RESEARCH ARTICLE
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Trade-offs in Root and Shoot Growth in Forage Pea [*Pisum sativum* (L.) *arvense*] with Foliar Applications of Synthetic Elicitor DPMP (2,4-Dichloro-6-{(E)-[(3-Methoxyphenyl) Imino] Methyl} Phenol) and SA (Salicylic Acid)

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ABSTRACT

Background: Climate change, abiotic and biotic stress pressure are forcing breeders and farmers to find alternative ways to improve and extend food production. Even though there are multiple ways to cope with stress conditions, alleviation of the stress by enhancing plant responses is one of the cheapest, environmentally safest and most direct ways. This study aimed to evaluate the effects of two common plant defense elicitors, 2,4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl} phenol (DPMP) and salicylic acid (SA) on plant growth and seedling vigor with forage pea [*Pisum sativum* (L.) *arvense*] as a model plant.

Methods: Two different chemicals, 100 μM SA and 10 μM DPMP were evaluated in response to Polyethylene glycol 8000 (PEG) treatment in a semi-hydroponic growth system. Root architecture and shoot growth parameters were evaluated. The experiment was designed according to completely randomized design with three replications and ten plants per replication.

Result: The effects of SA and DPMP foliar applications were significant on tap, lateral and total root lengths, number of lateral roots and root fresh weight. For most of the traits, SA and DPMP did not inhibit plant growth compared to control under treated and untreated conditions. Average lateral root length (aLatRL) was the noteworthy trait with significantly higher values in DPMP + unstressed conditions. Plants sprayed with DPMP had significantly higher (5.57 cm plant^{-1}) aLatRL values compared to SA (4.53 cm plant^{-1}) and control (3.01 cm plant^{-1}). The results of the current study suggest that SA and DPMP foliar spraying can be beneficial to reduce the effects of abiotic stresses at optimal doses defined for each species. DPMP can be a candidate as a sustainable pesticide alternative and growth-enhancing agent, similar to SA.

Key words: Elicitor, Forage pea, Osmotic stress, Root development, Salicylic acid.

INTRODUCTION

Plant species are naturally evolved to cope with environmental fluctuations. However, climate change and excessive abiotic and biotic stress pressure reduce plants' ability to be productive enough for the increasing population (Snowdon *et al.* 2021). Even though there are substantial developments on the resistance/tolerance to abiotic and biotic stresses, there is still a large gap between genotypic potential and current crop production levels (Jaggard *et al.* 2010). Drought and salt stress as two of the major limitations of agricultural production are becoming a growing threat worldwide, affecting an extending portion of the agricultural land (Yang and Guo, 2018). There have been a significant number of studies to reduce or eliminate the negative effects of osmotic stresses on plants (Nagel *et al.* 2014; Robin *et al.* 2021; Kouki *et al.* 2021), however, the success rate is quite limited due to the complex nature of the abiotic stresses and plants response mechanism (Witcombe *et al.* 2008; Tardieu and Tuberosa, 2010). Abiotic stresses such as ionic and osmotic stresses generally reduce or completely prevent photosynthesis and limit growth and production (Liang *et al.* 2018).

The plant defense system has a complex regulatory mechanism protecting plants against diseases (Tsuda *et al.* 2008; Sato *et al.* 2010). Salicylic acid (SA) is a phytohormone and one of the most important regulatory components of plant defense systems. Numerous reports showed the power of exogenous SA application to induce the plant defense system against a variety of pathogens (White, 1979; Bektas and Eulgem, 2015). Subsequent research demonstrated the activity of SA as a plant growth regulator. It enhances plant adaptation not only to biotic stresses but also against abiotic stresses such as drought,

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heavy metal and cold (Wani *et al.* 2016; Zhao *et al.* 2017). Moreover, increasing evidence has shown improved plant tolerance against drought by exogenous application of SA (Samota *et al.* 2017). Plant defense elicitors, aka 'Plant activators' are inducers of the plant defense system and protect plants against a variety of pathogens (Reddy, 2013; Bektas and Eulgem, 2015). Many studies have shown that in addition to SA, other characterized plant defense elicitors also increase plant adaptation to abiotic stresses. γ-aminobutyric acid (GABA) and β-aminobutyric acid (BABA), Acibenzolar S methyl (ASM), Sodium silicate and Saccharin were reported to enhance drought tolerance in broccoli (Jespersen, 2017; Venegas-Molina *et al.* 2020). Previous reports demonstrated that in addition to biotic stresses, plant defense elicitors may have the potential to increase abiotic stress tolerance and reduce the severity of stress factors including drought stress. 2, 4-dichloro-6-{(E)-[(3-methoxyphenyl) imino] methyl} phenol (DPMP) is a novel synthetic plant defense elicitor that has been shown to induce defense responses in *Arabidopsis thaliana* and tomato (Bektas *et al.* 2016) but its activity on abiotic stress tolerance has not been evaluated.

Forage plants are mostly legume or Poaceae species, that are mainly grown for fresh biomass and dry herbage production. Their sustainable production is the key to continuous farm animal and dairy production (Martin *et al.* 2017). Legume forage species are the most preferred forage group, due to their high protein content and nitrogen fixation advantage compared to Poaceae species (Chen *et al.* 2018). Forage pea [*Pisum sativum* (L.) *arvense*] is a legume species that is mostly used for fresh or dry herbage animal feeding (Çaçan *et al.* 2019). It is commonly grown around the world and is considered to be sensitive to salt stress (Grozeva *et al.* 2019). Improving abiotic and biotic stress tolerance levels of the current cultivars, or breeding new cultivars with better stress tolerance are considered to be the main paths to a climate-resilient production of forage pea. There have been several reports of forage pea seedling growth (Demirkol *et al.* 2019; Acikbas *et al.* 2021a), but none of the previous studies evaluated the effect of plant defense elicitors, such as SA and DPMP on osmotic stress tolerance and their role on growth in forage pea. Therefore, this study aimed to evaluate the effects of SA and DPMP foliar application on the osmotic stress tolerance, root-shoot growth and seedling vigor of forage pea, under controlled conditions.

MATERIALS AND METHODS

Forage pea cultivar GAP Pembesi is selected as the model plant for chemical application × osmotic stress tolerance interactions due to its known sensitivity to salt stress (Tekeli and Ateş, 2011) and known root developmental properties (Acikbas *et al.* 2021b). The study was conducted under controlled conditions in the Department of Agricultural Biotechnology, Siirt University, Siirt, Turkey (37°58′13.20″N -41°50′43.80″E). Mean temperature and relative humidity ranged between 25-27°C and 60-70%, respectively. The experiment was conducted under daylight conditions at 12:12h day/night. The study was established according to completely randomized design with three replications and ten plants per replication. Polyethylene glycol (PEG 8000) is applied as 10% and control treatment had no PEG treatment (0%).

SA was ordered from Sigma-Aldrich Chemie GmbH, Germany. DPMP was kindly obtained from Prof. Dr. Thomas Eulgem, University of California, Riverside, USA. SA and DPMP dissolved in 100% DMSO until 50 mM and 5 mM stock concentrations, respectively and then diluted with distilled sterile water to indicated concentrations. 100 µM SA, 10 µM DPMP, or control solution (0.2% DMSO) were applied as a foliar spray at the 7th and 10th days of growth. The growth system is a semi-hydroponic version of the modified cigar roll technique (Zhu *et al.* 2005; Acikbas *et al.* 2021a).

Seed surface sterilization was made with 70% ethyl alcohol (C_2H_5OH) and 5% sodium hypochlorite (NACIO) for 5 minutes and rinsed under running water for one minute. Seeds of similar size were placed between germination papers (60×40 cm) as ten seeds per germination paper, the method is slightly modified from (Hohn and Bektas, 2020). Each set of germination paper is covered with a second layer and rolled to fit in cylindrical containers filled with the specified solutions, 10% PEG 8000, or distilled water. The experiment was conducted on May 20th, 2021 and completed on June 7th, 2021.

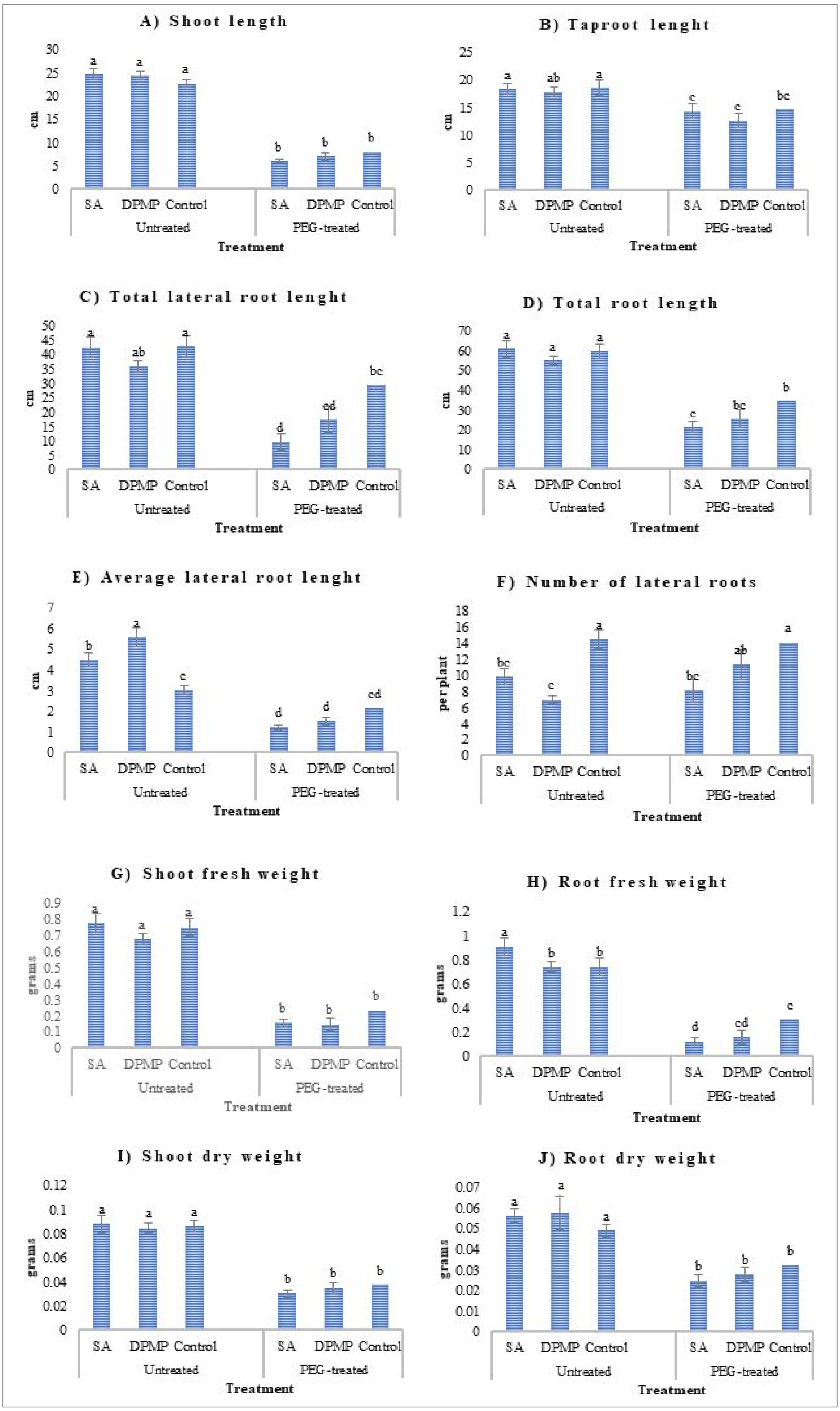
At the end of the experiment, each roll is separated and images of each plant are taken with a handheld scanner (Iscan Color Mini Portable Scanner) at 300 DPI resolution. Image analysis was performed manually using ImageJ (Rueden *et al.* 2017) software. Root and shoot growth (Acikbas *et al.* 2021a) and root architecture traits (Merrill *et al.* 2002; Acikbas, *et al.* 2021a) given in Table 1 were evaluated with image analysis. Stress tolerance-related calculations were made according to Moursi *et al* (2020).

The effects of SA and DPMP applications on osmotic stress tolerance and seedling growth vigor were analyzed with analysis of variance (ANOVA) using Statistix 10 software (Analytical Software; Tallahassee, FL, USA). Variance groupings were made using the Least Significant Difference (LSD) multiple comparison test (Steel *et al.* 1997).

RESULTS AND DISCUSSION

Root-shoot growth and seedling vigor

Shoot length (SL) under no stress conditions was similar between SA, DPMP and control. The SL under osmotic stress was also similar within the PEG treated group (Fig 1A). TapRL was the longest under SA and control compared to DPMP, while the control had better TapRL under osmotic stress. Similar results were also seen in TotalLatRL and TotalRL (Fig 1C and D). DPMP significantly improved aLatRL under untreated conditions, but it did not show the same effect under stressed conditions. NOLatR was better in control-treated and untreated conditions. SFW, SDW, RDW, SFW/RDW and SDW/RDW were similar



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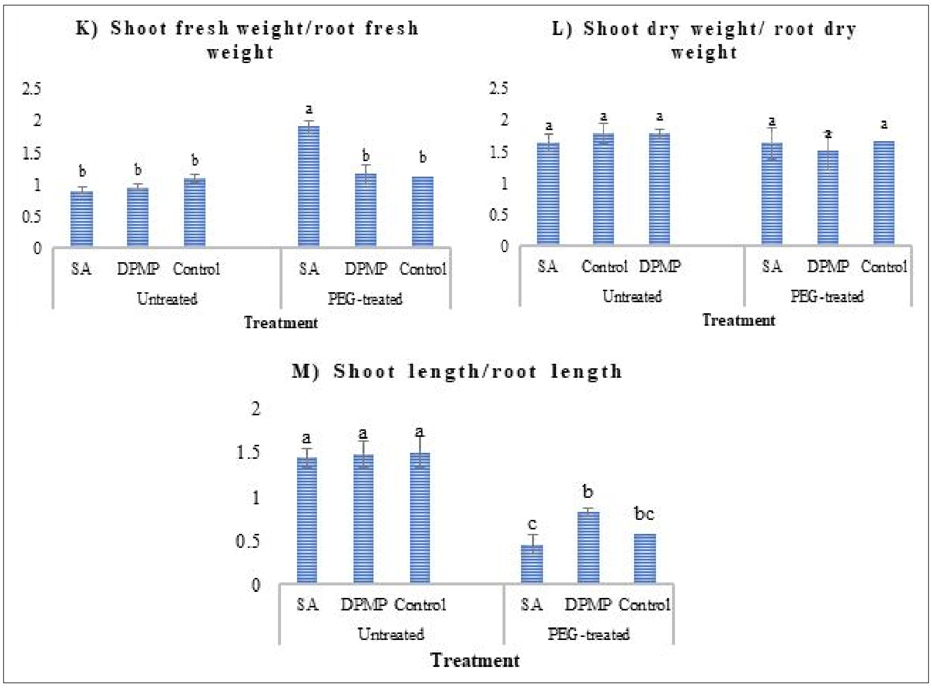

Fig 1: Effect of SA and DPMP foliar sprays on seedling development and vigor under osmotic stress (PEG 8000) and untreated conditions. For each trait, means followed by different letters are significantly different at a $p<0.05$ level according to the LSD test.

Table 1: Names, abbreviations and references of root and shoot traits evaluated.

Abbreviation	Trait name	Reference
NOLatR	Number of lateral roots	Merrill *et al.* (2002);
TotalRL	Total root length	Acikbas *et al.* (2021)
aLatRL	Average lateral root length	
TapRL	Taproot length	
TotalLatRL	Total lateral root length	
aLatRL	Average lateral root length (LatRL/ NOLatR)	
SL	Shoot length	
SFW	Shoot fresh weight	
RFW	Root fresh weight	
SDW	Shoot dry weight	
RDW	Root dry weight	
SL/RL	Shoot length/root length	
SFW/RFW	Shoot fresh weight/root fresh weight	
SDW/RDW	Shoot dry weight/root dry weight	
Reduction of RL	TotalRL contro-TotalRL stressed	Moursi *et al.* (2020)
Reduction of SL	SL control-SL stressed	
Reduction of SFW	SFW control-SFW stressed	
Reduction of SDW	SDW control-SDW stressed	
Reduction of RFW	RFW control-RFW stressed	
Reduction of RDW	RDW control-RDW stressed	
DTI	Drought tolerance index	
RL_DTI	(TotalRL stressed/TotalRL control)*100	
SL_DTI	(SL stressed/SL-control)*100	
RFW_DTI	(RFW stressed/RFW control)*100	
SFW_DTI	(SFW stressed/SFW control)*100	
RDW_DTI	(RDW stressed/RDW control)*100	
SDW_DTI	SDW stressed/SDW-control)*100	

within each group. On the other hand, RFW was higher in SA-untreated and control-untreated conditions. These results overall suggest that most of the root traits were similar within SA, DPMP and control untreated conditions and within treated conditions. We can suggest that SA and DPMP, neither inhibited nor enhanced some seedling root traits, except aLatRL, NOLatR, RFW and SFW/RFW ratio (Fig 1). Previous reports (Larqué-Saavedra and Martin-Mex, 2007) suggested growth-enhancing effects of SA can be seen under low SA doses. The dose we applied here was the spray dose used for biotic stress factors. So, the optimal SA dose for growth-enhancing effect and biotic stress tolerance may be different. Koo *et al* (2020) report significant biotic stress eliciting effects for SA, while results for plant growth under stress and optimum conditions were dose-dependent. SA becomes an inhibitory agent at high doses. The current dose we applied may be high for the growth enhancement of some root traits in forage pea. There is a need for the evaluation of different doses to define the right dose for growth enhancement in forage pea and other crops. These factors, tend to enhance plants' response to biotic stresses, but their effect on plant growth and vigor was not clear for some traits.

SA had higher values compared to DPMP and control on SL, TotalRL and SFW untreated and SFW/RFW treated conditions. DPMP had higher values in aLatRL, RDW and SDW/RDW untreated conditions (Table S1). However, some of these differences were not statistically significant (Fig 1). Pearson's correlation coefficients showed significant positive correlations between SL, SFW, RFW, SDW and RDW. TotalLatRL, aLatRL and TotalRL were in a positive correlation with shoot and root biomass traits (Table 2). Correlation analysis confirmed the above and below-ground growth interactions (Bektas *et al.* 2020).

Table 2: Correlation (Pearson) coefficients between root and shoot traits.

	SL	SFW	RFW	SDW	RDW	SFW/ RFW	SDW/ RDW	SL/RL	TapRL	NOLatR	Total LatR	aLatRL
SFW	0.86											
RFW	0.78	0.89										
SDW	0.74	0.79	0.73									
RDW	0.47	0.52	0.58	0.57								
SFW/RFW	-0.31	-0.25	-0.43	-0.31	-0.37							
SDW/RDW	0.09	0.10	-0.01	0.26	-0.26	0.33						
SL/RL	0.72	0.59	0.52	0.50	0.30	-0.23	0.12					
TapRL	0.20	0.26	0.28	0.22	0.18	-0.04	-0.08	-0.39				
NOLatR	-0.03	0.03	0.11	-0.01	0.04	-0.13	0.04	-0.09	0.16			
TotalLatRL	0.55	0.51	0.58	0.41	0.38	-0.31	-0.01	0.36	0.27	0.62		
aLatRL	0.57	0.46	0.49	0.39	0.34	-0.25	-0.03	0.40	0.20	-0.31	0.39	
TotalRL	0.55	0.52	0.60	0.47	0.39	-0.30	0.02	0.24	0.49	0.56	0.94	0.41

SL: Shoot length, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight, SFW/RFW: Shoot fresh weight/root fresh weight, SDW/RDW: Shoot dry weight/root dry weight, SL/RL: Shoot length/root length, TapRL: Taproot length, NOLatR: Number of lateral roots, TotalLatRL: Total lateral root length, aLatRL: Average lateral root length, TotalRL: Total root length.

Table S1: Descriptive traits for DPMP, SA and control under PEG treated and untreated conditions.

Variable	N	Mean	SD	SE Mean	Minimum	Maximum
			Treatment = DPMP-untreated			
SL	30	24.400	4.700	0.858	13.000	34.000
SFW	30	0.679	0.188	0.0343	0.100	0.960
RFW	30	0.741	0.230	0.042	0.150	1.020
SDW	30	0.085	0.021	3.83E-03	0.010	0.120
RDW	30	0.058	0.044	8.00E-03	0.020	0.280
TapRL	30	17.852	5.077	0.927	5.577	28.216
NOLatR	30	6.967	2.822	0.515	3.000	12.000
TotalLatR	30	35.915	10.449	1.908	13.350	54.050
aLatRL	30	5.574	2.385	0.435	2.120	10.810
TotalRL	30	54.928	11.222	2.049	31.950	77.350
			Treatment = DPMP-PEG			
SL	26	7.096	2.441	0.479	3.500	15.000
SFW	26	0.148	0.112	0.022	0.030	0.480

Table S1: Continue...

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RFW	26	0.164	0.143	0.028	0.020	0.460
SDW	26	0.036	0.016	3.10E-03	0.010	0.070
RDW	26	0.028	0.015	2.88E-03	0.010	0.050
TapRL	26	12.644	6.741	1.322	2.758	30.400
NOLatR	14	12.000	5.463	1.460	2.000	19.000
TotalLatR	14	19.669	9.759	2.608	4.150	37.540
aLatRL	14	1.701	0.473	0.126	1.080	2.850
TotalRL	26	26.875	14.755	2.894	7.510	66.680
			Treatment = Control-untreated			
SL	29	23.038	4.294	0.797	11.500	32.000
SFW	29	0.756	0.288	0.054	0.040	1.330
RFW	29	0.755	0.374	0.069	0.040	1.670
SDW	29	0.087	0.026	4.71E-03	0.03	0.130
RDW	29	0.050	0.016	3.04E-03	0.010	0.090
TapRL	29	18.589	7.375	1.370	6.956	33.924
NOLatR	29	14.586	6.214	1.154	2.000	27.000
TotalLatR	29	43.407	19.728	3.663	2.640	88.640
aLatRL	29	3.031	1.112	0.207	1.120	5.670
TotalRL	29	60.452	18.712	3.475	11.320	96.930
			Treatment = Control-PEG			
SL	28	8.050	3.774	0.713	1.000	18.000
SFW	28	0.229	0.196	0.037	0.070	0.900
RFW	28	0.304	0.305	0.058	0.020	1,430
SDW	28	0.038	0.024	4.52E-03	0.010	0,120
RDW	28	0.033	0.019	3.59E-03	0.010	0,090
TapRL	28	14.664	6.439	1.217	5.119	31,119
NOLatR	20	13.750	8.466	1.893	3.000	30,00
TotalLatR	20	28.672	20.254	4.529	1.540	78,340
aLatRL	20	2.005	0.713	0.160	0.510	3,380
TotalRL	28	35.142	25.431	4.806	5.120	103,200
			Treatment = SA-untreated			
SL	28	24.752	5.833	1.102	10.000	34.000
SFW	28	0.775	0.312	0.059	0.080	1.200
RFW	28	0.910	0.400	0.076	0.110	1.510
SDW	28	0.089	0.039	7.29E-03	0.020	0.210
RDW	28	0.057	0.019	3.57E-03	0.030	0.110
TapRL	28	18.252	4.926	0.931	10.136	33.949
NOLatR	28	9.893	5.174	0.978	3.00	24.000
TotalLatR	28	43.308	18.763	3.546	12.600	84.700
aLatRL	28	4.530	1.703	0.322	1.010	9.260
TotalRL	28	61.559	20.979	3.965	26.290	118.64
			Treatment = SA-PEG			
SL	27	6.147	2.797	0.538	2.500	13.500
SFW	27	0.151	0.141	0.027	0.051	0.667
RFW	27	0.120	0.140	0.027	0.016	0.576
SDW	27	0.030	0.022	4.23E-03	4.00E-03	0.113
RDW	27	0.025	0.015	2.95E-03	2.00E-03	0.065
TapRL	27	14.193	6.477	1.247	6.615	30.339
NOLatR	15	9.000	6.358	1.642	1.000	22.000
TotalLatR	15	13.605	13.493	3.484	1.403	51.815
aLatRL	15	1.377	0.446	0.115	0.641	2.591
TotalRL	27	22.312	15.135	2.913	8.051	76.349

Table 3: Drought tolerance index (DTI) comparisons for "SA PEG-SA untreated", "DPMP PEG-DPMP untreated" and "Control PEG- Control untreated" conditions.

Treatment	Reduction of TotalRL	Reduction of SL	Reduction of SFW	Reduction of SDW	Reduction of RFW	Reduction of RDW	RL_DTI	SL_DTI	RFW_DTI	SFW_DTI	RDW_DTI	SDW_DTI
SA Untreated -SA-PEG	39.25	18.61	0.62	0.057	0.79	0.031	36.24	24.83	13.16	19.48	44.28	34.58
DPMP Untreated -DPMP-PEG	28.05	17.30	0.53	0.049	0.58	0.028	48.93	29.08	22.10	21.72	50.53	41.81
Control Untreated -Control-PEG	25.31	14.99	0.53	0.049	0.45	0.018	58.13	34.94	40.39	30.26	63.53	43.92

TotalRL: Total root length, SL: Shoot length, SFW: Shoot fresh weight, SDW: Shoot fresh weight, RFW: Root fresh weight, RDW: Root dry weight, DTI: Drought tolerance index.

Above and below ground growth and vigor supports each other under normal and moderate stressed conditions, while under severe or long-term stress root-shoot relations may change based on genotypic potential and genotype × environment interactions (Ye *et al.* 2018).

Stress tolerance

The effects of SA and DPMP on plant growth and stress tolerance were evaluated under PEG-8000 treated and untreated conditions. To clearly see the effect of each treatment, a total of six different combinations were applied; SA, DPMP and control with and without osmotic stress. Therefore, we were able to compare the role of each elicitor under normal growth and stressed conditions. Reduction on TotalRL, RFW and RDW was the minimum on control, while reduction rates on SFW and SDW were similar in DPMP and control. Control untreated had the highest drought tolerance index (DTI) values compared to control-treated applications. It was followed by DPMP, which was close to control and SA had the least DTI values (Table 3). These results suggest that, even though SA and DPMP are common biotic stress response elicitors, they may not enhance plant growth under abiotic stress if the optimum dose is not applied (Hayat *et al.* 2010; Koo *et al.* 2020). Hayat *et al.* (2010) and references therein reported enhanced root and shoot growth at low doses of SA. Also, DPMP improved root length at lower concentrations while higher doses reduced root length on *Arabidopsis thaliana* (Bektas *et al.* 2016). The effect of this novel plant activator, DPMP, on plant growth and stress tolerance needs to be determined for defining appropriate doses for DPMP at foliar application.

CONCLUSION

This study aimed to evaluate the effects of two different biotic stress tolerance elicitors on seedling growth and development under osmotic stress and non-stressed conditions. It was seen that for some traits SA and DPMP did not inhibit or enhance plant growth compared to control under treated and untreated conditions, while their effects on aLatRL were noteworthy. Under untreated conditions plants sprayed with DPMP had significantly higher (5.57 cm plant^{-1} in DPMP, 4.53 cm plant^{-1}in SA and 3.01 cm plant^{-1} in control) aLatRL values. This was possibly due to inhibited NOLatR compared to control. Control had much higher (14.59 plant^{-1}) NOLatR than SA (9.89 plant^{-1}) and DPMP (6.97 plant^{-1}). Based on our results, these two defense elicitors can be applied as a foliar spray to enhance plant immune responses against pests and diseases, without any issues on growth limitation.

Conflict of interest: None.

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