) below the terminal flower bud (TFB) in indeterminate-type cultivar "Mini Carol" (a) and determinate-type cultivar "Suzukoma" (b) tomatoes. Axillary buds (AB) of the first node below TFB exist behind TFB. Flower bud removals are shown by bars (Source: Ohta and Ikeda [28]).

2.2. Effects of pinching treatment (shoot removal) on plant growth, flowering, and yield in determinate tomato

2.2.1. Experimental site, plant materials, growing conditions, and treatments

The determinate-type "Shuho" (Nagano Chushin Agricultural Institute Experimental Station, Shiojiri, Japan) was used for this experiment. Seeds were sown in plastic containers. All containers were placed in a greenhouse at Shimane University, Matsue, Japan. One plant was potted black plastic pots at a ratio of sandy loam:bark compost of 1:1 (v/v). After the third and sixth true leaves had expanded, the plants were pinched at the stem above the third and sixth true leaves (**Figure 2**). No pinching treatments were performed in the untreated control. The tomato plants were transplanted into the experimental field with the soil surface covered with black 0.02-mm polyethylene film at Yatsuka-cho, Matsue, Japan. The plants were arranged in a single 1.6 m wide row, with 0.8 m spacing between rows, 0.45 m spacing between plants, and a planting density of 1.39 plants m^{-2}. A randomized complete block design was used with three replicates. In total, eight plants per treatment were used. Six plants were used to measure the lateral shoot growth, flowering, and fruit yields, and the remaining plants were used to analyze the mineral nutrient contents.

2.2.2. Measurements

At 18 and 59 days after transplanting (DAT), the lengths of the lateral shoots generated from each node were measured. At 18 DAT, the plants were sampled and divided into stems, leaves on the main shoot, and lateral shoots, and then washed with deionized water. After being air-dried at 80°C for 72 h, the dried plants were ground using an electric mill (WB-1; AS ONE Corp., Osaka, Japan). Total nitrogen (N) contents were determined using a CN

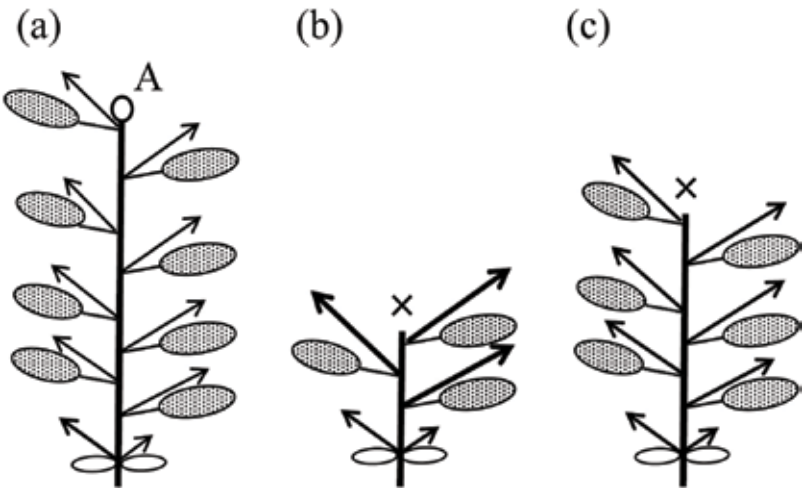

Figure 2. Pinching treatments (shoot removal) in determinate-type tomato (schematic diagram). Left is control (a), center is Pinch-3 (b), and right is Pinch-6 (c). Pinch-3 or -6 indicates pinching treatment with the plant left with three or six true leaves, respectively. A is terminal flower bud (TFB) of main stem. X is pinching position.

coder (Sumigraph NC-22F, Sumitomo Chemical Analysis Center Corp., Tokyo). The phosphorus (P) contents were measured by vanadomolybdate absorption spectrometry. The potassium (K), calcium (Ca), and magnesium (Mg) contents were measured by an atomic absorption spectrophotometer (AA-630, Shimadzu, Kyoto, Japan). The contents of mineral nutrient in each organ of plant were calculated from dry weight and mineral nutrient concentrations. The first flowering dates of the main stem and the lateral shoots were recorded, and the numbers of flowers, and the number of secondary and higher lateral shoots per primary lateral shoot were counted. Full ripe fruits were harvested twice per week during 6 weeks, and the number of fruits, fruit weight, and the number of marketable fruits were recorded. The soluble solids content (SSC) values of 20 marketable fruits were evaluated using a digital refractometer (APAL-1; AS ONE Corp., Osaka, Japan) to measure the Brix values of fresh juice samples.

3. Results

3.1. Lateral shoot elongation after TFB or shoot removal in indeterminate tomato

The lateral shoot length at the second node below TFB in the indeterminate-type cultivar "Mini Carol" was significantly suppressed by flower bud removal at 6 and 9 days after treatment, compared to that in untreated plants (**Figure 3**). On the other hand, lateral shoot lengths at the second node below TFB did not differ after shoot removal compared with untreated plants.

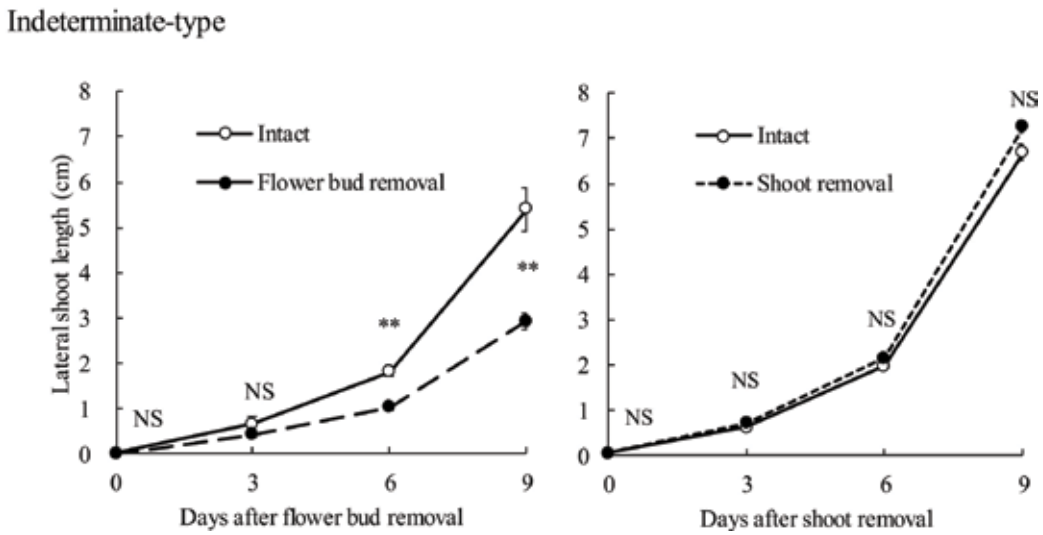

Figure 3. Lateral shoot length of the second node below the terminal flower bud (TFB) after flower bud removal and shoot removal at the upper position of second node below TFB of indeterminate cultivar, "Mini Carol". Significant difference was shown as **: $P < 0.01$, NS: not significant (t-test). Vertical bars indicate standard error (Source: Ohta and Ikeda [28]).

3.2. Lateral shoot elongation after TFB or shoot removal in determinate tomato

The lateral shoot length at the second node below TFB in the determinate-type cultivar "Suzukoma" was not significantly different between plants with flower buds removed and untreated plants (**Figure 4**). However, the lateral shoot length at the second node below TFB increased significantly at 6 and 9 days after shoot removal compared with that of untreated plants.

Figure 5 summarizes the results of **Figures 3** and **4**. Lateral shoot (C_1) growth at the second node below TFB was analyzed in indeterminate-type cultivars in the presence of either TFB (A_1) or AB (B_1). The growth of C_1 was suppressed in the presence of only B_1, and the growth of C_1 did not change even if both A_1 and B_1 were removed. Therefore, the presence of A_1 promoted the growth of C_1 in indeterminate-type cultivars. On the contrary, when the growth of lateral shoot (C_2) was analyzed in determinate-type cultivars in the presence of either TFB (A_2) or AB (B_2), the growth of C_2 in the presence of only B_2 did not change (growth was suppressed). However, the growth of C_2 was accelerated if both A_2 and B_2 were removed. Thus, the presence A_2 did not promote the growth of C_2 in determinate-type cultivars.

3.3. Effects of pinching treatment (shoot removal) on plant growth, flowering, and yield

At 18 DAT, the mean lateral shoot lengths in the three-true-leaf pinching treatment had extend significantly longer, at 14.7 cm, than those in the control, at 5.5 cm. CVs of mean lateral shoot length did not differ among the all treatments, at 50–55%. The lateral shoot lengths generated from the lower nodes in the six-true-leaf pinching treatment was no difference compared

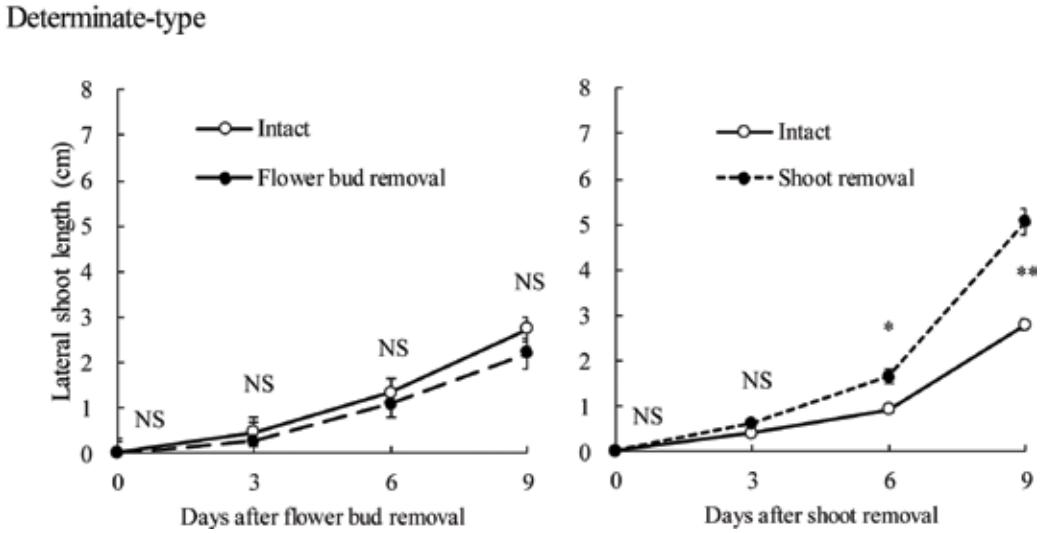

Figure 4. Lateral shoot length of the second node below the terminal flower bud (TFB) after flower bud removal and shoot removal at the upper position of the second node below TFB of determinate cultivar, "Suzukoma". Significant difference was shown as **: $P < 0.01$, *: $P < 0.05$, NS: not significant (t-test). Vertical bars indicate standard error (Source: Ohta and Ikeda [28]).

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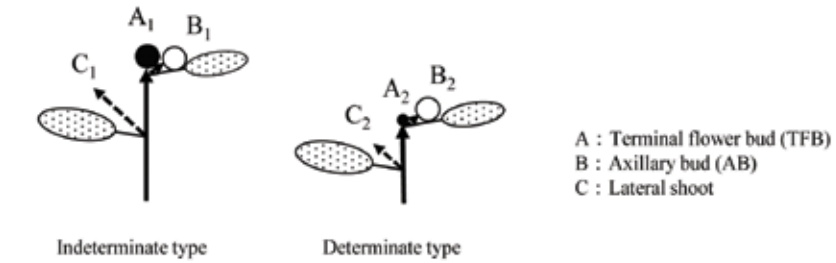

	Apical bud		C_1 growth
Indeterminate type	A_1 and B_1	→	Normal
	B_1 only	→	Suppressed
	None	→	Normal

Effect on the lateral shoot growth	
Terminal flower bud (A_1)	Axillary bud (B_1)
Promoted	Suppressed

	Apical bud		C_2 growth
Determinate type	A_2 and B_2	→	Normal
	B_2 only	→	Normal
	None	→	Promoted

Terminal flower bud (A_2)	Axillary bud (B_2)
Not promoted	Suppressed

Figure 5. Relationships among terminal flower bud (TFB) (A_1, A_2), axillary bud (AB) (B_1, B_2) and lateral shoot growth (C_1, C_2) of indeterminate and determinate-type tomatoes (Source: Ohta and Ikeda [28]).

with those in the control, however, the lateral shoots generated from the second to sixth true leaf nodes had extended significantly longer than those in the control (data not shown). At 59 DAT, the lateral shoot lengths in the pinching treatments showed the same tendencies as seen at 18 DAT. The mean lateral shoot lengths in the both pinching treatments were significantly longer, at 44.6 and 35.5 cm, than those in the control, at 27.8 cm. CV of the mean lateral shoot length in the three-true-leaf pinching treatment was smaller, at 28%, than the other treatments, at 33 and 37%.

Figure 6 shows the effect of pinching treatments (shoot removal) on the dry weight (DW) of the plants. Although total DW did not differ among the all treatments, DW in the stem in the three-true-leaf pinching treatment were significantly less compared with those in the six-true-leaf pinching treatment and the control. DW in the leaves in the three-true-leaf pinching treatment was significantly less compared with that in the control. However, DW in the lateral shoots in the three-true-leaf pinching treatment was highest among the all treatments.

Table 1 shows the effect of pinching treatments (shoot removal) on the content and distribution of N, P, K, Ca, and Mg at 18 days after transplanting (DAT) in each organ of plant. Although in the stem the contents of P and K in the three-true-leaf pinching treatment were significantly lower than that in the control, the contents of these mineral nutrients in the six-true-leaf pinching treatment did not differ compared with that in the control. In the leaves, the contents of all the mineral nutrients were no differences among the all treatments. In the lateral shoots, the contents of N, P, K, Mg, and Ca in the three-true-leaf pinching treatment were significantly increased compared with those in the control. In the lateral shoots, the

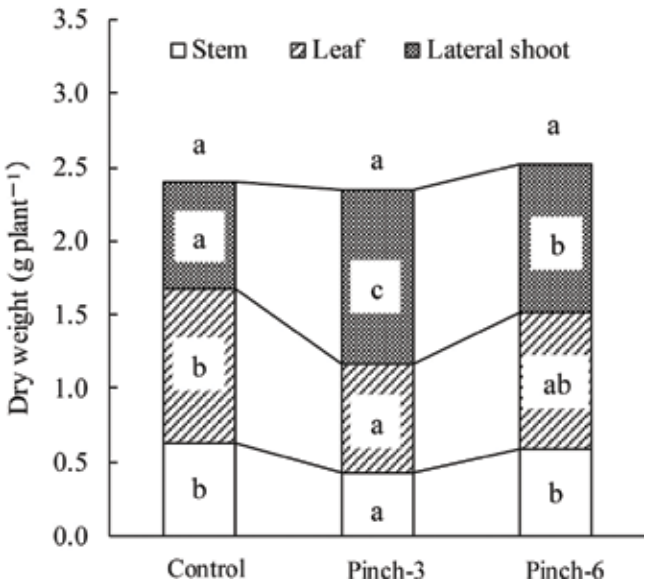

Figure 6. Effect of pinching treatments (shoot removal) on the dry weight (DW) at 18 days after transplanting (DAT) in determinate-type tomato. Pinch-3 or -6 indicates pinching treatment with the plant left with three or six true leaves, respectively. Different letters within each organ indicate significant difference at $P < 0.05$ (Tukey's test) (Source: Ohta and Ikeda [29]).

contents of N, K, and Ca in the six-true-leaf pinching treatment were significantly greater than that of the control. The total contents of N and Ca in the three-true-leaf pinching treatment were greater than those of the control. Although the distributions of P and K to the stem in the three-true-leaf pinching treatment were decreased compared with those in the control, the distributions of all the mineral nutrients to the lateral shoots in the three-true-leaf pinching treatment were increased compared with those in the control.

The first flowering days from sowing in the control was decreased, at 57.5 days, compared with those in the both pinching treatments, at 64.5 and 64.6 days, respectively. The number of days between the both pinching treatments and the control to the first flowering of the lateral shoots did differ. The number of days between the first and last flowering of the terminal flower truss of main and/or each the lateral shoots in the three-true-leaf pinching treatment was significantly lower, at 13.1 days, than that in the control, at 18.7 days, but the number of days between the first and last flowering of the terminal flower truss of each lateral shoot did not differ between the six-true-leaf pinching treatment and the control.

Table 2 shows the effect of pinching treatments (shoot removal) on the number of flowers per plant, per primary lateral shoot, and flowered lateral shoots. Although the number of flowers per whole plant in the six-true-leaf pinching treatment was significantly higher than that of the control, the number of flowers per plant in the three-true-leaf pinching treatment was significantly lower compared with that of the control. The total numbers of flowers per lateral shoot in both pinching treatments were significantly higher than that in the control. The number of flowers per primary lateral shoot did not differ among the all treatments; whereas, the

Organ	Treatment	N			P			K			Ca			Mg		
Stem	Control	7.9	a[a]	(13)[b]	2.9	b	(25)	16.5	b	(31)	17.8	a	(24)	4.3	a	(30)
	Pinch-3[c]	6.8	a	(8)	1.9	a	(16)	10.4	a	(19)	17.0	a	(19)	3.5	a	(21)
	Pinch-6	8.2	a	(11)	2.6	b	(20)	15.8	b	(26)	16.3	a	(23)	4.2	a	(29)
Leaf	Control	26.2	a	(42)	4.2	a	(36)	14.4	a	(27)	40.0	a	(55)	5.9	a	(42)
	Pinch-3	21.6	a	(25)	3.1	a	(25)	11.9	a	(22)	44.4	a	(49)	6.5	a	(40)
	Pinch-6	23.3	a	(31)	3.6	a	(28)	13.7	a	(23)	36.6	a	(50)	5.3	a	(36)
Lateral shoot	Control	28.2	a	(45)	4.6	a	(39)	22.4	a	(42)	15.0	a	(21)	3.9	a	(28)
	Pinch-3	56.8	c	(67)	7.2	b	(59)	31.8	b	(59)	29.1	c	(32)	6.3	b	(39)
	Pinch-6	42.9	b	(58)	6.7	b	(52)	30.3	b	(51)	19.9	b	(27)	5.2	ab	(35)
Total	Control	62.3	a	(100)	11.8	a	(100)	53.4	a	(100)	72.7	a	(100)	14.1	a	(100)
	Pinch-3	85.2	b	(100)	12.2	a	(100)	54.1	a	(100)	90.5	b	(100)	16.3	a	(100)
	Pinch-6	74.4	ab	(100)	13.0	a	(100)	59.8	a	(100)	72.8	a	(100)	14.7	a	(100)
P																
Organ (A)		NS			NS			NS			*			NS		
Treatment (B)		**			**			**			**			*		
A × B		NS			NS			NS			NS			NS		

[a]Different letters within each column indicate significant difference at $P < 0.05$ (Tukey's test).

[b]Values are the ratio of the nutrient amount in each organ to the total in each treatment.

[c]Pinch-3 or -6 indicates pinching treatment with the plant left with three or six true leaves, respectively.

*,**NS: significant at $P < 0.05$ and $P < 0.01$ or not significant, respectively (ANOVA).

Table 1. Effect of pinching treatments (shoot removal) on the content and distribution of N, P, K, Ca, and Mg (mg plant^{-1}) at 18 days after transplanting (DAT) in determinate-type tomato (Source: Ohta and Ikeda [29]).

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Treatment	Number of flowers per whole plant		Number of flowered lateral shoots per whole plant		Number of flowers per lateral shoot						Number of secondary and higher lateral shoots per primary lateral shoot	
					Total		Primary		Secondary and higher			
Control	198.5	b[a]	9.2	b	21.6	a	5.4	a	16.2	a	4.5	a
Pinch-3[b]	158.3	a	4.8	a	33.5	c	5.0	a	27.9	c	6.4	c
Pinch-6	239.6	c	9.0	b	26.8	b	5.1	a	21.8	b	5.4	b

[a]Different letters within each column indicate significant difference at $P < 0.05$ (Tukey's test).

[b]Pinch-3 or -6 indicates pinching treatment with the plant left with three or six true leaves, respectively.

Table 2. Effect of pinching treatments (shoot removal) on the number of flowers, flowering lateral shoots, flowers per lateral shoots, and secondary and higher lateral shoots per primary lateral shoot in determinate-type tomato (Source: Modified from Ohta and Ikeda [29]).

parameter per secondary and higher lateral shoot in the three-true-leaf pinching treatment was highest among the all treatments. The number of flowered lateral shoots per whole plant in the three-true-leaf pinching treatment was significantly lower compared with those in the other treatments.

Figure 7 shows the effect of pinching treatments (shoot removal) on the weekly marketable fruit yield. At 0 week after the start of the harvest (WAH), the weekly yield in the control was higher than those in both pinching treatments. However, at 1 WAH in the three-true-leaf pinching treatment was higher compared with that in the control. The weekly yield in the six-true-leaf pinching treatment at 2 WAH was also higher compared with that in the

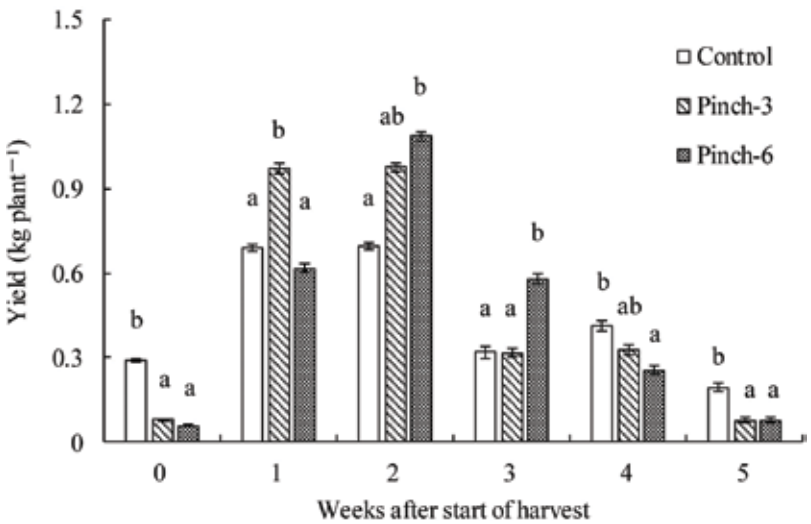

Figure 7. Effect of pinching treatments (shoot removal) on weekly marketable fruit yield in determinate-type tomato. Pinch-3 or -6 indicates pinching treatment with the plant left with three or six true leaves, respectively. Different letters within each week indicate significant difference at $P < 0.05$ (Tukey's test). Vertical bars indicate standard error (Source: Ohta and Ikeda [29]).

control. The harvest term in the both pinching treatments was shortened until 3 WAH compared with that in the control until 4 WAH. The fruit set ratio in the three-true-leaf pinching treatment was higher, at 20.4%, than in the other treatments, at 12.7 and 15.8%. However, the fruit yield per plant, at 2968–3018 g, the mean fruit weight, at 94.7–98.3 g, the number of harvested fruits per plant, at 30.3–31.7 fruits, the marketable fruits ratio, at 87.6–89.6%, and SSC, at 4.9–5.1°Brix, did not differ among the treatments. Although the numbers of flowers per whole plant in the six-true-leaf pinching treatment and the control were greater than those in the three-true-leaf pinching treatment, the numbers of harvested fruits were not different among the all treatments.

4. Discussion

Flower bud removal or shoot removal was carried out to clarify the roles of TFB and AB at the first node below TFB, and to clarify the reason that lateral shoots at the second node below TFB elongate. In indeterminate-type cultivar, the lateral shoot lengths at the second node below TFB were suppressed significantly at 6 and 9 days after flower bud removal, but these shoots did not elongate upon shoot removal (**Figure 3**). In determinate-type cultivar, growth of the lateral shoots at the second node below TFB was not suppressed by flower bud removal compared with untreated plants, but lengths of these shoots increased significantly at 6 and 9 days after shoot removal (**Figure 4**). Hence, these results suggest that TFB promoted the growth of lateral shoots at the second node below TFB in indeterminate-type cultivar, but not in determinate-type cultivar (**Figure 5**). In contrast, the presence of AB at the first node below TFB seemed to suppress elongation of AB at the second node in both types of cultivars. Because emergence of TFB occurred earlier than emergence of AB at the second node [28], the effect of TFB on lateral shoot growth might be stronger than that on AB in both types of cultivars.

In relation to the inner plant growth regulators, auxin is produced in the apical bud and young expanding leaves in *Arabidopsis*, Brussels sprouts, pea, and tomato [30–33]. In the indeterminate-type cultivars, if the auxin concentration that suppresses lateral shoot elongation decreases temporarily upon ablation of the apical meristem or emergence of TFB, the lateral shoot at the second node below TFB elongates due to high cytokinin concentrations in the main stem. According to Shimizu-Sato et al. [34], reduced auxin concentration in the apical organs is a factor involved in increased cytokinin concentrations. However, in determinate-type cultivars, emergence of TFB did not promote the growth of lateral shoots. The much shorter stem lengths in determinate-type cultivars compared indeterminate-type cultivars [28] suggests that auxin concentrations in the apical organs including TFB might differ much from those of non-flowering terminal buds. Furthermore, auxin concentrations in apical organs including TFB might be related to branching habit in tomato plants. Some researchers [35–39] reported that plant growth regulators such as auxin, cytokinin, and strigolactone are related each other to the outgrowth of AB in several plants. Further study is desired to clarify the differences between the two branching types in tomato and the fluctuations in plant growth regulator concentrations.

In the pinching treatments (shoot removal), the growth of lateral shoots, especially in the three-true-leaf pinching treatment, was greater compared with that in the control (**Table 1**), which would be due to the increase of mineral nutrients uptake since the distribution of some mineral nutrient elements was changed by the pinching treatment. The differences in lateral shoot lengths in the plants by the pinching treatment at four to six true leaves were larger than in the plants by the pinching treatment at zero to three true leaves in the determinate-type tomato "Wase Daruma" [14]. Almost the same result was obtained in regard to the lateral shoot lengths in the different pinching treatments in the present study. The shoot lengths of 3-scaffold shoots by pinching treatment were longer than those of 6-scaffold shoots because the nutrient competition among the remaining shoots reduced in watermelon (*Citrullus lanatus*) [39]. This might be the reason that at 59 DAT the mean lateral shoot lengths in the three-true-leaf pinching treatment were more uniform compared with those in the six-true-leaf pinching treatment. In this study, perhaps the emergence period of AB was shorter and the competition for absorbed mineral nutrients was reduced in the plants that underwent the three-true-leaf pinching treatment.

Since the flowering period in the three-true-leaf pinching treatment was significantly shorter than those in the other treatments, the decrease of fruit set ratio that could occur during periods of high air temperatures (over 35°C) might have been avoided by pinching treatment [40]. Although the number of flowers in the three-true-leaf pinching treatment was significantly decreased compared with the other treatments (**Table 2**), there was no difference in the total fruit yield among all the treatments because the fruit set ratio in the three-true-leaf pinching treatment was higher than that in the other treatments. The harvest term in the pinching treatments was shortened until 3 WAH compared with that in the control until 4 WAH (**Figure 7**). These findings are in agreement with those of earlier studies [26, 27, 41]. The possibility for both shortening the harvest term and increasing the early yield was recognized in the three-true-leaf pinching treatment. In particular, shortening of the harvest term would permit mechanical harvesting and save labor cost, as described previously [12, 42–44].

The number of flowers per primary lateral shoot was not different in all treatments, whereas the numbers of flowers per secondary and higher lateral shoots in the both pinching treatments were significantly higher compared with that in the control (**Table 2**). The flower numbers on the longer lateral shoots could be increased in processing tomato plants [45]. In eggplants, the flower numbers on pinched plants were higher than those on no pinched plants because the number of lateral shoots would be increased on the former [46]. Therefore, in this experiment, the increases in both the number of flowers and the number of secondary and higher lateral shoots in the both pinching treatments compared with the control might be due to the release of apical dominance in plants because of the extension of lateral shoots in the previous reports [17, 19, 20, 47].

Pinching (shoot removal) releases apical dominance and removes a metabolic sink in plants [38]. This results in decreased auxin production in the apical bud and increased nutrient distribution into and growth of the lateral shoots [48, 49]. The levels and distribution of N, P, and K were increased in the lateral shoots of bean plants in relation to apical dominance [50]. Ca, a structural component of the cell wall and membranes, is needed for tomato plant growth at early growth stages [51], and its uptake under high-growth conditions was increased in tomato shoots [52, 53]. Fukui et al. [13] also reported that increased the number of flowers were due to the relatively greater availability of photosynthetic products in tomato cultivars

with large leaf areas. The number of flowers in tomato plants is also increased by higher contents of N and P [54]. Decoteau [55] reported that topping enhanced axillary leaf development in processing tomato cultivars. Thus, pinching treatments likely increase the photosynthetic products and mineral nutrient uptake by increasing the leaf areas of lateral shoots, and also likely lead to increased numbers of flowers. Therefore, it was revealed that the numbers of dropped flowers in the control and six-true-leaf pinching treatments were greater than in the three-true-leaf pinching treatment because of the excessive number of flowers per plant.

5. Conclusion

In tomato plants, flower bud or shoot removal (pinching treatment) affected the branch formation and fruit yield. The emergence of TFB affected the growth of lateral shoots in indeterminate-type cultivar, whereas it did not affect the growth of lateral shoots in determinate-type cultivar. Therefore, it is suggested that the appropriate management of the lateral shoots would be necessary for improve fruit yield or fruit quality, and it would be different between indeterminate and determinate-type cultivars. In indeterminate-type cultivars, it would be important to consider both the position and timing of shoot pinching and the timing of lateral shoot removal. In determinate-type cultivars, it might be necessary to study the number of lateral shoots or the training direction of the vines in order to avoid plant diseases during the periods of high temperature and/or humidity conditions. The shortening of harvest term and increase of initial fruit production in the three-true-leaf pinching treatment would be due to elongated lateral shoots and shortening of the flowering periods per plant. Thus, the pinching treatment could permit machine harvesting and save labor costs for determinate tomato cultivation. From these results, further studies should be undertaken to elucidate the relationships among shoot growth of plant, number of flowers, and physiological factors such as the sink strength in each organ, the distribution of photosynthetic products, and the changes of nutritional status and some plant growth substances in plants after flower bud or shoot removal (pinching treatment).

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Chapter 5

Using Abrasive Grit for Weed Management in Field Crops

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Additional information is available at the end of the chapter

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Abstract

Abrasive grit, applied at high pressure and directed at plant base, can control weeds and increase yield. We evaluated fertilizer [pelletized turkey (*Meleagris gallopavo*) litter] and non-fertilizer [walnut (*Juglans regia*) shell] grits for maize and soybean in-row (IR) weed management. Grits were applied at V1 and V5 of maize, and V1 and V3 of soybean. Between-row weed cultivation was done alone (BR), or in combination with grit (I/B), after grit application. Small weeds (<4 cm) were controlled after grit treatment, but, larger broadleaf weeds, grass weeds (treated when growing points were below ground), and later emerging weeds resulted in IR weed biomass similar between season-long weedy (SLW) and IR treatments by August. In maize, fertilizer and nonfertilizer I/B treatments averaged 44 and 14% greater yields, respectively, than SLW ($p<0.01$) but each was similar to BR which averaged 23% greater yield ($p=0.63$). Maize grain had 16% higher N content in the fertilizer I/B treatment than SLW or nonfertilizer I/B ($p<0.003$). In soybean, I/B increased yield by 17% ($p=0.009$) over SLW yield, but was similar to the BR increase of 22% ($p=0.13$). Maize had a greater positive response to fertilizer than nonfertilizer grit, whereas soybean was less influenced by I/B treatment.

Keywords: maize (Zea mays), soybean (Glycine max), air-propelled grit, weed control

1. Introduction

The number and acreage of organic certified farms across the United States has increased [1] due to expanding organic foods sales [2], which has created premiums for organically grown commodities [3, 4] and alternative income streams for farmers. Crop fertility and weed

management, within the confines of the certified organic regulations [5], are major concerns in organic production systems, as methods other than synthetic chemicals must be utilized. Alternative organic-approved nutrient sources include manures [6–9] and seed meals [9–11], when derived from certified organic materials.

Weed control repeatedly has been ranked as very to extremely problematic [12–14] and as a top research priority [15] in organic producer surveys. Flexible systems that rely on cultural and mechanical methods are needed to prevent the creation of specialized weed communities [16, 17]. Cultural methods enhance the crop's competitive ability [16], reducing weed impact. Methods include alternating crops that vary in seasonal growth, fertilizing differentially [18], or speeding canopy closure by planting in narrow rows or at high densities [19–24]. Dense cover crop mulches, such as those created by rye grass (*Lolium* sp.) or hairy vetch (*Vicia villosa*), suppress weeds [25–29], although these may become major weed problems or immobilize N, negatively impacting yield, if not carefully managed [26, 28].

The most important time for weed control is during the early growth stages of a crop, also known as the critical weed free period, so that yield is not reduced [30–39]. Careful timing of physical and mechanical weed control operations [40–42], including deeper tillage or seedbed preparation that disturbs newly emerging weed seedlings (i.e. stale seedbed) [43–45] can provide weed control and lower in-season weed density [46, 47]. Burying the weeds to at least 1 cm deep, through rotary hoeing or rod weeding, or mowing the weeds at the surface also provides control [48, 49].

Cultivation, or flaming at high temperatures [50], are effective methods to control between-row weeds, however, in-row weed control is still a problem for organic growers [51]. In-row weeders, including harrows, finger and torsion weeders, and weed blowers, have been developed [52–55]. However, crop burial or injury [56] can result in yield reduction [57] so that accurate steering and slow driving are needed to minimize crop damage [51]. Despite advances in physical weed management, organic growers are not satisfied with the tools available nor the amount of weed suppression achieved [58]. Additional methods would provide alternatives to support these 'traditional' weed control techniques [51, 59–61].

Air-propelled abrasive grit application for weed control by tissue abrasion was proposed by Nørremark et al. [62]. Numerous types of grits made from agricultural (e.g., maize cobs and walnut shells), non-agricultural (e.g., sand), and organic fertilizer (e.g., soybean meal and corn gluten meal) materials controlled weeds in greenhouse and field settings [63–70] when sprayed at high pressure (800 kPa). In the field, two or three in-row grit applications, applied from V1 to V5 growth stage of maize, could reduce weeds and increase grain or silage yields [65, 69, 70].

Although Forcella [66] demonstrated that soybean could tolerate grit applications after the cotyledon (VC) growth stage, the influence of in-row grit application on weed control has not been field tested in this crop. In addition, organic fertilizers, such as pelletized turkey litter [71], have not been tested as abrasive grits for weed control in maize or soybean field studies. The hypotheses of this study were that 1) grits derived from different sources would result in similar weed control when applied at early crop/weed growth stages; and 2) crop yield would be increased by grits containing nitrogen [68, 72, 73].

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2. Air propelled abrasive grit influence on in-row weed control and crop yield

2.1. Materials and methods

2.1.1. Grits

Two grits, made from materials that are approved for organic production and that differ in fertilizer value, were used for in-row application and control of weeds. Sustane® (Sustane Corp., Cannon Falls, MN), is made from pelletized aerobically composted turkey litter [71], and had a fertilizer grade of 8-2-4 (N-P_2O_5-K_2O). Agra Grit (AgraLife), made from walnut shells, which has a high C:N ratio with little immediate nitrogen availability, provided a low N content comparison. Sustane and Agra Grit products have a hardness value of 3 on the Mohs scale of mineral hardness and varied in size from 0.56 to 0.85 mm.

2.1.2. Field experiments

Maize and soybean were planted from 2015 to 2017 in organic certified production fields at the SDSU Southeast Research Farm (Beresford, SD), in a non-certified transition area at the SDSU Research Field Station at Aurora, SD; and in conventionally managed fields at the at the Swan Lake Research Farm (Morris, MN). Soil types were silt loam complexes (Morris and Beresford) and a silty clay loam at Aurora.

Varieties used, relative maturity (RM), planting and harvest dates varied by year and location (**Table 1**). Swan Lake was the northernmost location and used shorter RM varieties. Southeast was the southernmost location and used longer RM varieties. Maize was seeded at 3.5-cm depth, when soil temperatures were 14°C. Soybean seeding rate varied by location and year (**Table 1**) and was planted at 2.4-cm depth when soil temperatures were 18°C. Row spacing was 0.76 m with four crop rows per treatment (~3-m width). Plot length varied from 3 to 9 m.

Sustane 8-2-4 and Agra Grit were applied in all trials. In-row (IR) grits were applied twice, at the V1 and V5 maize growth stages, and the V1 and V3 soybean growth stages (**Table 1**). About 800 kg ha^{-1} of grit was used for each application, which was applied using a propelled abrasive grit applicator [PAGMan] that sprays four rows simultaneously, with a nozzle on each side of the row [69, 70]. Distance of the nozzle tip to the base of the maize plants was between 10 and 15 cm, at a 45° contact angle. Spray pressure was 690 kPa and tractor speed was 2.5 km $hr.^{-1}$. After the final grit treatment each year, a single cultivation was used for between-row (BR) weed control using a John Deere® 866 spring tine cultivator at 5 km $hr.^{-1}$. In addition, other treatments all years included a single between-row cultivation, as described previously, to determine yield potential with only cultivation, season-long weedy (SLW) to estimate yield in nontreated conditions, and weed-free (hand-weeded weekly until canopy closure) to estimate maximum yield potential under weed-free conditions.

Weed species and density were recorded in each plot prior to and about 1 week after final grit applications. In mid-July to early September depending on crop and year (**Table 1**), weeds,

Year	Location	Crop	Relative maturity	Planting		Grit application		Weed biomass	
				Date	Rate	First	Second	Greenness	Harvest date
					(*1000)				
2015	Aurora	Soybean	1.4	June 9	395	June 30	July 8	September 8	October 22
	Beresford	Soybean	2.1	June 9	395	June 26	July 10	September 2	October 20
	Morris	Corn	93 d	April 16	79	May 16	June 2	August 27	October 1
2016	Aurora	Corn	99 d	May 7	79	May 17	June 15	July 15	October 5
	Aurora	Soybean	1.4	May 19	395	June 8	June 23	July 20	October 14
	Morris	Soybean	1.1	May 4	431	June 1	June 17	July 19	September 9
2017	Aurora	Corn	95 d	May 5	79	June 2	June 23	August 1	November 5
	Aurora	Soybean	1.7	May 30	395	June 15	June 29	July 31	October 11
	Beresford	Soybean	1.9	May 24	420	June 16	June 27	July 28	October 17
	Morris	Corn	93 d	May 11	86	June 6	June 26	July 25	October 31

Table 1. Locations, crops, relative crop maturity rating, planting dates and rates, dates of weed control applications, and measurements.

in- and between-rows, were harvested from 1/10 m^2 areas, separated by functional group (grass vs. broadleaf) and dried at 60°C until constant weight to quantify biomass by treatment. Relative greenness of the newest fully expanded maize leaf (SPAD meter, Konica Minolta, Japan) (10 to 20 plants per plot) was measured in 2016 and 2017 during this sampling time. At crop physiological maturity, the middle two rows of each treatment were harvested, and yield determined. Yield was corrected to 15.5% moisture for maize, and 13% moisture for soybean. Maize grain was tested for % N content, and soybean was analyzed for oil and protein contents.

2.1.3. Statistical analysis

Treatments [grit type followed by between-row treatment (I/B), between-row (BR) only, SLW, and hand-weeded] were replicated four times for each crop, year, and location in randomized complete block designs. Yields in hand-weeded and SLW treatments varied considerably among locations and years. To analyze main effect of grit treatments, the SLW treatment average yield by crop, location, and year was used as the 'base' yield, with grit type and BR treatment average yields for the crop, location, and year divided by the SLW value, providing a relative yield comparison with the SLW. This relative yield approach assisted in evaluating treatment comparisons among years and locations. The treatments by crop were the fixed effects, whereas location, blocks, and years were random effects. One-way ANOVAs (i.e., was the relative treatment yield greater than its SLW?) were calculated with significance of $p = 0.1$. In addition, a sign test for paired comparisons also was used as a non-parametric measure to determine if treatments differed.

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2.2. Results

2.2.1. Influence of treatments on in-row (IR) and between-row (BR) weed control

Broadleaf weeds observed in plots during the time of grit applications included common lambsquarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), common purslane (*Portulaca oleracea*); and grass weeds included green and yellow foxtail (*Setaria viridis* and *S. pumila*, respectively). Weed size ranged from <1 cm (V1 maize application), 2- to 6-cm tall (V1 soybean), and >10 cm tall (V3 soybean or V5 maize). Broadleaf weeds were greater in number, making up >60% (and at times, nearly 100%) of the weed profile.

In general at all locations, grit treatments tended to control all weeds in maize after the first (V1) application (data not shown) as weed densities were low, and most were small (<3 cm). When grit was applied in soybean, plots at Aurora and Beresford already had high weed densities (~700 plants m^{-2}) and weed height ranged from 2- to 6-cm at V1 and, while grits abraded leaf tissue, control ranged from 0- to 50%. Plots at Morris were rotary hoed prior to grit application and no weeds were present. Examining weeds before and after the second application indicated that emerged broadleaf weeds again were injured by abrasion, but if >4-cm tall, they were not controlled. Grass weeds, if emerged, were injured, but growing points were still below the soil surface, so that although defoliated, they were not controlled well.

Grit application, generally, did not influence IR weed biomass measured at R4, with weights similar to those recorded in SLW plots. At Aurora IR weed biomass was found to be nearly 100% grass in 2016 and averaged 300 kg ha^{-1} (SLW averaged 400 kg ha^{-1}), and in 2017, was about 60% grass and averaged about 4500 kg ha^{-1} (SLW averaged 4300 kg ha^{-1}). In Morris maize plots, nearly 100% of biomass was attributed to broadleaf species and averaged 100 kg ha^{-1} (SLW averaged 140 kg ha^{-1}) in 2015, and 2200 kg ha^{-1} (SLW averaged 2600 kg ha^{-1}) in 2017.

Total IR weed biomass in soybean treated with grit did not differ from the location/year SLW treatment except at Aurora in 2016, where grit treatments had reduced broadleaf biomass that ranged from 20 to 80% less (220 kg ha^{-1} in SLW) and about 50% less grass biomass (1100 kg ha^{-1} in SLW).

The BR cultivation in maize and four of six soybean site-years had few if any weeds remaining between rows and remained nearly weed-free through harvest. The exceptions were the 2015 soybean plots in Aurora where the BR treatment had 11,000 kg ha^{-1} between row weed biomass with grass weeds contributing about 80% of the total biomass, and 2015 soybean plots in Beresford, where BR weed biomass was 105 kg ha^{-1} with grass weeds accounting for all the biomass.

2.2.2. Crop yield

Maize yields in weed-free areas differed by location and year and ranged from 7588 (Aurora, 2017) to 12,690 (Morris, 2015) kg ha^{-1} in weed-free treatments. Yield losses in SLW compared

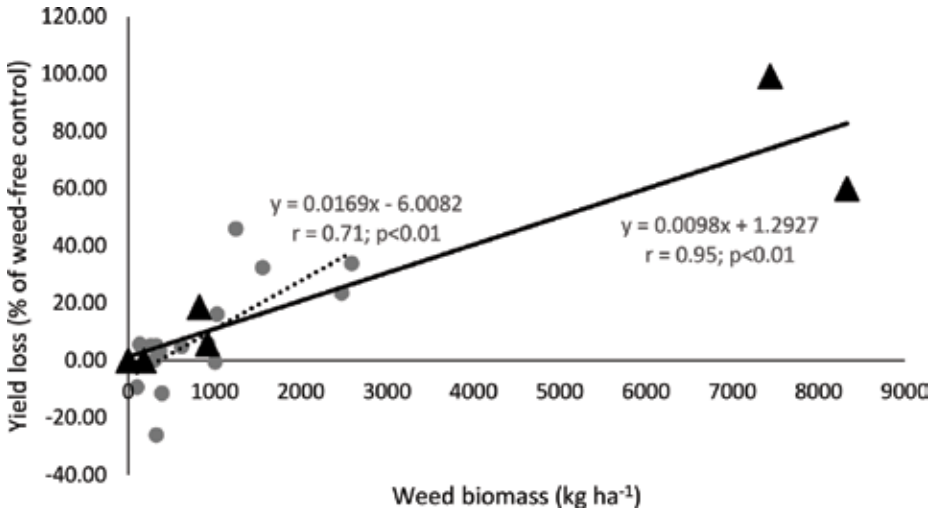

Figure 1. Maize yield loss (% of weed-free control) by total weed biomass at all locations and treatments. Triangles represent the season-long weedy treatments and the regression is shown by the solid black line ($r = 0.95$; $p < 0.01$). Circles represent yield loss based on weed biomass compared with the weed-free control plots of the I/B and BR treatments with the regression shown in the dotted line ($r = 0.71$; $p < 0.01$).

to the weed-free check ranged from 5% to nearly 100%. Maize yields in the SLW, I/B, and BR treatments were almost all lower than the weed-free check. In the SLW vs. weed-free treatments, the percent yield loss was positively correlated with total weed biomass (in-row plus between-row weeds) when examined across all studies ($r = 0.93$; $p = 0.008$) (**Figure 1**). Because the I/B and BR treatments had few between row weeds, the yield losses in these treatments were positively correlated with in row weed biomass ($r = 0.71$; $p = 0.001$). The slopes of each regression line were similar ($m \sim 0.01$), which indicated that percent yield loss was about 1% for each 100 kg ha^{-1} of weed biomass present at R4.

Although weeds were present in the row after I/B treatments, maize yields across years and locations were greater than the SLW, except in one case. On average, there was a 30% yield increase in grit application treatments compared within a year and location to its companion SLW treatment. Sustane treatments averaged 44% greater yield than SLW, whereas Agra Grit averaged 14% greater yield ($p = 0.1$). In addition, maize grain had 16% higher N content than grain from either the SLW or Agra Grit treatments ($p<0.003$). Sustane appeared to provide some nitrogen to the crop [74], as relative greenness, measured at R4 of maize, was similar to the weed-free check, and averaged 44% ($p < 0.01$) higher than greenness of plants in the SLW treatment. Agra Grit has a high C/N ratio, and actually slowed soil nitrogen mineralization in laboratory studies [74], although greenness values at R4 were about 30% greater than SLW plants. The BR treatment and I/B treatments averaged over all grits, however, had similar maize yield increases (23% vs. 30%, respectively; $p = 0.63$) compared to the SLW.

Soybean yield in weed-free treatments ranged from 1626 (Aurora, 2015) to 4856 (Aurora, 2016) kg ha^{-1}. Yield losses compared to weed-free treatments within location and year ranged from 2 to 43%. However, unlike maize yield losses, which were linearly related to total weed

biomass, soybean yield loss in SLW compared with weed-free was not correlated to total weed biomass ($r = 0.16$; $p = 0.69$), but was more correlated to in-row weed biomass ($r = 0.58$; $p = 0.12$). When examining relative yield compared with the SLW treatment, I/B treatments increased yield on average by about 16% ($p = 0.004$), which was similar to the BR treatment alone ($p = 0.48$). Soybean yield increases due to Agra Grit treatments compared with Sustane treatments were similar. Protein and oil content of soybean grain were similar among treatments as well. These data indicate that grit applications applied at V1 and V5 of soybean had minimal impact on soybean yield and weed biomass. Indeterminate growth of soybean may have been partially responsible for the inconsistency between weed biomass and soybean yield loss.

3. Conclusion

Weed management using grits was more effect on small broadleaf weeds than larger broadleaf or grass weeds. While the larger weeds and grasses were defoliated with the grit treatment, these regrew and by late season, biomass was similar in-row as the season-long weedy treatment. The in-row treatment, followed by cultivation between rows, tended to increase maize yield compared to no management, and grit with a higher N content tended to increase maize yield and nitrogen content more than a low N grit. Weed control in soybean was more challenging and, due to the size of the weeds even at V1 (one expanded trifoliate leaf), did not control weeds well, and by the second application (V5), weeds were likely too large for meaningful injury. Soybean yield loss was more related to in-row weed biomass than between row weed biomass. Thus, more research is needed to better control in-row weeds in soybean to limit yield loss.

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Chapter 6

Use of Some Bacteria and Mycorrhizae as Biofertilizers in Vegetable Growing and Beneficial Effects in Salinity and Drought Stress Conditions

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Additional information is available at the end of the chapter

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Abstract

Industrialization and rapid population growth, especially after the second half of the twentieth century, have also revealed significant environmental problems in the world. The consistent and alarming increase in the human population has again threatened the world's food security. It is becoming increasingly clear that conventional agricultural practices cannot sustain the production base, a healthy plant-soil system, for too long. There is a growing worldwide demand for compatible environmentally friendly techniques in agriculture, capable of providing adequate nourishment for the increasing human population and of improving the quality and quantity of certain agricultural products. For these reasons, the application of beneficial microorganisms is an important alternative to some of the traditional agricultural techniques which very often severely alter the agroecosystem balance and cause serious damage to health. Beneficial microorganisms can play a key role in this major challenge, as they fulfill important ecosystem functions for plants and soil. Utilization of these microorganisms affects plant's growth and yield in a positive way. Besides, their favorable effects on root growth help plants to deal with both biotic and abiotic stress factors. PGPR and mycorrhizae can influence higher plants response to abiotic stresses such as drought and salinity through different mechanisms.

Keywords: bio-fertilizers, PGPR, mycorrhizae, vegetable, abiotic stress, salinity, drought

1. Introduction

No matter chemical fertilizers or manures, using fertilizers for the purpose of improving the fertility of the soil and the productivity of the crops have caused that the biogeochemical

cycles in the nature have been affected negatively [1, 2], and the nutrients (specifically nitrogen (N) and phosphorus (P) were run off, which ultimately caused degradation in the environment [3, 4]. There are several underlying reasons for this situation some of which are the low use-effectiveness of fertilizers and the constant long-term use. Although there are damaging environmental effects, it is expected that the total fertilizer amounts that are used in the whole world will increase in future due to the ever-increasing world population, because there appears a need for producing more food by applying intensive agriculture, which necessitates a great amount of fertilizers [5, 6].

There are two objectives in modern horticulture that contradict with each other: the need to provide food for ever-rising population of the world; and the need for minimizing the damage done to the environment, which can affect horticulture in a negative way [7]. In this respect, horticultural industry and scientists face a major sustainability challenge [8]. In the past 10 years time period, there were some innovations in the field of technology to improve the sustainability of the production systems by reducing the use of chemicals. "Biostimulants" have been proposed as an effective tool in this context. As a result of the efforts made to reduce the harmful effects of fertilizers, plant growth promoting rhizobacteria (PGPR) and/or arbuscular mycorrhizae fungi (AMF) have been proposed as complements for fertilizers. "Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or to rhizosphere is to stimulate natural processes to enhance nutrient uptake, effectiveness, tolerance to abiotic stress, and crop quality, with no direct action on pests."

The rhizosphere is a soil volume under the effect of plant roots. Hiltner [9] defined "rhizosphere" as a maximum microbial activity zone. The microbial population that exists in this medium is different from the population that surrounds it because of the root exudates, which act as nutrition source for microbial growth [10]. The microorganisms may exist in the rhizosphere, rhizoplane, root tissue, and/or in a specialized root structure that is named "nodule." Among the plant, soil, and microorganisms that exist in the soil medium, significant interactions were reported [11]. These significant interactions can be beneficial, neutral, and/or harmful, and may affect growth of plants [12–14]. Usually there are bacteria, algae, fungi, protozoa, and actinomycetes in the microorganisms that colonize in the roots of plants. Evidence has been presented about the enhancement of plant growth and development by applying these microbial populations [15–19]. Bacterial population, i.e., fungi include a significant portion of soil rhizosphere microflora and affect plant growth. The togetherness of fungi and plant roots (mycorrhizae), which is symbiotic life, enhances the root surface area, and this enables the plant to absorb water and nutrients from big soil volume in a more efficient manner. Two mycorrhizae (ecto- and endo-mycorrhizae) types were reported in a few plant species. The mycorrhizae increase the availability of the nutrients and water, and in addition, protect the plant from some abiotic stresses [20, 21].

Agriculture is influenced greatly by the climate change; especially agriculture in tropical areas face increased stress because of natural and anthropogenic factors. In some major crops,

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increased abiotic and biotic stress is a major cause for productivity stagnation. It has been considered as a big difficulty to develop efficacious, low-cost, and easy-to-apply methods in abiotic stress management. Many studies have been conducted throughout the world for the purpose of developing tactics to deal with abiotic stress. In such studies, developing species that are tolerant to heat and drought, changing crop cultivation times, resource management, etc., were applied [22]. Many newly introduced technologies are cost-effective. Some studies conducted recently have reported that microorganisms could help crops fight against abiotic stress. It has long been recognized that microorganisms have effects on plant growth, nutrient management, and disease control. Some useful microorganisms invade the rhizosphere/endorhizosphere of plants. They enhance plants via some direct-indirect mechanisms [23]. In addition to these, the role of microbes in biotic and abiotic stress management has been focused on more in recent times. Soil supports plant growth through complex and dynamic systems. Plant growth and development are affected by some stresses which are major constraints for sustainable agricultural production in the soil environment. Biotic stresses include plant pathogens and pests (viruses, bacteria, fungi, insects, and nematodes). Abiotic stresses are salinity, drought, flooding, heavy metals, temperature, gases, and deficiency of nutrients or excessive nutrients. Abiotic stresses cause yield reduction, and their intensity changes according to the soil types and plant factors. Imbalance in hormones and nutritive elements, physiological disorders (epinasty, abscission, and senescence), and susceptibleness to diseases are some of the general impacts of these stresses [24–28].

2. Beneficial microorganisms against stress conditions PGPR and mycorrhiza

2.1. Plant growth promoting rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are useful bacteria that act on some soil types and facilitate that plants grow and develop in (in)direct ways. In a direct way, fixed nitrogen, phytohormones, iron isolated by bacterial siderophores, i.e., iron-carriers, and phosphate in soluble form are given to plants. In an indirect way, phytopathogens (biocontrol) are avoided resulting in plant growth enhancement. Such functions are performed by PGPR through several enzymes (like bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase) stimulating physiological changes at molecular level. ACC has an important effect on ethylene regulation, which is a plant hormone, resulting in modified plant growth and development. Bacterial strains with ACC deaminase may eliminate negative effects caused by stress and mediated by ethylene.

It was reported that there was ACC deaminase in some Gram-negative microbial bacteria, Gram-positive bacteria, rhizobia, endophytes, and fungi. It was investigated in some species of plant growth enhancing bacteria (*Agrobacterium genomovars* and *Azospirillum lipoferum*, *Alcaligenes* and *Bacillus*, *Burkholderia*, *Enterobacter*, *Methylobacterium fujisawaense*, *Pseudomonas*, *Ralstonia solanacearum*, *Rhizobium*, *Rhodococcus,* and *Sinorhizobium meliloti*, and *Variovorax paradoxus*).

The ACC of the root is metabolized into α-ketobutyrate and ammonia by the ACC deaminase. It also checks the ethylene production. If this process did not occur in this way, the growth of the plant would be inhibited via some mechanisms. If plants are treated with bacteria that have ACC deaminase, it is possible that they have extensive root growth because of less amounts of ethylene. In this way, plants may resist several stress sources. In recent years, using PGPR with ACC deaminase activity, to improve the growth of plants under stress and normal conditions, has been dealt with researchers as an interesting and new field. Also, cultivars' genetic manipulation with genes that express this enzyme has been dealt with recently by several authors. For this reason, focus must be laid on the further parts of this manuscript on late developments in this field of biotechnology.

Data on biosynthetic pathways of ethylene production in plants enabled us to elucidate the mechanisms by which plants regulate the endogenous ethylene level for their normal growth. It has been demonstrated that S-adenosylmethionine or ACC-degrading enzymes decrease ethylene levels in an efficient manner without changing plant physiology. For this purpose, researchers investigated some enzymes that aid to decrease ethylene levels in plants. In this respect, S-adenosylmethionine (SAM) hydrolase and SAM decarboxylase were examined less with regards to ethylene regulation in plants. ACC synthase and oxidase were examined more with several plants.

The ACC deaminase, which is a pyridoxal 5-phosphate (PLP)-dependent polymeric enzyme, was first investigated in a soil bacteria species *Pseudomonas* sp. strain. Bashan et al. [29] described structure for ACC deaminase and provided an understanding about the working of sole pyridoxal-5-phosphate that depends on cyclopropane ring-opening reactions of this enzyme in *Pseudomonas* sp. It was reported in [30] that there was a wide range (>100-fold) in ACC deaminase activity level in various organisms which show high ACC deaminase activity and typically bind to some plants. In this group, there are rhizosphere, phyllosphere organisms, and endophytes, which may behave as a sink-like structure for ACC that appear as a result of stress in plants. In addition, the abovementioned show little preference for one plant over another. However, the organisms that express low deaminase may only bind to some plants. They may also be expressed solely in some tissues; and do not reduce the level of ethylene in plants; but, they prevent a localized increase in the levels of ethylene. Glick reported that there are some rhizobia and ACC deaminases.

Glick et al. [31] investigated the model of PGPR which includes ACC deaminase. They examined how a bacterial ACC deaminase with a low relation to ACC could cope with plant enzymes and ACC oxidase that has high relation with the same substrate resulting in a reduction of endogenous ethylene concentration of a plant. They claimed that biological activity of PGPR was related with ACC deaminase ACC oxidase amounts. In order for PGPR to decrease ethylene levels in plants, the level of the ACC deaminase must be minimum from 100- to 1000-fold bigger than ACC oxidase level. For this to happen, the ACC oxidase expression must not be induced.

Indole-3-acetic acid (IAA) is synthesized and excreted by PGPR. IAA is adsorbed by the surface or roots of the seeds of plants by tryptophan and some molecules in seeds or root

exudates. Plants take up some IAAs that are synthesized recently, and IAAs may stimulate the cell proliferation and elongation of plants. In addition, SAM is converted into ACC by enzyme ACC synthetase stimulated by IAA. In the model of Glick et al., an important deal of ACC can be exuded from the roots or seeds of plants. It may also be taken up by soil microbes. It is also possible that it is hydrolyzed by vital microbial enzyme ACC deaminase to produce ammonia and α-ketobutyrate. This process causes that the ACC amount is reduced outside plants. In addition, the balance between internal-external ACC is kept stable via the exudation of more ACC into the rhizosphere. Soil microbial communities with ACC deaminase activity cause that plants biosynthesize more ACC than the plant could need and arouse ACC exudation from plant roots. Meanwhile, they will also provide microorganisms with nitrogen (ACC). As a result, microorganism with ACC deaminase growth is enhanced near roots of the plants. In this way, the ACC level is reduced in plants, and also, the ethylene (stress hormone) biosynthesis is inhibited. In some studies, PGPR inoculation with ACC deaminase was shown to change the endogenous ethylene levels, which ultimately lead to variations in plant growth.

Several chemicals (aminoethoxyvinylglycine (AVG), aminooxyacetic acid (AOA), and 1-methylcyclopropene (1-MCP)) were used to reduce the ethylene level in plants. They were also used to change the sensitivity to ethylene during fruit ripening and flower wilting. In many situations, these chemical substances are not cheap, not easily obtained, and are harmful for the environment. Using PGPR in a natural soil and plant environment is more economical and feasible and is more economical friendly because PGPR includes ACC deaminase activity. In addition, it has also some other advantages like the ACC deaminase trait being more common in some PGPR species that are native to rhizosphere and have a wide variety of survival potential in rhizosphere and rhizoplane. Moreover, PGPR has some other aspects (such as auxins, gibberellins, cytokines, and/or polyamines syntheses contributing directly to plant growth). These features cause that the selection of PGPR with ACC deaminase is more reliable than other alternatives.

2.2. Mycorrhizae

AMF were first described in the last years of nineteenth century. Albert Bernard Frank described the symbiotic associations between the plant roots and the fungi (mycorrhizae). Mycorrhizae means "fungal root." This association's basic principle is the nutrients taken up from the soil are exchanged with sugar. Lots of microorganisms form symbiosis with plants ranging on a continuous scale from parasitic to mutualistic. A typical example of these widespread mutualistic symbioses is the arbuscular mycorrhiza formed between AMF and vascular flowering plants [32]. Many scientists and mycologists researched the relations (associations) between mycorrhizae and the plants biology and their inoculation methods. This relation includes the structure of the root and mycorrhizal inoculation. Mycorrhizae are complex symbioses and the fungi produce some structures in the root. Quantification of the structures (hyphae, arbuscules, and vesicles) was standardized by the method suggested by Hungria and Vargas [33]. An arbuscular mycorrhiza has three important elements; the root, the fungal elements between the cells of the root and an extraradical mycelium in soil [34]. The most common type

of mycorrhizae is the arbuscular mycorrhiza occurring in about 90% of plant species infected with mycorrhiza. The most common type of mycorrhizae is the arbuscular mycorrhiza occurring in about 90% of plant species infected with mycorrhiza, approximately 83% of dicotyledons, 79% of monocot, and 100% of gymosperms. Most crop plants form mycorrhizae with the exception of the Brassicaceae (e.g., mustard, cabbage, and canola) and Chenopodiaceae (e.g., sugar beets and spinach).

AM fungi consists approximately 160 species belonging to three families. Glomaceae, Gigasporaceae, and Acaulosporaceae. More than 6000 fungal species can form mycorrhizae with about 240,000 plant species. AMF plants own bigger extraradical hyphae formation and soil aggregation. They enhance tilth and excrete hydrophobic protein called "glomalin." AMF produce more stress-resistant plants during production and for landscape, they reduce the pesticide usage, they increase the more drought and nutrient tolerant plants in landscape, and they potentially higher transplanting success and faster establishment. A symbiotic association formed by fungi with roots, exchanging for functioning as an extended root system, the fungi receives carbonhydrates from the host plant [35].

Arbuscular mycorrhizae fungi (AMF), which are useful organisms, have a significant role in performance and nutrition with plant mineral intake capacity [36]. AMF symbiosis is especially significant in improving the immobile uptake and indissoluble phosphate ions in soil with the interactions with bi/trivalent cations (especially Ca^{2+}, Fe^{3+}, and Al^{3+} [37, 38]. The main function in this mutualism is the capacity of AMF in developing external hyphae networks that may extend the surface area (up to 40 times) and the explorable soil volume for nutrient intake [39] by producing enzymes and/or excreting organic substances [40]. AMF can excrete phosphatases to hydrolyze phosphate from organic P-compounds [41–43], which enhance productivity under harsh conditions (deficiency of phosphorus; [44]). The extraradical hyphae are considered significant in terms of intake of ammonium, immobile micronutrients (Cu and Zn), and some mineral cations coming from the soil (K^{+}, Ca^{2+}, Mg^{2+}, and Fe^{3+}) [45, 46]. It was demonstrated that AMF enhance plant nutrition (biofertilizers), and interferes with the phytohormone balance of the plants, which in turn affects development of plant (bioregulators) and alleviates the influence of the environmental stresses (bioprotectors). This increases the biomass and yield, and causes shifts in some quality parameters [47].

The horticultural products have high phytochemical elements (carotenoids, flavonoids, and polyphenols) and therefore meet the desires of consumers and authors with their health/benefit influences [48]. Furthermore, AMF also bring tolerance to drought [49, 50] and salinity [51, 52], nutrient deficiency, heavy metal contamination [53] and in adverse soil pH [54, 55].

The AMF life cycle begins with asymbiotic stage (germination of the asexual chlamydospores). This depends on several physical factors (temperature and humidity). AMF retract the cytoplasm without the presence of a plant and turn to the dormant phase because they are obligate biotrophs. However, near the roots of the plants, the presymbiotic phase begins with the ramification of the primary germ tube [56]. Root exudates [57] and specific metabolites (strigolactones) may also induce this [58]. When there is a physical contact with the surface of the root, the

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fungi build up hyphopodia (appressoria) on the surface. On the other hand, a particular mycorrhizae-specific process occurs in epidermal cells underlying hyphopodia in the plant side. They constitute the pre-penetration apparatus, which is a transient intracellular structure used by the fungi to enter the root [59]. Fungal hyphae host the roots of the plant, firstly, between/through cells with linear/simple-coiled hyphae [60], and then build up high-branch hyphal structures that resemble a tree in plant cell apoplast (the arbuscules which gave the name). *Gramineae* members form vesicles rich in lipid as storage organs [61]. Parallel to the colonization of the root, fungi examines the soil around with its hyphae with which they uptake nutrients, interact with other microorganisms, and colonize roots of nearby plants of the same (or different) species. In this way, plants and their AM fungi are interrelated with each other in a network of roots and hyphae [62, 63]. They can exchange nutrients [64] or signals [65] in this way. Eventually, new chlamydospores are created in the extraradicular mycelium. The cycle of life is ended in this way.

3. The most effective environmental stress factors: salinity and drought

3.1. Salinity stress

Under saline conditions, the changes in soil-water potential cause that plant water intake is reduced as well as the nutritional and hormonal imbalance. In these conditions, proline, glycine betaine, trehalose, polyols, and similar organic solutes accumulate in the body of the plant to preserve the plant from the stress-induced effects with osmotic adjustment, with limiting water loss and diluting the toxic ion concentration [66, 68]. Such an accumulation makes it possible for the plant to maintain osmotic potential for improved water intake. For instance, proline accumulation preserves the plant by adjusting osmotic pressure and by stabilizing many functional units (e.g., complex II of the electron transport system, proteins, and enzymes [69, 70]. There are two mechanisms in which high-concentration soluble salts influence microbes: osmotic effect and specific ion effect. Osmotic potential (more negative) is increased by soluble salts and draws water out of the cells, which in turn, may kill microbes and roots via plasmolysis. Because of the low osmotic potential, it becomes more difficult for roots and microbes to eliminate water from the soil [71]. Plants, as well as microbes, can adapt to low osmotic potential through accumulating osmolytes. However, osmolyte synthesis necessitates large amounts of energy, which in turn, results in reduced growth and activity [72, 73]. Certain ions, including Na^{+}, Cl^{-}, and HCO^{-3}, are toxic for some plants when they are at high-concentrations [74]. In some previous studies, it was reported that salinity decreases microbial activity and microbial biomass and changes the structure of the microbial community [75–79]. The microbial biomass is decreased by salinity. The reason for this is that osmotic stress causes drying and cell lysis [80–86]. In previous studies, it was also reported that soil respiration was reduced with the increase in the soil EC [87–89]. Gerhardson [90] reported that soil respiration was decreased by more than 50% at EC1:5Z5.0 dS m1. However, according to Glick [91], soil respiration was not correlated at a statistically significant level with EC. However, they also reported that as EC increased, the metabolic quotient (respiration per unit biomass) also increased.

Microorganisms can adapt to/tolerate stress salinity stress by accumulating osmolytes [91–95]. Among the main organic osmolytes, there are proline and glycine betaine; and among the common inorganic solutes, there are potassium cations, which are used as osmolytes accumulated by saline-tolerant microbes [96]. However, high amount of energy is necessary for the synthesis of organic osmolytes [97, 98]. Inorganic salts accumulation (as osmolytes) may be toxic, and for this reason, it is limited to halophytic microbes which developed saline-tolerant enzymes to survive in highly saline medium. Fungi have a tendency for being more sensitive to salt stress than bacteria [99–102]. In this respect, the rate of bacteria/fungi may be increased in saline soils. When compared to nonsaline soils, salinity-tolerance differences among microbes cause those changes that appear in the structure of the community [103, 104].

3.1.1. PGPR help plants tolerate salinity stress

Salt stress enhances endogenous ethylene production in plants and mostly serves as a stress hormone. Probably decreasing the ethylene induced by salinity via any mechanism might reduce the negative effect of salt on the growth of plants. According to recent studies, plants inoculated with PGPR with ACC deaminase could cope with salinity stress with a normal growth pattern. According to Mayak et al. [105], *Achromobacter piechaudii*, which had ACC deaminase activity, increased fresh-dry weight of tomato seedlings at a great deal when grown in with NaCl salt (up to 172 mM). These bacteria decreased the ethylene production in tomato seedlings, and this situation would be stimulated if the seedlings were subjected to increased saline conditions. On the other hand, the sodium level in the plant could not be reduced, and phosphorus and potassium intake was increased. This situation may have enhanced the activation of the events that helped the relief of the side effects of the salt on the growth of the plants. In addition, these bacteria increased the water-use efficiency (WUE) under saline conditions. They also aided in relieving salt suppression of photosynthesis. According to Saravanakumar and Samiyappan [106], *Pseudomonas fluorescens* strain TDK1 that had ACC deaminase activity increased saline resistance of the groundnut plants. The strain also increased the yield when compared with *Pseudomonas* strain inoculation that lacked ACC deaminase activity. Glick et al. [107] verified that ACC deaminase bacteria provided plants with salt tolerance because they lower the salt-induced stress ethylene synthesis and enhance canola growth under saline conditions. We also saw similar results in maize under saline stress as a reaction to the inoculation with ACC deaminase PGPR. The results of research on the physiological effects of some vegetable species related to the benefits of PGPR in salt stress conditions are presented in **Table 1**.

3.1.2. Inducing salinity stress tolerance through inoculation of mycorrhizae

The symbiosis of AM has increased the resilience of the host plants to saline stress, maybe with bigger consistency than to drought stress. Compared to uninoculated controls, growth in saline soils was increased by the inoculation with *Glomus* spp., and with AM plants that had increased phosphate and decreased Na^+ concentrations in shoots [112, 113]. AM colonization in maize enhanced the salt resistance [114], and in mung bean [115] and in clover [116]. The AM influence had a correlation with enhanced osmoregulation/accumulation of proline. The inoculation of AM also enhanced NaCl resistance in tomato with extent of enhancement

References	Used plant growth promoting rhizobacteria (PGPR)	Vegetable species	Stress factor	Result
[108]	N-52/1, N-17/3, FE-43, F-21/3, 637 Ca, MfdCa1	Cucumber	Salinity	FE-43 increased yield 11%.
[109]	*Azotobacter* spp., *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus polymyxa*	Carrot	Salinity	*Azotobacter* spp. significantly increased phenolic content, antioxidant activity, total sugar and soluble solid content.
[110]	*Agrobacterium rubi (strain A16), Burkholderia gladii (strain BA7), Pseudomonas putida (strain BA8), Bacillus subtilus (strain OSU142) Bacillus megatorium (strain M3)*	Mint	Salinity	Root length was observed better in the cuttings were treated with BA7, A16 and M3 compared to the other treatments. Mint cuttings inoculated with M3 had more dry matter content than control and the other treatments.
[111]	N 52/1, N 17/3, Fe 43, F 21/3, 637 Ca	Pepper	Salinity	637 C and N 17/3 in bacteria have demonstrated positive results in practice. Both increased yield, nutrient element uptake and stem diameter.

Table 1. Summary of reported physiologic effects of plant growth promoting rhizobacteria (PGPR) under salinity stress conditions on different vegetables.

regarding the saline sensitivity of the cultivar [117]. AM enhancement of saline resistance was generally related with AM-related increase in P acquisition and plant growth in cucumber [118]. *Gigaspora margarita* colonization enhanced stomatal conductance in sorghum in drought stress in saline soils and also improved the survival dual-stress rates. Evelin et al. [19] investigated whether tomato ("Zhongzha" 105) with *F. mosseae* could increase its salt tolerance. They reported that mycorrhization facilitated salt-related reduction of growth and fruit yield, and also determined that the P and K concentrations were higher and Na concentration was lower in AMF in non-AMF tomato in 0, 50, and 100 mM NaCl. They also claimed that an improvement of the ROS-scavenging enzymes (such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX)) in leaves of salt-affected and control treatment accompanied AMF colonization.

Compared to non-mycorrhizae plants, the bigger antioxidant enzyme activity in plants inoculated with AMF was related with the lower lipid peroxidation accumulation, which indicates lower oxidative harm in the mycorrhized plants. In a similar manner, Habibzadeh et al. [119] reported that enhancement in tolerance to saline stress ("Behta" and "Piazar") of the tomato inoculated with *R. intraradices* was associated with a higher P, K, and Ca intake and with lower Na toxicity. The net photosynthesis enhanced mycorrhization through increasing stomatal conductance and protecting PSII [120]. It was claimed that the increased sink strength of AMF roots was the reason for the mycorrhizae promotion of stomatal conductance [121]. Furthermore, in [122], it was reported that the P, Cu, Fe and Zn accumulation was high in inoculated (*F. mosseae*) than in non-inoculated tomato plants in control and medium salinity groups. However, the Na concentration in the shoot was low in mycorrhized plants, which confirms that the tolerance of the plant to salt stress is enhanced by AMF colonization. Authors [123–125] reported that mycorrhizae pepper ("11B 14" and "California Wonder

300"), inoculated with *Rhizophagus clarum* and *R. intraradices*, had bigger biomass in shoots at different saline concentrations when compared to non-inoculated plants. In non-mycorrhizae plants, the lowest crop performance was reported to be associated with higher Na and lower N, P, K concentrations in leaf tissue and also with high leaf electrolyte leakage, but the effect of the saline stress on pepper shoot biomass varies among different fungi species at a significant level [126]. Cheng et al. [127] reported that inoculation with AMF (*R. intraradices*) might help to beat saline stress in zucchini-squash (*Cucurbita pepo* L. "Tempra"), which is a significant greenhouse vegetable. Enhanced nutrition (higher K and lower Na concentrations in leaf tissue) and the leaf water status might have helped plants to translocate minerals and assimilate to the sink, and alleviate the effects of saline stress on fruit production [128]. It was reported that onion (*Allium cepa* L.) and basil (*Ocimum basilicum* L.) inoculated with AMF could relieve deleterious influences of soil/water saline stress on the yield and growth of crop [129, 130]. About the leafy vegetables, in [131], it was reported that the DAOM 197198 isolate of *R. intraradices* might be accepted as a potential AMF candidate since it stimulated the growth of lettuce under two different saline concentrations. This influence was considered to be linked with higher leaf relative water content and lower ABA in roots, which show that AMF plants are less strained than nonmycorrhizal plants by saline conditions, which enables them to accumulate less ABA. Furthermore, in saline conditions, AM symbiosis improved the LsPIP1 expression, which involved in the transcellular water-flow regulation. A gene expression of this magnitude might contribute to regulate the root-water permeability to tolerate the osmotic stress caused by saline conditions better [132]. Hildebrandt et al. [133] reported in their study that AMF *R. irregularis* alleviated the deleterious influences of saline stress in lettuce ("Romana") by changing the hormonal profiles (higher strigolactone production) and affecting plant physiology in a positive manner, which allows lettuce to grow better under harsh conditions. Gadkar and Rillig [134] reported that AMF (*G. iranicum* var. tenuihypharum sp. nova) could alleviate the negative influence of irrigation with high saline water on physiological parameters (photosynthesis and stomatal conductance) in lettuce. The results of research on the physiological effects of some vegetable species related to the benefits of mycorrhizae in salt stress conditions are presented in **Table 2**.

3.2. Drought

Climate change is defined as the changes observed over many years in the average state of the climate regardless of its cause. Today's climate change depends on the greenhouse effect of gases released to the atmosphere due to fossil fuels, improper land use, deforestation, and industrial development, but it is not caused by natural factors, as it has been since the formation of the world. The primary effect of this change, in which the direct human factor plays a role, is the increase in mean surface temperatures, in other words global warming. Modeling efforts to understand global climate change predicts that the average global warming will increase by 1–3.5°C by 2100 and that there will be regional extreme temperatures, floods, and widespread and severe droughts all over the world. Drought is related to the amount of water that can be taken by the roots during the growth period of the plant which is added to the field rather than the total amount of rainfall that occurs throughout the year. Plants that are experiencing water deficiency during the growing period face with significant losses in terms of development and especially yield [143, 144]. Measures should be taken as soon as possible

Reference type	Used mycorrhizae species	Vegetable species	Stress factor	Result
[135]	*Glomus clarum*	Pepper	Salinity	Activity of catalase (CAT), glutathione reductase (POD), and ascorbate peroxidase (APX) in leaves of plants treated with mycorrhizae increased. Leaf water potential and osmotic potential has increased. Pepper plants inoculated with mycorrhizal fungi showed the highest chlorophyll content and leaf area in saline conditions. The interaction between mycorrhizal fungi and plants occur higher photosynthesis activities and transpiration rates pursuing with stomatal conductivity.
[136]	*Glomus deserticola*	Spinach	Salinity	*Glomus deserticola* increases K/Na ratio up to 54%.
[137]	*Glomus fasciculatum*	Tomato		MDX levels have increased in plants treated with *G. fasciculatum*.
[138]	*Glomus occultum*	Pepper		Increase in hormone levels of pepper plants with *G. occultum*
[139]	*Glomus fasciculatum*	Cucumber		*G. fasciculatum* caused important changes in the plant enzyme levels.
[140]	*Glomus mosseae*	Pepper		*G. mosseae* significantly increased yield and nutrient element uptake according to control.
[141]	*Glomus mosseae*	Radish		Caused important changes in the plant enzyme levels.
[142]	*Glomus mosseae*	Mint		More ACC deaminase has been detected in plants treated with *G. mosseae*.

Table 2. Summary of reported physiologic effects of mycorrhizae under salinity stress conditions on different vegetables.

to mitigate the effects of agricultural drought, since the available water resources are limited and the occupancy rate of these reserves is predicted to decrease rapidly due to the global warming-related rainfall and especially the decrease in the amount of snowfall that feeds groundwater resources. Although plant varieties belong to the same species, they may differ in their tolerance to drought.

Plants can adapt their growth and development mechanisms in such a way that they are least likely to be affected from environmental changes, and even adapt to environmental conditions when they grow in the same climatic conditions for long periods of time. Drought is one of the abiotic stress conditions which mostly affects the growth and development of plants [145]. Water constitutes 50% of the fresh weight of the trees and 89–90% of the other plants [146]. Plant growth is affected considerably in arid conditions. This effect in growth depends on the length of time the water stress is experienced. In the early stages of arid conditions, the plant slows elongation and triggers root development to reach more water. On the other hand, if arid conditions last long enough to cause damage to the plant, both stem and root growth will stop, leaf area and number of leaves will decrease, and even some leaves turn yellow. The decline in plant growth is due to the division of cells in the shoot and root meristems and the arrest of expansion of the cells. The disruption of cell division or enlargement is directly related to the decrease in the rate of photosynthesis due to water insufficiency [147]. When

the plants are exposed to drought stress, the water balance between the tissues is disturbed. In case of stress, cell growth is negatively affected by the loss of turgor, so the cells remain small. The decrease in cell growth also affects the synthesis of the cell wall. While protein and chlorophyll are adversely affected, it is observed that the seeds lose their germination ability [148–150]. Photosynthesis and respiration slow down and stop. Decrease in cell growth causes the leaves to shrink and the production of photosynthesis to decrease further [151]. Water deficiency causes the formation of various reactive oxygen derivatives (ROD) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH) and superoxide radical (O_2^-) [152]. ROD damages membrane lipids, nucleic acids, proteins, chlorophyll, and macromolecules in the cell. The effect of free oxygen radicals on the cell membrane depends on lipid peroxidation. Lipid peroxidation, which leads to cell membrane destruction, produces malondialdehyde (MDA) as a result of several reaction steps. Drought stress also has an important effect on enzyme activity and enzyme amount in plants. In addition, the amount of abscisic acid is 40 times higher in the leaves, while in other organs including the root, this increase is less. Abscisic acid prevents the transpiration of water by closing the stomata [153].

3.2.1. Inducing drought stress tolerance through inoculation of PGPR

Drought affects almost every climatic region in the world and more than half of it is prone to drought each year. Drought limits the growth and the production of crops as one of the most important stresses. The response to drought by plants is at cellular and molecular level. Drought stimulates the ethylene production in the tissues of plants as it is the case in some other environmental factors and also causes abnormal growth in plants. According to [154], ACC deaminase PGPR *Achromobacter piechaudii* ARV8 increases the fresh-dry weights in tomato and pepper seedlings at a great deal under transient water stress. Also, these bacteria decreased the ethylene production in tomato seedlings under water stress. In water stress, the bacteria had no effects on the water content of plants, and enhanced the recovery of plants if irrigation was started again. It is interesting that when bacteria were given to the tomato plants, the plant growth continued under water stress and also when irrigation was started again. Giri et al. [155] investigated the physiological response of peas (*Pisum sativum* L.) to inoculation with ACC deaminase bacteria *Variovorax paradoxus* 5C-2 in moisture stress and watering conditions. Bacterial effects were more obvious and consistent in controlled soil drying process (moisture stress conditions). In trials that had short time periods, it was seen that ACC deaminase bacteria had positive influences on root-shoot biomass, leaf area, and plant transpiration. In trials that had long time periods, it was seen that the plants that were inoculated with ACC deaminase bacteria produced more seed yields (25–41%), seed numbers, and seed nitrogen accumulations than the plants that were uninoculated. In addition to these, the inoculation caused that the nodulation in pea plants under drought was restored to uninoculated plant levels that were well-watered. In recent years, similar results were reported. According to the recent reports, the inoculation with ACC deaminase bacteria eliminated the influences of water stress on growth, yield, and ripening of *Pisum sativum* L.—although partly—pot and field experiments. The results of the physiological effects of some studies related to the benefits of PGPRs on vegetables in drought stress are given in **Table 3**.

References	Used plant growth promoting rhizobacteria (PGPR)	Vegetable species	Stress factor	Result
[156]	52/1 and E43, 21/3F, 17/3 N, E43 F, 637Ca, MFD Ca1, 52/1, 21/3 + 637Ca, 52/1 Zeatin	Tomato	Drought	21/3F, 21/3 + 637 Ca and 17/3 N bacteria races applications had positive effects on yield and yield components of tomato.
[157]	*Agrobacterium rubi, Pseudomonas putida, Pseudomonas fluorescens, Pantoea agglomerans, Bacillus subtilis, Bacillus megaterium*	Garlic	Drought	*Bacillus subtilis* caused important changes in the plant enzyme levels.
[158]	*Bacillus megaterium* TV-3D, *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK- 92 and *Bacillus megaterium* KBA-10	Broccoli	Drought	PGPR treatments increased seedling length, stem diameter, leaf area, and leaf dry matter at ratios of 7.85%, 42.56%, 18.12% and 41.98%, respectively, compared to the control. Except for Na, the mineral element content was also increased with PGPR treatments.
[159]	*Bacillus megaterium var. phosphaticum*	Tomato	Drought	Plant growth, total and marketable yield increased by *Bacillus megaterium var. phosphaticum*.

Table 3. Summary of reported physiologic effects of plant growth promoting rhizobacteria (PGPR) under drought stress conditions on different vegetables.

3.2.2. Drought stress tolerance through mycorrhizae

Arbuscular mycorrhizae (AM) symbiosis is associated with enhancing the resistance to water and drought stress despite the change of plant physiology and the expression of plant genes [120, 160]. It was reported in previous studies that AM-related increase in drought tolerance involved increased dehydration and dehydration tolerance [161]. AM fungi inoculation was able to reduce the leaf content of malondialdehyde and soluble protein and improve the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which resulted in enhanced osmotic adjustment and drought tolerance of mycorrhizae citrus-grafting seedlings [162]. Inoculation of *Glomus versiforme* in citrus plants enhanced the osmotic adjustment of the plant in drought stress via improved levels of non-structural carbohydrates, $K(^{+})$, $Ca(^{+})$, and $Mg(^{2+})$, which resulted in improvement of drought tolerance [163].

It was reported that the role of abscisic acid (ABA) was behind the AM-related stress response in plants [164]. When exogenous ABA was added, the ABA content was improved in shoots of non-AM plants, concomitant with the expression of the stress marker genes Lsp5cs and Ls1ea and the gene Lsnced. However, when exogenous ABA was added, the ABA content in AM shoots decreased, and this addition did not cause more improvement of the expression. Co-inoculation of lettuce with PGPR *Pseudomonas mendocina* and *G. intraradices* or *G. mosseae* improved an antioxidative catalase in serious drought, which shows that they might be used in inoculants to relieve the oxidative harm [165]. A 14-3-3 protein encoding gene from *Glomus intraradices* growing in vitro and subjected to drought stress was identified [166]. The role of these proteins regulating the signaling pathways and effector proteins was claimed to impart

the protection to the host plants against drought stress. Glutathione and ascorbate have a significant effect in conferring the protection and maintaining metabolic function of plants in water deficit conditions.

AMF are known to have an efficacious and sustainable mechanism. With this mechanism, tolerance to drought is enhanced in vegetables [167, 168]. AMF cause changes in the roots of plants, especially in length, density, diameter, and number of lateral roots [169]. Improved root structure in mycorrhizae plants allows the extraradical hyphae to extend beyond depletion zones of plant rhizosphere, which makes the water and low-mobile nutrient intake (P, Zn, and Cu) more efficiently under water stress [170].

The AM symbiosis effectiveness in improving drought tolerance was also investigated in vegetables. Open-field tomato (*Solanum lycopersicum* L.) inoculated with AMF (*R. intraradices*) influenced the agronomical and physiological responses of exposure in different drought intensities [171]. Compared to non-inoculated ones, the fruit yield of inoculated plants in severe-moderate-mild drought stresses was high at a statistically significant level by 25, 23, and 16%, respectively. It was reported in this study that high crop performance in inoculated plants was associated with better nutritional status (higher N and P) in connection with the maintenance of leaf water status. Ikiz et al. [172] confirmed this effect on tomato. They showed that the colonization of processing tomato "Regal 87-5" plants by *F. mosseae* and *G. versiforme* might increase marketable yield by 20% and 32%, respectively, when compared with those of non-inoculated plants under mild-heavy drought stress. Greenhouse melon (*Cucumis melo* L. "Zhongmi 3") plants (inoculated with three *Glomus* species: *G. versiforme* and *R. intraradices* and, especially, *F. mosseae*) showed higher tolerance to drought stress than non-inoculated plants. This situation was determined in plant heights, root lengths, biomass production, and net photosynthetic rates [173]. They claimed that the increase in drought tolerance and better crop performance might be associated with the antioxidant enzyme production (SOD, POD, and CAT) and the soluble sugar accumulation by AM symbiosis. Lucy et al. [174] examined the mechanisms which affected the relief of drought by a mixture of *Glomus* spp. from Mexico ZAC-19 (*G. albidium*, *G. claroides*, and *G. diaphanum*) in Chile ancho pepper (*C. annuum* L. San Luis). They reported that ZAC-19 had the potential to be incorporated into Chile pepper transplant systems to relieve the harmful effect of drought in open-field production in Mexico, which was shown by high root-to-shoot rate and leaf water potential. In a similar manner, in [175] it was reported that drought enhanced bigger extraradical hyphae development of *G. deserticola* in bell pepper, and as a result, a high water intake, when compared to non-mycorrhizae plants. It was also reported that AMF symbiosis enhanced lettuce (*Lactuca sativa* L. "Romana") tolerance to drought and recovery. This enhancement was achieved via the modification of the plant physiology and the expression of plants genes [176, 177]. Lettuce, which was inoculated with the AMF *R. intraradices*, gave high root hydraulic conductivity and low transpiration in drought, when it was compared with non-inoculated plants. Authors [178, 179] also emphasized that the plants inoculated with AMF could regulate their abscisic acid (ABA) concentrations in a better and quicker manner than non-inoculated plants, which allows a better balance between leaf transpiration-root water movement in drought stress and recovery [180, 181]. It was reported that inoculation with AMF enhanced WUE in watermelon [182], which shows that AMF improved water intake and resulted in the host plant making

Reference type	Used mycorrhizae species	Vegetable species	Stress factor	Result
[185]	*Glomus mosseae*	Muskmelon	Drought	K/Na ratio has increased in several plant tissues.
[186]	*Glomus mosseae*	Watermelon		Water use efficiency, Leaf water content and leaf osmotic potential has increased.
[187, 188]	*Glomus mosseae*	Lettuce		Endogenous auxin and cytokinin levels are increased in the presence of *G. mosseae*.
[189]	*Glomus occultum*	Cabbage		Yield and quality increased with mycorrhizae.
[190]	*Glomus fasciculatum*	Lettuce		L-arabinose (L Ara), ribose (Rib); D-xylose (D Xyl), L-xylose (L Xyl), adonitol (Ado), beta-methyl-D-xyloside (Mdx) levels increased.
[191]	*Glomus mosseae*	Aubergine		Water use efficiency, Leaf water content and leaf osmotic potential has increased.
[192]	*Glomus caledonium*	Pepper		Activity of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) in leaves of plants treated with *Glomus* increased.
[193]	*Glomus mosseae*	Melon		Water-use efficiency, leaf water content, and leaf osmotic potential has increased.

Table 4. Summary of reported physiologic effects of mycorrhizae under drought stress conditions on different vegetables.

use of water in a more efficacious manner [183]. This was associated with the mechanisms that could increase transpiration and stomatal conductance [184], and also improve the availability of the nutrients [183]. The results of the physiological effects of some studies related to the benefits of mycorrhizae on vegetables in drought stress are given in **Table 4**.

4. Conclusion

Today, the utilization of natural resources in agriculture comes to the forefront because of improving environmental awareness. The evaluation of the use of natural resources, such as mycorrhiza and a cleaner environment, is important both for economic reasons. Resources are often used as a source of plant nutrition in hydroponics. Given the chemical, the use of mycorrhiza in agriculture is very important in soil. Particularly with the use of mycorrhiza, the use of chemical fertilizers especially consisting phosphorus, can be reduced. As a conclusion, mycorrhizae are important for the growth of agricultural crops as well as healthy ecosystem functions. Many benefits of mycorrhizal symbiosis can be enhanced by changing agricultural practices which may decrease colonization and mycorrhizal abundance [194].

Hydraheaded stress caused by biotic and abiotic reasons is threatening modern agriculture. Several stress types explained in this chapter emphasize ethylene biosynthesis, which prevents plant growth by some tools at molecular level. In this chapter, for the purpose of regulating

the plant ethylene, application of PGPR with ACC deaminase is crucial. Several roles of PGPR in saline conditions, in drought, waterlogging, biocontrol, temperature and nutritional stresses and in cut-flower industry and nodulation in legumes were not investigated in detail by researchers. In commercial terms, applying PGPR with ACC deaminase in agriculture may be useful. It may also be an important progress to obtain sustainable crop production and conservation. Because of several drawbacks, genetic modification of plant species is not probable (for example, proprietary rights, trade agreements among countries for genetically modified (GM) crops, and due to the limitations in DNA recombinant technology in some areas in the world). Because of all these reasons, using PGPR with ACC deaminase activity and similar innovations may be a cost-effective and environment-friendly way for sustainable agriculture.

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Chapter 7

High-Voltage Methods for Mushroom Fruit-Body Developments

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Additional information is available at the end of the chapter

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Abstract

High-voltage electrical stimulation is effective for promotion of fruit-body development in mushroom cultivation. The high voltage applying to cultivation bed of mushroom generates intense electric field inside the bed substrate. The intense electric field accelerates the hypha move owing to the electrostatic force. As a result, some parts of hyphae are cut and scratched. The cutting and scratching of hypha work as stimulation for promotion of the fruit-body development. The promotion effect of high-voltage stimulation to sawdust-based substrate of *L.* and natural logs hosting *Lentinula edodes*, *Pholiota microspora* and *Hypholoma lateritium* are confirmed through the experiment in the cultivation field. The fruit-body formation of mushrooms increases 1.3–2.0 times in terms of the total weight. The accumulated yield of *L. edodes* for four cultivation seasons is improved from 160 to 320 g by applying high voltage of 50 or 100 kV. However, the yield decreases from 320 to 240 g upon increasing applied voltage from 100 to 130 kV. The yield of the other types of mushrooms shows tendencies similar to those of *L. edodes* by applying high voltage. An optimal voltage exists for efficient fruiting body induction.

Keywords: fruit-body development, mushroom cultivation, high-voltage methods, electrical stimulation, *L. edodes*, *Pholiota microspora*, *Hypholoma lateritium*

1. Introduction

Mushrooms such as *Agaricus bisporus* (white button mushroom), *Pleurotus ostreatus* (oyster mushroom), *L. edodes* (shiitake mushroom), *Flammulina velutipes* (enokitake or winter mushroom) are globally cultivated for fresh food or dried food. Some other mushrooms such as

Ganoderma lucidum are cultivated for special medicinal mushroom. Mushroom is fruiting body mainly in basidiomycetous fungi and some ascomycetous fungi. Therefore, mushrooms are developed for spore formation at reproductive growth phase. Mushroom farming is mainly based on two methods: log-grown or fungus bed-culture which using a pot fill with sawdust-based substrate. The later method offers controllable conditions so that effective mushroom growth can be expected. Biological efficiency has been improved by optimizing various factors, such as substrate formula, strain type, culture maturity, water condition and other environmental conditions of the cultivation room.

Physical phenomena in cells caused by external pulsed electromagnetic energy have a variety of applications on biotechnologies [1]. The electrical stimulation can either destroy the cells and plants or promote its growth rate, depending on the degree of stimulation. In nature, mushrooms extraordinary grow-up around a hit point of a lightning have been reported by some mushroom farmers. Early studies of mushroom growth promotion by artificial lightning were carried out on edible mushroom cultivation using an impulse generator [2]. The output voltage of the impulse generator was more than 500 kV. After that, the high-voltage pulsed power supplies were designed to generate an output voltage from 50 to 130 kV for the electrical stimulation on mushroom cultivation bed. The promotion effects of high-voltage stimulation on sawdust-based substrate of *L. decastes* and natural logs hosting *L. edodes*, *Pholiota microspora* and *Hypholoma lateritium* were evaluated using the developed compact pulsed power generator [3]. Typical stimulation effects are shown in **Figure 1** as a photograph of cultured *L. edodes* taken on the same day. The upper bed-log was used in cultivation without the high-voltage stimulation. The lower bed-log was used in cultivation and a 50 kV voltage was applied 50 times as stimulation. *L. edodes* in the stimulated log grew faster than that in the bed-log without stimulation. The high-voltage electrode is located on the left side of the log. The fruiting bodies mainly grow near the high-voltage electrode. In this chapter, the effect of high-voltage electrical stimulation on induction of fruiting body of mushroom is described.

Figure 1. Typical photograph of the cultured *L. edodes* with (**bottom**) and without (**top**) electrical stimulation.

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2. Mushroom cultivation and stimulation for fruiting body development

Mushroom is fruiting body mainly in basidiomycetous fungi and some ascomycetous fungi. Therefore, mushrooms are developed for spore formation. Multiple environmental factors such as light, temperature, nutrient, gaseous components influence fruiting body induction and development. These environmental factors are used for sensing appropriate conditions for spore formation and dispersal.

Condition for fruiting body induction is one of critical factor for mushroom cultivation. To establish high yield cultivation method, it is very important to understand effects of environmental factors for fruiting body induction. Environmental factors for fruiting body induction are classified into physiological and physical factors. Gaseous condition and nutrient, or hormones are classified as physiological factor, and wounding or striking as physical factors. Light is one of the important factors for fruiting body induction, and blue light is the effective wavelength. For example, light promotes fruiting body induction in *L. edodes*. In contrast, some species can induce fruiting body in complete darkness. Therefore, light can promote fruiting body development in some species, but not really necessary. Temperature is one of the critical factors for fruiting body induction in basidiomycetes. Especially, down shift of temperature stimulates fruiting body induction in many mushroom species. For example, fruiting body of *F. velutipes* can induce temperature down shift (e.g. 23 → 16°C) in complete darkness. Interestingly, fruiting body formed in complete darkness has tiny cap on its head [4]. It is revealed that proteins expressed specifically during fruiting body formation are regulated by temperature but not by light in *F. velutipes*. Nutrient is another critical factor for fruiting body induction. Especially, high concentration of carbon and nitrogen sources inhibits fruiting body induction. Wood decay fungi are major species for commercially cultivating mushrooms, therefore, wood decay is closely related to fruiting body induction.

Wounding or striking are used for commercial cultivation in several mushroom species. For example, scrapping mycelia on surface of the media (so called Kinkaki in Japanese) is used for fruiting body induction in several mushrooms. Striking log wood is used for stimulation of fruiting body induction especially in *L. edodes*. Electrical stimulation is also a physical factor for fruiting body induction similar to Kinkaki or striking. Japanese farmers have their elders' wisdom that lightning comes crashing into the ground provokes a plentiful mushroom harvest. Electrical stimulation used for stimulating fruiting body induction by mimicking the effect of lightning in nature.

3. History of electrical stimulation for mushroom fruiting body development

The application of a pulsed high voltage to improve the yield in edible mushroom cultivation has also been attempted by some research groups. The fruiting capacity of shiitake

mushroom (*L. edodes*) was remarkably promoted by applying a high voltage to cultivation bed-log (wood) [3]. This effect was also recognized in *L. edodes* fruiting on a mature sawdust substrate [5, 6]. The fruiting body (sporocarp) yield in the electrically stimulated substrate was observed to be 1.7 times more than that without the electrical stimulation [6]. This effect was also confirmed in the fruiting body development of edible mushrooms: *Grifola frondosa*, *P. microspora*, *F. velutipes*, *Hypsizygus marmoreus*, *P. ostreatus*, *P. eryngii*, *P. abalones* and *Agrocybe cylindracea* [7, 8]. The fruiting body yield in the electrically stimulated substrate was observed to be 130–180% greater than that without the electrical stimulation [7]. The high-voltage stimulation technique was also applied to ectomycorrhizal fungi such as *Laccaria laccata* and *Tricholoma matsutake* [9, 10].

Many types of electrical power supplies have been employed to provide electrical stimulation. A large scale 1 MV high-voltage impulse generator was used to stimulate *L. edodes* log wood [2]. High-voltage AC was used to stimulate an *L. edodes* sawdust substrate [5]. Inductive energy storage (IES) pulsed power generators have favorable features for mushroom-cultivating applications, for example, they are compact, cost effective, light, and have high-voltage amplification compared with capacitive energy storage generators such as the impulse generator [11]. The yield of *L. edodes* fruiting bodies was improved with high-voltage stimulation generated by the IES pulsed power generators. The effect of the pulsed voltage stimulation on some other types of mushroom such as *P. microspora* and *L. decastes* was also confirmed using an IES generator developed for the improvement of yield of mushroom production [8]. The harvested weight from log wood and/or sawdust substrates for mushroom cultivation was increased by applying a pulsed voltage as an electrical stimulation.

The mechanism driving the increase in the fruiting body formation by applying high voltage is not clear, but researchers have suggested two possible explanations. One is that the mushroom hyphae are ruptured by applying a high voltage. Physical damage to the hypha stimulates fruiting body formation in mushrooms [5, 7]. The other explanation involves the activation of enzymes. Some enzymes are activated by applying a high voltage, and consequently, mushroom fruiting bodies develop abundantly [2]. Some effects of the high-voltage stimulation were recognized using microscopic observation and chemical analysis. A scanning electron microscope observation indicated that the synthesis of crump connections was accelerated with electrical stimulation [2, 5]. Some types of enzymes, including *laccase* and *protease*, were activated by the electrical stimulation [3, 5, 9].

4. Laboratory test using impulse generator

Early stage of the study on mushroom fruiting promotion and large scale impulse generators was used as artificial lightning for stimulation on the mushroom fruiting promotion. In this section, the laboratory test of artificial lightning stimulation for fruiting body induction using impulse voltage is described.

Figure 2 shows typical photograph of an impulse generator [12]. The impulse generator consists of 10–20 capacitors, gap switches and damping resistors [13]. The capacitors are connected

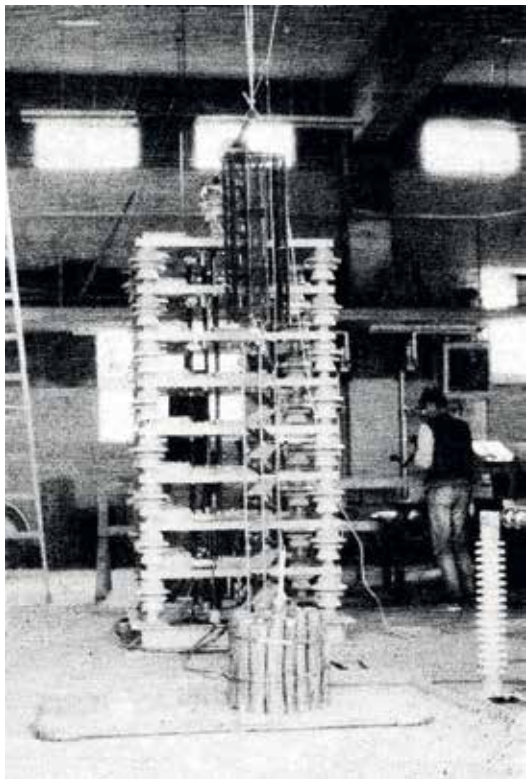

Figure 2. Photograph of impulse generator at stimulation on shiitake mushroom cultivation bed-log [12].

in parallel at charging phase. After charging up the capacitors, the connection of the capacitors is changed from parallel to series using the gap switches. As a result, the output voltage is multiplied by changing the connection of the capacitors. Typical output voltage is in range from 250 kV to 1 MV. The rise time of the output voltage is controlled around the microsecond-order as an artificial lightning stroke voltage. The example of the applied voltage to the bet-log is shown in **Figure 3** [2]. The peak voltage of 288 kV is generated by operating the impulse generator. The rise time of the voltage is close to 0.5 μs as shown in **Figure 3**. In experiments, the bed-logs are connected to high-voltage electrode as shown in **Figure 2**. The bed-logs (Konara oak; *Quercus serrata*) have dimension of 1 m length. The bed-logs 5–9 are bundled or connected in parallel as shown in **Figure 4** for the high-voltage stimulation by impulse generator. The impulse high voltages are applied to the bed-logs bundle or top of the bed-logs connected in parallel. After the stimulation, the bed-logs are cultivated for fruiting body formation. The yielding rates of the fruiting bodies on the bed-logs are monitored for each stimulation condition.

Typical results of the stimulation on yielding rate of *L. edodes* fruiting bodies are shown in **Tables 1** and **2** for various amplitudes of applied voltage. The numbers of the bed-logs are 24 and 21 for each experimental condition. The number of fruiting body formation and total harvested yield increase by stimulating high voltage. In both cases, the fruiting body

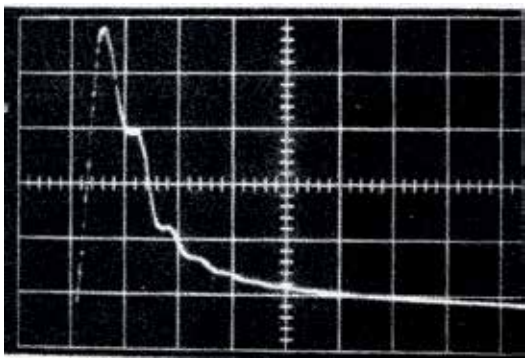

Figure 3. 288 kV output voltage of an impulse generator [2]. X: Time (1 μs/div.), Y: Voltage (50 kV/div.).

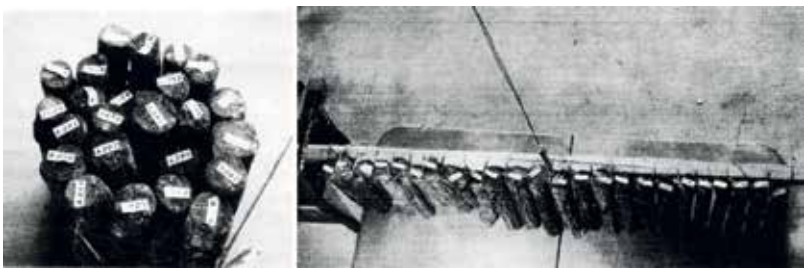

Figure 4. Photographs of setup of bed-logs for impulse high-voltage stimulation [12].

Exp. group.	Number of exp. bed-logs	Fruit-body yield (per 1 m^3 of wood)	Dry wt (g)
		Number	
144 kV	2 4	505.3	1337.0
288 kV	2 4	770.1	2171.4
576 kV	2 4	121.6	558.4
Contd.	2 4	16.9	55.2

Bed-log age: 38 months after inoculation (Yakult haru 2). Water content of bed-logs: 38.9% (mean value of six samples). All exp. groups had 34 mm rainfall in a week after discharge.

Table 1. Fruit-body yield of *L. edodes* of bed-logs using high-voltage stimulation without submergence treatment [2].

yields increase by applying impulse high voltages as stimulation for fruiting body forming. However, the optimum amplitude of impulse voltage for improving fruiting body yield exists as **Tables 1** and **2**. The fruiting body yield at 288 kV impulse voltage is larger than those at 144 and 576 kV applied voltage as shown in **Table 1**. When an electrical field E is generated by applying impulse high voltage to the bed-logs, hyphae will thus be subjected to a Coulomb force f ($f = qE$; q means total charge of the hypha) from the electrical field. As a result, the

Exp. group.	Number of exp. bed-logs	Fruit-body yield (per 1 m^3 of wood) Number	Dry wt (g)
288 kV	2 1	650.8	2100.0
576 kV	2 1	485.8	1648.9
720 kV	2 1	453.8	1427.4
Cont.	2 1	276.2	840.6

Bed-log age: 38 months after inoculation (Yakult haru 2). Water content of bed-logs: 42.3% (mean value of six samples).

Table 2. Fruit-body yield of *L. edodes* of bed-logs using high-voltage stimulation with submergence treatment [2].

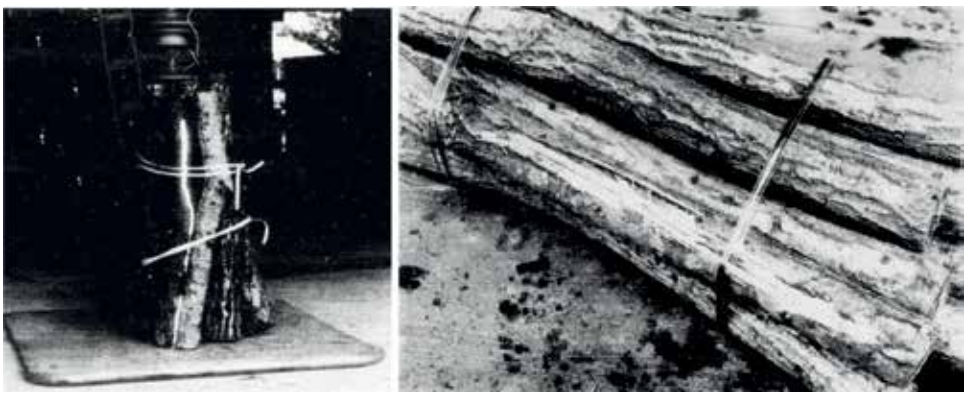

Figure 5. Photographs of electrical discharge on surface of the bed-log and crack of the bed-logs by impulse high-voltage application [12].

hyphae are accelerated towards the positive electrode according to the equation $f = ma$, where m and a mean mass of the hypha and acceleration of the hypha, respectively. The application of electric pulses, resulting in hyphal displacement and sometimes damage, can be considered as a form of physical stress. The physical stress works as trigger to promote the fruiting body formation. However, when the applied voltage is too high compared with the optimum condition, the physical damage of the hypha is too much for stimulation of fruiting body promotion. Sometimes the bed-logs are also damaged by the high pressure wave (shockwave) caused by electrical discharge and impulse high current as shown in **Figure 5** [12].

The frequencies of the fruiting body yield by impulse high-voltage stimulation under same condition with **Table 1** are shown in **Figure 6** [2]. In the control case (without high-voltage stimulation), the fruiting body cannot be harvested for 20 bed-logs (83%). One fruiting body can be harvested from four bed-logs (17%). However, the fruiting bodies can be harvested from 21 bed-logs (except 3 bed-logs; 12%) at 288 kV impulse voltage applying. The decrease of number of the bed-log without *L. edodes* fruiting bodies mainly contributes to increasing yield of mushroom by applying high-voltage shown in **Table 1**.

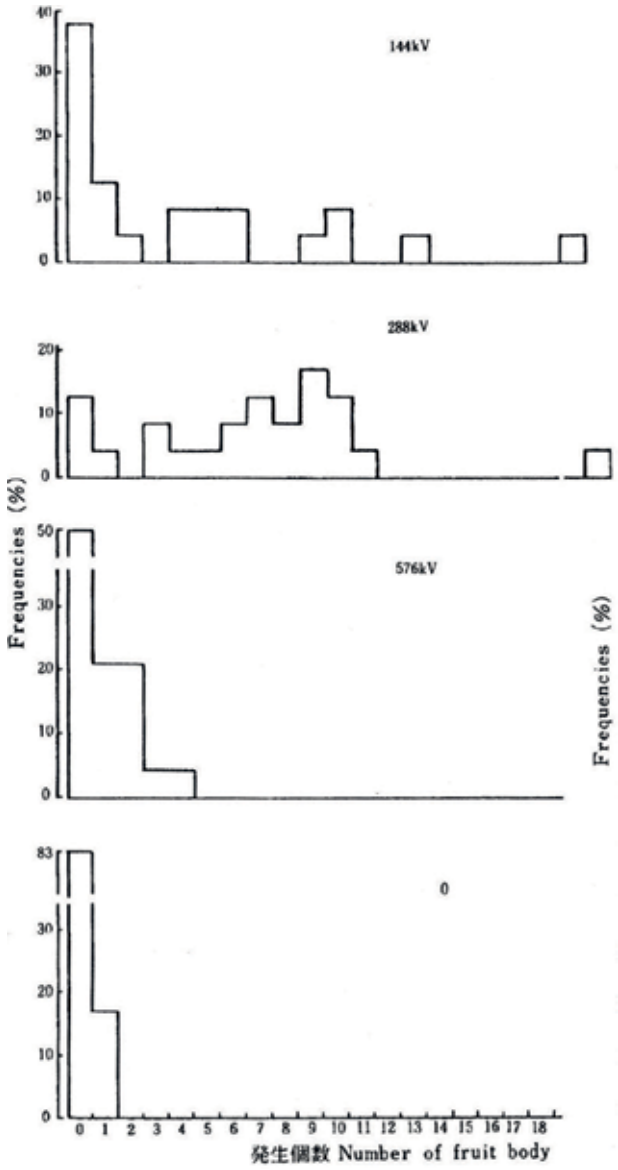

Figure 6. Frequencies of the fruit-body yield by impulse high-voltage stimulation to *L. edodes* of bed-logs without water submerged treatment [2].

5. Field test using compact high-voltage generator

The impulse generator has huge size for utilization in mushroom-cultivating field as shown in **Figure 2**. Some types of compact high-voltage pulse generator were developed for promotion of the fruiting body formation on bed-logs or sawdust bed-blocks (substrate) of mushroom cultivation.

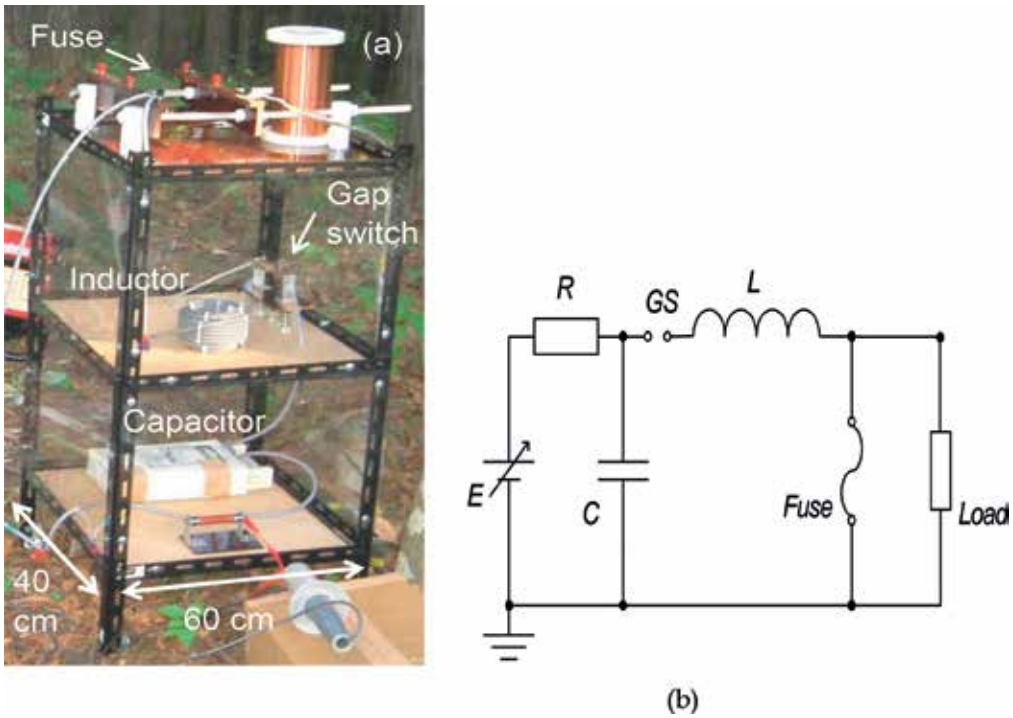

Figure 7. IES pulsed power generator with fuse opening switch; (a) photograph and its circuit. (*C*: Primary energy storage capacitor, *L*: Secondary energy storage inductor) [8].

Figure 7(a) and **(b)** shows photograph and equivalent circuit of a compact pulsed power generator used for promotion of fruit-body formation in natural-log based mushroom cultivation [8]. An inductive energy storage (IES) system consists of a primary energy storage capacitor C, a closing switch GS, a secondary energy storage inductor L and an opening switch. A thin copper fuse is used as the opening switch to interrupt large current in short time. **Figure 8(a)** shows typical circuit current and output voltage waveforms at 12 kV charging voltage. The 8 cm-length fuse and the 15 μH-inductance secondary energy storage inductor are used. The current starts to flow after closing the switch GS with LC oscillation. The circuit current is interrupted after fuse melting phase within 50 ns. The output voltage increases rapidly and has a 120 kV maximum voltage. This output voltage corresponds to 10 times amplification. The high voltage pulse is produced by the total circuit inductance and rapid current interruption produces a high-voltage pulse expressed as

$$v = V_0 - \frac{1}{C}\int i dt - L\frac{di}{dt} \approx -L\frac{di}{dt}, \tag{1}$$

where i means the circuit current. The output voltage waveforms for various charging voltages are shown in **Figure 8(b)**. The peak voltage increases from 80 to 130 kV with increasing charging voltage from 10 to 16 kV. These values correspond to 8.0 and 8.1 of voltage amplification factors.

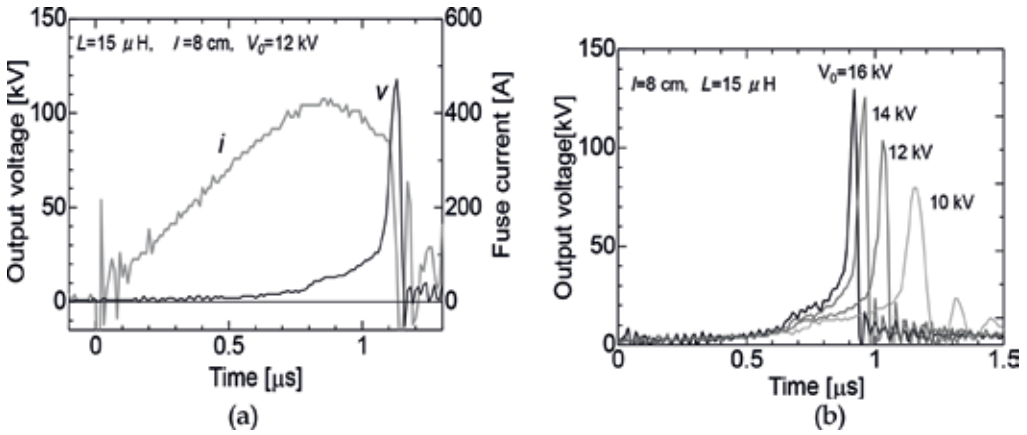

Figure 8. Typical waveforms of (a) circuit current though the fuse and output voltage at 12 kV charging voltage and (b) output voltage for various charging voltages [8].

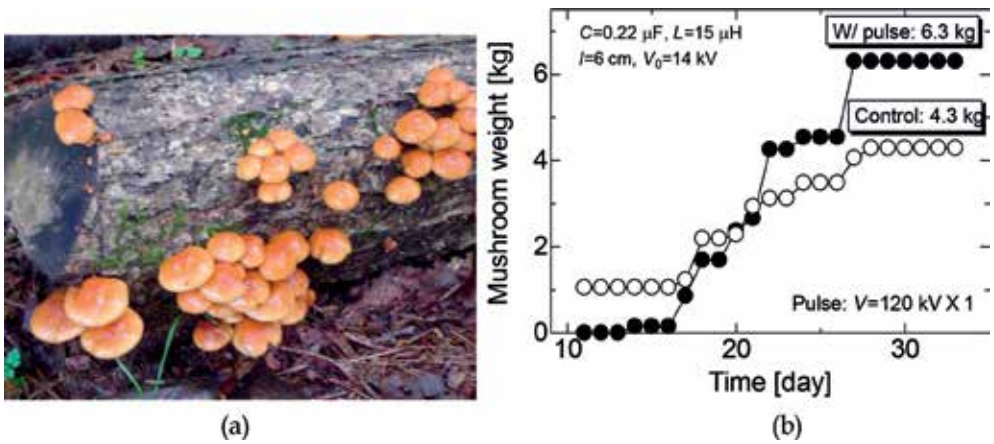

Figure 9. The cultured *P. microspora;* (a) photograph of fruiting-bodies and (b) its total weight yield as a function of days from the stimulation of 120 kV applied voltage [8].

Figure 9(a) shows the total weight of *P. microspora* mushroom cropped by 15 logs as a function of days from the high-voltage stimulation [8]. The logs of applying voltage group are stimulated with the pulsed voltage of 120 kV. The 15 logs of the control group are not stimulated. **Figure 9(b)** shows the photograph of cultured *P. microspora*. The *P. microspora* start to appear about 2 weeks after the stimulation and stop to appear at day 26. The yield of *P. microspora* is improved with the pulse voltage stimulation. The total weight of the cropped *P. microspora* with the high-voltage stimulation is 6.3 kg. This value is 1.5 times larger than 4.3 kg total weight under condition without the stimulation.

Figure 10(a) and **(b)** shows photograph and equivalent circuit of a compact pulsed power generator based on combining IES with Marx circuit to reduce the primary charging voltage [14]. After charging up the four primary energy storage capacitors, the gap switches GS are

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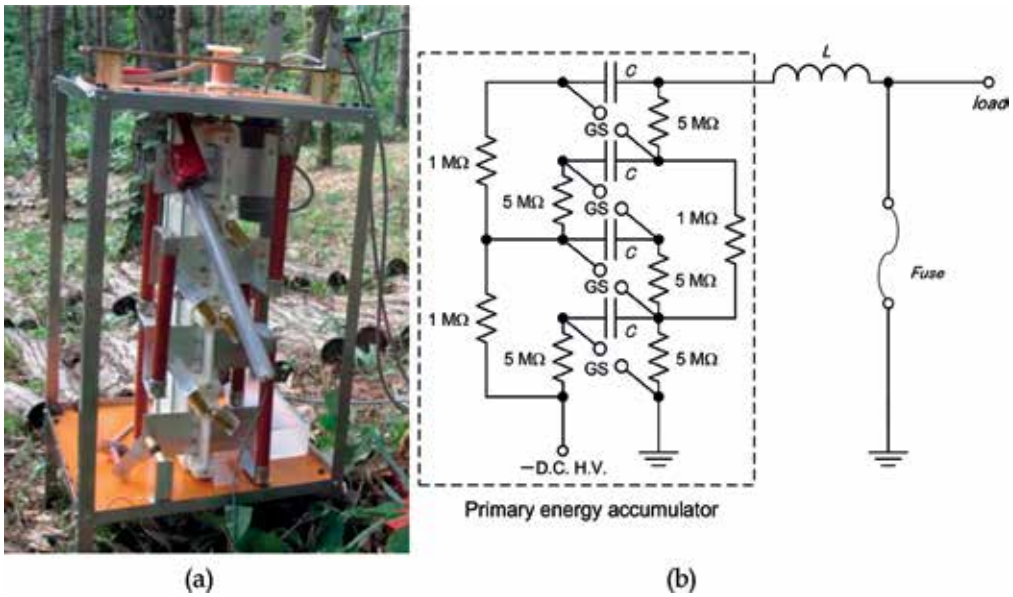

Figure 10. Marx-IES pulsed power generator with fuse opening switch; (a) photograph and (b) its circuit with fuse opening switch. (*C*: Primary energy storage capacitor, *L*: Secondary energy storage inductor) [14].

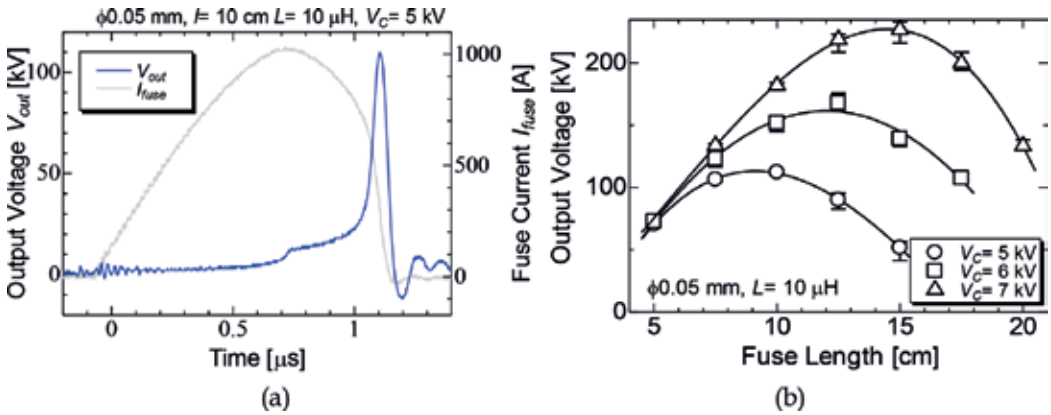

Figure 11. Typical waveforms of (a) circuit current though the fuse and output voltage at 5 kV charging voltage and (b) output voltage as a function of fuse length for various charging voltages of the primary energy storage capacitor [14].

triggered externally. The closing switch GS changes the connection of the capacitors from parallel to series. As a result, the voltage is multiplied from V_C to 4 V_C in same manner to the Marx generator. **Figure 11(a)** and **(b)** shows typical waveforms of the circuit current and output voltage at 5 kV charging voltage and peak voltage as a function of fuse length for various charging voltages of the primary energy storage capacitor, respectively. The circuit current starts to flow after closing the switch GS with LC oscillation. The circuit current is interrupted after fuse melting phase. The output voltage increases rapidly and has a peak voltage of 110 kV. This peak voltage corresponds to 22 amplification defined as the ratio of

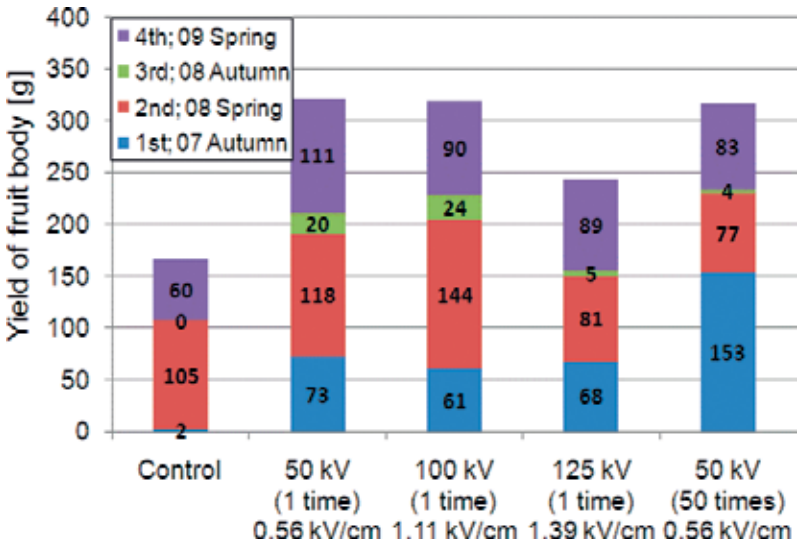

Figure 12. Total weight of cultured *L. edodes* for various electrical stimulation conditions. The total yield are 167, 322, 319, 243 and 317 g for control, 50 kV-1 time, 100 kV-1 time, 125 kV-1 time and 50 kV-50 times, respectively [3].

the maximum output voltage to the charging voltage. The peak voltage increases from 110 to 230 kV with increasing the charging voltage from 5 to 7 kV. These values correspond to 22 and 33 of voltage amplification, respectively.

Figure 12 shows the *L. edodes* yield for different applying voltages. One group is cultured without high-voltage stimulation (control group). Three groups are stimulated by a single high-voltage pulse (one time application) at three different amplitudes: 50, 90 and 125 kV. The last group is stimulated 50 times with a 50 kV pulsed voltage. The yield of the fruit body is evaluated as the total weight harvested during four seasons. It includes the crops from all 15 logs, appropriately averaged without statistical analysis. The yield of the control group was only 2 g in the first harvesting season, autumn of 2007, because the *L. edodes* species used in the present experiment mainly fruits in the spring. In this case, the 30 g weight of fruit bodies is harvested from only one log. Therefore, the standard deviation is 7.5 g, which is larger than the 2 g average weight. This result indicates that the mushroom species employed in the experiment usually does not develop fruit bodies. However, the yield from the first season increased from 2 to 73 g when a 50 kV pulsed voltage is applied. The yield increased from 73 to 153 g when the number of pulses increased from 1 to 50. In this case, the standard deviation is determined to be 73.0 g, which is lower than the 153 g average weight. This result indicates that the mushrooms develop fruit bodies as the result of applying high voltages. The total harvested weight over four seasons is 167 g in the control group. The yield increases to 322 and 319 g when pulsed voltages of 50 and 100 kV are applied, respectively. However, the yield decreases to 243 g at 125 kV voltage applying. This result indicates that optimum voltage amplitude exists and is estimated in range from 50 to 100 kV/m.

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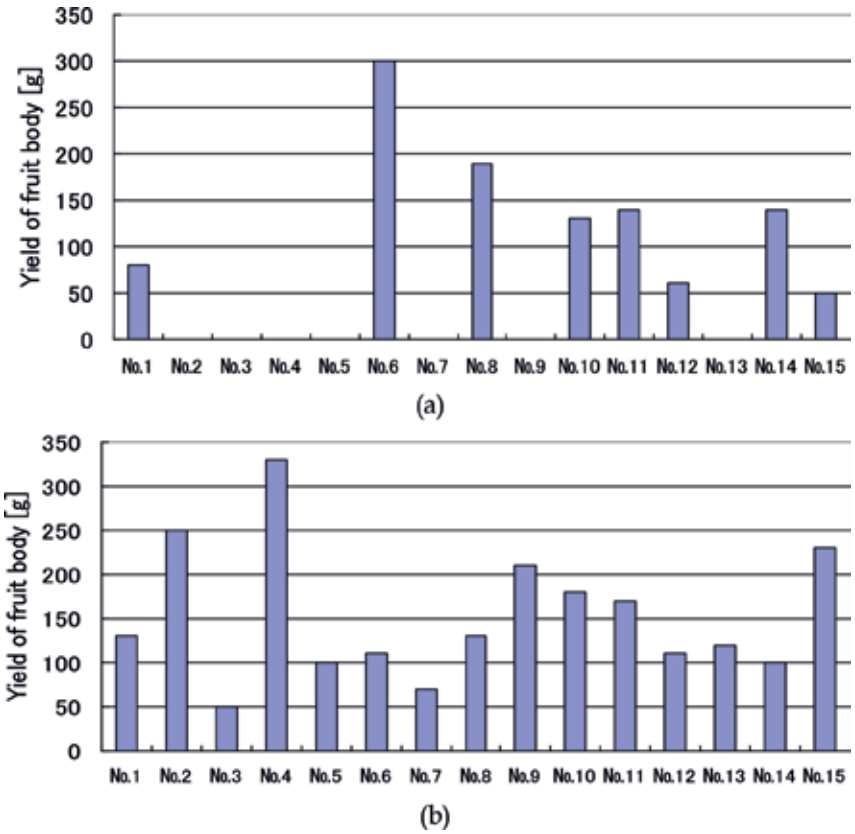

Figure 13. Difference in the yield of fruit bodies of *L. edodes* based on the number of 50 kV applied voltage treatments received. No. 1–15 indicates labels for each cultivation log. (a) One-pulse stimulation; (b) 50-pulse stimulation [3].

Figure 13 shows the weights of *L. edodes* harvested from each log at two different numbers of pulse voltage stimulation. The applied voltage was 50 kV in all cases. The total weight from the logs after 50-pulse stimulation was 2.29 kg (=153 g × 15), as shown in **Figure 5**, which is larger than the 1.09 kg (=73 g × 15) harvested after a one-pulse stimulation. The maximum value of the harvested fruiting body from one log after a one-pulse stimulation was 300 g, which is similar to the 320 g obtained after 50-pulse stimulations. Although there were no logs observed without fruiting body formation for 50-pulse stimulation, after a one-pulse stimulation, seven logs contained no fruiting bodies. The average yield for one log was approximately 73 g (=1090/15) after a one-pulse stimulation. Only 6 logs showed a yield larger than the 73 g average value, whereas 14 logs showed a yield larger than 73 g in the case of 50-pulse stimulation. This result indicates that on particular logs, use of the pulsed voltage decreased the deviation in the mushroom formation. The standard deviations are 27 and 19 g at one- and 50-pulse stimulations, respectively.

Figure 14 shows the time history of the amount of mushrooms cultured under various stimulation conditions in the spring of 2009. The yield is normalized by the total crop weight for one harvesting season and is evaluated as an aggregate of all crops. The total crop weights

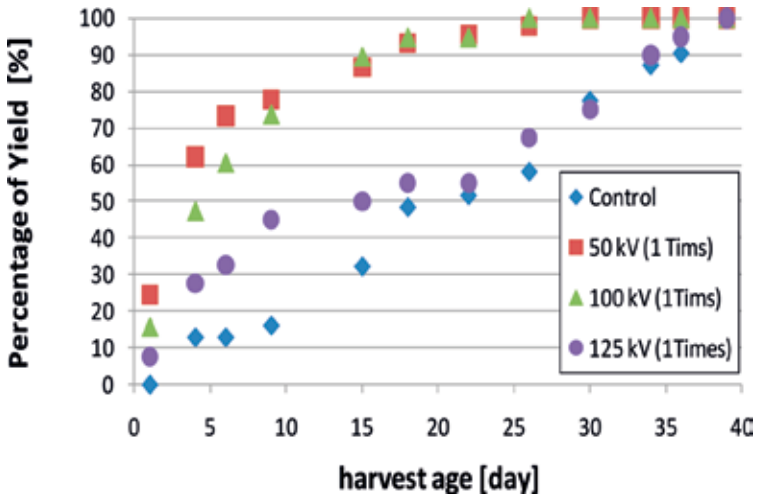

Figure 14. Time-history of the total amount of harvested fruit bodies for various stimulation voltages [3].

were 60, 111, 90 and 89 g in the control, 50, 100 and 125 kV stimulation groups, respectively. Compared with the control group, the total yield increased when applying a voltage of 50 and 100 kV. The harvested weight for 15 days after the first crop (day 18) was approximately 50% of the total in the control group. However, the crop weight during this period increased to 86% of the total when applying voltages of 50 and 100 kV. This result indicates that the mushrooms can be harvested in fewer days by applying high voltage as electrical stimulation.

Figure 15 shows the crop weight of *L. decaste* stimulated with three different voltage amplitudes: 50, 90 and 130 kV. The yield of the fruiting body at the first flash in substrate cultivation

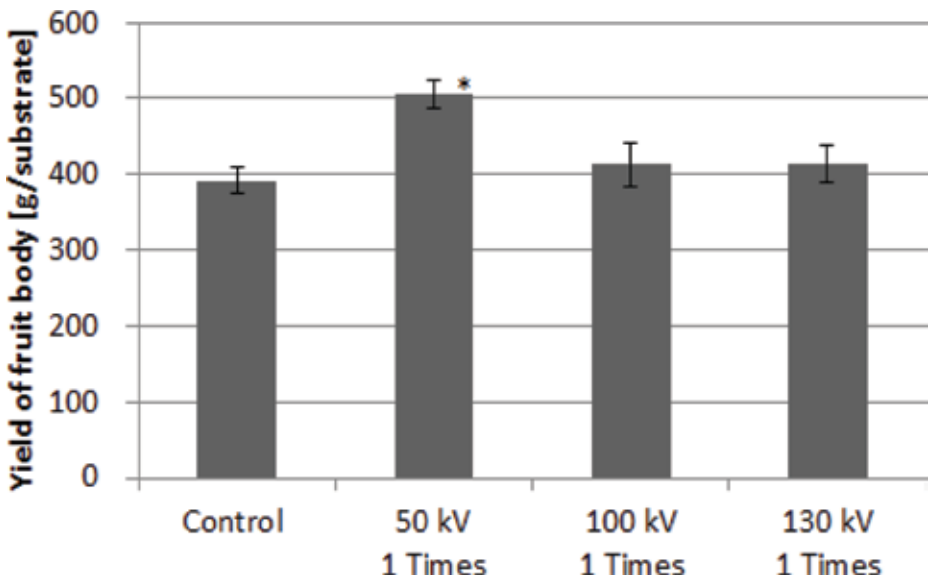

Figure 15. Yield of *Lyophyllum decastes* fruit bodies for various stimulation conditions. Vertical bars indicate the standard errors of the mean (number of samples; $n = 20$). Asterisks indicate the significant differences at $p < 0.05$ (*) [3].

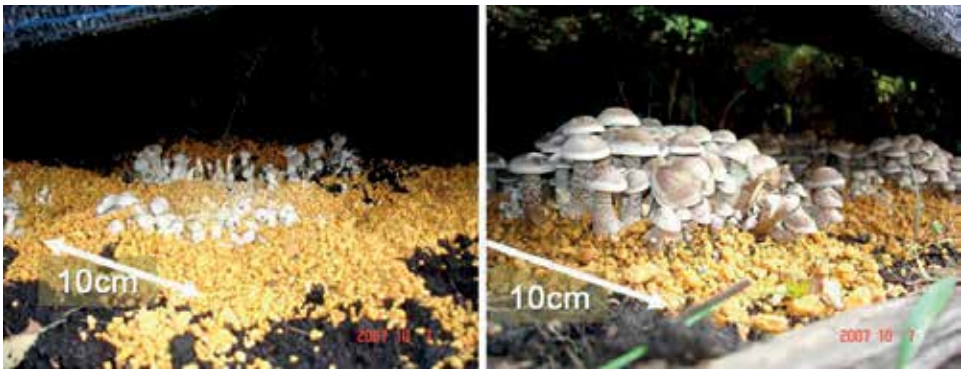

Figure 16. Typical photographs of the cultured *L. decastes* without (left) and with (right) electrical stimulation [3].

was used. The average yield was obtained using the total weight harvested from 20 substrate beds. The average yield of the control group is approximately 392 (±17) g/substrate. The average yield increased to 505 (±19) g/substrate by applying a voltage of 50 kV. The yield was 1.3 times larger than that of the control group with statistical significance of $p < 0.05$. The applied voltage of 100 kV corresponds to 3.57 kV/cm in an averaged electric field. **Figure 16** shows photographs of cultured *L. decastes* taken the same day. The *L. decastes* in the stimulation group grew faster than those in the control group.

6. Morphological changes after electrical stimulation

It is very difficult to reveal how electric stimulation affects fruiting body induction in mushroom species. Because molecular mechanisms for fruiting body induction in mushroom species have not still been well understood yet. Therefore, we focused on morphological changes after electrical stimulation.

Figure 17(a) and **(b)** shows images of *L. edodes* hyphae before (a, red) and after (b, blue) application of electric pulses. **Figure 17(c)** shows a superimposed image of (a) and (b) with purple (red + blue) indicating that hyphae retained the same position before and after applying the pulsed electric fields. Red and blue colored hyphae in **Figure 17(c)** show displaced hyphae. Displacement can be explained by the slightly negative charge of mushroom hyphae. When an electrical field E is applied, hyphae will thus be subjected to a Coulomb force f ($f = qE$; q means total charge of the hypha) from the electrical field. As a result, the hyphae are accelerated towards the positive electrode according to the equation $f = ma$, where m and a mean mass of the hypha and acceleration of the hypha, respectively. The application of electric pulses, resulting in hyphal displacement and sometimes damage, can be considered as a form of physical stress. Other physical stresses such as scrapping of surface hyphae (Kinkaki) have been known to induce fruiting body formation in several mushrooms, suggesting that electric pulses that induce fruiting body formation act through a similar mechanism. **Figure 17(d, e)** shows scanning electron microscope (SEM) images of hyphae before and after applying an electrical pulse of 10 kV between wire electrodes with a gap length of 9 cm. It was observed in the SEM image that after

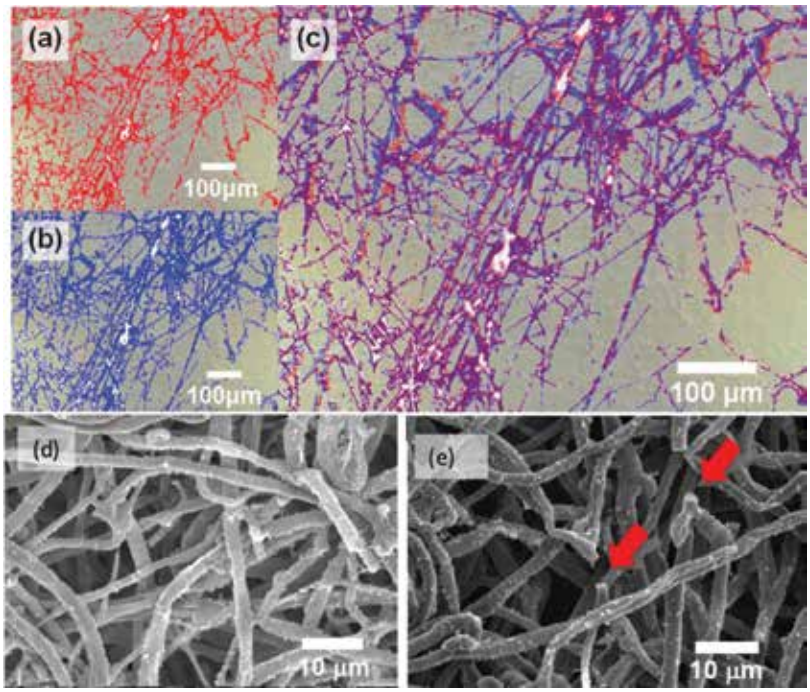

Figure 17. Microscopic images of *Lentinula edodes* hypha (a) before and (b) after applying 5 kV/cm pulse electric field with pulse width of 100 ns and 500 times of repetition. (c) Superimposed image of two images (a) and (b). (d) and (e): SEM images of *L. edodes* hyphae before (d) and after (e) applying 10 kV pulse voltages. White bar indicates 100 μm in (a), (b), (c) and 10 μm in (d) and (e).

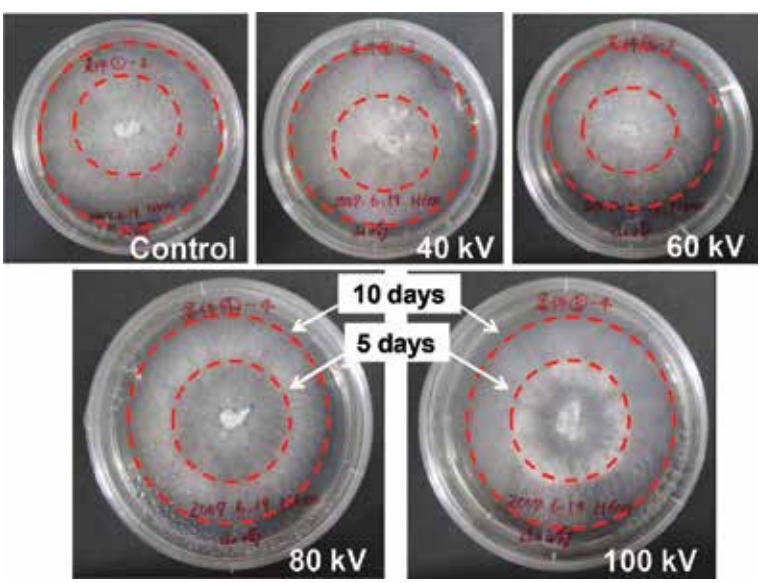

Figure 18. Influence of the pulsed voltage stimulation on hypha growth in agar medium cultivation. The diameter of the petri dishes is 10 cm in the all cases. The inner and outer dotted circles indicate growth positions of hyphae at 5- and 10-days cultivation, respectively [3].

some hyphae were broken by the electric pulse (**Figure 17(e)** arrow). This suggests that the electric pulse will be a similar stimulation as scratching mycelia on the surface of the sawdust media for mushroom production. Furthermore, it would be possible that new hyphae will be generated after electric pulse stimulation and Kinkaki. Hydrophobin, which is involved in hyphal structure and architecture in fungi [15, 16], would be involved in new hyphae generation after pulse stimulation.

Figure 18 shows typical photographs 10 days after cultivation at various amplitudes of the applied voltage. The pulsed voltage was applied after 5 days of cultivation of *L. edodes* hyphae. The tip positions of the hyphae after 5 days of cultivation were marked by the inner dotted circles. The hyphae grew from the inner to the outer circle positions after 5 days cultivation from the pulse voltage stimulation. From the microscopic observation, the growth direction of the hyphae changed perpendicular to the surface of the agar medium between the inner and the outer dotted circles as the result of applying a high voltage.

7. Conclusions

High-voltage electrical stimulation on fruiting body formation in cultivating mushrooms was described. The compact high-voltage pulsed power supplies were developed for the electrical stimulation to promote fruiting body formation on cultivation bed-logs and sawdust substrate (bed-block). The promotion effects of high-voltage stimulation of sawdust-based substrate of *L. decastes* and natural logs hosting *L. edodes*, *P. microspora* and *H. lateritium* were confirmed through the evaluation using a developed compact pulsed power generator. The fruiting body formation of mushrooms increases 1.3–2.0 times in terms of the total weight. The accumulated yield of *L. edodes* for four cultivation seasons was improved from 160 to 320 g by applying voltages of 50 or 100 kV. However, the yield was decreased from 320 to 240 g upon increasing the applied voltage from 100 to 130 kV. The yield of the other types of mushrooms show tendencies similar to those of *L. edodes* when voltage was applied. An optimal voltage was confirmed for efficient fruiting body induction.

Securing profitability of the electrical stimulation is important for the widespread to the mushroom famers. The pulse voltage stimulation systems for improvement of mushroom yield have been developed and sold by some companies. Typical price of the stimulation system is around 5000 USD. The increment of *L. edodes* yield is around 155 g/(1-log, 2-year) at 50 kV. The price of the *L. edodes* is around 20 USD/1-kg at natural-log cultivation in Japan. If the mushroom farmer uses 1612 logs, the initial cost of 5000 USD can be recovered with increment of the mushroom yield.

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The authors of this chapter confirm that they have received permission to reuse all the tables and figures in their current work.

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Chemical additives used for increasing plant productivity can contaminate the raw materials used in food production. Physical methods represent alternative promising sources for stimulating plant and mushroom development and increasing vegetable production. Many physical factors are currently used for plant treatment, including electromagnetic waves, optical emission, laser, magnetic field, gamma rays and ultrasound and ionizing radiation. This book discusses these physical methods for stimulation of plant and mushroom development and seed invigoration. Current research trends, future research directions and challenges are also discussed. This book will be of interest to many readers, researchers and scientists who can find this information useful for the advancement of their research works towards a better understanding of physical methods in plant and mushroom development.

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