

# Interaction impact of drought stress, nutrient-deficient water, and seed-borne pathogen (*Alternaria alternata*) on germination and vigor of two tomato varieties

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**Summary:** Tomato (*Solanum lycopersicum*) is considered one of the leading vegetable plants in the world. This study evaluated the germination and vigor capabilities of 'Marmande' and 'Kecskeméti Jubileum' varieties under different conditions, including drought stress, nutrient-deficient water, and the effect of seed-borne disease caused by *Alternaria alternata* when prime and non-prime with salicylic acid. The experiment was conducted in the laboratory in 2023 at Agricultural and Food Sciences and Environmental Management Faculty of University of Debrecen. Results indicated that the 'Kecskeméti Jubileum' variety exhibited a superior strength to 'Marmande' in the seedling's dry weight, seedling growth rate, and vigor index under nutrient-deficient water. Nevertheless, when applying levels of drought, the 'Marmande' variety had a higher viability rate of 62.5% in comparison with another variety, and the germination rate of the two tomato varieties is at 85.5% in 5% concentration but decreased progressively when exposed to a higher drought concentration of 10%. When using 3 ml of salicylic acid during germination stages, the seedling vigor index of 'Marmande' shows a greater index at 165 compared to 'Kecskeméti Jubileum', just 108 under the infection of *Alternaria alternata*. The results of the examination of drought stress, and the effect of *Alternaria alternata*, one cause of seed-borne pathogens, showed that the percentage germination and vigor ability of the 'Marmande' variety performed better than 'Kecskeméti Jubileum' under the same conditions.

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**Key words:** abiotic stress, biotic stress, tomato variety, *Alternaria alternata*

## Introduction

Tomato (*Solanum lycopersicum*) is a member of the Solanaceae family and is one of the key crop plants following potato. Nutrients in tomatoes such as minerals, vitamins, proteins, monounsaturated fatty acids, and carotenoids (Chaudhary et al., 2018). Lycopene plays an important role in protecting against dangerous diseases such as cancer, cardiovascular illnesses, cognitive dysfunction, and osteoporosis (Ali et al., 2020). Drought is known as a typical and common factor of osmotic pressures. Drought is one of the most vital factors negatively influencing crop yield (Khan et al., 2015). To clarify, drought stress hinders plant respiration, photosynthesis, and stomatal movement, which harms plant growth and physiological metabolism (Yang et al., 2021). Under drought pressures, plants expand the roots to uptake more water, thus minimizing stomatal loss of water when there is a water inadequacy (Martínez-Vilalta & Garcia-Forner, 2017). Seed is the most significant agricultural input. Several plant diseases are seed-borne, resulting in massive crop losses (Azma, 2018). *Alternaria* spp. can also be classified as a seed-borne pathogen, causing tomato disease (Chrapacienne et al., 2022). Furthermore, seed germination can be negatively influenced by *Alternaria* spp. because of the presence of the fungus on the seed's surface (Gaur et al., 2020; Zhange et al., 2020). However, the fungal disease can be controlled by several solutions including resistant varieties, production technology, seed treatments and dressings,

and soil disinfection (David, 2004). To alleviate seed-borne diseases, seed treatment is regarded as a low-cost and highly effective method (Mekonnen, 2020). Salicylic acid is a phenolic compound that is involved in plant processes that contain fruit maturity and senescence (Khan et al., 2015). Salicylic acid occurs naturally in plants and has a key role in plant development, stress tolerance, and defense against pests and pathogens (Gomma, 2021; Zhang et al., 2017). Also, salicylic acid constitutes an integral component of the innate defense mechanism employed by host plants to counteract the detrimental effects of pathogens across a diverse range of interactions between hosts and pathogens (Narasimhamurthy et al., 2019). This study aims to compare the germination and vigor of two tomato varieties 'Marmande' and 'Kecskeméti Jubileum' under drought stress, nutrient deficient water and examine the application of salicylic acid on mitigating seed-borne disease caused by *Alternaria alternata*.

## Materials and methods

The experiments were conducted in 2023 in the phytopathology laboratory at the Institute for Plant Protection of the University of Debrecen, Hungary. As part of the studies, two types of tomatoes ('Marmande' and 'Kecskeméti

Jubileum') were chosen randomly for the investigation. This study was divided into three experiments.

#### ***Effect of the drought stress on germination and vigor of two tomato varieties experiment***

The experiment was conducted over 12 days and involved irrigating with a solution of polyethylene glycol 6000 (PEG) (ACROS Organic Germany) at 5% in the first test and 10% in the second trial. Tomatoes were grown in a completely randomized design (CRD) split, such that the two varieties of tomatoes and all treatments were randomly selected and planted in Petri dishes with a diameter of 9 mm on two sheets of filter paper. Each Petri dish was watered once on the first day with 10 ml of the polyethylene glycol 6000 solution at 5% and 10% concentration and then left to set at room temperature. On the twelfth day of each investigation, the researcher recorded the number of seedlings that had grown (complete shoot structure, and roots).

#### ***Impact of the nutrient deficient water irrigation on germination and vigor of two tomato varieties experiment***

The experiment lasted for 12 days and involved the use of pure distilled water for irrigation. In a split completely randomized design (CRD), each five-seed tomato variety was randomly replicated four times. For the study, five tomato seeds were randomly selected from each variety and placed on two pieces of filter paper in Petri dishes with a diameter of 9 mm. A volume of 3-cm<sup>3</sup> of distilled water was used to irrigate each Petri dish and the dishes were covered and kept at a room temperature of 24.3 °C and a relative humidity of 56.8% (every day, the mean laboratory temperatures and humidity levels were noted using a sensor (SENCOR SWS 5051)). After 12 days, the healthy seedlings with complete shoot, root, and terminal bud structures were collected. The entire fresh mass of the five seedlings was recorded, and the total dry mass was measured after drying at 70 °C for 48 hours in a vented oven. In the molecular biology lab, the digital scale of (OHAUS adventurer) was utilized.

#### ***Influence of the seed-borne pathogen (*Alternaria alternata*) on germination and vigor of two tomato varieties***

Each set of five seeds was pre-treated with a solution of salicylic acid at a concentration of 100 ppm, with approximately 3 ml applied, for 48 hours. Subsequently, they were subjected to priming with an inoculum of *Alternaria alternata* under the same conditions of rate and duration. Besides, five seeds randomly selected cultivars were incubated with *Alternaria alternata* directly without priming with salicylic acid. In this study, five tomato seeds were treated and non-treated randomly chosen from each variety and arranged on filter paper inside Petri dishes with a 9 mm diameter. Each Petri dish received 3 cm<sup>3</sup> of distilled water for irrigation, after which they were covered and maintained at a room temperature of 21.9 °C and air moisture of 54.7%. The SENCOR SWS 5051 devices were employed to record the daily readings of the mean laboratory temperature and humidity. After a span of 12 days, a collection was made of the vigorous seedlings that exhibited undamaged shoot, root, and terminal bud formation. Their overall fresh weight was recorded and subsequently, they were dried in a well-ventilated oven at 70 °C for 48 hours. This trial is a factorial design (variety versus salicylic priming) with four replicates.

#### ***Data collection***

Germination (viability) percent was recorded. The OHAUS adventurer scale was utilized to determine the dried mass of all five seedlings. Additionally, the guideline was also taken from the AOSA publication (1983) which was used as a guide on measuring seedling growth rate (SGR). The determination of tissue water content (TWC) for the group of five seedlings was carried out based on Black and Pritchard's formula (2002):  

$$TWC = \frac{(\text{Fresh Weight} - \text{Dry Weight})}{\text{Fresh Weight}} \times 100$$

The vigor of the seedlings was determined using the formula proposed by Abdul-Baki & Anderson (1973):

$$\text{Seedling vigor index} = \text{Germination\%} \times \text{Seedling dry weight.}$$

#### ***Analysis of data***

An analysis of variance (ANOVA) with replication was conducted using Microsoft Excel 365. In CRD trials (1-way ANOVA was used), while in factorial trials (2-way ANOVA was used). To assess the differences in means across multiple groups, a Post Hoc Tukey-Kramer Test in Minitab (version 20) with a significance level set a 5%. This test was utilized to examine variations among the means of the different groups.

## **Results and discussion**

#### ***The impact of dryness stress on germination and vigor of two tomato varieties***

##### *The growth seeds percentage of two tomato varieties*

The germination percentages of seedlings that have grown for two different types of tomato cultivars among all tested osmotic stress levels. The results indicate a significant difference between the 'Marmande' and 'Keckskeméti Jubileum' varieties, with the 'Marmande' variety showing a higher percentage of germination at 62.5% compared to the 'Keckskeméti Jubileum' variety under the drought conditions.

Besides, the germination rate of the seeds at a 5% drought concentration was much higher at 85.5% compared to the germination rate at a 10% drought concentration. This finding suggests that as the concentration of drought increases, the germination capacity of the tomato seeds decreases. Additionally, the root structure that develops after germination is also less pronounced, resulting in weaker and stunted seedlings. Similarly, the dehydration tolerance of both seed germination and seedling development was evaluated by subjecting them to different concentrations incorporated 0%, 2%, 4%, 6%, 8%, 10%, 12% and 16%. Interestingly, the outcomes revealed that the maximum germination rate was accomplished at a reduced PEG concentration. However, when the PEG concentration reached 12% there was a noteworthy reduction in the germination rate. Furthermore, not one of the tomato varieties tested could sprout at this concentration of PEG (Basha et al, 2015).

##### *Regression optimizer of parameters of two tomato varieties under drought stress*

**Tables 1-2** indicate various maximum and minimum responses for different tomatoes. A variety called 'Marmande' performed higher at a 5% drought level than another did. However, **Table 4** shows that the 'Keckskeméti Jubileum' variety recorded one of the

lowest percentages of germination under 10% drought stress. Based on the previous study evident that drought stress occurring during the vegetative or early reproductive stages typically diminishes crop yield through a decrease in seed quantity, size, and quality. An appraisal is crucial to determine the consequences of drought stress on tomato seed yield, quality, and overall growth (Pervez et al., 2009).

**Table 1:** The germination parameter of two tomato varieties under different drought stress conditions.

Solution	Goal	Lower	Target	Upper	Weight	Important
Germination (%)	Maximum	0	100	-	1	1
	Minimum	-	0	100	1	1

**Table 2:** Multiple maximum and minimum response optimization for germination percentage at different drought levels of two tomato varieties.

Level	Solution	Variety *	Drought *	G% Fit
Maximum	1	1	1	98.75
Minimum	1	2	2	3.75

Variety\*: 1 - 'Marmande' variety, 2 - 'Keckskeméti Jubileum'.

Drought level\*: 1 - 5% of drought level, 2 - 10% of drought level.

### The effect of nutrient-deficient water irrigation on germination and vigor of two tomato varieties

The seedling fresh weight, dry weight, and growth rate of two tomato varieties

**Table 3** presents the value of seedlings that have grown from two tomato cultivars in the same irrigational condition. The seedling's fresh mass reveals that there is no significant distinction between the 'Marmande' and 'Keckskeméti Jubileum' varieties. Especially, when subjected to identical conditions, the Marmande variety displays a significantly lower dry weight, measuring 0.65 mg, in comparison to the 'Keckskeméti Jubileum' at 1.15 mg. Similarly, Jubileum's seedling growth rate of 1.27 mg was greater than Marmande's 0.79 mg. This results are suitable with the result of Helves experiment, the 'Keckskeméti Jubileum' tomato variety under irrigation giving the highest yield, and having the good average weight, dry matter content in comparison with the other variety in the same condition (Helves et al., 1998).

**Table 3:** The data of fresh weight, dry weight, and growth rate of two tomato varieties under the same germination condition.

Genotype	Seedling fresh mass (mg) seedlings <sup>-5</sup>	Seedlings dry mass (mg) seedlings <sup>-5</sup>	Seedling growth rate (mg) seedling <sup>-1</sup>
'Marmande'	19.75 a	0.65 b	0.79 b
'K. Jubileum'	20.25 a	1.15 a	1.27 a
Significance	NS	S	S

Values in columns sharing the same letters are not significant at the 5 percent (%) HSD probability level. NS: Non-Significant & S: Significant.

### The percentage of seedling vigor index and tissue water content for two tomato varieties

Based on the data presented in **Table 4**, it is evident that the effects of the two varieties are differently significant on the seedling vigor index. In addition, the seedling vigor index of 'Keckskeméti Jubileum' variety was much higher at 105.00 compared to 55.03 in 'Marmande'. This implies that the 'Keckskeméti Jubileum' variety could have inherent

characteristics regarding genetic factors that contribute to its superior vigor compared to 'Marmande' under nutrient shortage. The outcome is consistent with research by Geshnizjani et al. (2020), which highlighted genetic factors contributes to seed germination and seedling vigor. On the other hand, the percentage of the tissue water content of seedlings suggests that there is no statistically significant distinction between the 'Marmande' and 'Keckskeméti Jubileum' varieties under identical conditions. The insignificant difference in terms of tissue water content could be explained by comparable levels of hydration in the two varieties despite their disparity in seedling vigor. Thus, this may indicate that seedling vigor is more greatly influenced by factors other than tissue water content, such as nutrient uptake efficacy and metabolite rate.

**Table 4:** The percentage of seedling vigor index and tissue water content on two tomato varieties.

Genotype	Seedling Vigor Index	Tissue Water Content (%)
'Marmande'	55.03 b	76.53 a
'K. Jubileum'	105.00 a	83.27 a
Significance	S	NS

Values in columns sharing the same letters are not significant at the 5 Percent (%) HSD probability level. NS: Non-Significant & S: Significant

### Influence of the seed-borne pathogen (*Alternaria alternata*) on germination and vigor of two tomato varieties

#### The seedling fresh weight

From **Table 5** it seems that there is a statistical difference in salicylic priming. It was observed that when 3 ml of salicylic acid was used it produced a higher fresh weight of 33.95 mg at the germination process as compared to when no treatment was used at 21.45 mg in *Alternaria alternata* affected condition. The results therefore imply that salicylic acid is observably influential in seed germination although one cannot detect the real difference in the interaction of the two variants by themselves. According to research indicated that salicylic acid affects different plants differently. As an example, the use of a lower concentration of salicylic acid on pepper leads to an increase in the elongation of seedlings as well as higher fresh weight. Such revelations indicate that salicylic acid has a broad effect on various plants (Li & Liu, 2022). Besides, salicylic acid plays a major role in secondary signalling that activates defense response genes when plants are attacked by pathogens (Dolatabadian et al., 2008).

#### The seedling dry weight and seedling vigor index

Data presented in **Table 6** show that the 'Marmande' variety has a high dry weight of 1.29 mg while the 'Keckskeméti Jubileum' variety records a significantly low dry weight of 0.97 mg. Another similar pattern appears for dry weight where the addition of salicylic acid leads to the dry weight by 1.5 mg but showed 0.76 mg recorded when salicylic acid was not applied during seed germination. The observation implies that salicylic acid is considered an ingredient that affects the fresh and dry weights, especially concerning the 'Marmande' variety. Based on the previous study, the seedlings developed from grain that were pre-treated with salicylic acid exhibited increase leaf count, as well as fresh and dry mass per plant (Hayat et al., 2005).

**Table 5:** The fresh weight (mg) data of two types of tomato varieties under *Alternaria alternata* infection.

Concentration	Genotypes		Average of genotypes
	'Marmande'	'Kecskeméti Jubileum'	
3 ml salicylic acid	38.55 a	29.35 a	33.95 a
0 ml salicylic acid	22.55 b	20.35 b	21.45 b
<b>Average of concentration</b>	30.55 a	24.85 a	
<b>P-value (Genotypes)</b>		NS	
<b>P-value (Concentration)</b>		S	
<b>P-value (Interaction)</b>		NS	

Values in columns sharing the same letters are not significant at the 5 Percent (%) HSD probability level. NS: Non-Significant & S: Significant.

**Table 6:** The dry weight (mg) data of two types of tomato varieties under *Alternaria alternata* infection.

Concentration	Genotypes		Average of genotypes
	'Marmande'	'Kecskeméti Jubileum'	
3 ml Salicylic acid	1.65 a	1.35 ab	1.50 a
0 ml Salicylic acid	0.93 bc	0.59 c	0.76 b
<b>Average of concentration</b>	1.29 a	0.97 b	
<b>P-value (Genotypes)</b>		S	
<b>P-value (Concentration)</b>		S	
<b>P-value (Interaction)</b>		NS	

Values in columns sharing the same letters are not significant at the 5 Percent (%) HSD probability level. NS: Non-Significant & S: Significant.

**Table 7:** The seedling vigor index of two types of tomato varieties under *Alternaria alternata* infection.

Concentration	Genotypes		Average of genotypes
	'Marmande'	'Kecskeméti Jubileum'	
3 ml Salicylic acid	165.0 a	108.0 b	136.5 a
0 ml Salicylic acid	55.8 c	45.4 c	50.6 b
<b>Average of concentration</b>	110.4 a	76.7 b	
<b>P-value (Genotypes)</b>		S	
<b>P-value (Concentration)</b>		S	
<b>P-value (Interaction)</b>		S	

Values in columns sharing the same letters are not significant at the 5 Percent (%) HSD probability level. NS: Non-Significant & S: Significant.

**Table 8:** The seedling the growth rate of two types of tomato varieties under *Alternaria alternata* infection.

Concentration	Genotypes		Average of genotypes
	Marmande	Kecskeméti Jubileum	
3 ml Salicylic acid	1.65 a	1.68 a	1.67 a
0 ml Salicylic acid	1.3 ab	0.78 b	1.04 b
<b>Average of concentration</b>	1.48 a	1.23 a	
<b>P-value (Genotypes)</b>		NS	
<b>P-value (Concentration)</b>		S	
<b>P-value (Interaction)</b>		NS	

Values in columns sharing the same letters are not significant at the 5 Percent (%) HSD probability level. NS: Non-Significant & S: Significant

As shown in the statistics in **Table 7**, there were significant differences between the treatment groups and genotypes. It is obvious that for the 'Marmande' variety, seedling vigor index was about 110.4 compared to 76.7 for 'Kecskeméti Jubileum' variety. Also, upon application of 3 ml of salicylic acid, the seedling vigor index is elevated to 136.5 as compared to just 50.6 without the use of salicylic acid in similar conditions. Moreover, the combination of 'Marmande' with 3 ml of salicylic acid gives surprisingly more vigor 165.0 against 108.0 of 'Kecskeméti Jubileum' variety in response to the same dose. There does seem to be a non-substantial difference between the two varieties in the case of 0 ml salicylic acid. The effect of salicylic acid has a strong influence on the 'Marmande' variety making its percentage significantly higher than 'Kecskeméti Jubileum' especially *Alternaria alternata* was also present.

Based on the study of Hayat & Ahmad (2012) salicylic acid plays two distinct roles in curtailing pathogen invasion. It increases plant immunity, thus developing more vigorous reactions towards them, and, secondly, prevents the propagation by these pathogenic bacteria and fungi. Salicylic acid has been demonstrated to fulfil a significant signalling function in the initiation of diverse plant defense mechanisms subsequent to pathogen assault. Such mechanisms encompass the stimulation of both localized and systemic resistance against diseases, the enhancement of programmed cell death in host organisms, and the restriction of pathogen proliferation (Dempsey et al., 1999)

#### The seedling growth rate

As per **Table 8**, there seem to be statistically significant variations among the salicylic acid concentration. Similarly, as was noted under these assessments earlier, the application of 3 ml salicylic acid increases the seedling growth rate by about 1.67 mg against seedlings without salicylic acid, which grew at the rate of 1.04 mg. This shows that salicylic acid use still has effects on the development of seedlings' growth by increasing growth rate. However, there was no considerable difference between the different varieties. This is similar to the result of some previous study on the soybean and maize indicated that salicylic acid stimulating the seedling growth in vegetative period of these crops, and having the ability enhance the shoot and root development (Vicente & Plasencia, 2011).

#### Regression optimizer of parameters of two tomato varieties

The regression analysis from **Tables 9-10** shows that the 'Marmande' variety at 3 ml salicylic acid had an improved germination percentage compared to other varieties. Furthermore, there was better seedling dry weight and an improved seedling vigor index than the 'Kecskeméti Jubileum' variety. By contrast, the 'Kecskeméti Jubileum' variety has more disadvantages in terms of non-treatment with salicylic acid under the same condition (*A. alternata* contagion). The observation that the application of salicylic acid has greater and more noteworthy impacts on the stimulation of sprouting, the dry weight of seedlings and the vigor index of seedlings of the 'Marmande' variety is a significant finding. The seedling vigor index plays an important role in developmental plants and also gauges the capacity of those seeds to generate healthy seedlings when faced with suboptimal or challenging growth conditions (Collins & Edmisten, 2016).



**Table 9:** The germination, seedling dry weight, seedling vigor index parameter of two tomato varieties under primed and non-primed salicylic acid solution.

Solution	Goal	Lower	Target	Upper	Weight	Important
Germination (%)	Maximum	60	100	-	1	1
	Minimum	-	60	100	1	1
Seedling dry weight (mg)	Maximum	0	2.3	-	1	1
	Minimum	-	0	2.3	1	1
Seedling vigor index	Maximum	0	180	-	1	1
	Minimum	-	0	180	1	1

**Table 10:** Multiple maximum and minimum response optimization of two tomato varieties under primed and non-primed salicylic acid solution.

Level	Solution	Variety*	Priming*	G% Fit	SDW Fit	SVI Fit
Maximum	1	1	1	91.25	1.66	153.4
Minimum	1	2	2	66.25	0.6	33.8

Variety\*: 1 – ‘Marmande’; 2 – ‘Keckskeméti Jubileum’.

Priming\*: 1 – 3 ml of Salicylic acid; 2 – 0 ml of salicylic acid.

## Conclusions

In this study, a comprehensive experiment was conducted to investigate the effects of drought stress, nutrient deficiency water, and the presence of the seed-borne pathogen *Alternaria alternata* on the germination and vigor of two tomato varieties including ‘Marmande’ and ‘Keckskeméti Jubileum’. The results have meaningful implications for the responses of tomato plants to stressors and diseases commonly encountered in the field. This shows that drought stress has a major influence on tomato plants, a 5% concentration resulted in a low rate of germination while at a 10% of drought, there was no germination at all. Additionally, ‘Marmande’ variety is tolerable to drought and shows high seed germination compared to ‘Keckskeméti Jubileum’ under the same conditions. Tomato plants that were irrigated with filtered water had a stable seedling vigor index. Observed fact imply that, good water quality affects not only tomatoes' health but also the entire plant life. This study established that while both the ‘Keckskeméti Jubileum’ and ‘Marmande’ cultivars could be grown in pure water conditions, the former grew better than the latter under these specific environmental conditions. Some visual symptoms of leaf spot disease and stunted growth were recorded because of the infection of the seed-borne pathogen *Alternaria alternata*. The research findings indicated that the ‘Marmande’ tomato variety outperforms the ‘Keckskeméti Jubileum’ variety in terms of germination and seedling vigor index when selecting cultivars under fungal infection conditions, especially when using 3 ml salicylic acid during germination stages. This suggests that the ‘Marmande’ shows greater resilience and growth potential when facing the challenges posed by the presence of *Alternaria alternata*, rendering it a preferable selection for cultivation as opposed to the ‘Keckskeméti Jubileum’ under such circumstances.

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