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Effects of Chemical Seed Priming on Germination and Antioxidant Enzymes Activity of Two Cucumber (Cucumis sativus L.) Cultivars

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ABSTRACT

Seed priming is one of the most important measures in propagation of plants by seed, which leads to increase of percentage and rate of germination. This study was conducted as a factorial arrangement in a completely randomised design on two farm cucumber cultivars ('Impress' and 'Emperor'). Priming treatments were silicic acid (SA) and ascorbic acid (AsA) at three levels of 50, 100 and 150 mg L-1, pyridoxine at three levels of 0.02%, 0.04% and 0.06% and compound treatment (SA 75 mg L-1 + AsA 75 mg L-1 + pyridoxine 0.03%). There was a positive and significant correlation between the Catalase (CAT) enzyme activity in the radicle and plumule and Polyphenol oxidase (PPO) in the radicle and germination rate and percentage. In the 'Impress' cultivar, the highest CAT activity in the radical and plumule was observed after using of 150 mg L-1 AsA. The highest activity of Polyphenol oxidase (PPO) in the radical was detected after using of 0.04% pyridoxine. In the 'Emperor' cultivar, the highest CAT enzyme activity was detected in the radical and plumule, respectively by using of 75 mg L-1 AsA, 75 mg L-1 SA, and 0.03% pyridoxine. AsA (50 mg L-1) induced the highest activity of PPO enzyme in the radicle. Finally, to increase the antioxidant enzyme activity in cucumbers against unfavourable environmental conditions, our results confirmed the effectiveness of 150 mg L-1 AsA and 0.04% pyridoxine for the 'Impress' cultivar and the compound treatment and 50 mg L-1 AsA for the 'Emparator' cultivar.

Introduction

Cucumber (Cucumis sativus L.) is an annual plant from Cucurbitaceae family. It usually has a creeping or climbing growth habit, and some cultivars have a bushy growth habit. The root system is extensive, but usually with low depth. Its fruit is used freshly and also processed as pickles in the food basket (Daneshvar, 2001).

Emperor cucumber seed is marketed by one of the largest companies that are called Seminis which produces high quality seeds. The fruits produced from the cucumber seeds of the hybrid Emperor are dark green, very market friendly and high quality. The plants produced by this seed are considered strong. Due to the high marketability and exceptional quality of fruits, this cultivar in the wholesale market results in a high price. This cultivar of cucumber with high yield and high profitability can be one of the most popular options among farmers.

'Empress' cucumber seed is one of the outdoor cucumber cultivars. 'Impress' Cucumber Seed is a product of the French company Wilmourin and is marketed in boxes which contain 5000 seeds. 'Impress' outdoor cucumber fruit is mostly with a length of 16 to 17 cm and a diameter of 3.5 to 4 cm and dark green color. This cultivar is one of

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the earliest open cucumber cultivars, which is preferred by many farmers.

Cucumber (Cucumis sativus L.) has epigeal germination that needs energy and a high metabolic rate during germination. To reach a maximum rate in germination, different methods such as seed priming are used. In the priming method, the seeds are allowed to absorb some water so that germination stages are achieved before the radicle has emerged. In other words, the seed is advanced to the second stage of imbibition but does not enter the third stage, i.e. the radicle does not emerge (McDonald et al., 1999). Multiple studies have revealed that priming causes an increase in the percentage, rate and uniformity of germination and also the emergence of seedlings (Ashraf and Rauf, 2001; Murungu et al., 2003). The priming technique allows for early transcription of DNA, increases in RNA and synthesis of protein in the seeds and increases the growth of the embryo, repair of the damaged parts of the seed and decreased metabolite secretions. These factors can improve the number and uniformity of seed germination and the emergence of seedlings (Omidi et al., 2005). For these reasons, priming treatments are used both under normal and stressful conditions (Basra et al., 2004).

Rehman (2011) concluded that treatment of seeds with 50 mg L-1 SA could be successfully used to improve germination and seedling growth in cucumber. The results showed a trend of tolerance to the effects of copper at different concentrations on seed germination of Cucurbita pepo. The hydro-priming improved the morphology of germinated seeds in Cucurbita pepo. Similarly, it enhanced the tolerance index and the T/50 in the no stressed seeds by copper. Priming with GA3 at low dose had no effect on seed germination of this species (Bankaji et al., 2017).

The plain area of Khuzestan province is one of the plains of Iran that in the middle and southern part of it salinity and soil heaviness causes a severe reduction in seed germination. In this regard, it has been reported that the priming technique increases the germination range in stressful environmental conditions such as salinity and drought (Ashraf and Foolad, 2005). Priming has also led to increased yield in plants (Harris et al., 2001). It has been shown that priming with SA or AsA on pumpkin seedlings grown under saline (10 dS m-1) conditions reduced the severity of the salt stress, and the amelioration was obtained due to 30 mg L-1 AsA or 30 mg L-1 SA treatments as these treatments showed best results on seedling growth, fresh and dry matter production under non-saline and saline environments. Rafique et al. (2011) also reported that priming with SA or AsA in pumpkin seed ameliorates the adverse effects of salt stress.

It has been reported that the AsA treatment improves germination percentage, speed of germination and the vigour index compared with the control, and increases length of shoot, primary and seminal root and number of seminal roots. The best result was achieved by 55 mM AsA. The result of field experiment showed that interactions were not always significant and 55 mM AsA increased the seedling height, the number of leaves and leaf area but it had no effect on the water deficit and root length (Hamama and Murniati, 2010). Seed priming with either 20 mg L-1 or 40 mg L-1 solutions of H2O2, AsA and SA causes maximum seed invigoration and better performance in maize through inducing superoxide dismutase (SOD) activity and improves nutrient contents in root and shoot. Application of 20 mg L-1 of H2O2, AsA and SA seem to be suitable concentration for seed priming (Gul et al., 2015).

Pyridoxine-priming treatments improved seed germination and early seeding growth traits included germination percentage, coleoptiles and radical length, seeding dry matter accumulation, mean germination time (MGT), germination index (GI), vigour index (VI), and time to 50% germination (T50) of wheat genotypes. Seed pyridoxine priming duration of 12 h produced maximum value for most of the germination and early seedling growth characteristics of wheat inbred lines of PBW-154 and PBW-343. These results have practical implications in the pre-sowing seed treatment with pyroxene solution, which could enhance the seed germination and early seedling growth characteristics of wheat plant (Zanjan and Asli, 2012).

Hydro-priming increased the percentage and speed of seed germination and accelerated the growth of two types of cucumbers that had low and high germination percentages (Rukui et al., 2006). Seed priming with AsA and hydro-priming increased CAT and SOD activity (Burguieres et al., 2007). The highest percentage and germination rate, normal seedling percentage and seedling dry weight were related to the concentration of 100 ppm AsA.

In this study, effects of different concentrations of AsA, pyridoxine and SA on seed germination, morphological characteristics and activity of some antioxidant enzymes in two cultivars of cucumber were evaluated. Therefore, the aim of this study was to demonstrate the effect of seed priming and germination stimulants on some

antioxidant enzymes during the germination of two cucumber cultivars.

Materials and Methods

Plant materials and treatments

To study the effects of seed priming on germination features and seedling growth of farm cucumbers, an experimental was conducted using a completely randomised design with a factorial arrangement and three replications. The first factor was cultivar including: 'Impress' and 'Emparator' (obtained from Vilmorin and Seminis companies, respectively); and the second factor was priming including control (no priming), salicylic acid (SA) and ascorbic acid (AsA) at concentrations of 50, 100, and 150 mg L-1; and pyridoxine at concentrations of 0.02, 0.04, and 0.6%; and combined treatment with 75 mg L-1 of SA, 75 mg L-1 AsA and 0.03% pyridoxine (Table 1). The seeds were immersed in different solutions for 16 h at 25°C. Then the seeds were placed in petri dishes between two layers of filter paper (20 seeds per replicate at 25 °C) and incubated in the incubator (At a temperature of 25 to 27 °C and a relative humidity of 75%).

Table 1. Treatments of silicic acid, pyriodoxine, and Control in experiment

Treatment No.	Explanation of treatment
T1	Control
T2	50 mg L^{-1} Salicylic acid
T3	100 mg L^{-1} Salicylic acid
T4	150 mg L^{-1} Salicylic acid
T5	50 mg L^{-1} Ascorbic acid
T6	100 mg L^{-1} Salicylic acid
T7	150 mg L^{-1} Salicylic acid
T8	2% Pyriodoxine
T9	4% Pyriodoxine
T10	6% Pyriodoxine
T11	75 mg L^{-1} Ascorbic acid + Pyriodoxine 3% + 75 mg L^{-1} Salicylic acid

Counting of germinated seeds was done daily until the time when there were no more germinated seeds. Then germination parameters such as germination percentage and rate, mean daily germination, pick value and germination value were calculated.

The 'Emperor' plant has a high yield potential, is very productive, fruitful and tasty. 'Emperor' cucumber is very tolerant to some of the common plant pathogens, such as: Cucumber mosaic virus (CMV), Watermelon mosaic virus, Zucchini yellow mosaic virus (ZYMV), Papaya ring spot virus (PRSV), Powdery Mildew, and Downy Mildew. Beit Alpha hybrid Empress Cucumber is suitable for outdoor cultivation (spring, summer, and early autumn cultivation). It is very productive, with medium to strong vegetative vigor and adaptation to difficult environmental conditions. 'Empress' cucumber with dark green fruit skin with a suitable and marketable groove, very early ripening, is resistant to true whitefly fungal disease, false whitefly fungal disease, also resistant to cucumber mosaic virus and zucchini yellow mosaic virus, papaya ring spot virus, watermelon mosaic virus. It has been the best-selling seed in Iran in recent years. The number of seeds consumed per hectare is 60000, which is consumed in pairs in each pit with two seeds or seedlings.

Furthermore, vegetative traits such as radicle length, plumule length (using digital calliper with an accuracy of one tenth of a millimetre), radicle weight and plumule weight (using Kern model digital scale with an accuracy of 0.001 g) were measured. Germination value was calculated by multiplying the mean germination (MDG) by the maximum value (PV). To calculate the daily germination average, the number of germinated seeds was divided by the number of days. The maximum value was calculated by determining the germination peak point (in which the highest germination percentage took place) and the total number of germinated seeds until that day divided by the day this number of seeds germinated.

Germination rate was also calculated from the following equation, in which the unit of Germination rate is number divided by day (Maguire, 1962):

Germination rate = No. of germinated seeds per day/No. of days (1)

Evaluating the activity of antioxidant enzymes

Seven days after pre-treatment, extraction of enzymes was performed for the radicle and plumule. To prepare an enzymatic extract for measuring antioxidant enzymes, 0.5 g of plant

sample powder was poured into a micro tube and 1.5 mL of phosphate buffer (0.1 µM) with pH=7 was added to the plant sample and centrifuged in refrigerated centrifuge (Hitachi model). Then after settling, the supernatant was used as an extract to measure enzyme activity.

Measurement of superoxide dismutase (SOD) activity

SOD activity was measured by the method described by Giannopolitis and Ries (1977). The amount of enzyme in leaf samples in terms of µM per gram of fresh tissue was calculated based on the following equation:

$$SOD_{activity} = \frac{A_{560}(control) - A_{560}(sample)}{A_{560}(control)} \times 100 \quad (2)$$

Where, A_{560}(control) and A_{560}(sample) are the light absorption values of the control solution respectively (control composition: sample composition without enzyme extract, which has also been treated with light) and sample (including 300 µL of enzyme extract, 300 µL riboflavin (1 µM), 300 µL Nitroblobotrazolium chloride (*NBT*) (75 µM), 300 µL L-Methionine (12 mM), 300 µL sodium carbonate (50 mM), 1500 µL phosphate buffer (pH=7) (50 mM) at 560 nm wavelength.

Measurement of Ascorbate Peroxidase (APX) activity

APX activity was measured by the method described by Huang et al. (2009). Absorption at 290 nm was read twice with a Varian 0.5 spectrophotometer at two-min intervals. The final reaction composition includes 2200 µL phosphate buffer (100 mM), 300 µL ascorbate (10 mM), 300 µL hydrogen peroxide (100 mM), 200 mL enzyme extract. Blunk contains all of the above except the enzyme extract. The amount of this enzyme in the samples was calculated in µM of ascorbate per minute in mg of protein.

Measurement of Polyphenol oxidase (PPO) activity

For PPO activity, the absorption at 410 nm was read twice with a "Varian 0.5" spectrophotometer at two-minute intervals. Plant samples include (1800 µL phosphate buffer (100 mM), 600 µL Catechol, 600 µL enzyme extract). Blunk contains all of the above except the extract. This enzyme was evaluated by Solvia et al. (2001) and its amount was calculated in terms of absorption changes in mL of enzyme extract.

Measurement of Catalase (CAT) activity

For CAT activity, the absorption at 240 nm with the "Varian 0.5" spectrophotometer was read twice at two-minute intervals. Samples include 2400 µL phosphate buffer (50 mM) (pH=7), 100 µL enzyme extract, 500 µL oxygenated water (10 mM). Blunk contains all of the above except enzymatic extract. CAT was measured by the method of Aebi (1983) and its amount was calculated in mM of hydrogen peroxide reduction per mg of protein per min (Aebi, 1983).

Statistical Analysis

The data were analysed using SAS 9.1 software and the means were compared with the Duncan test at a 5% level. Correlation coefficients between germination traits and enzyme activity were calculated using SPSS 16.0 software.

Results

The results of ANOVA showed that the interaction effects of cultivar and chemical priming on radicle weight, plumule weight, total seedling weight, APX activity in radicle and plumule, CAT activity in radicle, SOD in radicle, and radicle PPO were significant. Interaction of cultivar and treatment on germination rate, plumule length, radicle weight, APX activity in radicle and plumule, CAT in radicle and plumule, SOD in radicle and plumule, PPO in radicle and plumule were also significant. Simple effect of cultivar was significant on mean daily germination, germination value, and radicle length (Tables 2, 3, and 4).

Table 2. Analysis of variance of data related to traits of germination of cucumber seeds as affected by chemical priming and cultivar

S.V	D.F	Mean Square (MS)				
		Germination percentage	Germination velocity	Peak value (PV)	Mean daily germination (MDG)	Germination value (GV)
cultivar (A)	1	1.52^{ns}	10.58**	22.8^{ns}	95.0**	1016149**
Treatment (B)	10	62.73**	81.80**	2255.9**	49.9**	830138**
AB	10	4.85^{ns}	2.60**	47.8^{ns}	17.9^{ns}	189154^{ns}
Error	44	5.68	0.79	58.4	12.2	119183
C.V%		2.4	5.0	8.9	29.8	32.8

$^{ns, *, **}$: Not significant and significant at $p<0.05$ and 0.01, respectively.

Table 3. Analysis of variance of data related to seedling vegetative growth traits as affected by chemical priming and cultivar

S.V	D.F	Mean Square				
		Radical length	Plumule length	Radical weight	Plumule weight	Total weight
Cultivar	1	5245.1**	305.7**	0.0083**	0.00030ns	0.0054**
Treatment	10	1023.3**	29.1 ns	0.0030**	0.00263**	0.0103**
Cultivar ×Treatment	10	103.4 ns	26.9 ns	0.0008**	0.00045*	0.0022**
Error	44	104.0	14.6	0.0001	0.00020	0.0004
C.V %		10.4	11.9	8.4	6.6	5.8

ns, *, **: Not significant and significant at $p<0.05$ and 0.01, respectively.

Table 4. Analysis of variance of data related to antioxidant enzymes in cucumber seedlings as affected by chemical priming and cultivar

S.V	D.F	Mean Square							
		APX		CA		SOD		PPO	
		radical	plumule	radical	plumule	radical	plumule	radical	plumule
Cultivar	1	123.8**	0.2 ns	2.411**	7.76ns	1696.0**	49750.4**	0.057**	0.0123**
Treatment	10	9.5**	206.7**	1.466**	31.18**	1.5**	60.0**	0.021**	0.1445
Cultivar × Treatment	10	7.9**	178.3**	0.333**	5.07 ns	0.6**	35.3**	0.010**	0.1378**
Error	44	1.9	1.6	0.101	3.12	0.1	4.2	0.002	0.0004
C.V %		20.7	11.0	17.9	20.2	2.8	3.6	11.4	6.1

ns, *, **: Not significant and significant at $p<0.05$ and 0.01, respectively.

Percentage and rate of germination

In the two investigated cultivars with regards to germination percentage, there was a significant difference between priming treatments and the control group. There were no significant differences among treatments for the 'Impress' cultivar; however, for the 'Emperor' cultivar, pyridoxine 0.04% significantly reduced the germination percentage in comparison to other treatments and it caused a higher germination percentage in comparison to control. With regards to germination rate, in the 'Impress' cultivar, treatment with 100 mg L^{-1} SA had the highest rate of germination followed by treatment with 50 mg L^{-1} SA. There were no significant differences between these two treatments. However, in the 'Emperor' cultivar, treatment with pyridoxine 0.02% had the highest germination rate and after those treatments with SA at 50, 100 and 150 mg L^{-1}, and AsA at 50 and 100 mg L^{-1} increased germination rates. There were no significant differences among the above mentioned treatments. The control had the lowest rate of germination and there was a significant difference from all other treatments (Table1). In both cultivars, different treatments had various effects, which are due to the genetic differences between the two cultivars.

Radicle weight, plumule weight and total seedling weight

In the 'Impress' cultivar, treatments with SA at 150 mg.L^{-1} produced the highest radicle weight followed by treatment with pyridoxine 0.02%. There was no significant difference between these two treatments; however, the control group had the lowest radicle weight. In the 'Emparator' cultivar, treatment with AsA at 50 mg L^{-1} had the highest radicle weight after compound treatment and pyridoxine 0.06%, but there was no significant difference between these two treatments. In the 'Impress' cultivar, treatment with 150 mg L^{-1} SA had the highest plumule weight followed by treatment with pyridoxine 0.04%; but there was no significant difference between these two treatments. With regards to plumule weight, in the 'Impress' cultivar, treatment with 50 mg L^{-1} AsA had the highest weight followed by treatment with 100 mg L^{-1} SA; but there was no significant difference between these two treatments. The control group had the lowest plumule weight with a significant difference from all treatments. In the 'Impress' cultivar, treatment with 150 mg L^{-1} SA had the highest weight of the total seedlings followed by treatment with 150 mg L^{-1} AsA and a significant difference was observed between these two treatments. The control had the lowest weight among all seedlings, which was significantly different from all other treatments.

In the 'Emperor' cultivar, treatment with 50 mg L^{-1} AsA produced the highest seedling weight. The control showed the lowest total seedling weight and it had a significant difference from all treatments (Table 5). The reason for a lower weight in the control treatment can be attributed to the positive effects of priming treatments, because seed priming improves seedling growth as well as germination. There was a strong correlation between CAT enzyme activity in the radicle and plumule and PPO in the radicle with the germination percentage and rate (Table 6).

Antioxidant enzyme activity in the 'Impress' cultivar

In the 'Impress' cultivar, treatment with 150 mg L^{-1} SA showed the highest activity of APX activity in the radicle. Treatments with 150 mg L^{-1} AsA, pyridoxine 0.04% and pyridoxine 0.02% caused the highest CAT activity in the radicle and plumule. Compound treatment resulted in the highest SOD activity in the radicle. After treatment with pyridoxine 0.02%, the highest amount of SOD activity was observed in the plumule followed by treatments of compound, SA 75 mg L^{-1}, AsA 75 mg L^{-1} and pyridoxine 0.03%. Treatment with 150 mg L^{-1} AsA resulted in the highest activity of PPO in the plumule (Table 7).

Antioxidant enzyme activity in the 'Emperor' cultivar

In the 'Emperor' cultivar, treatment with pyridoxine 0.02% resulted in the highest APX enzyme activity in the radicle. Treatment with 100 mg L^{-1} AsA resulted in the highest APX enzyme activity in the plumule. Compound treatment led to the highest CAT enzyme activity in the radicle and plumule. Treatment with 150 mg L^{-1} AsA resulted in the highest SOD activity in the radicle. However, treatment with 100 mg L^{-1} AsA resulted in the highest SOD activity in the plumule. Treatment with 50 mg L^{-1} AsA resulted in the highest PPO activity in the radicle and plumule (Table 8).

Table 5. Effect of chemical priming and cultivar on germination and vegetative growth characteristics of two field cucumber cultivars

Parameter / Treatment	Germination %		Germination velocity (No./day)		Radicle weight(g)		Plumule weight(g)		Total weight (g)	
	'Impress'	'Emperor'	'Impress'	'Emperor'	'Impress'	'Emperor'	'Impress'	'Emperor'	'Impress'	'Emperor'
Control	†90.0^{b}	88.3^{c}	4.3^{c}	8.9^{c}	0.071^{e}	0.076^{g}	0.164^{d}	0.149^{c}	0.235^{d}	0.225^{f}
50 mg L^{-1} Salicylic acid	100.0^{a}	100.0^{a}	19.0^{ab}	19.2^{ab}	0.103^{d}	0.126^{ef}	0.205^{c}	0.211^{b}	0.308^{c}	0.337^{de}
100 mg L^{-1} Salicylic acid	100.0^{a}	100.0^{a}	19.2^{a}	19.2^{ab}	0.103^{d}	0.148^{bc}	0.214^{bc}	0.224^{ab}	0.317^{bc}	0.371^{b}
150 mg L^{-1} Salicylic acid	100.0^{a}	100.0^{a}	18.8^{ab}	19.2^{ab}	0.171^{a}	0.153^{bc}	0.255^{a}	0.219^{b}	0.425^{a}	0.372^{b}
50 mg L^{-1} Ascorbic acid	100.0^{a}	100.0^{a}	18.7^{ab}	19.2^{ab}	0.109^{cd}	0.173^{a}	0.217^{bc}	0.245^{a}	0.327^{bc}	0.417^{a}
100 mg L^{-1} Salicylic acid	100.0^{a}	100.0^{a}	18.8^{ab}	19.2^{ab}	0.121^{bcd}	0.133^{de}	0.217^{bc}	0.213^{b}	0.338^{bc}	0.346^{cde}
150 mg L^{-1} Salicylic acid	100.0^{a}	100.0^{a}	18.5^{ab}	18.0^{b}	0.126^{bc}	0.140^{cd}	0.231^{ab}	0.205^{b}	0.357^{b}	0.346^{cde}
2% Pyriodoxine	98.3^{a}	100.0^{a}	18.9^{ab}	19.8^{a}	0.134^{b}	0.140^{cd}	0.211^{bc}	0.216^{b}	0.345^{bc}	0.356^{bcd}
4% Pyriodoxine	100.0^{a}	95.0^{b}	17.8^{ab}	18.2^{b}	0.103^{d}	0.117^{f}	0.230^{abc}	0.218^{b}	0.333^{bc}	0.335^{e}
6% Pyriodoxine	100.0^{a}	100.0^{a}	17.4^{b}	18.5^{ab}	0.110^{cd}	0.154^{b}	0.208^{bc}	0.206^{b}	0.318^{bc}	0.360^{bc}
75 mg L^{-1} Ascorbic acid + Pyriodoxine 3% + 75 mg L^{-1} Salicylic acid	98.3^{a}	100.0^{a}	18.4^{ab}	19.0^{ab}	0.119^{bcd}	0.157^{b}	0.209^{bc}	0.207^{b}	0.327^{bc}	0.364^{bc}

†Means having the same letter in each column are not different according to DMRT ($p < 0.05$)

Table 6. Correlation coefficients among germination characteristics and enzymatic activity

	Germination %	Germination velocity (No./day)	Radicle APX	Plumule APX	Radicle CAT	Plumule CAT	Radicle SOD	Plumule SOD	Radicle PPO	Plumule PPO
Germination %	1									
Germination velocity (No./day)	0.787**	1								
Radicle APX	-0.104	-0.150	1							

	Germination %	Germination velocity (No./day)	Radicle APX	Plumule APX	Radicle CAT	Plumule CAT	Radicle SOD	Plumule SOD	Radicle PPO	Plumule PPO
Plumule APX	0.206	0.207	0.262*	1						
Radicle CAT	0.511**	0.596**	-0.188	0.138	1					
Plumule CAT	0.473**	0.590**	0.000	0.241	0.716**	1				
Radicle SOD	0.101	-0.034	0.362**	0.049	-0.253*	-0.077	1			
Plumule SOD	0.099	-0.037	0.385**	0.065	-0.255*	-0.082	0.997**	1		
Radicle PPO	0.437**	0.549**	-0.369**	0.057	0.512**	0.367**	-0.296*	-0.296*	1	
Plumule PPO	0.207	0.220	-0.115	0.068	0.388**	0.452**	0.084	0.070	0.139	1

*, ** Significant at $p<0.05$ and $p<0.01$, respectively. APX: ascorbate peroxidase, CAT: catalase, SOD: superoxide dismutase, PPO: polyphenol oxidase

Table 7. Comparison of the effect of treatments on enzyme activity in plumule and radicles of cucumber 'Impress' cultivar

Parameter / Treatment	Radicle APX µM/mg/min	Plumule APX M/mg/min	Radicle CAT mM/mg/min	Plumule CAT mM/mg/min	Radicle SOD µM/g	Plumule SOD µM/g	Radicle PPO mg/ml	Plumule PPO mg/ml
Control	†9.42^{abc}	6.22^{d}	0.62^{c}	3.94^{e}	15.96^{d}	79.22^{f}	0.24^{c}	0.14^{d}
50 mg L^{-1} Salicylic acid	10.92^{ab}	17.46^{a}	0.78^{c}	6.87^{d}	16.74^{c}	83.46^{bcde}	0.44^{a}	0.18^{d}
100 mg L^{-1} Salicylic acid	8.75^{bcd}	9.56^{c}	0.95^{c}	7.22^{d}	17.10^{b}	84.84^{ab}	0.27^{c}	0.14^{d}
150 mg L^{-1} Salicylic acid	11.18^{a}	12.35^{b}	1.61^{b}	69.7^{cde}	17.28^{ab}	84.04^{bcd}	0.30^{bc}	0.24^{c}
50 mg L^{-1} Ascorbic acid	10.36^{ab}	19.30^{a}	1.87^{ab}	10.94^{ab}	17.15^{b}	82.95^{cde}	0.40^{a}	0.29^{b}
100 mg L^{-1} Salicylic acid	5.94^{ef}	13.32^{b}	1.65^{ab}	9.13^{bcd}	17.29^{ab}	83.81^{bcd}	0.39^{ab}	0.31^{b}
150 mg L^{-1} Salicylic acid	7.00^{def}	13.62^{b}	2.24^{a}	12.72^{a}	17.08^{b}	81.99^{e}	0.37^{ab}	1.24^{a}
2% Pyriodoxine	5.41^{f}	14.44^{b}	2.01^{ab}	8.53^{bcd}	17.30^{ab}	86.36^{a}	0.44^{a}	0.31^{b}
4% Pyriodoxine	7.73^{cde}	9.84^{c}	2.16^{ab}	9.98^{bc}	17.29^{ab}	82.78^{de}	0.46^{a}	0.30^{b}
6% Pyriodoxine	5.22^{f}	6.60^{d}	1.63^{b}	7.09^{d}	17.09^{b}	84.10^{bcd}	0.42^{a}	0.31^{b}
75 mg L^{-1} Ascorbic acid + Pyriodoxine 3% + 75 mg L^{-1} Salicylic acid	5.58^{ef}	5.52^{d}	1.82^{ab}	8.38^{bcd}	17.46^{a}	84.49^{bc}	0.46^{a}	0.31^{b}

†Means having the same letter in each column are not different according to DMRT ($p < 0.05$). APX: ascorbate peroxidase, CAT: catalase, SOD: superoxide dismutase, PPO: polyphenol oxidase

Table 8. Comparison of the effect of chemical priming on enzyme activity in plumule and radicle of the 'Emperor' cultivar

Parameter / Treatment	Radicle APX µM/mg/min	Plumule APX M/mg/min	Radicle CAT mM/mg/min	Plumule CAT mM/mg/min	Radicle SOD µM/g	Plumule SOD µM/g	Radicle PPO mg/ml	Plumule PPO mg/ml
Control	†6.68^{b}	6.09^{e}	0.50^{d}	2.86^{d}	5.35^{c}	18.80^{c}	0.29e	0.20^{d}
50 mg L^{-1} Salicylic acid	0.68^{d}	0.70^{g}	2.08a^{bc}	8.65^{bc}	6.91^{b}	22.84^{bc}	0.48^{b}	0.34^{b}
100 mg L^{-1}	0.66^{d}	6.62^{de}	1.70^{c}	7.83^{bc}	7.38^{ab}	32.95^{a}	0.47^{bc}	0.34^{b}

Parameter / Treatment	Radicle APX µM/mg/min	Plumule APX M/mg/min	Radicle CAT mM/mg/min	Plumule CAT mM/mg/min	Radicle SOD µM/g	Plumule SOD µM/g	Radicle PPO mg/ml	Plumule PPO mg/ml
Salicylic acid 150 mg L^{-1}	0.68^{d}	13.23^{c}	1.75^{bc}	9.70^{abc}	6.93^{b}	25.26^{b}	0.44b^{cd}	0.31^{bc}
Salicylic acid 50 mg L^{-1}	3.44c	3.51^{f}	2.03^{abc}	10.65^{ab}	7.33^{ab}	33.49^{a}	0.54^{a}	0.37^{a}
Ascorbic acid 100 mg L^{-1}	8.00ab	33.24^{a}	2.30^{a}	10.77^{ab}	7.30^{ab}	34.08^{a}	0.45^{bc}	0.33^{b}
Salicylic acid 150 mg L^{-1}	4.35^{c}	29.20^{b}	2.10^{abc}	9.81^{abc}	7.88^{a}	32.30^{a}	0.46^{bc}	0.31^{bc}
Salicylic acid 2% Pyriodoxine	10.62a	12.12^{c}	2.21^{ab}	9.72^{abc}	7.01^{b}	31.48^{a}	0.45^{bc}	0.33^{b}
4% Pyriodoxine	6.85^{b}	8.32^{d}	2.03^{abc}	10.76^{ab}	7.35^{ab}	30.28^{a}	0.39^{d}	0.30^{c}
6% Pyriodoxine	4.34^{c}	7.73^{de}	2.40^{a}	6.79^{c}	6.99^{b}	30.50^{a}	0.43^{cd}	0.31^{bc}
Ascorbic acid + Pyriodoxine 3% + 75 mg L^{-1} Salicylic acid	10.29^{a}	6.16d^{e}	2.47^{a}	12.48^{a}	5.79^{c}	22.04^{bc}	0.43^{bcd}	0.32^{bc}

†Means having the same letter in each column, are not different according to DMRT ($p < 0.05$). APX: ascorbate peroxidase, CAT: catalase, SOD: superoxide dismutase, PPO: polyphenol oxidase

Discussion

In the two investigated cultivars with regards to germination percentage, there was a significant difference between priming treatments and the control group. There were no significant differences among treatments for the 'Impress' cultivar; With regards to germination rate, in the 'Impress' cultivar, treatment with 100 mg L^{-1} SA had the highest rate of germination. However, in the 'Emperor' cultivar, treatment with pyridoxine 0.02% caused the highest germination rate, the control had the lowest rate of germination and there was a significant difference with all other treatments (Table 5).

Priming performs in some vegetables seeds including cucumber to augment the germination rate, total germination, and seedling uniformity etc.; mainly under unfavourable environmental conditions. It is a useful technique to exploit seed potential in arid and desert ecosystem. Also the knowledge gained on the repair mechanisms that take place upon various priming treatments has been used in many crops of seed industry in cucumber seed priming improved seed germination and vigour significantly over un-primed ones. The response of low vigour (aged) seeds to seed priming was much higher when compared to high vigour (un-aged) seeds. Priming with chemicals (KH_2PO_4 and K_2HPO_4) appears beneficial as they increase the vigour when compared to priming with water (hydro-priming) (Pandey et al., 2017).

Improved germination rate, uniformity of germination, and early seedling growth in melons by reducing germination time, 50% germination time, mean germination time and improving final emergence percent, emergence index, root and shoot length and number of roots by pre-sowing SA seed treatment have been reported (Basra et al., 2007). Improved germination rate and percentage by ascorbate and sodium form of SA in wheat (Triticum aestivum L.) has been reported by Al-Hakimi and Hamada (2001). Increase in germination percentage after treatment might be the consequence of breakdown of dormancy as fresh seeds were used during the investigations. The earlier and synchronized germination might be attributed to increased metabolic activities in treated seeds (Shakirova et al., 2003).

In the 'Impress' cultivar, treatments with SA at 150 mg L^{-1} produced the highest radicle weight; however, the control group had the lowest radicle weight. In the 'Emperor' cultivar, treatment with AsA at 50 mg L^{-1} had the highest radicle weight. In the 'Impress' cultivar, treatment with 150 mg L^{-1} SA had the highest plumule weight with regards to plumule weight,

in the 'Impress' cultivar, treatment with 50 mg L^{-1} AsA had the highest weight, and the control group had the lowest plumule weight with a significant difference with all other treatments.

In the 'Impress' cultivar, treatment with 150 mg L^{-1} SA had the highest weight of the total seedlings. The control had the lowest weight among all seedlings and there was a significant difference with all other treatments. In the 'Emperor' cultivar, treatment with AsA 50 mg L^{-1} produced the highest seedling weight. The control showed the lowest total seedling weight and it had a significant difference from all treatments (Table 5).

Seed priming of cucumber with SA improved germination rate, uniformity and growth rate of seedlings. The maximum freshness of seeds and dry weight were reported in the treatment of 100 mg L^{-1} SA. Seed treatment with 50 mg L^{-1} SA can be used successfully to improve germination and seedling growth in cucumber (Rehman et al., 2011).

In the 'Impress' cultivar, treatments with 150 mg L^{-1} AsA, pyridoxine 0.04% and pyridoxine 0.02% had the highest CAT activity in the radicle and plumule. Treatment with 150 mg L^{-1} AsA resulted in the highest PPO enzyme activity in the plumule (Table 7). In the 'Emperor' cultivar, compound treatment led to the highest CAT enzyme activity in the radicle and plumule. Treatment with 50 mg L^{-1} AsA resulted in the highest PPO activity in the radicle and plumule (Table 8).

CAT acts as an antioxidant enzyme and plays an important role in removing hydrogen peroxide produced in peroxisomes and reducing the destructive effects of reactive oxygen species (Simova Stovilova et al., 2008). Seedlings obtained from primed seeds of melon (*Cucumis melo* L.) showed higher catalase activity than seedlings grown from un-primed seeds (Farhoudi et al., 2011). Plants have different mechanisms to reduce the destructive effect of reactive oxygen species, including the antioxidant defence system through antioxidant enzymes such as PPO (Agarwal and Pandy, 2004).

Normally, plants kept high activity of PPO enzyme to perform normal physiological processes, but under stress condition the activity of this enzyme would be more elevated (Siosemardeh et al., 2014). Increased activity of this enzyme is due to the increase of its substrate, including active oxygen compounds, and shows the important role of this enzyme in counteracting the oxygen free radicals created in these conditions. In the reproductive stage, PPO activity under stress conditions was less than that under non-stress conditions (Siosemardeh, 2014).

Afzal et al. (2009) reported that germination and seedling vigour can be enhanced by priming whit Spermidine and Spermidine in tomato cultivars through maintaining higher level of antioxidant enzyme like SOD and CAT for eliminating excessive total peroxide. It is recommended that in order to increase the activity of antioxidant enzymes and the germination percentage and rate, treatments of 150 mg L^{-1} AsA and pyridoxine 0.4% for the 'Impress' cultivar and compound treatments and 50 mg L^{-1} AsA for the 'Emperor' cultivar should be applied. Faster germination increases plant vigour and establishment so it can use the resources better, and it increases ultimate yield (Bradford, 2017).

Conclusion

Different priming treatments increased the rate and percentage of germination in cucumber, and also produced seedlings with higher weights than the control seedlings. The reactions of cultivars to different priming treatments were various, so that the highest rate of germination in the 'Impress' cultivar was obtained with 100 mg L^{-1} SA and in the 'Emperor' cultivar by treatment with pyridoxine 0.02%. Furthermore, there was a positive correlation among germination percentage, germination rate and CAT enzymes in the radicle and plumule. In Cucurbitaceous plants, soaking of seeds is done as a common method before planting. To accelerate germination, the best treatments of this experiment can be applied in the water for soaking in order to accelerate seed germination. Seed priming increases the amount of nucleic acids, proteins, and also increases mobility of material which is stored in the seed; as a result, the seed germinates faster and the seedling appears on the soil surface.

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Conflict of interest

The authors indicate no conflict of interest for this work

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