African Journal of Biotechnology Vol. 12(11), pp. 1223-1228, 13 March, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1941
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Effects of hydropriming on seed germination and seedling growth in sage (*Salvia officinalis* L.)

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Accepted 16 October, 2012

The germination of *Salvia officinalis* L. (sage) seeds is a problem of great concern that may be overcome by employing seed priming techniques. Seed priming is an efficient technique for improvement of seed vigor, increasing germination and seedling growth. Little information has been reported on seedling development of sage subsequent to seed priming. The influence of hydropriming treatments on seed parameters of *S. officinalis* L. was investigated. Seeds of sage were treated by hydropriming at three temperatures 10, 20, 30°C for 0, 12, 24 and 48 h. The hydropriming clearly improved the final germination percentage (FGP), mean germination time (MGT) and synchronized the germination of seeds at each three temperature. All the seed treatments resulted in germination enhancement except hydroprimed seeds for 48 h at temperature 30°C. However, hydropriming (12 h at 30°C) was most effective in improving seed germination that FGP was increased by 25.5% as compared to that of non-primed seeds.

Key words: Germination, hydropriming, *Salvia officinalis* L., seed priming.

INTRODUCTION

Salvia officinalis L. plant belongs to Lamiaceae family, having 900 species around the world (Kandemir, 2003; Joshi and Pant, 2010). Sage is a perennial hardy subshrub, native to the Mediterranean regions. It grows naturally in many places throughout the world (Khan et al., 2011). *S. officinalis* is cultivated for culinary, medicinal and ornamental purposes and hence has ethno-pharmacological and economic importance (Khan et al., 2011; Mossi et al., 2011). Sage dried leaves are used as raw material in medicine, perfumery and food industry (Ali and Aboud, 2010). Also, *S. officinalis* L. is one of the richest sources of antioxidants such as rosmarinic acid, caffeic acid, carnosic acid and oligomers of caffeic acid with multiple catechol groups (Bors et al., 2004). It is propagated sexually through seeds and vegetatively through stem cuttings. Because of unsynchornization and unviability, the germination percentage of commercial seeds is generally low. Its final germination percentage is 60% seeds and it germinates between 4 and 21 days after sowing (Budvytyte, 2001; Mossi et al., 2011; Nicola et al., 2005).

For increasing final germination percentage, improving germination rate, accelerating the synchronized seed germination, vigorous seedling establishment, stimulating vegetative growth and crop yield, various seed priming techniques (McDonald, 2000; Farooq et al., 2005, 2006, 2007) have been applied in many plants such as mustard (Srivastava et al., 2010), chickpea (Kaur et al., 2002), muskmelon (Nascimento, 2003), sunflower (Wahid et al., 2008; Hussain et al., 2006), wheat (Basra et al., 2003; Ahmadi et al., 2007; Yari et al., 2010), cotton (Afghani Asl and Taheri, 2012), galbanum (Rahnama and Tavakol, 2007), maize (Moradi et al., 2008), grain sorghum (Moradi and Younesi, 2009), rice (Farooq et al., 2005,

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Abbreviations: FGP, Final germination percentage; **MGT,** mean germination time; **GI,** germination index; **Z,** synchrony of the germination; **SVI,** seedling vigor index.

2006, 2007; Mokhberdoran et al., 2009) and sugarcane (Patade et al., 2009).

Various seed priming techniques have been developed to improve seeds vigor, such as hydropriming, osmopriming, matripriming, hardening, osmohardening, and hormonal priming (Basra et al., 2003). Hydropriming is a special type of seed priming in which seeds are soaked in water followed by drying of seeds, but the emergence of the radicle is prevented (Cantliffe et al., 1984; Farooq et al., 2006). This technique is a common method that can increase rate, percentage and uniformity of germination of seed (Farooq et al., 2005, 2006; Srivastava et al., 2010).

Since cultivation and production of this medicinal plant is essential and there are some major difficulties to yield high production in this plant such as the lack of synchronized crop establishment, relatively little information is available on the seed germination requirements of this plant and the absence of literatures on suitable methodology like seed priming techniques. Therefore, the objective of this study was to survey the effects of hydropriming on sage seed germination and seedling growth quantity parameters.

MATERIALS AND METHODS

The experiment was conducted at ROOYESH Research Laboratory, Isfahan, Iran in 2011. Seed of *S. officinalis* L., cultivar SHIRAZ, was used. The seed was obtained from PAKAN BAZR Company. Moisture content was determined by grinding the seeds and drying at 107°C for 17 h. Sage seeds were dipped in 5% NaClO (sodium hypochloride) for 10 min to avoid fungal invasion and again washed thoroughly with sterilized water under aseptic conditions. Hydropriming was carried out in distilled water, without aeration, at three different temperature of 10, 20, 30°C for 0, 12, 24 and 48 h.

The distilled water was sterilized for primed seeds. Sage seeds were fully immersed in sterilized water and kept for different time intervals (12, 24 and 48 h) at three temperature 10, 20, 30°C. The seeds were then taken out from distilled water (as priming solutions) and dried on a filter paper at room temperature. Dry Salvia seeds were considered as a control treatment (non-primed).

Four replicates of 50 seeds (50 × 4 = 200 seeds) were uniformly planted in 9.5 cm Petri dish on Wathman No.1 paper surface and watered with distilled water as necessary. The experiment consisted of 10 treatments. Therefore, the total amounts of seeds exposed to hydropriming were 2,000. Seeds were incubated in germinator chamber with 12/12 h light/dark regime at 21 ± 0.1°C and germination counts were made every day for 14 days according to the methods of the Ellis et al. (1985). The time to reach 50% germination (T_{50}) was calculated according to the following formula of Coolbear et al. (1984) modified by Farooq et al. (2005):

$$T_{50} = t_i + [(N / 2 - n_i) (t_i - t_j)] / n_i - n_j$$

Where, N is the final number of emergence and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j, respectively when $n_i < N / 2 < n_j$. Final germination percentage (FGP) was calculated with the following formula:

$$FGP (\%) = n / N \times 100$$

Where, n is number of germinated seeds and N is number of total seeds. Also, seedling vigor index (SVI) was calculated by the following formula:

$$SVI = FGP \times DW$$

Where, DW is seedling dry weight. Mean Germination Time (MGT) was calculated using the formula of Ellis and Roberts (1981):

$$MGT = \Sigma Dn / \Sigma n$$

Where, n is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination. The germination index (GI) was calculated as described in the Association of Official Seed Analysts (AOSA, 1983) by following formula:

$$GI = G_1 / T_1 + G_2 / T_2 + \ldots + G_n / T_n$$

Where, G1, G2,..., Gn: number of germinated seed on the first count, second count, and so on until the last count (n), respectively, and T1, T2,..., Tn: number of days between sowing and the first count, between the sowing and the second count, and so on until the last count (n), respectively. Synchrony of germination is calculated by the expression:

$$Z = \Sigma C_{ni, 2} / N,$$

Being $C_{ni, 2} = n_i(n_i - 1) / 2$ and $N = \Sigma n_i (\Sigma n_i - 1) / 2$,

Where, $C_{ni, 2}$ is combination of the seeds germinated in the time i, two together, and n_i is number of seeds germinated in the time i. Then, Z = 1 when the germination of all seeds occur at the same time and Z = 0 when at least two seeds could germinate, one at each time (Ranal and Santana, 2006) according to Primack (1980).

The experiment was conducted with a factorial arrangement based on completely randomized design with four replications. Root and shoot length, and seedling fresh and dry weights were recorded 14 days after sowing. Seedling dry weight of each replication was determined by drying normal seedling obtained at 14 days after sowing in an oven at 107°C for 17 h. The data were analyzed with the analysis of variance using SPSS software. Mean separations were obtained using a Duncan's New Multiple Range Test (DMRT) at the 0.05 probability level.

RESULTS

Analysis of variance of data showed that effects of temperature, duration of hydropriming, and their interactions were significant for traits of mean germination time (MGT), seedling vigor index (SVI), root/shoot ratio, seedling fresh and seedling dry weight (Tables 1 and 2). Also, the effects of duration of hydropriming and its interaction with temperature on final germination percentage (FGP), germination index (GI) and synchrony of the germination (Z) process were significant, whereas the effect of temperature was not significant on these traits (Table 1). Characters of root and shoot length were affected significantly by temperature and its interaction with hydropriming duration, but the effect of hydropriming duration was not significant (Table 2). T_{50} was not affected by temperature, duration of hydropriming, and their interactions (Table 1).

Table 1. Analysis of variance of the effects of hydropriming duration and temperature on the evaluated traits in sage.

Source of variance	df	Mean of square				
		FGP	MGT	GI	T_{50}	Z
Temperature (TP)	2	113.8^{ns}	0.772*	0.544^{ns}	7.619^{ns}	0.004^{ns}
Time (T)	2	1723.8**	0.977**	22.834**	1.263^{ns}	0.042**
TP*T	4	432.2**	2.706**	8.774**	2.949^{ns}	0.057**
Error	30	66.2	0.164	0.948	4.126	0.003

FGP, Final germination percentage; **MGT,** mean germination time; **GI,** germination index; T_{50}, time to 50% germination; **Z,** synchrony of the germination process. **ns, * and **,** non-significant and significant at the 0.05 and 0.01 levels of probability, respectively.

Table 2. Analysis of variance of the effects of hydropriming duration and temperature on the seedling vigor in sage.

Source of variance	df	Mean of square					
		SVI	Shoot length (cm)	Root length (cm)	Root/shoot ratio	Seedling fresh weight (g)	Seedling dry weight (g)
Temperature (TP)	2	39.369**	3.909**	2.975**	0.267**	0.384**	0.008**
Time (T)	2	42.624**	0.496^{ns}	0.276^{ns}	0.361**	0.259**	0.003**
TP*T	4	8.621**	1.572**	2.106**	0.478**	0.160**	0.001**
Error	30	1.75	0.156	0.454	0.045	0.013	0.000

SVI, Seedling vigor index; **ns, * and **,** non-significant and significant at the 0.05 and 0.01 levels of probability, respectively.

Table 3. Effect of hydropriming treatments on the germination of *Salvia officinalis* L.

Hydropriming temperature	Time (h)	FGP (%)	MGT (days)	GI	T_{50} (days)	Z
Control		60.00^{a}	7.07^{a}	4.74^{ab}	4.48	0.185^{de}
10°C	12	72.50^{abc}	5.90^{b}	6.83^{c}	3.39	0.223^{de}
	24	81.50^{c}	4.83^{c}	8.98^{de}	3.83	0.429^{a}
	48	66.50^{ab}	6.26^{b}	6.00^{bc}	4.23	0.190^{de}
20°C	12	77.00^{bc}	5.85^{b}	7.34^{c}	4.48	0.261^{cd}
	24	75.00^{bc}	4.89^{c}	8.08^{d}	4.10	0.359^{ab}
	48	65.00^{ab}	5.18^{c}	6.73^{c}	4.66	0.317^{bc}
30°C	12	85.50^{c}	4.76^{c}	9.50^{e}	3.82	0.438^{a}
	24	77.50^{bc}	6.34^{b}	7.29^{c}	3.40	0.232^{de}
	48	46.50^{d}	6.29^{b}	4.13^{a}	2.81	0.170^{e}

FGP%, Final germination percentage; **MGT,** mean germination time; **GI,** germination index; T_{50}, time to 50% germination, **Z,** synchrony of the germination process. Figures not sharing the same letters in the same column differ significantly at the 0.05 level of probability. The columns without letters have no significant differences at the 0.05 level of probability.

Hydropriming treatments improved FGP, MGT, GI and Z (Table 3). According to this data, maximum FGP (85.50%), GI (9.50) and Z (0.438) were recorded for seeds hydroprimed for 12 h at 30°C, followed by hydropriming for 24 h at 10°C. Minimum FGP (46.50%), GI (4.13) and Z (0.170) were observed in seeds hydroprimed for 48 h at 30°C, followed by control. FGP of control was 60%. Enhancement of hydropriming duration up to 24 h in each three temperature resulted in more FGP and GI as compared with non-primed seeds, whereas it was decreased by increasing of hydropriming duration at 30°C. Lowest MGT (4.76 days) was obtained in case of seeds hydroprimed for 12 h at 30°C. More uniform and earlier germination was observed as indicated by lower values of MGT while higher GI expresses the power of germination that is, germination spreads over the time. As shown in Table 4, the highest SVI (9.13) was attained from 12 h hydropriming duration at 10°C followed by 12 h at 30°C. The effects of hydropriming on T_{50}, shoot and root length, root/shoot

Table 4. Effect of hydropriming treatments on the seedling vigor of *Salvia officinalis* L.

Hydropriming temperature	Time (h)	SVI	Shoot length (cm)	Root length (cm)	Root/shoot ratio	Seedling fresh weight (g)	Seedling dry weight (g)
Control		5.38^{bc}	3.31	3.85	1.16	0.917	0.090
	12	9.13^{a}	3.11	3.14	1.01	0.850	0.126
10°C	24	8.35^{a}	3.58	3.06	0.85	1.028	0.103
	48	8.41^{a}	3.56	3.59	1.01	0.945	0.129
	12	7.64^{a}	3.42	3.77	1.10	0.960	0.099
20°C	24	4.85^{c}	3.12	3.34	1.07	0.580	0.065
	48	2.77^{d}	2.85	3.03	1.06	0.330	0.043
	12	8.59^{a}	3.48	3.44	0.99	0.993	0.101
30°C	24	7.18^{ab}	3.70	4.20	1.14	1.066	0.093
	48	2.90^{d}	3.71	4.03	1.09	0.706	0.060

SVI, Seedling vigor index. Figures not sharing the same letters in the same column differ significantly at the 0.05 level of probability. The columns without letters have no significant differences at the 0.05 level of probability.

ratio, and seedling fresh and seedling dry weight were not significant.

DISCUSSION

In this study, seed priming has been demonstrated as a successful and effective strategy for improving the germination of *S. officinalis* L. seeds. Comparisons between FGP of control (60.0%) and hydropriming treatment for 12 h at 30°C (85.5) showed that FGP was increased by 25.5% (Table 3). Significantly, higher germination percentage and rate of germination observed in hydroprimed seeds as compared to non-primed seeds indicated a positive effect of seed priming in synchronizing the seed germination process (Table 3).

Twelve hours of soaking duration at 30°C was found to be optimum time because maximum germination (85.50%), GI (9.50) and Z (0.438) were recorded, and lowest MGT (4.76) as compared to other soaking periods. Interactions between temperature 30°C and 12 h of soaking duration have provided germination optimum time. Also, 30°C temperature could have facilitated the imbibitions of water by the seeds and hence improved germination process (Berchie et al., 2010). These positive effects are probably due to the stimulatory effects of hydropriming on the early stages of germination process. When a dry seed is soaked in water, the uptake of water occurs in three stages (Varier et al., 2010). Stage (I) is imbibitions where there is a rapid initial water uptake due to the seed low water potential. Also, proteins are synthesized and mitochondria are repaired. In stage (II), there is a less increase in water uptake, but physiological activities related with germination are initiated, including synthesis of proteins by translation of new mRNAs and synthesis of new mitochondria. In stage (III), there is a rapid uptake of water where the process of germination is completed with radicle emergence (Bewley, 1997; Varier et al., 2010).

The probable reason for enhancement of percentage and uniformity of germination of the hydroprimed seed may be due to the completion of pre-germination process such as repair and synthesis of nucleic acids (DNA and mRNA), protein, repair of membranes (Bewley, 1997; McDonald, 2000; Jowkar et al., 2012) and induction of a range of biochemical changes enzymes activation (Wattanakulpakin et al., 2012).

Also, it seems the temperature could be improving these effects. Seed germination is very sensitive to hydration process. Therefore, it seems in parallel to increase of hydropriming time to 24 h, increasing FGP and GI in primed seeds as compared with non-primed seeds, is due to time duration of water uptake in hydropriming time. In other words, water uptake rate in priming period is slow and seeds had enough time to complete the pre-germination process (Varier et al., 2010).

Uniformity of primed seed germination could be due to synchronization of G_2 phase of the cell cycle and cell division in all the cells of germinating seeds. Furthermore, priming causes synchronization of the metabolism of all the germinating seeds, enhancement of the number of mitochondria and ATP production (Varier et al., 2010). Effect of higher temperature (30°C) in improving germination might be due to increase in activity of enzymes like α-amylase, protease and lipase. These enzymes play an important role in hydrolysis of macromolecules for growth and development of seed embryo (Ahmadi et al., 2007; Noorbakhshian et al., 2011a).

The improvement of germination could largely depend upon the activity of antioxidative systems (Ahmed et al., 2012). It is generally accepted that antioxidant enzymes play a role in preventing lipid peroxidation and seed damage occurs during hydration (Umair et al., 2012). In fact, increased level of antioxidant enzymes protects the cell against the oxidative damage by removal of free radicals or reactive oxygen species (Ahmed et al., 2012). Hydropriming increased activities of antioxidant enzymes like superoxide dismutase, peroxidase, catalase and ascorbate peroxidase in cucumber seed (Huang et al., 2006) and wheat (Ahmed et al., 2012). Peroxidase and polyphenoloxidase were also increased significantly by seed priming of amaranth (Moosavi et al., 2009). Delayed and poor FGP, MGT, GI and Z, in primed seeds for 48 h could be due increased timing for hydropriming (Pazdera, 2003). In stages (I) and (II) of uptake of water, seeds are tolerant to drying. However, in stage (III) where root start to appear, there was no tolerance to drying. This poor performance may be due to the start of stage (III) of germination process in hydroprimed seeds for 48 h (Bewley, 1997; Moosavi et al., 2009). Another reason could be that soaking for longer periods may have resulted in excess water been trapped within seed, causing suffocation and death of the embryo due to lack of oxygen (Finch-Savage et al., 2004). Furthermore, excess water uptake might have led to physiological seed damaging (Murungu, 2011).

Hydropriming have increased crude protein in moringa leaves (Nouman et al., 2012) and total seed protein of amaranth (Moosavi et al., 2009), chickpea (Zarei et al., 2011) as compared to the control. Beneficial effects of hydropriming on grain yield were reported in tomato (Khalil and Moursy, 1983), maize (Murungu et al., 2004), sunflower (Hussain et al., 2006), chickpea (Ghassemi-Golezani et al., 2008; Zarei et al., 2011) and pinto bean (Ghassemi-Golezani et al., 2010). Hydropriming ensure early flowering in wheat (Ahmadi et al., 2007) and maize (Murungu et al., 2004), and has reduced time from emergence to flowering and flowering to bloom in chickpea (Zarei et al., 2011). Also, seed priming of tomato resulted in significant increases in number of flowers and fruit set (Khalil and Moursy, 1983).

These findings support the earlier studies on rice (Farooq et al., 2006, 2007), sunflower (Hussain et al., 2006), wheat (Ahmadi et al., 2007), bean (Abebe and Modi, 2009; Ghassemi Golezani et al., 2010), sorghum (Moradi and Younesi, 2009), lentil (Saglam et al., 2010), tomato (Amoaghaie et al., 2010), basil (Aliabadi and Maroufi, 2011), bromus (Tavili et al., 2011), cowpea (Singh et al., 2011) and sainfoin (Noorbakhshian et al., 2011b) that have reported hydropriming improve germination percentage and rate. In conclusion, it may be concluded that seed hydropriming is a very effective technique in improving seed germination and the growth of seedlings of *S. officinalis* L. Hydropriming for 12 h at temperature 30°C was the most effective treatment, while hydropriming for 48 h at this temperature resulted in lowest germination.

Improved seed germination due to hydropriming may be explained by an increased water uptake and rate of cell division. However, more detailed investigation is required to reveal the mechanism of priming-mediated increase in the seed germination process.

ACKNOWLEDGEMENTS

The authors would like to thank PAKAN BAZR Company for providing seed material of *Salvia officinalis* L. They are also thankful to ROOYESH Research Laboratory for providing facilities.

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