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Exogenous Salicylic Acid Application During Germination of Silage Maize (*Zea mays* L.) Exposed to PEG-Induced Drought Condition

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A B S T R A C T

The aim of study was to investigate the effects of salicylic acid on germination and seedling parameters of maize cultivars (*Zea mays* L.) under drought stress conditions. The research was carried out in a factorial experiment design with four replications in random blocks. In this study, three different silage maize cultivars were used (Side, Pehlivan and Burak). Drought conditions were established using Polyethylene glycol-6000 (PEG-6000) at three different levels (0.-0.4 MPa and -0.8 MPa). Salicylic acid applications were calculated at three different doses of 0-0.1-0.2 mM. The parameters examined in Side cultivar gave superior results exposed to drought conditions compared to other cultivars. Differences were determined in the response of maize cultivars to drought stress, and statistically noteworthy diminishes were also observed as the drought level enhanced. It was displayed that salicylic acid applications generally boosted germination and seedling parameters exposed to drought conditions compared to control. The maximum shoot lenght was detected at 0.2 mM SA dose with 2.30 cm but that did not exhibit significant numerical differences. SA applications, on the other hand, did not have an effect on root length. Moreover, the best result of shoot fresh weight was recorded in 0.1 mM SA application, as root fresh weight gave the best in 0.2 mM SA application. Furthermore, when a correlation is made between the specified parameters, the highest relation was markedly positive and linked between GR and GI (r: 0.99, $p<0.01$). In this study, it was found that the growth deficiency that may occur under drought stress conditions that maize seeds may encounter during the germination period can be reduced and even improved by salicylic acid applications.

1. Introduction

Water restriction stress is a universal matter, restricting crop fertility and the last climate change scenarios got it more essential and imperiled food security (Abd El-Mageed et al., 2016). Water restriction is also in the substantial restrictions impressing product productivity (Anjum et al., 2011) and yield comprising significant cereals (Golbashy et al., 2010; Joshi et al., 2016; Hasanuzzaman et al., 2020). Drought conditions induce physiological, biochemical, and molecular modifications in plants (Shao et al., 2009), influencing cereal metabolism, growth, and yield fertility (Paupi`ere et al., 2014; Liang et al., 2020).

Water deficit seriously impresses plant physiology, modifying processes, such as osmotic potential, stomatal conductance (Brilli et al., 2019), carboxylation efficiency, photosynthesis rate (Wu

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et al., 2020; Xu et al., 2020), water potential (Taiz and Zieger, 2002), transpiration rate, growth (Sattar et al., 2021; Chakma et al., 2021), germination (Gökkaya and Arslan, 2023) osmolyte accumulation, and the statement of specific genes (Furlan et al., 2012; Abid et al., 2018). Furthermore, below in vitro situations, Polyethylene glycol (PEG), a non-ionic water polymer, is not anticipated to pass through plant tissue rapidly, is completely utilized to cause water stress (Macar et al., 2009). Since PEG does not go into the apoplast, water is drawn from the cell wall. Therefore, PEG solutions imitate dry soils in well than the others which infiltrate the cell wall (Verslues et al., 1998).

The damage extent to the physiological, cellular and molecular responses of plants to drought stress varies depending on the plant growth stage. Seed germination is the most noteworthy biological processes in the plant varieties cycle and immensely susceptible to its existing environment. Successful seed germination mostly affects the yield favorably. In addition as known, weak germination is common cause of decrease in plant yield in arid and semi-arid areas (Shatpathy et al., 2018; Yilmaz and Kizilgeçi, 2022).

Successful planting of normal seedlings, particularly under unfavorable environmental situations, is immediately addicted on the balanced synthesis of plant hormones (Gharbi et al., 2018; Shatpathy et al., 2018). These hormones, which are synthesized endogenously in plants, are considered as defense function compounds (Mohaddes Ardebili et al. 2019) and those that reduce lipid peroxidation (Nazari et al. 2020) in stress situations. External application of plant hormones is one of the most widely used techniques to reduce the effects of environmental stress on germination (Eisvand et al. 2010; Hajiabbasi et al. 2020).

Salicylic acid (SA) functions in the regulation of various physiological processes in plants (Shakirova et al., 2003). These tasks are to evolve plant growth (Metwally et al., 2003; Khodary, 2004, Wang et al., 2013), transpiration rates, stomatal regulation and photosynthesis (Khan et al., 2003), ion uptake and transport (Gunes et al., 2005), flowering and protein synthesis (Zaki and Radwan, 2011; Ullah et al., 2012), inhibition of ethylene synthesis (Ghassemi-Golezani et al., 2015) especially in drought conditions (Latif et al, 2016).

Selection and breeding is known to be essential to produce stress tolerant crop plants, but besides, exogenous application of osmoprotectants, growth promoting compounds to plants has been considered as a temporary solution to mitigate the negative effects of different stresses on plants in the last decade. The aim of study was to conducted with the effects of salicylic acid on germination and seedling parameters of maize (*Zea mays* L.) under drought stress conditions.

2. Material and Methods

This experiment was carried out in the forage crops laboratory, Department of Field Crops, Akdeniz University, Turkey during the autumn of 2022. The maize (*Zea mays* L.) seeds were obtained from Western Mediterranean Agricultural Research Institute. Three varieties were selected as Side, Pehlivan and Burak, that were provided as genetic material, that materials were harvested in 2022. Ten seeds from each cultivar were chosen and placed in 9 mm petri dishes, two Whatman filter papers were lined in. The petri dishes were settled in a growth chamber at 20 ºC under photoperiodic condition 16 hours light 8 hours dark. The experiment was carried out in four replications with factorial arrangement according to the randomized blocks design. Observations were recorded daily. Three drought stress levels causing 0, -0.4 MPa and -0.8 MPa were calculated by the equation of Michel and Merrill (1973) using PEG 6000 concentration. Salicylic acid was administered at doses of 0-0.1-0.2 mM due to its therapeutic effect. 10 ml of solution was used for moistening in each application. The study ended on the seventh day.

Germinatcion tests were carried out according to ISTA rules (2017). The seed of germination (MGT) was calculated using formulas described by Majda et al. (2019). Germination rate (GR) was calculated according to Xia et al. (2019). Germination index (GI) and seedling vigor index (SVI) were counted by the method of Xia et al. (2019). The root/shoot ratio (R/S ratio) was calculated as the following equation (Shtaya et al., 2021).

$$\text{MGT(day)}=\sum \frac{\text{number of seeds germinated on the } i^{th} \text{ day}}{\text{number of days to count the } n^{th} \text{day}} \quad (1)$$

$$GR(\%)=\frac{\text{number of germinated seed/}}{\text{total number of seed tested}}*100 \quad (2)$$

$$GI=\sum\frac{\text{the number of germinated seeds in day}}{\text{day of counting seed germination}} \quad (3)$$

$$SVI=\frac{\text{germination percentage*average seedling lenght}}{100} \quad (4)$$

$$R/S\ ratio=\frac{\text{roots lenght}}{\text{shoot lenght}} \quad (5)$$

Data obtained for the investigation subjected to analysis variance using R (ANOVA) and means were compared by one-way ANOVA and post hoc test of Duncan in the agricolae, which differed significantly at 0.05 levels. (4.3.19) package program.

3. Results and Discussion

According to variance analysis of the plant growth parameters of maize cultivars exposed to drought conditions with effects of salicylic acid application and were given in Table 1 and Table 2.

Based on variance analysis, maize cultivars gave a statistically noteworthy effect on experimental parameters except MGT, GR and GI ($p<0.01$). Similarly, boosting drought conditions substantially affected the growth parameters ($p<0.01$), yet only root fresh weight was significantly influenced ($p<0.05$) except mean germination time, germination rate, germination index, seedling vigor index. Moreover, increasing salicylic acid application caused notable ($p< 0.01$) effect on root length. The mean germination time, shoot length, root fresh weight and root/shoot ratio were noteworthy influenced by the cultivars and enhancing drought level interactions. Closely, cultivars and increasing salicylic acid doses caused a statistically ($p<0.01$) substantial change in germination and growth parameters of maize. Enhancing drought and salicylic acid interaction conditions showed a meanful effect on parameters examined in the study, except root parameters. Furthermore, that application interactions did not cause a statistically noteworhty change in the SVI and RL. (Table 1 and 2).

Table 1. Results of variance analysis on germination and growth parameters of salicylic acid doses in maize cultivars exposed to drought stress levels

Source of Variance	df	Mean Germination Time	Germination Rate	Germination Index	Seedling Vigor Index	Shoot Length	Root Length
C	2	0.44	94.52	49.86	14.10**	15.36**	126.22**
DL	2	1.63	499.60	743.85	0.31	29.93**	11.21*
SA	2	2.71	545.83	836.50	0.43	0.924	15.71**
C*DL	4	2.61*	391.60	575.75	0.32	3.15**	6.42
C*S	4	4.07**	680.96**	1038.65**	0.63**	3.90**	18.01**
DL*SA	4	3.45*	744.63**	1066.59**	0.35*	1.41**	0.82
C*DL*SA	8	2.35*	487.58*	698.43*	0.19	2.73**	3.29

*Significant at the 0.05 probability level.**Significant at the 0.01 probability level. (Cultivar: C, Drought level: DL, Salicylic acid: SA)

Table 2. Results of variance analysis on growth parameters of salicylic acid doses in maize cultivars exposed to drought stress levels

Source of Variance	df	Shoot Fresh Weight	Root Fresh Weight	Total Biyomass	Root/ Shoot Rate
C	2	4704.56**	48207.30**	88454.20**	35.20**
DL	2	58221.45**	15150.19*	110460.07**	47.71**
SA	2	2181.80	4529.44	6267.86	1.31
C*DL	4	1675.62	9297.07*	13173.95	14.38**
C*S	4	5424.96**	13991.86**	26207.77**	5.95*
DL*SA	4	3367.59**	7564.95	17746.17*	8.99**
C*DL*SA	8	7122.54**	6931.35*	22977.06**	8.24**

*Significant at the 0.05 probability level.**Significant at the 0.01 probability level. (Cultivar: C, Drought level: DL, Salicylic acid: SA)

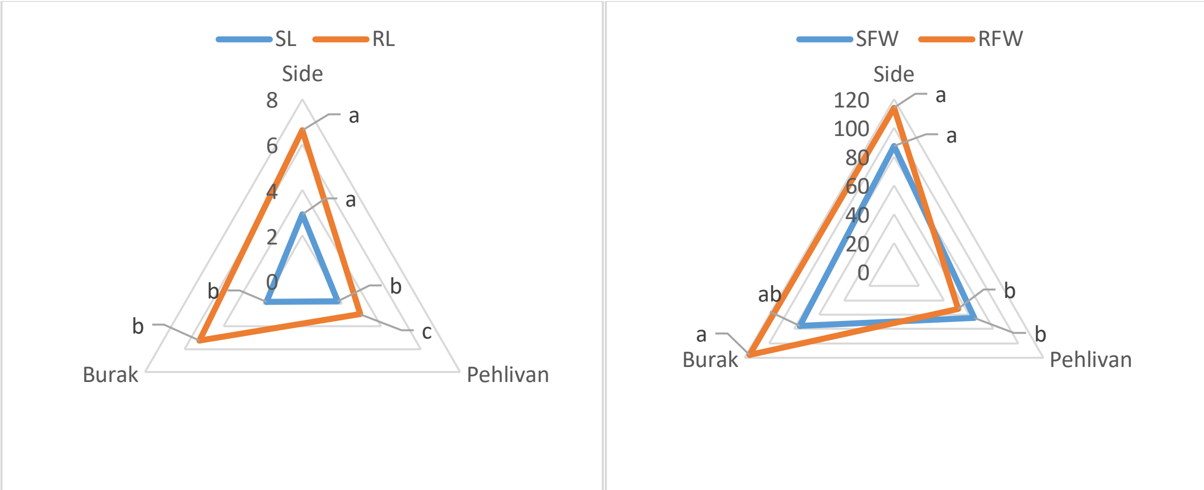

Figure 1. Effects of salicylic acid doses on growth of maize cultivars under drought conditions (Shoot length: SL, Root length: RL, Shoot fresh weight: SFW, Root fresh weight: RFW).

The effects of salicylic acid doses on germination and growth of maize cultivar exposed to drought stress were given in Figure 1 and Table 3. In this experiment investigated on silage maize cultivars, a distinction was recorded as expected. The unfavorable effects of drought stress on the growth, improving, and yield of maize is attached to the intensity of water deficit, growth stage and genotype (Ghassemi-Golezani et al. 2018).

In general, the best results were obtained in the Side cultivar. In this cultivar, shoot lenght was found almost half times higher than the others. Moreover, the root lengths were determined to be much longer than the shoot lengths. And, the root lengths were determined to be much longer than the shoot lengths. When examined in terms of fresh weight, the lowest averages were detected in Pehlivan, as expected based on observations of their length (Figure 1). The responses of the cultivars under stress conditions differ in terms of the parameters studied (Vishnupradeep et al., 2022)

Side was observed as an major cultivar from the standpoint of both component characteristics and germination speed, cause its mean germination time was the maximum. Germination rate and germination index were determined close and did not show a statistically substantial difference. It was stated that the difference between the root and shoot lengths of the Burak cultivar was high and therefore the R/S ratio was the highest, nevertheless the lowest total weight was found in this cultivar (Table 3).

Table 3. The effects of salicylic acid doses on growth parameters of maize cultivars exposed to drought stress levels

Cultivars	Mean Germination Time (day)	Germination Rate (%)	Germination Index (%)	Seedling Vigor Index (%)	Root/ Shoot Rate	Total Biyomass (mg)
Side	5.71	83.80	100.00	2.46a	2.66b	201.33a
Pehlivan	5.93	80.56	99.99	2.45a	2.08b	103.96b
Burak	5.88	82.18	97.96	1.37b	4.01a	168.76a

Different letters next to values indicate statistically different means at $p<0.05$ level, and $p<0.01$ levels.

The germination and growth parameters of maize cultivars under drought stress were displayed in Figure 2 and Table 4. As predicted, whilst the means were recorded at the superiorly in the control application, it was determined as the least in the PEG application at the dose of -0.8 MPa. The drought levels boosted as the growth declined. Some symptoms of drought stress lead to diminished leaf water content, loss of turgor pressure and stomatal closure, resulting in decreased cell and plant growth (Estaji and Niknam 2020). These negative effects adversely affect germination.

The parameters most affected by PEG-induced drought stress were noted as shoot length and fresh weight. Notwithstanding no large numerical difference was recorded in root lengths, it was found to be statistically noteworthy. Moreover, the maximum and minimum root fresh weight were obtained as 110.12 and 70.80 mg, respectively (Figure 1). The decreasing results of experiment were similar to the studies on plant height (Ye et al., 2016), fresh weight (Ghazi 2017; Shemi et al., 2021; Naz et al., 2021), root lenght (Bijanzadeh et al., 2019; Tanveer et al., 2023; Baltacier et al., 2023).

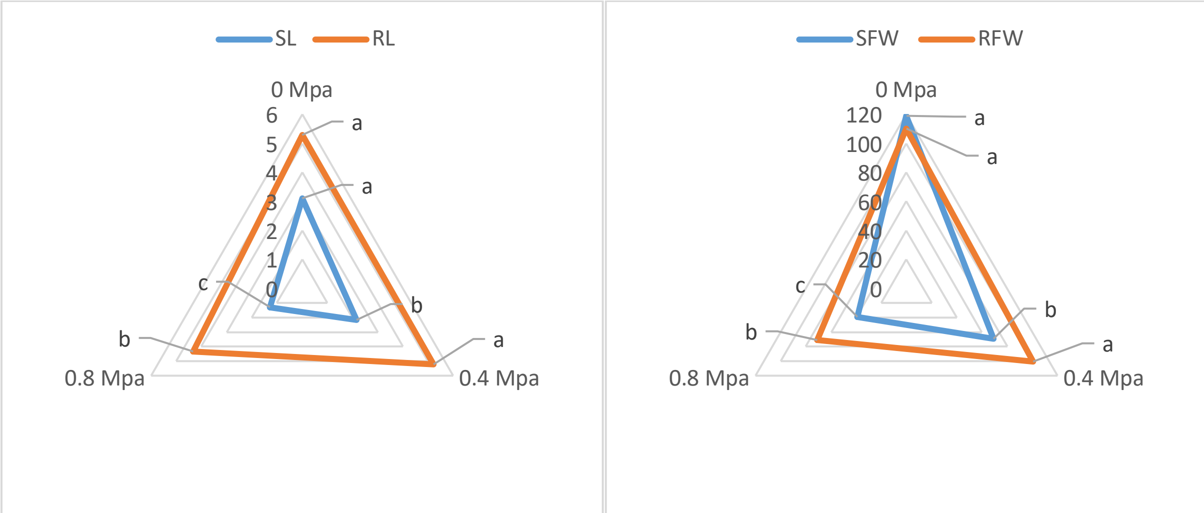

Figure 2. Effects of drought conditions on growth of maize cultivars (Shoot length: SL, Root length: RL, Shoot fresh weight: SFW, Root fresh weight: RFW).

Table 4. The growth parameters of maize cultivars exposed to salinity

Drought Level (MPa)	Mean Germination Time (day)	Germination Rate (%)	Germination Index (%)	Seedling Vigor Index (%)	Root/ Shoot Rate	Total Biyomass (mg)
0 MPa	5.73	86.34a	104.39a	2.20	1.82c	214.78c
-0.4 MPa	5.70	79.17b	95.60b	2.02	2.81b	155.16b
-0.8 MPa	6.08	81.02ab	97.95ab	2.20	4.12a	104.11c

Different letters next to values indicate statistically different means at $p<0.05$ level, and $p<0.01$ levels.

Interestingly, germination time was detected close to non-drought conditions with PEG at -0.4 MPa level, and even a little faster mean germination time in drought condition. On the contrary, the slowest germination was observed at the level of -0.8 MPa. The negative effects of drought conditions on plant growth criteria were determined, even the root/shoot ratio was approximately one in non-drought stress conditions, as it enhanced fourfold in the drought highest dose. Furthermore, this was another proof that the roots lengthen meanwhile the trunk shortens under stress conditions (Table 4). Droght produced a extraordinary reduction in germination percentage (Yilmaz and Kizilgeçi, 2022), germination index and seedling vigor index compared to control. Similar results of drought stress on germination were noted by Shatpathy et al. (2018), Ilyas et al. (2020) and Tanveer et al., (2023).

The corrective effect of low salicylic acid doses on maize cultivars germination and growth exposed to drought stress conditions were exhibited in Figure 3 and Table 5. The supreme means were obtained at 0.2 mM SA level. It was displayed that low doses impressed the growth criteria. The maximum shoot length was detected at 0.2 mM SA dose with 2.30 cm but that did not exhibit significant numerical differences. SA applications, on the other hand, did not have an effect on root length. Moreover, the best result of shoot fresh weight was recorded in 0.1 mM SA application, as root fresh weight gave the best in 0.2 mM SA application (Figure 3). The curative effect of salicylic acid in drought conditions was determined close to this research (Ghazi, 2017; Koo et al., 2020; Sohang et al., 2020; Shemi et al., 2021).

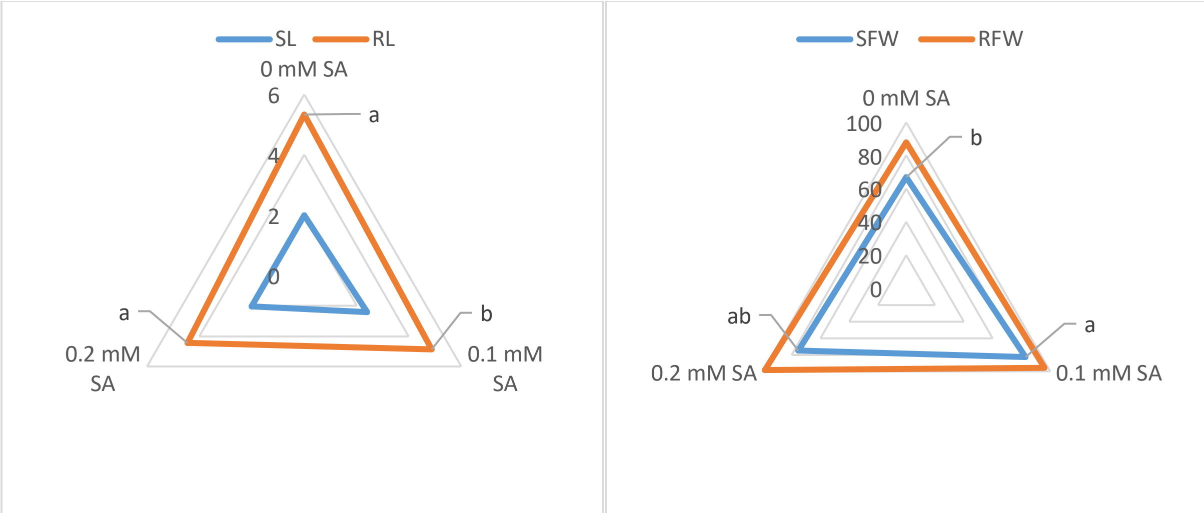

Figure 3. Effects of salicylic acid doses on growth of maize cultivars (Shoot length: SL, Root length: RL, Shoot fresh weight: SFW, Root fresh weight: RFW).

Table 5. The effects of salicylic acid doses on growth parameters of maize cultivars

Salicylic acid (mM)	Mean Germination Time (day)	Germination Rate (%)	Germination Index (%)	Seedling Vigor Index (%)	Root/ Shoot Rate	Total Biyomass (mg)
0 mM	5.99	85.19a	103.11a	2.19a	3.06	145.95
0.1 mM	6.00	83.57ab	100.94ab	2.12ab	2.70	156.00
0.2 mM	5.52	77.78b	93.89b	1.97b	3.00	172.10

Different letters next to values indicate statistically different means at p<0.05 level, and p<0.01 levels.

SA applications did not have much effect on mean germination time, germination time was found to be 5.84 days on average. The highest GR and GI levels occurred without SA administration. Moreover, the root/shoot ratios were not statistically different in general and were found to be approximately as 3 (Table 5). The application of low doses of salicylic acid reduces the effect of inhibiting germination in drought conditions, is also supported by other studies (Miura and Tada, 2014; Kulak et al., 2021). Salicylic acid increases water absorption in seeds. The use of salicylic acid prevented the destructive effects of drought stress on germination (Shatpathy et al., 2018; Bahrabadi et al., 2022) and growth (Tanveer et al., 2023; Sohang et al., 2020).

Table 6. Correlation of germination and growth parameters in maize cultivars

	MGT	GR	GI	SVI	SL	RL	R/L	SFW	RFW
GR	0.891**								
GI	0.899**	0.995**							
SVI	0.558**	0.622**	0.662**						
SL	-0.168	0.044	0.024	0.177					
RL	0.122	0.273**	0.219*	0.100	0.412**				
R/L	0.207*	0.095	0.068	-0.226*	-0.518**	0.327*			
SFW	-0.077	0.124	0.100	0.090	0.790**	0.308**	-0.491**		
RFW	-0.076	0.079	0.037	-0.119	0.500**	0.580**	-0.069	0.526**	
TB	-0.008	0.183	0.151	0.072	0.685**	0.552**	-0.267**	0.791**	0.854**

*Significant at the 0.05 probability level.**Significant at the 0.01 probability level. (Mean germination time: MGT, Germination Rate: GR, Germination index: GI, Seedling Vigor index: SVI, Shoot length: SL, Root length: RL, Root/shoot rate:R/S, Shoot fresh weight: SFW, Root fresh weight: RFW, Total biomass: TB).

The values of Pearson's correlation coefficients between germination and growth parameters of salicylic acid doses in maize cultivars exposed to drought stress levels were given in Table 6. RL had the most association with other parameters. The maximum relation was markedly positive and linked between GR and GI (r: 0.99, $p<0.01$). MGT was positively correlated with GR (0.891**), GI (0.899**), SVI (0.558**) and R/L (0.207*). Similarly, GR observed noteworthy positive correlations with GI, SVI and RL ($p<0.01$). In addition, a strong positive correlation was determined between GI and SVI (r: 0.662, $p<0.01$) and also moderate relation was reported between GI and RL (0.219*). Moreover, SL showed high positive association with RL, SFW, RFW and TB. Obviously, it substantially negatively correlated with R/L. That parameter did not have any significant correlation with germination parameters. Furthermore, R/L, SFW, RFW and TB exhibited strong relation with RL. A marked negative correlation was detected between SFW and TB. And also, RFW and TB showed noteworthy with SFW ($p<0.01$, Table 6).

4. Conclusion

As conclusion, PEG-6000 induced drought stress reduced germination and growth parameters. And, cultivars also were found to be different responses. The parameters examined in Side cultivar gave superior results exposed to drought conditions compared to other cultivars. Furthermore, noted that salicylic acid applications produced an boost in the parameters investigated under improving drought conditions. Thereby, thought that the different levels of drought and salicylic applications effect applied in the experiment on growth during the germination period may be beneficial for future research.

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Researchers Contribution Rate Declaration Summary

The authors declared that they have contributed to the article equally. All authors discussed the results and contributed to the final manuscript.

Statement of Conflict of Interest

Author has declared no conflict of interest.

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