Physiological enhancement of medicinal pumpkin seeds (*Cucurbita pepo*. var. styriaca) with different priming methods

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Abstract

In order to evaluate the effects of priming materials and duration of priming on the seed physiological enhancement and biochemical traits of germination in pumpkin seeds a factorial experiment was conducted based on a completely randomized design with three replications at the University of Mohaghegh Ardabili. Treatments were priming with water, NaCl (3%), gibberellic acid (20 mg L^{-1}), and ascorbic acid (20 ppm) for 8, 16, and 24 hours. Seed mass as a control for comparison of different pretreatment methods was measured. Results showed that priming treatments except gibberellin application, caused an increase in radicle length. Increase in priming duration increased the plumule length of seedlings. With increasing duration of priming, radicle length also increased except for seeds primed with NaCl. Seedlings from primed seeds with gibberellin had the highest dry weight (1.08 g) when compared to other priming treatments and control. Percentage and germination rate in the seeds primed with water, gibberellic acid, and ascorbic acid increased with increasing duration of priming and it was observed that the rate of increase was even higher in ascorbic acid treatment. Superoxide dismutase, catalase, and peroxidase in sodium chloride and gibberellic acid treatments had the highest and lowest activity, respectively. The activity of these enzymes had upward trend except in halo-priming, and hormone-priming with gibberellin for 24 h was the best treatment considering enzyme activity.

Keywords: antioxidant enzymes; gibberellic acid; priming; pumpkin

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Introduction

Seed quality is a broad term and encompasses several aspects of seed germination and seedling emergence. Seedbed has direct effect on seed germination and plant establishment and germination may be seriously limited under high stress conditions. To produce the transplant and in direct seed planting, high quality seeds should be taken to ensure the appropriate establishment of plant. Seed quality can be improved by various methods of hydration before planting, so the seeds planted under

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optimal levels can achieve the highest percentage of emergence (Copland and McDonald, 2000).

Enhancement includes several methods that speed up seed germination and seedling growth. All these methods may be applied before planting. Soaking the seeds in water, soluble inorganic salts, organic osmotic solutions, seed treatments at high and low temperatures, seed wetting using biological treatments, and solid matrix seed priming has been recognized as important techniques (Khaje Hosseini et al., 2003; Ashraf and Foolad, 2005). Kauret al.(2002 a) showed that pre-treatment of pea seeds with water and mannitol (4%) caused the seedlings have more protein and amylase, invertase, sucrose synthase, and sucrose phosphate synthase enzymes in the shoot of the pre-treated seeds than control. McDonald (2000) found that water treated seeds after seven hours completed the repair mechanisms of the cell membrane. Seed pre-treatment partially restored degenerative changes and increased the longevity of seeds (Villiers and Edgecumbe, 1975). Documents relating to the repair of DNA (Dell' Aquila and Tritto, 1990; Sivritepe and Dourado, 1994), RNA (Dell' Aquila and Tritto, 1991; Kalpana and Madhava Rao, 1997), protein (Petruzzelli, 1986; Dell' Aquila and Tritto, 1991), cell membrane (Petruzzelli, 1986), and enzymes as a result of seed priming are present.

Germination and plant growth is a complex and dynamic process in which a number of metabolic processes change the storage phase to a phase which causes the mobility and transport of materials (Bewley and Black, 1982). Seed germination is composed of three known stages. First, the rapid absorption of water in which water is absorbed by the seed, although little is known about the metabolic process. Second, delay during which water absorption is low, but significant metabolic activity is associated with activation of enzymes and increased respiration. During the third step, the water content of seeds increases root growth and its emergence coincides with its characteristic division and enlargement of cells (Bradford, 1995).

Reports indicate many factors related to both improvement in germination and mean germination time (Lee and Kim, 2000). Kaur et al. (2002 a) showed that pea seeds treated with water and mannitol (4%) had root and shoot length of seedlings after 7 days, three to four times higher than the control. Mean seed germination time in pre-treated lettuce seeds were reduced by approximately 61% (Tarquis and Bradford, 1992). Basra et al. (2005) concluded that wheat seeds pre-treated for 24 hours in aqueous solution had impact on accelerating high buds. Caseiro et al. (2004) found that the most effective method for improving seed germination of onion was water treatment. Bradford et al. (1990) and Harris et al. (1999) found that pre-treatment is a way to accelerate and enhance seed germination and establishment of seedlings in field conditions. Pre-treatment has the great impact on germination rate and seedling emergence, but the efficiency is different depending on the plant species and pretreatment conditions. Increased seedling emergence after pre-treatment may be due to the initiation of metabolic activity in the seeds. Various reports suggest that pre-treatment could increase the rate and uniformity of emergence (Murungu et al., 2003; Demir Kaya et al., 2006). Also, it has been reported that this technique increases the range of emergence under stressful environmental conditions such as salinity, drought, and temperature (Ashraf and Foolad, 2005; Demir Kaya et al., 2006). Zheng et al. (1994) also found that pre-treatment of seeds and seedling emergence rate increases in several varieties of oilseed rape.

The present experiment was conducted to investigate the effect of seed priming on pumpkin seed germination and seedling growth.

Materials and Methods

To study the effect of different pre-treatment methods on some biochemical and physiological characteristics of pumpkin seeds, a factorial experiment was conducted based on completely randomized design with three replications at Faculty of Agricultural Sciences, University of Mohaghegh Ardabili, and Ardabil, Iran. Seed sample was divided into five sub-samples. One of the sub-samples was considered as control (unprimed) and the other four sub-samples were prepared for priming treatments.

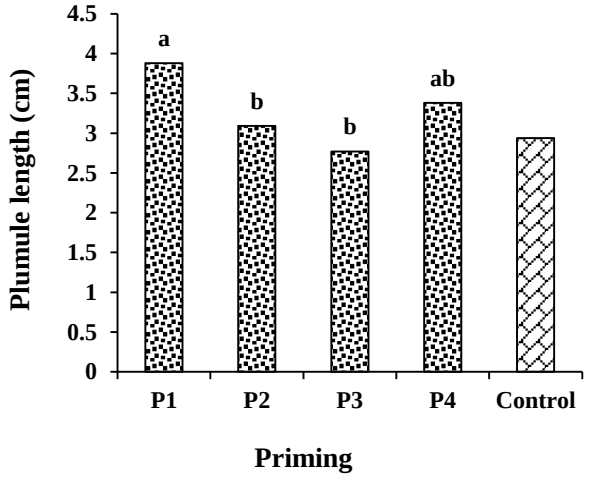

Fig. I. Effect of seed priming on plumule length of pumpkin seedling; P_1, P_2, P_3, P_4,and control: pre-treated with water, sodium chloride, gibberellic acid, ascorbic acid, and without pre-treatment, respectively.

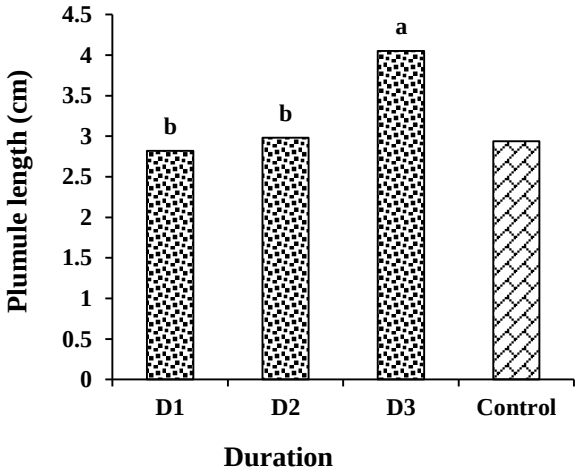

Fig. II. Effect of priming duration on plumule length of pumpkin seedling; D_1, D_2, and D_3: pre-treated for 8, 16, and 24 hours, respectively.

The sub-samples were primed by soaking the seeds in distilled water (P_1), solutions of NaCl (3%) (P_2), gibberellic acid (20 mg per liter) (P_3), and ascorbic acid (20 ppm) (P_4) for 8, 16, and 24 hours (D_1, D_2, and D_3, respectively). All priming treatments were performed in an incubator adjusted on 20±1°C under dark conditions. For pre-treatment, pumpkin seeds were prepared and immersed in solutions at specified times. After the pre-treatment periods, the solutions were removed and the seeds were washed several times with distilled water and then dried to the original moisture level.

Germination test was carried out using paper towel method in four replications of 100 seeds at a temperature of 20° C for eight days (ISTA, 2008) and filter paper (Boeco-Germany) size 58 × 58 was used. Germination rate was calculated using the following formula (Ellis and Roberts, 1980):

$$GR = \frac{\sum \mathrm{n}}{\sum \mathrm{Dn}}$$

where GR = germination rate(number of germinated seeds per day), n = number of seeds germinated on day D, and D = number of days elapsed since the start of the experiment.

Radicle and plumule length and seedling dry weight were measured at the end of the experiment from 25 normal seedlings. For the evaluation of the activity of antioxidant enzymes, primary leaves of seedlings were frozen in liquid nitrogen and 1 g of the frozen samples was homogenized in mortar with 5 ml of 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 1 mM dithiotreitol and 2% polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 15,000 g for 25 min and the supernatant was used for superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) assay (Sairam et al., 2002).

The activity of SOD (EC 1.15.1.1) was determined according to Beyer and Fridowich (1987). In small glass tubes, 20 µL of enzyme supernatant were added to 50 mM potassium phosphate buffer (pH 7.8), 9.9 mM L-methionine, 57 µM nitro blue tetrazolium (NBT), and 0.025% triton-X-100. Reaction was started under fluorescent light for 10 minutes by adding 10 µL of riboflavin solution. Absorbance of the solution was measured at 560 nm for both blank and control. SOD activity was expressed as Unit mg^{-1} DW.

The activity of CAT (EC 1.11.1.6) was assayed according to Chance and Maehly (1955). A 1.5 mL reaction mixture containing 30 µL water, 50 µL 1M Tris-HCl buffer (pH 8.0), 5 mM EDTA, and 900 µL 10 mM H_2O_2 was added to 20 µL of enzyme supernatant. The decrease in the absorbance at 240 nm was recorded for 60 seconds. CAT activity was expressed as absorbance in mg protein per min.

The activity of POD (EC 1.11.1.7) was determined by spectrophotometer at 470 nm according to Yamane et al. (1999) in a 3 mL reaction mixture containing 1.5 mL 0.1 M potassium phosphate buffer (pH 7.0), 600 µL 10 mM guaiacol, 800 µL 4 mM H_2O_2, and 100 µL

Table1
Analysis of variance of the seed priming and duration on pumpkin seedling parameters

SOV	df	Mean of Squares							
		Germination percent	Germination Rate	Radicle Length	Plumule Length	Seedling Dry Weight	Superoxide Dismutase	Catalase	Peroxidase
Priming (P)	3	100.09**	1584**	33.24**	1.99*	0.061**	37.24**	10.13**	23.92**
Duration (D)	2	34.88**	1017.33**	180.56**	5.35**	0.022**	19.51**	1.8**	2.72**
P×D	6	11.96**	254.66*	52.03**	0.304	0.001	2.59**	0.7**	2.45**
Error	24	1.21	70.22	6.18	0.42	0.0004	0.043	0.048	0.056
CV (%)	-	15.02	12.95	14.01	19.91	2.11	0.33	1.36	0.28

*, **: significant at p≤0.05 and p≤0.01, respectively

crude enzyme. POD activity was expressed as µ M of guaiacol oxidized to tetraguaiacol by a unit of enzyme per min.

After testing the normality of data distribution and homogeneity of variance, factorial analysis was conducted in a completely randomized design. All statistical analyses and comparisons were performed using SAS9.1 software.

Results

The interaction of priming and priming duration on characteristics of germination, root length, superoxide dismutase, catalase, and peroxidase was significant at 1% probability level and the germination rate was significant at the 5% level. The interaction between the two treatments on shoot length and seedling dry weight was not significant. Simple effects of priming on shoot length and seedling dry weight were significant at 1% level. Duration of priming was significant on shoot length and seedling dry weight at 1% level (Table 1).

In the seeds primed with sodium chloride, gibberellic acid and ascorbic acid (P_1, P_3, and P_4, respectively), germination percentage increased with increasing duration of priming. The highest germination percentage (94.66%) and germination rate (13.23) were recorded for the pre-treatment with ascorbic acid at 24 and 8 hours, respectively. Among the priming treatments NaCl (P_2) had the lowest value at all times (Table2).

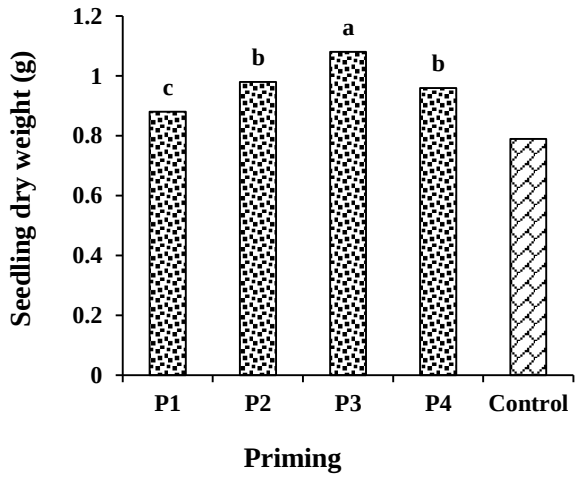

Fig. III. Effect of seed priming on dry weight of pumpkin seedling; P_1, P_2, P_3, P_4, and control pre-treated with water, sodium chloride, gibberellic acid, ascorbic acid ,and without pre-treatment, respectively.

Results showed that root length of pumpkin seedlings in hydro-priming (P_1) was significantly greater than that of the other priming treatments. However, the difference in the seeds treated with vitamin C (P_4) was not significant. All priming treatments except for hormone (P_3) increasingly influenced the control of shoot elongation (Table1).

Table 2
Comparison of means for some traits of pumpkin seedlings under different priming and duration treatments

Priming	Duration	Germination percent	Germination Rate (seed day^{-1})	Radicle Length (cm)	Superoxide Dismutase (mg min^{-1})	Catalase (mg min^{-1})	Peroxidase (mg min^{-1})
P_1	D_1	70.66 bcd	11.59 ab	18.27 abcd	56.6 g	15.33 f	81.56 e
	D_2	57.33 de	6.05 f	20.11 abc	62.33 e	15.83 e	81.86 e
	D_3	65.33 cde	6.6 ef	22.49 ab	63.4 d	16.2 de	81.9 e
P_2	D_1	32 f	1.29 h	16.73 cd	59.56 g	15.23 f	80.73 f
	D_2	53.33.e	3.07 gh	18.27 abcd	59.63 g	14.76 g	80.36 f
	D_3	58.66 de	3.43 g	17.05 dc	59.33 g	14.7 g	79.53 g
P_3	**D_1**	57.33 de	10.46 bc	14.08 d	62.63 e	16.53 dc	82.4 d
	D_2	58.66 de	5.45 f	17.77 bcd	64.56 b	17.43 b	84.43 a
	D_3	81.33 ab	9 cd	21.38 abc	66.23 a	18.3 a	85.43 a
P_4	**D_1**	69.33 bcde	13.23 a	14.58 d	60.43 f	15.9 e	81.96 e
	D_2	77.33 bc	8.44 de	19.72 abc	62.56 e	16.43 d	82.73d
	D_3	94.66 a	9.55 cd	22.61 a	63.93 c	16.96 c	83.46 c
Control		85.33	10.24	15.83	58.8	14.63	78.2

Different letters at each column indicate significant difference at p≤0.05. P1, P2, P3, P4and control: pre-treated with water, sodium chloride, gibberellic acid, ascorbic acid and without pre-treatment, respectively. D1, D2 and D3: pre-treated for 8, 16 and 24 hours, respectively.

Superoxide dismutase activity demonstrated that, except in the case of P_2, with increasing duration of priming the enzyme significantly increased in the seedling. In general, priming treatment with gibberellic acid for 24 hours had the highest activity of SOD (66.23). All the treatments increased the amount of the enzyme activity. Therefore, priming has a positive effect on superoxide dismutase activity (Table2). All four types of priming in this experiment led to an increase in catalase activity compared to the control. Apart from saline pre-treatment (P_2), with increasing soaking time catalase levels fell in the pre-treatment process was reversed. Pre-treatment with the hormone (P_3) at all times because of its superior offering was better compared to the other treatments (Table 2).

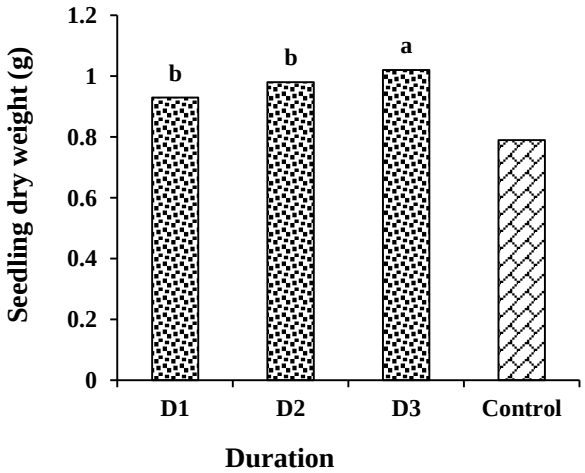

Fig. IV. Effect of priming duration on dry weight of pumpkin seedling; D_1, D_2, and D_3: pre-treated for 8, 16, and 24 hours, respectively.

Discussion

In this experiment priming treatments increased seed germination in comparison with

control and priming with ascorbic acid had the highest value. Results of water pre-treatment on lettuce (Parera and Cantliffe, 2002) showed that seed priming increases germination of seeds compared to control, but this increase is not statistically significant. Gray et al. (1991) reported that pre-treatment with water is low efficient for seed germination.

Priming increased the activity of antioxidant enzymes. This increase can lead to optimizing defense mechanisms during seed germination. Gibberellin synthesis inhibitors such as paclobutrazol application increased SOD enzyme activity in the stem of sesame (Somasundaram et al., 2009). Pre-treatment of corn seeds and foliar application of salicylic acid and brassinolid on salt stressed plants increased the activity of antioxidant enzymes and endogenous hormones like auxin, gibberellin, and Zeatin (El-Khallal et al., 2009).

Conclusion

Priming for 24 hours had the highest positive effect on germination and other related traits. Gibberellin and ascorbic acid were the best materials for seed priming in pumpkin seeds and can be used for seed enhancement.

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