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EFFECT OF PRIMING TYPES ON GERMINATION OF *Nigella sativa* UNDER OSMOTIC STRESS

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ABSTRACT. *In order to study the effect of priming types on germination of Nigella sativa under osmotic stress, a factorial experiment was conducted in completely randomized design with three replications in Seed Technology Laboratory of Yasouj University. The main factors of the experiment included six priming levels (2 osmopriming levels with potassium nitrate (1 and 3 %), two salicylic acid levels (0.2 and 0.5 mM), hydro-priming with distilled water and a control or no priming treatment), and three osmotic stress levels (osmotic pressures of zero, -2, and -6 (bars) with polyethylene glycol 6000). Results indicated that interaction of osmotic stress and seed priming had significant effect on all traits under study. Also, in no-stress condition, the maximum germination percentage, seed vigor, plumule and radicle lengths, radicle weight, conversion efficiency of mobilized seed reserve to seedling tissue, were obtained by potassium nitrate 3 %. The maximum germination rate, weight of mobilized seed reserve and seed reserve depletion percentage were observed as a result of applying hydropriming and 0.5 mM salicylic acid treatments. At osmotic pressure of -3 (bars), priming with 0.2 mM salicylic acid led to enhancement of germination rate and percentage, seed vigor, and weight of mobilized seed reserve. The largest radicle weight and length were observed by 1% potassium nitrate application. Increasing osmotic potential to -6 (bars), priming of 0.2 mM salicylic acid improved germination percentage and rate, seed vigor, and radicle length. Priming with 0.5 mM salicylic acid gave up the maximum plumule length and conversion efficiency of mobilized seed reserve to seedling tissue. Also, the* *largest values for radicle weight and seed reserve depletion percentage were observed by hydro-priming application.*

***KEYWORDS**: black cumin; hydropriming; osmopriming; potassium nitrate; salicylic acid; seed reserve.*

INTRODUCTION

The seed of *Nigella sativa* L. (Ranunculaceae), commonly known as black seed or black cumin, has been an important nutritional flavoring agent and natural remedy for many diseases for centuries in traditional systems of medicine centuries like Unani, Tibb, Ayurveda, Siddha, Chinese and Arabic Medicines as herb and pressed into oil in Asia, Middle East, and Africa. Many active components have been isolated from *N. sativa*, including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine and alpha-hederin. It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders. Also, active components have been identified to include immune stimulation, anti-inflammation, hypoglycemic, antiasthmatic, antimicrobial, antiparasitic, antioxidant and anticancer effects (Ahmad et al. 2013; Randhawa & Alghamdi 2011). Due to its miraculous power of healing, *N. sativa* has got the place among the top ranked evidence based herbal medicines.

Despite the fact that eugenic treatments of plant seeds have been successful in influencing the yield to some extent through improvement of germination under unfavorable conditions, but two decades after knowledge of seed preparation methods, the respective procedures have been always regarded among the common and prevailing methods for accelerating the germination process and enhancing uniformity of emergence in field conditions and also improving the tolerance of seeds against adverse environmental conditions. In this respect, it is needed to deploy a procedure which can strengthen the germination and seedling establishment, enabling further use of soil moisture, nutrients, and solar radiation for the plant. Under such conditions, the plant will be capable of completing its development stage prior to occurrence of premature stresses.

Osmotic stress is one of the most important environmental factors that affect germination and seedling establishment. Resistance against stress is significant throughout the plant life stages, and obviously, germination is the first stage. Since both qualitative and quantitative yields depend upon rate and percentage of germination, seedling emergence and also its uniformity, the germination stage of plants therefore is a sensitive and crucial stage which can play a substantial role in production process through providing favorable seedling establishment. It is to a great extent dependent on biochemical and physiological structures of the seed, and hence, seeds with high vigor are required for achieving this goal; because seed vigor and strength is one of the most important qualitative attributes which influence the plant growth (Rashed Mohasel & Kafi 1992).

Some plants have evolved mechanisms to preclude hormonal imbalances under osmotic stress. However, in plants lacking the inherent ability to maintain optimum levels of plant growth regulators (PGRs) under osmotic stress, exogenous application of PGRs may overcome their deficiency (Ashraf et al. 2011). Sharafizad et al. (2013) found that wheat seed soaking treatment with salicylic acid (SA) in low concentration at low level of drought stress could decrease the germination time and increase the germination percentage. Application of SA for seed priming of different crops to improve seed germination and reduce the effects of soil salinity has been reported in many studies (Entesari et al. 2012, Erdal et al. 2011). Farahmandfar et al. (2013) reported that seed priming of fenugreek by salicylic acid improved the dry weight and length of plumule and radicle under salt stress. Farhbakhsh (2012) also reported that application of 0.25 and 0.5 mM of salicylic acid for seed priming of fennel were effective on germination percentage and rate, seed stamina index, hypocotyl and radicle length, fresh and dry weight under different water potentials (0, -2, -4 and -6 bar). Dry weight of seedlings of wheat raised from seeds primed with SA improved as compared to the seeds non-treatment of SA under non salinity and salinity conditions (Bahrani & Pourreza 2012). Nasiri et al.,

(2014) reported that 0.5 mM salicylic acid improved all traits except mean germination time as compared to control. Salicylic acid (0.5 mM) improved radicle length under 0, 40 and 80 mM NaCl salinity levels as well as increased plumule length at the 0 and 40 mM NaCl salinity conditions.

Moreover, hydropriming increased germination and seedling growth under salt and drought stresses (Kaya et al. 2006). The former research results revealed the fact that seed priming leads to increased germination and emergence in different grasses. Hydropriming of barley seeds before planting led to earlier germination of seeds under different ambient conditions including in unfavorable environmental circumstances (Rashid et al. 2004).

Makizadeh Tafti (2006) declared that the osmotic potential of the solution significantly affected germination percentage at osmotic pressure of -8 (bars) in borage seeds osmoprimed with polyethylene glycol solution. Tzortzakis (2009) expressed that priming with KNO_3 improved rate of germination and seedling emergence, and stated that priming is a practical method with economic benefit for producers. Beside, KNO_3 primed seeds excelled over all other priming agents including NaCl (Abdollahi & Jafari 2012). Jumsoon et al. (1996) studied the effect of priming (150 mM KNO_3 at 20°C for 4 days) of tomato seeds under water or saline stress. They concluded that primed seeds had higher percentage germination than unprimed seeds at 15 or 20°C under both water and saline stress.

Although priming improves the rate and uniformity of seedling emergence and growth particularly under stress conditions (Parera & Cantliffe 1991), the effectiveness of different priming agents varies under different stresses and different crop species (Iqbal & Ashraf 2005).

For this purpose, effects of hydropriming, osmopriming, and hormone priming are studied on germination of *Nigella sativa* under osmotic stress in the present experiments.

MATERIALS AND METHODS

In order to study the effects of different types of priming on germination and growth of *Nigella sativa* under varied osmotic stress levels, a factorial experiment was carried out in Seed Technology Laboratory of Yasouj University. This experiment was conducted as completely randomized design and the main factors included six priming levels (1% potassium nitrate, 0.5 mM salicylic acid for 6 hours, and 3% potassium nitrate for 24 hours, 0.2 mM salicylic acid and hydro-priming with distilled water for 12 hours and control treatment without priming) and 3 osmotic stress levels (osmotic pressure of zero (control), -3, and -6 bars with polyethylene glycol 6000).

To select the best primes at different osmotic stress levels in laboratory, 50 perfect and healthy seeds were chosen after resting inside the priming solutions for a needed duration (Ahmadpour Dehkordi 2012) and drying them in absence of light for 24 hours. The selected seeds were transferred to sterilized petri dishes after disinfection with 1.5% sodium hypochlorite for 2 minuets. The petri dishes were placed in germinator at fixed temperature of 25 ˚C and the germinated seeds were counted every day (Ghassemi-Golezani & Dalil 2011). The seeds with radicles protruding 2 mm were considered as germinated seeds. The required moisture of the seed was provided by polyethylene glycol solution. In the 10^{th} day, after counting the number of germinated seeds, plumule and radicle were separated and after measuring their lengths with millimeter ruler, their dry weighs were determined by placing them inside an oven at 75 ˚C for 24 hours. Germination percentage (G) and germination rate (GR) were evaluated through counting the number of germinated seeds (Soltani 2007). Furthermore, seed vigor, weight of mobilized seed reserve, conversion efficiency of mobilized seed reserve to seedling tissue and seed reserve depletion percentage were calculated using the following formulas (Soltani et al. 2006, 2002).:

1) Weight of mobilized seed reserve (mg) = [Initial seed weight (mg) – weight of seed coat (mg)]

2) Conversion efficiency of mobilized seed reserve to seedling tissue = [(dry weight of plumule (mg) + dry weight of radicle (mg) / Weight of mobilized seed reserve (mg))]

3) Seed reserve depletion percentage = [Weight of mobilized seed reserve (mg) / initial seed weight (mg)] × 100

4) Seed Vigor = [(germination percentage/100) × (plumule length (cm) + radicle length (cm))]

Data obtained from experiment were subjected to analysis variance (ANOVA) using SAS 9.1 software. The mean values of the main factors were compared by Fisher’s least significant difference (LSD) test at p-value of 5%, and in the case of significance of interactions (by ANOVA), sliced analysis was carried out and means values were compared using least square means test (Soltani, 2007).

RESULTS and DISCUSSION

Germination Percentage

Interaction of priming and osmotic stress was significant on germination percentage at p-value of 1%. In sliced analysis table, effect of priming was significant on germination percentage for different osmotic potential levels at p-value of 1%. Maximum germination percentage (92%) in no-stress conditions was observed for 3% potassium nitrate and 0.2 mM salicylic acid treatment which exhibited 12% incremental germination compared to non-primed control case. At osmotic pressure of -3 bars, the highest germination percentage belongs to 0.2 mM salicylic acid, which is not significant, compared to other primed treatments except for hydropriming. The difference is significant in comparison with the control treatment. Also, at osmotic pressure of -6 bars, the largest germination percentage is observed for 0.2 mM salicylic acid and exhibit significant difference with other treatments, and, a 58% increase was achieved compared to the control treatment (Table 1).

The results indicated that germination percentage significantly decreased with increase in osmotic stress; this value was improved as a result of priming application. Increase in germination percentage as a result of seed priming with the aid of materials which provide low water potentials was reported by some researchers for pea (Musa et al. 2001), corn, rice, and melon (Harris et al. 2001). Also, Murugu et al. (2004) reported that difference between pretreating materials in terms of germination is associated with different availability levels of water for seeds during pretreatment. Application of salicylic acid prevents from reduction of auxin and cytokinin in the seedlings encountered with stress, resulting in improvement of cellular division in meristem of root tip, accordingly, contributing to enhancement of plant growth and production. Hus and Sung (1997) and Bewly & Black (1994) reported that priming leads to increase of antioxidant enzymes including glutathione-ascorbate in the seeds. These enzymes reduce activity of lipid peroxidation during germination, and as a result, lead to augmentation of germination percentage.

Nasiri et al. (2014) showed that seed pretreatment with 0.5 mM SA

Table 1. Mean comparison of osmotic stress and seed priming type's interactions on Black cumin germination traits.

Osmotic stress	seed priming types	Germination percentage	Germination rate (seed/day)	Seed vigor	Plumule length (mm)	Radicle length (mm)
Control (0 bar)	No-priming	79 d(b)	16 e (e)	4.73 d (c)	29 cde (c)	30 e (d)
	KNO_3 1%	91 a (a)	31 a (a)	6.17 b (bc)	30 cd (c)	37 abc (ab)
	KNO_3 3%	92 a (a)	30 ab (ab)	11.7 a (a)	90 a (a)	38 ab (a)
	Salicylic acid 0.2 mM	92 a (a)	27 c (c)	6.44 b (b)	37 b (b)	33 d (c)
	Salicylic acid 0.5 mM	90 ab (a)	28 bc (c)	6.57 b (b)	36 b (b)	37 bc (ab)
	Hydro-priming	84 cd (b)	23 d (d)	6.29 b (b)	40 b (b)	35 cd (bc)
-3 bar	No-priming	62 f (c)	14 f (c)	2.92 f (d)	22 f (c)	24 f (d)
	KNO_3 1%	83 cd (a)	27 c (a)	5.52 c (a)	28 de (b)	37 abc (a)
	KNO_3 3%	82 cd (a)	22 d (b)	4.18 e (c)	28 de (b)	23 f (d)
	Salicylic acid 0.2 mM	85 bc (a)	29 bc (a)	5.54 c (a)	31 cd (ab)	33 d (b)
	Salicylic acid 0.5 mM	82 cd (a)	27 c (a)	5.11 cd (ab)	32 c (a)	30 e (c)
	Hydro-priming	67 e (b)	20 d (b)	4.75 d (b)	30 cd (ab)	40 a (a)
-6 bar	No-priming	0 i (d)	0 i (d)	0.00 i (d)	0 h (d)	0 i (d)
	KNO_3 1%	38 g (b)	10 g (b)	0.20 i (d)	1 h (d)	4 h (c)
	KNO_3 3%	41 g (b)	12 fg (b)	1.08 h (c)	13 g (c)	14 g (b)
	Salicylic acid 0.2 mM	58 g (a)	17 e (a)	2.56 f (a)	21 f (b)	23 f (a)
	Salicylic acid 0.5 mM	40 g (b)	9 gh (bc)	1.96 g (b)	26 e (a)	23 f (a)
	Hydro-priming	31 h (c)	8 h (c)	0.85 h (c)	12 g (c)	16 g (b)
LSD		5.25	2.20	0.53	3.72	2.61

Means in each column, fallowed by similar letter(s) are not significantly different at 5% probability level, using LSD (out of the parentheses) and L.S. Means (inside the parentheses) test. The letters out of the parentheses and inside the parentheses shows mean comparison of total interaction and sliced effects, respectively

significantly increased germination percent and germination rate compared to control. The mechanism that salicylic acid enhances seed germination is not yet clearly understood (Jamshidi-Jam et al. 2012), but according to Nun et al. (2003), salicylic acid can inhibit the activity of catalase. Reduction of catalase activity lead to increased hydrogen proxide that it can improve some seeds germination. It is possible that salicylic acid stimulates the seed germination via bio-synthesis of GA and acts as a thermogenesis inducers (Shah 2003). These results are in agreement with those obtained by other researchers such as Farahbakhsh (2012) on fennel, Bahrani & Pourreza (2012) on wheat, Dallali et al. (2012) on Sulla (*Hedysarum carnosum* and *H. coronarium*).

Germination Rate

According to ANOVA (Analysis of Variance) of germination traits under osmotic stress, interaction of priming and osmotic stress is proved to be significant at p-value of 1%. According to sliced analysis, effect of priming at different osmotic potential levels is observed to significant on germination rate (Table 1). In stress-free conditions, significant contrast was observed in 1% potassium nitrate treatment with highest germination rate compared to control case and other treatments except for 3% potassium nitrate. At osmotic potential of -3 bars, 0.2 mM salicylic acid exhibits a higher germination rate (13.7 seed per day) compared to the control case, indicating a significant difference. This treatment was classified in the same statistical group with 1% potassium nitrate and 0.5 mM salicylic acid treatments. Here, the control treatment yields the lowest germination rate. At osmotic potential of -6 bars, 0.2 mM salicylic acid treatment results in higher germination rate and a significant difference was observed in comparison with the control and other treatments (Table 1).

In both stressed and stress-free conditions, priming increased the germination rate compared to the control treatment (no priming). Germination rate is among the essential parameters in determining the quality of crop seeds, and normally, has direct relation to plant growth and amount of products. Results demonstrated that osmotic stress affects the germination time which is an index of seed germination rate, leading to delay in germination time and reduction of germinated seeds per day. Improved germination rate has been also reported in corn, rice and pea seeds as a result of priming (Harris et al., 2001). K^+ content in embryo of treated tomato seeds in KNO_3 is 50% larger than the non-treated seed embryos, which indeed contributes to osmotic water absorption by seeds (Alvardo & Bradford 1988). It must be mentioned that germination rate parameter is more sensitive to osmotic stress compared to germination percentage; slight variations in water potential reduces the germination rate. This result was consistent with findings of Baalbaki et al. (1999) who stated that germination rate was more sensitive to water deficiency stress compared to germination percentage in wheat cultivars.

These results are in agreement with Farhbakhsh (2012) also reported that application of 0.25 and 0.5 mM of salicylic acid for seed priming of fennel were effective on germination percentage and rate, seed stamina index under different water potentials (0, -2, -4 and -6 bar).

Seed Vigor

Interaction of water stress and priming is also significant under osmotic stress for seed vigor (Table 1). According to sliced analysis, effect of priming was significant on seed vigor at p-value of 1% for different levels of osmotic potential (Table 1). Potassium nitrate at 3% in stress-free state exhibits more favorable seed vigor compared to the control and other treatments such that the difference with control treatment is around 7.4%, suggesting a very significant difference. Hydropriming, 0.2 and 0.5 mM salicylic acids and 1% potassium nitrate treatments are not significantly different from each other. At osmotic potential of -3 bars, 0.2 mM salicylic acid yields the highest seed vigor, indicating an improvement compared to control treatment (2.92%). No significant contrast was observed in comparison with 1% potassium nitrate and 0.5 mM acid salicylic. At osmotic pressure of -6 bars also, 0.2 mM salicylic acid exhibits higher value for seed vigor compared to the control and other treatments, and, the lowest value is observed for 1% potassium nitrate and control treatments (Table 1).

Seed vigor is evaluated in terms of germination percentage and seedling length and has direct relationships with both parameters. Low seed vigor might affect the plant yield in two ways: first, percentage of emargenced seedlings in the field is less than expected, and consequently, plant density falls below the desired level, and second, the growth rate of seedling in such plants might be lower than the growth rate of plants produced from strong seeds (Roberts & Osei-Bonsu 1988). Eisvand & Alizadeh (2003) indicated that premature aging lowers seed vigor index of *Dracocephalum moldavica* more than germination percentage. Increase in the respective index could be regarded as a promising sign of quality trend of aged seeds. In a study conducted on improvement of physiological quality of hormone priming, it was observed that vigor index in both moisture states of the respective experiments can be effectively improved by hormone priming in aged seeds (Eisvand et al. 2008).

Nasiri et al. (2014) showed that seed vigor of Milk Thistle increased with priming of seeds by 0.5 mM SA significantly compared to other treatments in osmotic stress. But treatments of Hydro priming and 1 mM SA did not significantly increase the seed vigor compared to the control.

Plumule and Radicle Length

Mutual impact of osmotic stress and priming is significant on plumule length trait (Table 1). According to sliced analysis, effect of priming was significant on plumule length at p-value of 1% for different levels of osmotic potential (Table 1). In stress-free conditions, 90 mm was the largest average plumule length which belongs to the seedling treated with 3% potassium nitrate and was significant different (60 mm) from the control treatment; the difference was significant compared to other treatments as well. At osmotic pressure of -3 bars, priming with 0.5 mM salicylic acid assumes the largest plumule length which was significantly different from the control treatment but the respective treatment is classified in the same statistical group as 0.2 and 0.5 mM salicylic acid treatments. At osmotic pressure of -6 bars also, 0.5 mM salicylic acid leads to more significant difference compared to the control treatment (Table 1).

Plumule length was reduced by decrease in osmotic potential. Former research demonstrated that seed priming altered radicle and plumule growths; the level of changes differs depending on the species and priming conditions. Difference in radicle and plumule growths between the primed and non-primed seeds was evident such that primed seeds feature larger radicle and plumule lengths (Riyazi et al. 2007).

ANOVA suggests that interaction of osmotic stress and priming is significant on radicle length (Table 1). According to sliced analysis, effect of priming is observed to be significant on radicle length for different osmotic pressure levels at p-value of 1% (Table 1). Priming with 3% potassium nitrate featured a larger radicle length in stress-free state compared to non-primed control treatment and had no significant difference in comparison with 1% potassium nitrate and 0.5 mM salicylic acid pretreatments. At osmotic pressure of -3 bars, hydropriming yields greater radicle length, indicating significant difference with respect to other treatments. At osmotic pressure of -6 bars, 0.2 mM salicylic acid causes larger radicle length and is classified in the same statistical group only with 0.5 mM salicylic acid treatment (Table 1).

Nasiri et al. (2014) also showed that seed priming with 0.5 mM SA significantly increased radicle length (RL), seedling weight (SW) and seed stamina index (SSI) compared to control. But in the case of plumule length (PL) both hydro priming and salicylic acid (0.5 and 1 mM) were led to a significant increase in this trait. Enhance of those traits may be owing to early emergence induced by pretreatment as compared to un-primed seeds (Khan et al., 2009). These results are also in verification with Farahbakhsh (2012) and Ghoohestani et al. (2012) showed that seeds primed with salicylic acid resulted to improve of plant growth characters. These positive effects are probably due to the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds (Sivritepe et al. 2003).

Dry Weight of Plumule and Radicle

Mutual impact of priming and osmotic stress was significant on dry weight of plumule (Table 2). In sliced analysis, effect of priming in different osmotic potentials was observed to be significant on dry weight of plumule at p-value of 1% (Table 2). Potassium nitrate at 1% in stress-free state yields the maximum plumule weight (0.64 mg) which was significantly different from the control treatment (0.29 mg); the difference, however, was not

Table 2. Mean comparison of osmotic stress and seed priming type's interactions on Black cumin seedling growth traits.

Osmotic stress	Seed priming types	Plumule weight (mg)	Radicle weight (mg)	WMSR[a] (mg)	CEMSR[b]	SRDP[c] (%)
Control (0 bar)	No-priming	0.35 ij (c)	0.28 ef (b)	1.04 ef (a)	0.60 h (d)	52.6 ef (a)
	KNO_3 1%	0.64 ef (a)	0.22 fg (c)	1.04 ef (a)	0.83 ef (bc)	52.4 ef (a)
	KNO_3 3%	0.59 fg (ab)	0.38 b (a)	0.99 fg (c)	0.98 d (a)	49.9 fg (a)
	Salicylic acid 0.2 mM	0.33 ij (c)	0.35 bc (a)	0.91 h (b)	0.74 fg (c)	46.1 h (b)
	Salicylic acid 0.5 mM	0.56 g (b)	0.35 bc (a)	1.04 ef (a)	0.87 e (b)	52.6 ef (a)
	Hydro-priming	0.38 i (c)	0.38 b (a)	0.92 h (b)	0.83 ef (bc)	46.2 h (b)
-3 bar	No-priming	0.48 h (c)	0.36 b (b)	1.09 de (b)	0.77 efg (e)	55.1 de (b)
	KNO_3 1%	1.0 a (a)	0.44 a (a)	0.95 gh (d)	1.56 a (a)	47.9 gh (d)
	KNO_3 3%	1.0 a (a)	0.35 b (b)	1.02 f (c)	1.38 b (b)	51.4 f (c)
	Salicylic acid 0.2 mM	0.91 b (b)	0.31 cd (c)	1.13 cd (b)	1.09 c (d)	56.8 de (b)
	Salicylic acid 0.5 mM	0.29 b (b)	0.25 ef (d)	1.12 d (b)	0.48 i (f)	56.3 d (b)
	Hydro-priming	0.91 b (b)	0.43 a (a)	0.93 h (d)	1.20 b (c)	46.9 h (b)
-6 bar	No-priming	0.00 l (f)	0.00 h (d)	0.43 i (d)	0.00 k (d)	21.6 i (d)
	KNO_3 1%	0.4 i (d)	0.43 a (a)	1.18 bc (b)	0.7 g (b)	59.3 bc (b)
	KNO_3 3%	0.8 c (a)	0.23 f (b)	0.99 fg (b)	1.04 cd (a)	50.1 fg (c)
	Salicylic acid 0.2 mM	0.71 d (b)	0.19 g (c)	1.27 a (a)	0.71 g (b)	64.1 a (a)
	Salicylic acid 0.5 mM	0.22 k (e)	0.19 g (c)	1.18 bcd (b)	0.34 j (c)	59.6 b (b)
	Hydro-priming	0.67 ge (c)	0.44 a (a)	1.02 de (c)	1.09 c (a)	51.6 f (c)
LSD		0.06	0.03	0.05	0.10	2.81

Means in each column, fallowed by similar letter(s) are not significantly different at 5% probability level, using LSD (out of the parentheses) and L.S. Means (inside the parentheses) test. The letters out of the parentheses and inside the parentheses shows mean comparison of total interaction and sliced effects, respectively

[a]WMSR: weight of mobilized seed reserve; [b]CEMSR: conversion efficiency of mobilized seed reserve to seedling tissue; [c]SRDP: seed reserve depletion percentage

significant compared to 3% potassium nitrate. Potassium nitrates at 1% and 3% featured greater plumule weights at osmotic pressure of -3 bars and exhibited a significant contrast with the control and other treatments. At osmotic pressure of -6 bars, the maximum plumule weight was observed in 3% potassium nitrate having significant difference compared to other treatments (Table 2).

Mutual impact of priming and osmotic stress was proved to be significant on radicle weight (Table 2). According to sliced analysis, effect of priming was observed to be significant for different osmotic potentials on dry weight of radicle at p-value of 1% (Table 2). In stress-free state, hydropriming and 3% potassium nitrate yielded the maximum radicle weight; significant difference was observed in comparison with the control and 1% potassium nitrate treatments. 1% potassium nitrate at osmotic potential of -3 bars yielded greater radicle weight, suggesting significant difference compared to the control and other treatments except for hydropriming. Hydropriming also yielded the best result at osmotic potential of -6 bars, which was not significantly different from other treatments. It was worth mentioning that 0.2 and 0.5 mM salicylic acid treatments were classified in the same statistical group (Table 2).

Increase in dry and wet weights of radicle as a result of seed pretreatment in peas was reported by Satvir et al. (2003). They also showed that the seeds pretreated with NaCl produced lower dry weight of radicle. Joudi & Sharifzadeh (2006) believed that increase in seed root length and dry weight of radicle is probably due to stimulation of metabolic activities inside the embryo.

The former researches demonstrated that seed priming alters radicle and plumule growths through causing a series of variations in water absorption by primed seeds in comparison with non-primed ones. The variation level differs depending on the species and priming conditions (Riyazi et al. 2007). Length of hypocotyl axis decreases due to presence of large molecules and also the property of polyethylene glycol in inducing negative potential which impairs water absorption through the root and causes the plant to dry out. Length of hypocotyl axis assumes remarkable reduction under severe water deficiency stress compared to the control treatment. For this case, it can be stated that polyethylene glycol prevents from enlargement of hypocotyl (Memar & Nasirzadeh 2006).

Our results are in confirmation with Nasiri et al. (2014) that showed the greatest increase in seed dry weight was observed in seed priming with SA at concentration of 0.5 mM as 9.3 % compared to non-primed seedlings. Also in agreement by result of Bajehbaj (2010) evaluated the effects of priming with KNO_3 on the germination traits and seedling growth of four *Helianthus annuus* L. cultivars under salinity conditions and reported that germination traits of primed seeds was greater than that of un-primed seeds.

Weight of mobilized seed reserve

Mutual impact of priming and osmotic stress was significant on weight of mobilized seed reserve (Table 2). In sliced analysis, effect of priming in different osmotic potentials was observed to be significant on weight of mobilized seed reserve at p-value of 1% (Table 2). The largest value of mobilized seed reserve (1.04 mg) in stress-free state belonged to 1% potassium nitrate, control and “0.5” mM salicylic acid treatment which were significantly different from other treatments. At osmotic potential of -3 bars, 0.2 mM salicylic acid mobilized seed reserve compared to other treatments but had insignificant difference with the control case and was classified in the same statistical group as 0.5 mM salicylic acid. At osmotic potential of -6 bars, the non-germinated control and 0.2 mM salicylic treatments exhibited the maximum amount of mobilized seed reserve and were significantly different from 1% potassium nitrate (Table 2).

Seed germination consists of two distinctive metabolic processes: 1) Enzyme hydrolysis of seed reserve, and 2) formation of new seedling structures. Synthesis of hydraulic enzymes including amylase and ribonuclease, protease, phosphatase, and 1-3 gluconase is activated in seed germination. These enzymes undertake hydrolysis of accumulated matters including carbohydrates, lipids, proteins, and phosphorous compounds. Hydrolysis products are consumed in synthesis of seedling tissues and dry weight of seedling during germination is lower than the weight of mobilized matters due to partial consumption of the matters for respiration. In addition, heterotrophic growth of seedlings can be divided based on two elements: weight of mobilized or transferred supplies and conversion efficiency of mobilized seed supplies into the seedling tissues (Soltani et al. 2006). Reduction in dry weight of seedling can be attributed to impairment of mobility level of seed supplies and/or reduction of conversion efficiency of mobilized supplies. Also, Soltani et al. (2002) reported that reduction in seedling growth as a result of osmotic stress is mainly because of reduction in depletion of seed supplies.

Conversion efficiency of mobilized seed reserve to seedling tissue

Mutual impact of priming and osmotic stress was significant on conversion efficiency of mobilized seed supplies (Table 2). According to sliced analysis, effect of priming in different osmotic potentials was observed to be significant on conversion efficiency of mobilized seed reserve at p-value of 1% (Table 2). In stress-free conditions, the highest conversion efficiency of mobilized supplies was 0.98% which belonged to 3% potassium nitrate pretreatment and was significantly different from the control treatment. The respective treatment was not classified in the same group with any of the treatments. At osmotic potential of -3 bars, 1% potassium nitrate exhibited the highest efficiency among all treatments including the control case. The highest conversion efficiency of mobilized reserve was observed for hydropriming at osmotic potential of -6 bars compared to other treatments; the difference was insignificant only compared to 3% potassium nitrate treatment (Table 2).

Stress affects seedling growth rate, conversion efficiency rate of seed supplies, and conversion efficiency of seed reserves in pea (Soltani et al. 2002). Furthermore, they demonstrated that reduction in wheat seedling growth caused by both osmotic and salinity stresses results from reduction in seed depletion and conversion efficiency is not affected by the stresses. The efficiency is higher in stress-free state, and, larger amount of matters was devoted to generation of tissues; yet, with increase in stress level, conversion efficiency of the supplies was allocated to maintenance respiration.

Seed reserve depletion percentage

Significance of interaction effect of priming and osmotic stress was observed on seed reserve depletion percentage in ANOVA (Table 2). In sliced analysis, effect of priming in different osmotic potentials was seen to be significant on seed reserve depletion percentage at p-value of 1% (Table 5). In absence of osmotic stress, the control and 0.5 mM salicylic acid treatments yielded the greatest value of seed reserve depletion percentage (52.56%); the difference was not significant with respect to 1 and 3% potassium nitrate treatments. At osmotic pressure of -3 bars, 0.2 mM salicylic acid exhibited higher values and was classified in the same statistical group with the control and 0.5 mM salicylic acid treatments. 0.2 mM salicylic acid assumed the largest percentage of mobilized supplies at osmotic pressure of -6 bars; the difference was observed to be significant compared to the control and other treatments (Table 2).

CONCLUSIONS

It is well established that seed priming especially hormonal seed priming by SA and halo priming by KNO_3 improved all germination traits under osmotic and non osmotic stress. Also, in no-stress condition, the maximum germination percentage, seed vigor, plumule and radicle lengths, radicle weight, conversion efficiency of mobilized seed reserve to seedling tissue, were obtained by potassium nitrate 3 %. The maximum germination rate, weight of mobilized seed reserve and seed reserve depletion percentage were observed as a result of applying hydropriming and 0.5 mM salicylic acid treatments. Our finding also showed that at osmotic pressure of -3 (bars), priming with 0.2 mM salicylic acid led to enhancement of germination rate and percentage, seed vigor, and weight of mobilized seed reserve. The largest radicle weight and length were observed by 1% potassium nitrate application. Increasing osmotic potential to -6 (bars), priming of 0.2 mM salicylic acid improved germination percentage and rate, seed vigor, and radicle length. Priming with 0.5 mM salicylic acid gave up the maximum plumule length and conversion efficiency of mobilized seed reserve to seedling tissue. Also, the largest values for radicle weight and seed reserve depletion percentage were observed by hydro-priming application.

Moreover, from results of this study, we conclude that Germination components declined linearly with increase in intensity of osmotic stress. Although germination rate also decreased in the primed seeds as osmotic stress increased; but germination rates or primed seeds were higher than in control seeds at all osmotic stress levels. Taking into account the fact that primed seeds have higher germination rates than the control case, more dry matter was accordingly produced under osmotic stress compared to the control seeds during a certain time period.

In the present research, the best priming treatments under osmotic stress were 0.2 mM salicylic acid and 3% potassium nitrate. It is probably due to the fact that potassium nitrate was capable of enhancing the germination rate and percentage as well as other seed germination components through nutritional strengthening of the seed. Here, KNO_3 concentration of 3% compared to concentration of 1% had higher influence on the germination attributes of *Nigella sativa*.

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