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Seedling's Vigor of Tomato and Paprika Genotypes under a Simulated Model of Multiple Abiotic Stresses and Lower Dosage of Salicylic Acid ($C_7H_6O_3$)

Mohunnad Massimi*, Laszlo Radocz

Institute of Plant Protection, University of Debrecen, Debrecen, Hungary
Email: *mohunnad.massimi@agr.unideb.hu

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Abstract

Seedling vigor in tomato and paprika is affected by variety. Genotype selection under environmental stresses and its effects on seedling vigor was investigated. During the year 2021, the study was carried out and it revealed an appropriate selection for open field gardeners. The selection of two drought-tolerant, and one non-drought tolerant cultivars was the initial stage. Six commercial genotypes were evaluated (three for tomato and three for paprika). The second phase involved planting cultivars till the cotyledon leaves phase, and the first set of true leaves. Seedlings were investigated under simulated environmental stresses of non-ideal temperatures, low humidity, closed spacing, minimum light dose, nutrient-deficient water, and spraying a lower dosage of salicylic acid. The evaluation of seedling's growth was performed by measuring germination percentage, seedling length, shoot length, root length, seedling fresh weight, seedling dry weight, and seedling vigor index. Tomato (Mobil) and paprika (Carma) seedlings outperform other varieties, possibly because of the variety's vigor under various stress conditions. These findings reveal that tomato (Mobil) and paprika (Carma) had a positive impact on development and may be raised under optimal conditions of nurseries and then be transferred to open-air environmental and biological exposed conditions in Hungary.

Keywords

Environment, Environmental Stress, Foliar, Genotypes, Paprika, Tomato

1. Introduction

Cultivation and protection of vegetables are considered one of the most important technologies that contribute to reducing the damage of environmental factors [1] such as high temperature, drought, salinity, cold, frost, light stress, and lack of nutrients. In this regard, the methods of integrated pest control and organic agriculture have developed in conjunction with its predecessor to produce safe food from environmental and food-health standpoints.

However, the most prominent question in this regard is how to conduct open cultivation while ensuring the management of climate change factors and the resistance of plants using integrated pest management methods. Škrubej *et al.* [2] demonstrated, through its scientific experiments on tomato plants, the ideal conditions for the germination and growth of seedlings, use germination incubators at temperatures 30˚C during the day (8 hours) and 20˚C at night (16 hours), while maintaining a constant level of relative humidity of 75% and an intensity of illumination of 750 lux. On the other hand, Sanjuan-Martínez *et al.* [3] experimented (*Capsicum annuum* L.) plants that were grown for (14 hours) under fluorescent light at a temperature of 25˚C and 85% - 90% relative humidity. It can be concluded from the fact that climate change and open cultivation constitute hostility to each other, especially that high-temperature extremes lead to drought and salinization.

Drought stress affects plants when it becomes difficult to deliver water to the roots or when the plant's transpiration rate becomes very high [4]. The abiotic pressures of rising temperatures, reduced irrigation water supplies, and salinity are the major limiting factors in maintaining and improving vegetable output. Extreme climatic conditions can also harm soil fertility and exacerbate soil erosion. To maintain productivity, further fertilizer application or enhanced nutrient-use efficiency of crops will be required [4]. It is obvious to say that the weakness of plants in front of the environmental conditions will produce plants with a low health condition and prone to infection with various pests and diseases, which affects production in quantity and quality.

It can be found that the plant's susceptibility to insects, viral, bacterial, fungal, and weed infections, is influenced by soil moisture, salinity, soil temperature, and air temperature surrounding the plant. Private agricultural companies and scientific researchers seek to find varieties and genetic structures capable of growing and producing in non-ideal environmental conditions. However, this idea is not enough, as finding solutions from natural and organic extracts such as salicylic acid extracted from the willow plant species, where researchers found its ability to generate acquired resistance for the plant against pathogens. Here, the purpose can be achieved by open-air vegetable cultivation through varieties that have resistance and the ability to produce in unfavorable environmental conditions.

Sajyan *et al.* [5] reported that even though salicylic acid did not affect fruiting, it did boost plant growth, root development, and blooming characteristics in salt-stressed tomato plants. Najafian *et al.* [6] found that salicylic acid in aqueous solutions, enhanced stem diameter, and the number of leaves. The favorable effect of salicylic acid on tomato plant stem diameter could also be due to accelerated cell division [7]. This acid possesses a stimulatory action like the auxin plant growth regulator that stimulates cell elongation.

Salicylic acid is present naturally in plants and plays a key role in growth and stress tolerance, as well as controlling the defensive response of plants to pests, pathogens, and environmental stimulations [8]. When given exogenously as a foliar spray, salicylic acid promotes stress tolerance by improving the plant's physiological response to salt and temperature extremes, as well as altering antioxidant, nutrient, and chlorophyll levels. It was reported that the salicylic acid as foliar concentration reached its maximum (48 hours) after the first application in greenhouse-grown tomatoes [8].

In contrast to these positive results, other studies prove the opposite. Salehi *et al.* [7] reported the importance of the tomato cash crop in Iran. As a result, a pot experiment was carried out to see how salicylic acid affected tomato growth under salt stress. The findings suggest that the salicylic acid concentrations utilized in this experiment, as well as the experimental site's climatic circumstances, did not have the expected beneficial effects on tomato plants (cv. Viva). Treatment had no significant effects on the assessed properties (shoot fresh and dry weights) within each salinity level (0, 4, 8, and 12 $dS \cdot m^{-1}$). According to these findings, salicylic acid did not improve tomato salt tolerance under these experimental conditions.

Haytova (2013) [9] remarked on the advantages of foliar application. The technique encourages quick mineral nutrient absorption while avoiding soil interactions that decrease root uptake by immobilizing the soil. It can aid with physiological difficulties caused by nutritional deficiencies, such as pepper and tomatoes blossom end rot.

Massimi and Radocz [10] (2020) highlighted and shed light on the possibility of the spread of powdery mildew disease in drought conditions in tomato and pepper crops by comparing a group of scientific sources. Their small review article in the Hungarian Agricultural Engineering journal advocated looking into the link between the development of powdery mildew and drought stress on tomatoes and peppers in Hungary and Jordan [10]. Because of poor drought shocks, Kafle *et al.* [11] used tomato as a model plant to strengthen its resistance to powdery mildew disease (*Oidium* spp. and *Leveillula* spp.) by stimulating its innate resistance system at an early seedling stage. Based on the foregoing, it's reasonable to conclude that salicylic acid, which has an auxin-like effect, regulates metabolic processes, and maintains osmotic potential, is advantageous at all stages of growth.

Some pioneer applied studies for spraying pepper with salicylic acid (measured in $mg \cdot L^{-1}$) to increase growth and production indicated that the dosage must not be less than 50 $mg \cdot L^{-1}$ [12], and [13]. Also, when a tomato is cultivated under moderate to ample soil moisture availability, exogenous use of salicylic acid at 150 $mg \cdot L^{-1}$ as a foliar spray may be recommended [14]. Furthermore, several informal and extension findings suggest that the salicylic acid dose should not be less than 175 $mg \cdot L^{-1}$ to achieve the aim at least two days after spraying.

Consequently, this study aims to evaluate the tomatoes and paprikas genotypes vigor under simulated multiple environmental stresses and a very low dosage of foliar spraying of salicylic acid. The simulation model of varieties' performance was carried out in unsuitable environmental conditions in terms of temperature, humidity, lighting, competition, lack of nutrients, and minimum spraying dosage. The germination percentage, dry and fresh weight, length of seedlings, length of shoots, root length, and seedling vigor index were measured at the end of the test. The study aims to select the superior vigor cultivars for raising in nurseries and transferring to open field gardens.

2. Materials and Methods

2.1. Experimental Description

Seed and seedling tests were conducted in the full germination, cotyledon, and first set of true leaves stage under non-optimal environmental conditions. This is to simulate environmental stress conditions such as temperature, air humidity, lack of nutrients, and minimum lighting dose, as well as scheduled irrigation to facilitate the selection of varieties suitable for environmental and biological stress conditions.

In the plant pathology laboratory of the Institute for Plant Protection at the University of Debrecen's school of horticulture, a three-stage experiment was carried out. The three stages included full germination (17 days), cotyledon leaves phase (20 days), and the first set of true leaves stage (30 days).

(Mobil, Korall), two drought-tolerant tomato varieties, and (Carma and Fokusz), two drought-tolerant sweet pepper cultivars, were planted in a completely randomized design (CRD) with three replicates in all stages. One cultivar for each plant that was not resistant to drought was also used. Namely the tomato (Tyking F1), and paprika (Bobita F1) were drought sensitive.

2.2. Germination Stage

For each plant, 10 seeds were randomly selected from each variety and planted in 9 mm diameter Petri dishes on 2 layers of filter paper. To minimize uncontrolled and excessive relative humidity, fungal infections, and overcrowding of seedlings after growth, each variety was replicated three times by planting it on 2 plates at a rate of 5 seeds per plate.

The plants in the germination stage received total irrigation water of 35 mL, with an average of 7 mL per seed for 17 days. Germination was complete for all kinds with variable speeds after 17 days of seeding. Because the seedlings in the Petri dishes are submerged in distilled irrigation water, symptoms of fungal molds began to arise.

2.3. Cotyledon Leaves Phase

After merging seedlings of two plates of each variety in 2 holes in a plastic tray filled with organic soil, perforated from the bottom, only 10 superior vigor seedlings of each variety were transferred, distributed into 2 holes, 5 per hole.

The total amount of irrigation per hole was 157 mL during the second stage (average 12 mL per seedling for 20 days). During this stage (cotyledon leaves phase), plants were subjected to competition pressure due to the presence of 5 seedlings per hole.

2.4. First set of Trues Leaves

After the seedlings began to produce true leaves, only 2 superior and vigor seedlings of each variety were randomly selected, transferred, and planted with three replications in 3 pots. Only 2 seedlings of each variety were placed in each pot, spaced about 1.5-cm apart. Each 15 cm-diameter container receives 900 mL of potting mix.

In this stage, irrigation was scheduled at a rate of 2 mL per plant once every (72 hours) based on the amount of evaporation from the soil and experimental conditions.

A thinning procedure was carried out and 1 more vigor seedling was selected for each pot on day 20 of the last stage. The experimental design was a completely randomized design (CRD) with three replicates. On the 28th day of the true leaves stage, every plant with two or three true leaves was sprayed with 4 mL of salicylic acid 50 mg·L^{-1}, then seedlings were irrigated with 2 ml of pure distilled water.

2.5. Recording of Multiple Environmental Stressors

Using a model (SENCOR SWS 5051, hygro-thermometer 433 MHz used) weather station, the average laboratory temperature and air relative humidity were recorded every day, with the average temperature and humidity being (22˚C) and (40%), respectively in the first stage, (23.5˚C) and (33.3%) in the second, and (22.4˚C) and (43.8 %) in the true leaves stage (**Figure 1**). It appears from the averages that the plants did not obtain the ideal temperatures and humidity ratios for growth during the three stages.

The plants were also exposed to light stress, as the lighting was turned on only during the true leaf stage. The (Led28, model 38MM5730) light source has (380 - 780 nm) was used. The light source is 5 Watts, 120 Lumens per Watt, for a total of 600 Lumens, which was enough for 18 pots (0.4675 m^{-2}) at 1277 lux (3 pots for each tested variety). The plants were exposed to minimal light daily for only seven hours (from 9 AM to 4 PM) using an electronic auto-timer switcher (type: Nedis Programme, Time 02/Time 02 E).

Distilled water without nutrients was used for irrigation in the three-stage experiment. The total dissolved salts (TDS) and acidity (pH) in the nutrient-deficient irrigation water were measured using (Pancellent, and VWR PH/ CO 1030) digital sensors with four replicates, respectively. Water contains 1 ppm (average electrical conductivity 2 μs·cm^{-1}, and pH of 7).

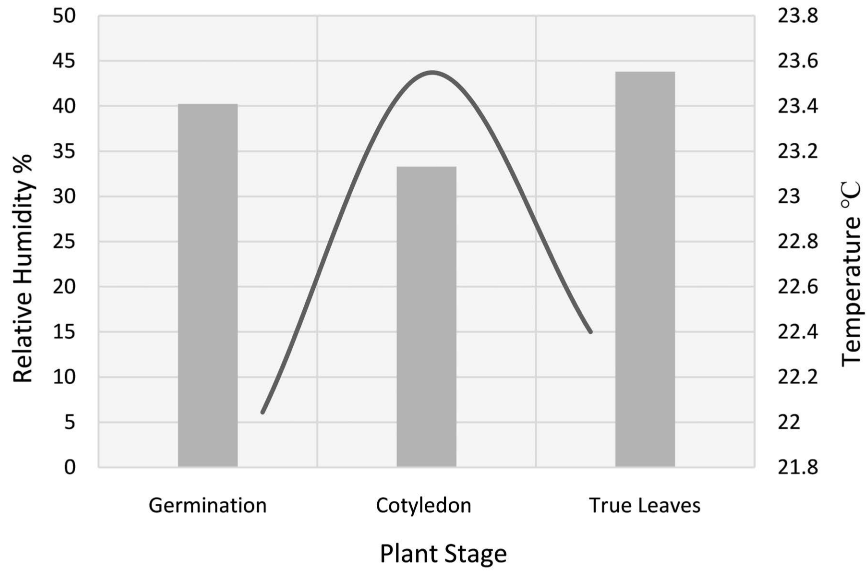

Figure 1. Average temperatures (curve) and relative humidity (bars) in the laboratory during the growing stages.

Electronic sensors of various types (Luster Leaf 1835, and Pancellent) were used to measure the chemical and physical properties of the organic bio-humus that was used in the second stage, and a substance of potting mix was used in the true leaves stage (**Table 1**).

2.6. Data Collection and Statistical Analysis

At the end of the germination test, normal seedlings, abnormal seedlings, and dead seeds were counted. Normal seedlings were determined to calculate germination recorded as a germination percentage with 3 replicates.

Three normal seedlings from each variety were taken after 48 hours of salicylic acid spraying and before the next irrigation period, and the shoot length (cm), root length (cm), seedling length (cm), and seedling fresh weight (gm) were measured.

The seedlings were then dried at 70˚C for (48 hours) in a vented oven, and the seedling dry weight was measured (gm). In the molecular biology lab, the (OHAUS Adventurer) digital scale was utilized. The data of seedling vigor index were extracted by multiplying germination percentage with seedling fresh weight for the first time, and with the seedling length for the second time.

The single factor (genotype) analysis of variance (ANOVA-One Way) was used. At a probability level of 0.05, means were separated using the Honestly Significant Difference (HSD). The Post Hoc Test (Tukey-Kramer a Post Hoc Test in Excel) was used to analyze gaps in the means of various groups.

3. Results and Discussion

The average percentage of germination at the end of the germination stage is shown in (**Table 2**). The means for seedling length (cm), shoot length (cm), and

Table 1. Chemical and physical traits of bio-humus organic and potting mix.

Substance	Stage	Acidity (1-14)	Temperature (˚C)	Fertility (N-P-K) (ppm)	Total Dissolved Salts (ppm)	Electrical Conductivity (μs·cm^{-1})
Bio-humus organic	Cotyledon leaf	7	21.7	50-4-50	202	403
Potting Mix	True leaves stage	7	22.8	50-4-50	526	1115

Each record represents a mean of three replicates.

Table 2. Overall mean for the impact of genotype on germination percentage, seedling length, shoot length, root length, seedling fresh weight, and seedling dry weight after a simulated test.

Tomato	Germination %	Seedling Length	Shoot Length	Root Length	Seedling Fresh Weight	Seedling Dry Weight
Mobil	90	**13.2 A**	**9.5**	**3.7**	**0.10**	**0.010**
Tyking F1	100	10.3 AB	7.6	2.7	0.08	0.008
Korall	93.3	9.20 B	6.0	3.2	0.05	0.005
Significant	NS	YES	NS	NS	NS	NS
Paprika	Germination %	Seedling Length	Shoot Length	Root Length	Seedling Fresh Weight	Seedling Dry Weight
Carma	100	**14.3**	**7.0**	**7.3**	**0.23**	**0.022**
Fokusz	100	11.4	6.9	4.4	0.12	0.013
Bobita F1	100	11.7	6.5	5.3	0.13	0.019
Significant	NS	NS	NS	NS	NS	NS

root length (cm) are also shown, as well as seedling fresh weight (gm), and seedling dry weight (gm). The table shows that tomato cultivar (Mobil) and paprika cultivar (Carma) surpass the others quantitatively, but there are no significant differences except for the seedling length of tomatoes (Table 2). Unless the germination percent, the tomato variety (Mobil) and the paprika (Carma) appear to have produced the longest seedlings (cm), shoots (cm), roots (cm), fresh weight (gm), and dry weight (gm) at the end of uniform multiple environmental stresses test imposed.

In Figure 2, the seedling vigor index (SVI) is calculated in two ways: the first is based on the product of multiplying the percentage of germination by the weight of fresh seedling, and the second is based on the multiplying of germination percentage along the entire length of the seedling, where the tomato variety (Mobil) has a significant advantage. Despite the numerical superiority of paprika variety (Carma) in the two measured parameters of seedling vigor Figure 3, all paprika varieties were not significantly different.

It has been discovered that plants' growth is slowed by environmental stress. Under optimal conditions, tomatoes germinate in 10 days and peppers in up to 21-days [15]. In 17 days, the seeds have fully germinated (Table 2). The end of the cotyledon leaves period and the start of true leaves was delayed in this experiment due to homogeneous and interfering stress sources.

This experiment represents a real model for simulating non-ideal environmental conditions in the open field, which represents the reality of open-air gardening.

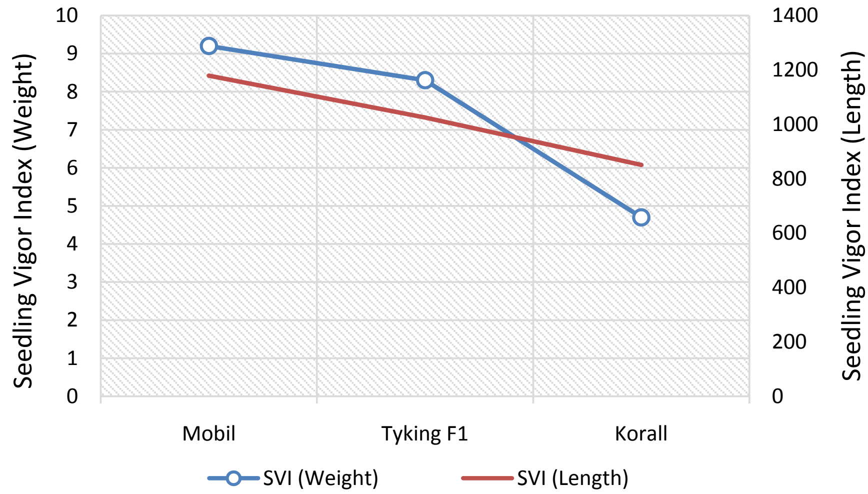

Figure 2. The seedling vigor index (based on fresh weight) (blue curve) and seedling vigor index (measured to seedling length) (red curve) combined for 3 tomato genotypes.

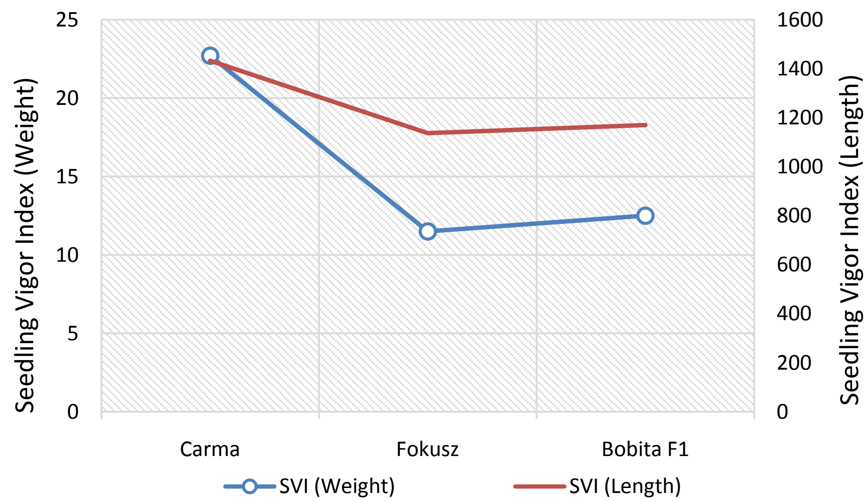

Figure 3. The seedling vigor index (based on fresh weight) (blue curve) and seedling vigor index (measured to seedling length) (red curve) combined for 3 paprika genotypes.

The temperature of the laboratory, the site of the experiment, did not rise above 25˚C. The relative humidity ranged from 30% to 45% during the three growth stages which are germination, cotyledons, and true leaf stage (**Figure 1**). Both research for Škrubej *et al.* [2] and Sanjuan-Martínez *et al.* [3] describe the ideal temperatures and humidity for the cultivation of tomatoes and peppers, respectively, in ideal incubators conditions. Škrubej *et al.* [2] reported that tomato plant demands temperatures of (20˚C - 30˚C) during germination and early growth, as well as a high relative humidity of 75%. The second research for Sanjuan-Martínez *et al.* [3] conducted an ideal experiment for pepper growth at a temperature of (25˚C) and relative humidity of (85% - 90%). The temperature and humidity parameters in the current experiment are considered inadequate for germination and growth, as the temperature did not surpass (23.5˚C), and the humidity did not exceed (44%). The ideal relative humidity of tomato cultivation should be increased to (65% - 85%) [16].

Further, the plants in this test were provided with artificial lighting suitable for photosynthesis during the true leaf stage only and at a minimum duration of 7 hours. However, tomatoes need 8 hours, and pepper 14 hours [2] and [3]. During the germination stage, it is recommended that artificial lighting be placed over the seedlings for 24 hours [15]. Tomatoes require (6 - 12 hours) of sunlight per day, whereas peppers require (8 - 10 hours) [15].

In addition, there was an imposed competition between plants during the cotyledon leaves stage and irrigation with pure nutrient-deficient water during all stages of the experiment. Calikim, (2020) [15] stated that to limit competition for nutrients and water, seedling spacing (approximately 60-cm) should be maintained on average. It is noted from (**Table 1**) that the culture media in the two stages of cotyledons and true leaves contained the minimum amounts of major nutrients, where a device (Luster leaf 1835) can record the highest ideal measurements of the three major elements of (N-P-K) by (200-14-200).

Integrated pest management is a philosophy that is founded on the principle of implementing the best agricultural practices to ensure plant health in terms of irrigation and nutrition requirements [10]. Chemical spraying should be the last resort if a specific pest develops, to ensure plant immunity and productivity. The production success factors in this test were insufficient and inappropriate, and the salicylic acid spray dosage was insufficient to accelerate growth and to recover from environmental stress factors' complicated impact.

Based on the foregoing, any superiority of any variety under difficult environmental conditions will be the result of the strength (vigor) of the variety and its genetic makeup. A tension pressure method is an excellent tool, even though private companies have tremendous marketing and publicity resources, scientific study and testing are a better strategy to secure results.

4. Conclusion and Recommendation

Plant genetic structure and its potential to create resistance in the face of environmental circumstances and pests can be tested using environmental models. This study covers a wide range of plant physiology topics and their applications in ecology. Tomato (Mobil) and paprika (Carma) seedlings outperformed other types in this trial, presumably due to the variety's vitality under stress. These studies show that tomato (Mobil) and paprika (Carma) have a favorable impact on development and may be grown in nurseries under ideal environmental conditions before being transplanted to open garden environments in Hungary. Spraying (Carma) seedlings with an appropriate dose of salicylic acid before transplanting is a good idea because salicylic acid promotes stress tolerance by improving the plant's physiological response to drought, salt, and temperature extremes, as well as changing antioxidant, nutrient, and chlorophyll levels. Experiments in optimal circumstances of growth incubators are required to evaluate plant health status, and a factorial design is recommended to be used to choose varieties, spraying materials, and most appropriate spraying rates by combining two factors such as plant genotype and spraying material/dosage.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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