A laboratory and glasshouse evaluation of ascorbic and salicylic acid effect on germination traits and grain yield of safflower cultivars

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Abstract

Information regarding the evaluation of ascorbic and salicylic acid on safflower cultivars germination traits is not available. The study, consisting of two experiments (laboratory and glasshouse), was conducted as a factorial experiment with randomized design and three replications. Factor A included six priming treatments (50 mg L^{-1} salicylic acid [SA], 100 mg L^{-1} SA, 50 mg L^{-1} ascorbic acid [ASA], 100 mg L^{-1} ASA, hydro-priming and control) and factor B included three safflower cultivars ('Faraman', 'Sina' and 'IL 111'). Results showed that the highest shoot length (4.71 cm) was observed in the 50 mg L^{-1} SA treatment and the lowest length was 1.24 cm, in the control treatment. The longest (3.71 cm) and shortest (1.06 cm) radicles were observed in hydroprimed 'Faraman' and 'IL111' ASA100 treatments, respectively. Also, the highest (8.32 mg) and lowest (1.96 mg) radicle weights were observed in 'Faraman' SA50 and 'IL111' AsA100 treatments, respectively. For all cultivars, as SA and ASA concentration increased, the emergence rate index decreased. The highest grain yield (16.2 g per plant) was observed for the 'Faraman' cultivar.

Key words: ascorbic acid; germination; safflower; salicylic acid; vigour index.
Abbreviations: ASA, ascorbic acid; SA, salicylic acid.

Introduction

Oil seeds have a special place in industry and food security of the human population throughout the world. Safflower (*Carthamus tinctorius* L.) is a member of the family Compositae that is cultivated for its oil seed. One of the most important factors for oil seed cultivation is seed product with high vigour and rapid seed germination, because these factors are critical to crop production under different conditions. Rapid germination and emergence are important determinants of successful stand establishment. On the other hand, germination and emergence are important issues in plant production and they have significant effect on the next stages of plant growth in the field. Seed priming is a technique of controlled hydration (soaking in solution) and drying that result in more rapid germination when the seeds are re-imbibed. Stratification is also important, whereby seeds are placed in a refrigerator to simulate a winter cooling period. Priming of seeds in growth regulators such as salicylic acid (SA), ascorbic acid (ASA) and in water (hydro-priming) has been reported to be a simple and safe technique for increasing the capacity of seed to osmotic adjustment and enhancing seed germination (Afghani Asl, Taheri 2012; Ghasemi Lemrasky, Hosseini 2012; Nautiyal, Shukla 2013).

Ascorbic acid (vitamin C) is an important metabolite involved in many cellular processes, including cell division (De Gara et al. 2003). Several reports indicate that a large amount of ascorbic acid is utilized during the initial stages of germination by both zygotic and somatic embryos (Stasolla, Yeung 2001). Along with its role in scavenging reactive oxygen species, ASA is also involved in regulating photosynthetic capacity by controlling stomatal movement and is also an important co-factor of some enzymes or protein complexes that are involved in the regulation of photosynthesis (Ahmad et al. 2012). Salicylic acid plays an important role for increasing resistance to environmental stress (Raskin 1992). It has been reported that SA increases salinity tolerance (Jamshidi Jam et al.,2012) and resistance to water deficit (Bezaukova et al. 2001) in seedlings.

Thus, it seems that ascorbic acid, salicylic acid and water are promising materials for seed treatments. In the present study, the effects of seed priming with different concentration of ascorbic acid, salicylic acid and hydro-priming were investigated on germination traits of safflower cultivars.

Materials and methods

Laboratory and glasshouse assays were conducted in 2013 in the Islamic Azad University of Sanandaj, located in Kurdistan province of Iran. To achieve this, a factorial experiment with completely randomized design and three replications was used. Factor A included six priming treatments (50 and 100 mg L^{-1} SA, 50 and 100 mg L^{-1} ASA, hydro-priming and control) and factor B included three safflower cultivars ('Faraman', 'Sina' and 'IL 111').

In laboratory study, for priming safflower seeds (25 seeds in each replication) were soaked in aerated solution of respective osmoticum having concentration 50 and 100 mg L^{-1} of SA and ASA and water for 24 h in a germination box at 25 °C. Untreated dry seeds were taken as the control. After 24 h priming treatment, the seeds were washed with distilled water for 2 min and surface dried on absorbent paper. Then, seeds were surface sterilized using 0.05% sodium hypochlorite to remove microorganisms. Then, they were placed on a piece of clean germination paper, allowing dehydration in a drying oven at 25 °C to retrieve the original seed moisture before priming treatment. The growth conditions of seeds in the germination box were 30 °C temperature, 40 J m^{-2} s^{-1} light intensity and 12 h day^{-1} photoperiod. Germinating seeds were counted every day during 1 to 10 days after treatment. Ten days after treatment, seedling height and root length were measured and the dry weights of seedling shoot and root were measured after pre-drying at 90 °C for 10 min and continuous drying to constant mass at 70 °C. According to the methods described by Sun et al. (2010) the emergence percentage was calculated using the formula: number of germinated seeds with in first 10 days / total number of seeds × 100. To measure the emergence rate, the petri dishes were daily visited and the emerged seedlings were recorded. The emergence rate index was calculated using the following equation

$$\text{Emergence rate index} = \sum_{1}^{i} \frac{n_i}{d_i},$$

where n_i is the number of emerged seedlings on day i, and d_i is the number of days after sowing. Vigour index was calculated by multiplying the mean seedling length by emergence percentage divided by 100 (Sun et al. 2010).

In glasshouse study, air-dried soil was placed in 20-cm-tall plastic pots with 18-cm diameter and gently tamped to create a 15-cm-deep soil layer with a bulk density of 1.25 Mg m^3. After 24 h priming treatment, the seeds were washed with distilled water, and 25 seeds were sown in each pot. Emergence was measured by counting all individual seedlings at 24-h intervals beginning 7 days after planting and continuing until no further emergence occurred. After 21 days, only three plants were kept in each plot and other plants were removed. At harvest time, grain yield of safflower was evaluated.

The data collected in this study were subjected to analysis of variance (ANOVA) using PROC GLM of the SAS statistical program and the least significant difference was used to compare means of traits ($p < 0.05$).

Results and discussion

Laboratory study

Priming treatments and safflower cultivars had a significant effect on shoot length and weight. No interactions between priming and cultivars on shoot length and weight were found. The highest shoot length (4.71 cm) was in the 50 mg L^{-1} SA treatment and the lowest length (1.24 cm) in the control treatment. The 'Faraman' cultivar had the longest seedling shoot compared to other cultivars (Table 1). The highest seedling shoot weight (9.71 mg) was in the SA50 treatment and the lowest seedling length (1.18 mg) in the control treatment. Priming treatments improved shoot length and weight; the largest effect was in the SA50 and ASA50 treatments. In SA50 and ASA50 treatments the shoot length was 379 and 339% of the control, respectively. Also, in SA50 and ASA50 shoot weight was 822 and 713% of the control treatment, respectively. Among the cultivars the 'Faraman' cultivar had longest seedling shoots (Table 1). These observations are similar with the report of Ghoohestani et al. (2012), who showed that shoot fresh and dry weight and root fresh weight of sugar beet were increased in seedlings raised from seeds primed with 50 mg L^{-1} SA. This is in agreement with the results of Ahmad et al. (2012) for maize and Eisavand et al. (2011)

Table 1. Effects of priming and cultivar on germination traits of safflower. Mean values in each priming treatment with the same letter are not significantly different using LSD tests at 5% of probability

	Treatment	Shoot length (cm)	Shoot dry mass (mg $plant^{-1}$)	Emergence (%)	Emergence rate (seedlings per day)	Grain yield (g $plant^{-1}$)
Cultivars	'Faraman'	3.67 a	5.91 a	89.9 a	7.41 a	16.21 a
	'Sina'	3.12 ab	5.04 ab	71.2 b	7.19 a	15.32 b
	'IL111'	2.87 b	4.53 b	75.2 b	7.18 a	15.25 b
Priming	SA50	4.71 a	9.71 a	92.1 a	11.18 a	15.81 a
	SA100	2.71 c	3.17 d	62.1 c	5.78 c	15.61 a
	ASA50	4.21 ab	8.42 b	82.4 b	9.49 b	15.57 a
	ASA100	2.65 c	3.29 d	61.2 c	4.71 d	15.66 a
	Hydro-priming	3.84 b	5.21 c	88.7 b	9.60 b	15.41 a
	Control	1.24 d	1.18 e	86.4 b	2.81 e	15.52 a

for carrot plants treated with salicylic acid. SA in plants has significant impact on plant growth and development, photosynthesis, transpiration, ion uptake and transport and also induces specific changes in leaf anatomy and chloroplast structure. It is supposed that the protective and growth promoting effect of SA are due to increased level of cell division within the apical meristem of seedling root, which caused an increase in plant growth. SA is recognized as an endogenous signal, mediating in plant defense and against environmental stress (Jamshidi Jam et al. 2012). The authors also reported that pre-treatment with SA leads to increased dry and fresh weight of leaves.

Priming treatments and cultivars had a significant effect on radicle length and weight. There was a significant interaction of both factors on radicle length and weight. The longest (3.71 cm) and shortest (1.06 cm) radicles were observed in hydroprimed 'Faraman' and 'IL111' ASA100 treatments, respectively (Fig. 1). Also, the highest (8.32 mg) and lowest (1.96 mg) radicle weights were observed in 'Faraman' SA 50 and 'IL111 ASA100 treatments, respectively (Fig. 2). Findings of Eisavand et al. (2011) showed an increase in respiration activities, ATP production, induced RNA activity and protein synthesis in primed seeds, as well as enhanced radicle length and weight of primed sunflower seed. Ahmad et al (2012) observed that germination percentage, root and shoot length, root-shoot ratio and catalase activity decreased with increasing salinity, while treated seeds with 20 mg L^{-1} ascorbic acid alleviated the salinity stress. Also, Sanches et al. (2001) reported that the root length of cucumber and pepper increased due to hydro-priming.

The results showed that the priming and cultivars had a significant effect on safflower emergence percentage. Seed primed with SA50 had the maximum emergence percentage (92.1%) and ASA100 had the lowest emergence percentage (61.2%; Table 1). In treatments with increased SA and ASA concentration (100 mg L^{-1}) the emergence percentage decreased. In all cultivars ASA and SA enhanced the emergence in lower concentrations. The 'Faraman' cultivar had the highest emergence percentage. Mazaherie Tirani and Manouchehri (2005) reported that high concentrations of SA reduced *Brassica napus* seed germination compared to hydro-priming which is in accordance with the findings of the present study. Also, the hydro-priming treatment increased the emergence percentage compared to the control treatment.

Priming had a significant effect on emergence rate index of safflower seed, but the cultivars did not had significant effect on emergence rate index. The highest (11.18 seedlings a day) and lowest (2.81 seedlings a day) emergence rate indexes were observed in SA50 and control treatments, respectively (Table 1). Several reports related to applications of SA and ASA have been presented for the germination and its subsequent stages, but their specific roles are not explicit yet. This means that both SA and ASA trigger and maximize specific responses such as germination at lower concentrations and have negative influence at higher concentrations (Ahmad et al. 2012). Increasing SA concentration enhances ABA synthesis, which can inhibit seed germination. Also, hydro-priming causes some physiological changes including changes in the sugar content, organic compounds and cumulated ions in the seed, root and finally in the plant leaves leading to high rate of germination and enhanced resistance to unfavorable conditions (Farooq et al. 2012). In all cultivars, as ASA concentration increased, the emergence rate index declined. The hydro-priming treatment increased the emergence rate more than the 100 mg L^{-1} ASA, 100 mg L^{-1} SA and control treatments. Habibi and Abdoli (2013) reported that low concentrations of SA increased the germination percentage, but this increase was not significant compared to the control treatment. Findings of Nun et al. (2003) showed that SA can inhibit the activity of catalase. Reduced catalase activity leads to increased hydrogen peroxide (H_2O_2) production, which can improve seed germination.

There was a significant interaction of priming and cultivar effect on vigour index. The highest (6.72) and lowest (1.44) vigour indexes were found for 'Farraman' SA50 and 'IL111' ASA100 treatments, respectively (Fig. 3). In all cultivars, the highest vigour index was observed for the 50 mg L^{-1} SA treatment. In all cultivars the vigour index decreased with increasing SA and ASA concentrations from 50 to 100 mg L^{-1}.

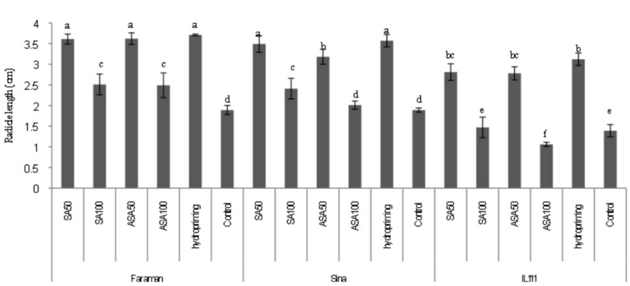

Fig. 1. The interaction of priming and cultivar effect on radicle length of safflower. Mean values in each priming treatment with the same letter are not significantly different using LSD tests at 5% of probability. Bars indicate the standard error of means (n = 3).

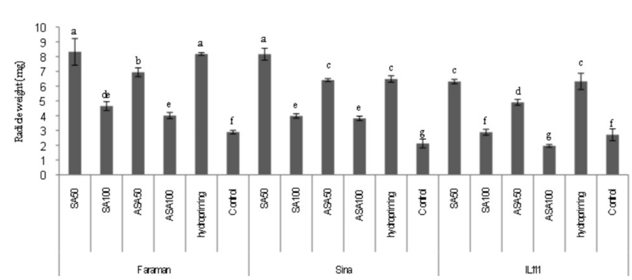

Fig. 2. The interaction of priming and cultivar effects on radicle weight of safflower. Mean values in each priming treatment with the same letter are not significantly different using LSD tests at 5% of probability. Bars indicate the standard error of means (n = 3).

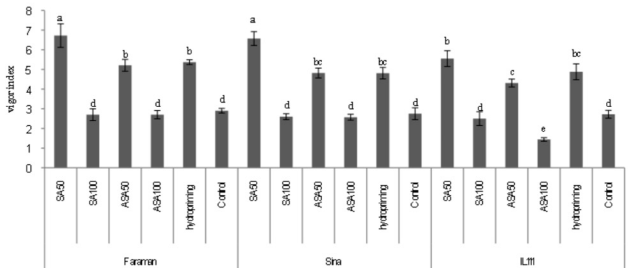

Fig. 3. The interaction of priming and cultivar effect on vigour index of safflower. Mean values in each priming treatment with the same letter are not significantly different using LSD tests at 5% of probability. Bars indicate the standard error of means (n = 3).

Glasshouse study

The response of emergence percentage in glasshouse study was quite similar to these in laboratory study. Seed primed with SA50 had a maximum emergence percentage (88.9%) and ASA100 had a lowest emergence percentage (51.2%) (data not shown). The 'Faraman' cultivar showed the highest emergence percentage (91.6%).

Only cultivars had a significant effect on safflower grain yield. Comparison of means showed that the highest grain yield (16.2 g per plant) was by the 'Faraman' cultivar. Although priming had no significant effect on grain yield, the highest grain yield was observed in the SA50 treatment (15.8 g per plant). It is possible that salicylic acid enhances endosperm cell division and cytokinin activity. Findings of Sakhabutdinova et al. (2003) showed that treatment of seeds with SA completely prevented water deficit-induced decline in concentration of indole acetic acid and cytokinins in seedlings and reduced accumulation of abscisic acid, which is a prerequisite for acceleration of growth resumption of seedlings after withdrawal of a stressor from the medium. Also, Zaki and Radwan (2011) observed greater wheat grain yield and higher quality under salinity conditions when treated with SA. Furthermore, the beneficial effect of SA on grain yield may be due to translocation of more photo-assimilates to grain during grain filling, thereby increasing grain weight (Aldesuquy et al. 2012).

Conclusions

Priming can be useful for production of safflower seedlings, because this technique increases seed germination and seedling quality. This is of importance, as primed seeds germinate faster and this will increase grain yield and safflower competition power against the weeds. Also, priming increases vigour index, which is important for coping with a soil crust and other unfavorable conditions in the field. Priming with ASA and hydro-priming was less effective than priming with SA. Among the safflower cultivars, 'Faraman' had the best germination traits and grain yield. In further research study using very low concentration of ASA and SA would be an informative strategy to elucidate their effects on seed germination.

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