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Research article

EFFECT OF PRE-TREATMENT OF LEMON BALM (*MELISSA OFFICINALIS* L.) SEEDS ON SEED GERMINATION AND SEEDLINGS GROWTH UNDER SALT STRESS

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ABSTRACT: High germination rate and vigorous early growth under salty soils is preferred because, seedlings establishment at early growth stages of crop plants is severely affected by soil salinity. A study germination and seedling growth of Lemon Balm (*Melissa officinalis* L.) was assessed using factorial experiment based on a completely randomized design with three replications. This experiment was carried out at the laboratory of Horticulture faculty of Gorgan Agricultural and Natural Resources University, Iran, March 2014. The seeds divided into five groups, equally. The first group was soaked for 6h in distilled water at 20C to serve as control while four other groups were soaked in 0.1, 0.5, 1 and 1.5 mM of salicylic acid respectively (Senaranta et al, 2002). Afterward, seeds air dried at room temperature for 36h. After a preliminary test for low water potential tolerance (caused by NaCl), four water potential levels were selected and used for germination trials. The drought solutions had water potential of zero (control), −2, −4 and −6 Bar. The results showed that effect of salt stress on germination percentage, germination rate, radical length and weight and hypocotyls length and weight were significant. Effect of Salicylic acid (SA) and interaction between salt stress and SA on germination rate were significant only. Germination percentage and germination rate was significantly increased by SA under salinity conditions compared to non treatment of SA. Priming with 1.5 mM SA showed maximum radicle and hypocotyl length. Priming with SA could improve radical length and radical dry weight in all salinity levels. However, treated seeds with SA produced the higher hypocotyl length in all salinity levels than untreated seeds. SA decreased radicle length, while increased hypocotyl length relatively Dry weight of hypocotyl was decreased due to salinity stress but seedlings raised from seeds primed with SA improved dry weight of seedlings as compared to non treatment of SA. All traits responded negatively with lowering water potential but using salicylic acid ameliorated the harmful effect of induced stress on measured traits. The concentration of 1.5 mM of salicylic acid on measured traits was more effective compared with the other levels. Therefore, seed pre-treatment with salicylic acid could be a suitable tool for improving germination characteristics of Lemon Balm under low water potential.

Key words: Lemon Balm, salicylic acid and salt stress

INRODUCTION

Seed germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Several environmental factors such as temperature, salinity, light, and soil moisture simultaneously influence germination [5]. Successful establishment of plants largely depends on successful germination [5]. In arid and semi-arid areas in almost all the regions of the world, saline soils are becoming a major problem, due to a variety of natural and man caused factors [10]. Seed priming is a technique for controlling seed slow absorption and post-dehydration ([8]. After treatment with initiators, plant seeds exhibit not only enhanced emergence rate and even emerge of seedlings [7] but also improved resistance or tolerance to cold, drought [21] and salt [14]. Initiator has obvious effects on seed germination and osmotic stress resistance [20], during which the contents of compatible solutes, proline and soluble sugar, and the activity of protective enzymes [4].

Salicylic acid (SA) plays an important role in abiotic stress tolerance, and more interests have been focused on SA due to its ability to induce a protective effect on plants under adverse environmental conditions. SA may affect directly on specific enzymes function or may activate the genes responsible for protective mechanisms [9]. Shruti and Singh [18] showed that salt-induced deleterious effects in maize seedlings were significantly eliminated by the pretreatment of SA. It is concluded that 0.5 mM salicylic acid improves the adaptability of maize plants to NaCl stress. Pre-soaking seeds in SA can protect the plants against abiotic stresses [19] but the background of this effect is not well understood. Gunes et al, [6] reported that SA could be used as a potential growth regulator to improve plant salinity tolerance.
This study was conducted to evaluate the effects of different water potential, salicylic acid concentration, and their interaction on germination percentage, germination rate and seedling growth of Lemon Balm (*Melissa officinalis*).

MATERIALS AND METHODS

A factorial experiment based on a completely randomized design with three replicates was carried out at the laboratory of Horticulture faculty of Gorgan Agricultural and Natural Resources University, Iran, March 2014. Seeds of Lemon Balm (*Melissa officinalis* L.) used in the experiment were provided from seed and plant research center. The seeds were less than 9 months old and had been previously stored in paper bags under conditions of 4C and 20% relative humidity. Seeds were surface-sterilised with a 3% sodium hypochlorite solution, rinsed in distilled water for three times and dried 36h before the experiment. Afterwards, the seeds divided into five groups, equally. The first group was soaked for 6h in distilled water at 20C to serve as control while four other groups were soaked in 0.1, 0.5, 1 and 1.5 mM of salicylic acid respectively [16]. Afterward, seeds air dried at room temperature for 36h. After a preliminary test for low water potential tolerance (caused by NaCl), four water potential levels were selected and used for germination trials. The drought solutions had water potential of zero (control), −2, −4 and −6 Bar. 25 seeds of each lot evenly placed on Whatman filter paper No.1 in sterilized 9-cm Petri dishes separately and 10 ml of each solution were added to related treatment. All Petri dishes were sealed to prevent the loss of moisture and avoid contamination, and then placed in a Conviron PGR-15 plant growth chamber for 12 days. The seed were allowed to germinate at 20C with 16/8-h light/dark periodicity. The photosynthetic photon flux density was 340-mol m^{-2} s^{-1}, provided by metal halide lamps, with a relative humidity of 45%. Germination was determined by counting the number of germinated seeds at 24-h intervals over a 12-d period and expressed as total percent germination. Seeds were considered to be germinated at the emergence of the radicle [2]. Radicle and hypocotyl lengths were measured 12 days after germination. The root and stem dry weight were determined by drying the plant material in an oven at 80C for 24-h prior to weighing.

Germination percentage and germination rate and were calculated using following formula.
G% = (n/N)*100 [3]
RG =∑(Ni /Di) [3]
G: germination percentage, n: number of seeds germinated, N: total number of seed in each petri dishes,
RG: rate of germination (seed /day), Ni: germinated seeds in each numeration, Di: day of each numeration,
The data were processed using the GLM procedure of statistical analysis system (SAS), [15].

RESULTS AND DISCUSSIONS

Germination percentage

The results showed that the effect of salt stress on germination percentage was significant only (Table 1). Mean comparison table showes that the germination percentage (GP) reduced with decreasing water potential so that the lowest GP was recorded for the -6 bar water potential. SA at the levels of 1.5 mM caused a significant increase in germination percentage at the salt stress levels. The same response was observed at 0 and -2 bar water potential (Table 2).

Table 1. Analysis of variance of Lemon Balm seeds, primed with salicylic acid under salt stress

S.O.V	DF	GP	GR	Radicle length	Hypocotyl length	Radicle DW	Hypocotyl DW
R	2	72	193	68	6	0.0000001	0.00000001
S	3	1073**	5418**	2569**	141**	0.000015**	0.000009**
SA	4	73	193**	12.9	0.55	0.0000002	0.00000015
S*SA	12	89	83**	6.3	0.28	0.0000003	0.00000013
Error	38	53	29	8.5	0.69	0.0000007	0.0000009
CV%		8.5	7.6	20.2	13.1	5.8	19.6

**, * and ns: Significant at the 1% and 5% levels of probability S= Salt stress, SA= Salicylic acid

Germination Rate

The effect of salt stress, SA and interaction between them on germination rate were significant (table 1). With increase in use of SA level germination rate increased (table 2). Interaction between SA and salt stress showed that maximum germination rate obtained in 0.5 mM SA and control level of salt stress (Figure 1).This figure showed that minimum of germination rate obtained at no use of SA in -6 bar water potential. The reason of reduction in GP at the lower water potential caused by NaCl may be due to slower rate of imbibition. From present investigations, it is quite clear that seeds primed with various concentrations of salicylic acid proved to be effective in inducing stress tolerance at the germination stage. Priming presumably allowed some repairs of damaged to membrane caused by deterioration. It has been reported that primed seeds showed better germination pattern and higher vigor level than non- primed [14].

Table 2. Means comparison of Lemon Balm seeds, primed with salicylic acid under salt stress

Treatment	GP(%)	GR(%)	radicle length(mm)	hypocotyl length(mm)	radicle DW(g)	hypocotyl DW(g)
Salt stress (bar)						
0	90a	89a	33a	10a	0.0026a	0.0051a
-2	91a	81b	12b	5b	0.0018b	0.0054a
-4	87a	67c	7c	4c	0.0010c	0.0056a
-6	73b	46d	4d	3d	0.0002d	0.0039b
LSD	5.4	4	2.16	0.61	0.0006	0.0007
SA(mM)						
0	84ab	57b	16a	6a	0.0015a	0.005a
0.1	82b	67b	13b	6a	0.0015a	0.005a
0.5	86ab	73a	14ab	6a	0.0015a	0.0049a
1	86ab	73a	14ab	6.4a	0.0013a	0.005a
1.5	89a	75a	16.6a	6.6a	0.0016a	0.0051a
LSD	6	4.5	2.42	0.68	0.0007	0.0008

Means with at least one similar letter in each column have no signification difference at %5 of probability level
S= Salt stress, SA= Salicylic acid

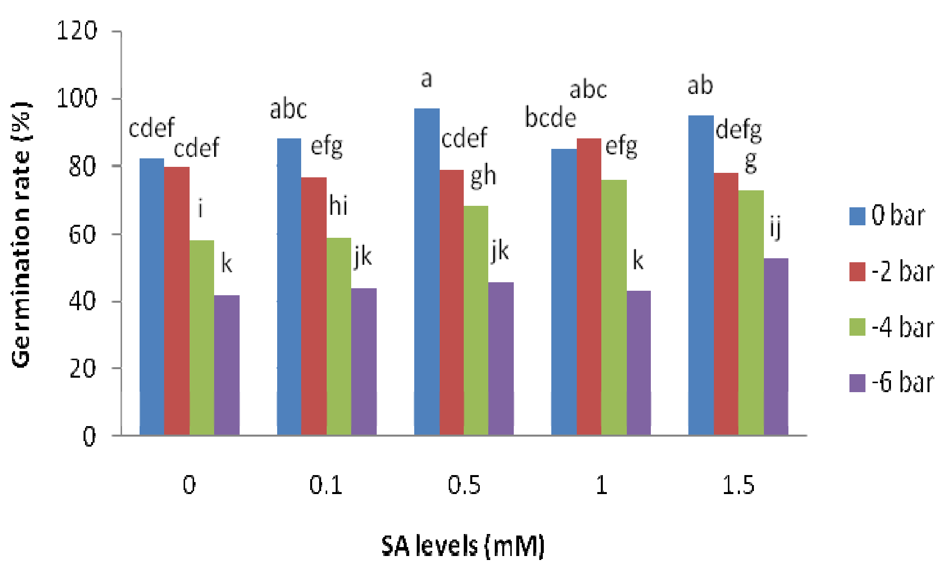

Figure 1. The effect of priming on germination rate of Lemon Balm seeds under salt stress.
Means with at least one similar letter in each column have no signification difference at %5 of probability level.

Radicle length

The effect of salt stress on radicle length was significant and the other treat were not significant on it (table 1). In salt stress levels the maximum radicle length (33 mm) obtained at zero water potential while the minimum of this trait resulted from the lowest level of water potential (-6 bar) in all levels of SA application (Table 2). In SA levels the maximum radicle length (16 mm) obtained at 1.5 mM while the minimum of this trait resulted from the 0.1 SA applications (Table 2). Elevated salinity (lower water potential) slowed down water uptake by seeds, thereby inhibited their germination and root elongation.

Hypocotyl length

The effect of salt stress on hypocotyl length was significant and the other treat were not significant on it (table 1). The response of hypocotyl length to different levels of water potential and SA was almost the same as radicle length. The response of this trait varied at different levels of water potential with different concentrations of SA. Hypocotyl length decreased with reducing the water potential at the all levels of SA. In salt stress levels the maximum hypocotyl length (10 mm) obtained at zero water potential while the minimum of this trait resulted from the lowest level of water potential (-6 bar) in all levels of SA application (Table 2). In SA levels the maximum radicle length (6.6 mm) obtained at 1.5 mM SA while the minimum of this trait resulted from the 0, 0.1 and 0.5 mM SA application (Table 2).

Seedling dry weight (SDW)

The seedling fresh and dry weight, respond differently to different levels of water potential. The response of radical dry matter to different levels of water potential and SA was almost the same as hypocotyl dry matter. The effect of salt stress on radicle and hypocotyl dry matter were significant and the other treat were not significant on them (table 1). The response of this trait varied at different levels of water potential with different concentrations of SA. Radical and hypocotyl dry matter with reducing the water potential at the all levels of SA. In salt stress levels the maximum radicle and hypocotyl dry matter obtained at zero water potential while the minimum of this trait resulted from the lowest level of water potential (-6 bar) in all levels of SA application (Table 2). In SA levels the maximum radicle and hypocotyl dry matter obtained at 1.5 mM SA while the minimum of this trait resulted from the 1 and 0.5 mM SA application respectively (Table 2). The stimulatory effect of SA levels on SDW was almost the same with SFW. The results showed that the concentration of 1.5 mM SA were more effective than other levels in improving the harmful effects of low water potential caused by NaCl. Shoot fresh and dry weight and root fresh weight were increased in seedlings raised from seeds primed with 50 ppm salicylic acid. It was also found that SA application increased the dry mass of wheat seedlings under water stress.

DISCUSSION

There are conflicting reports about the effects of salicylic acid on seed germination. Rajaskaran et al, [13] showed the external application of salicylic acid stimulates seed germination. Madah, [11] reported that low concentrations of salicylic acid increased the germination percentage, but this increase was not significant compared to control treatment. Also, Shakirova et al, [17] reported soaking wheat seeds at a low concentration of salicylic acid (0.05 mM) for 3 hours activate germination. The results of our study more consistent with Madah's [11] results. According to Nun et al, [12] salicylic acid can inhibit the activity of catalase. Reduction of catalase activity leading to increased hydrogen proxide that it can improve some seeds germination.

Application of all level of SA affected significantly the germination rate of seeds in all levels of water potential and it could lessen the effects of low water potential on these traits. If the emergence rate is slow or seedlings could not emerge, it is possible seedlings attacked and damage by present microorganisms in the soil and emergence percent decrease dramatically. In general, the highest germination rate recorded for 0.5 mM of SA at the zero water potential (no salinity). However, SA pre treatment had a different effect on germination rate at different levels of water potential (Figure 1). Osmotic stress is major factors which affect seed germination. It reduces germination rate. In high intensity stresses, in addition to germination rate, germination percentage is also affected. Afzal [1] reported that reduction of water absorption by seeds due to increase in osmotic pressure in salinity and drought stress have been reduced the physiological and metabolic processes. The mechanism of seed priming is to initiate the repairing system for membrane and the metabolic preparation for germination through controlling water absorption rate of seed [4]. As a result, the germination capability and resistance to unfavorable conditions of seed can be promoted obviously. The improvement in germination and vigor of normal/low-vigor seed might be due to reserve mobilization of food material, activation and resynthesis of some enzymes DNA and RNA synthesis start during priming. Priming can repair some damages that have been arisen from seed erosion and improve seed quality.

The probable reason for early emergence of the primed seed maybe due to the completion of pre-germination metabolic activities making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with untreated dry seed. Like germination percentage, prime seeds had higher hypocotyl and radical length compared with un-primed seeds. 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 16]. The protective and growth promoting effect of SA are presumably due to increased level of cell division within the apical meristem of seedling root, which caused an increase in plant growth.

Seed priming is a regular step before sowing in a few vegetables and flower crops in some countries. As a result, the germination capability and resistance to unfavorable conditions of seed can be promoted obviously. However, osmopriming has been shown to activate processes related to germination, for instance, by affecting the oxidative metabolic such as increasing superoxide dismotase and peroxidase or by the activation of ATPase as well as acid phosphatase and RNA syntheses. Our finding revealed that inhibition of germination at lower water potential probably resulted from osmotic effect. Seeds primed with SA gave better performance than control (unprimed) under all water potential. It seems priming increased the tolerance of seeds of Lemon Balm to water stress, therefore it can be concluded that priming is a simple, cheap and unsophisticated tool that has a practical importance and could be recommended to farmers to achieve higher germination and uniform emergence under field conditions.

In conclude, the result of this research shows that priming, induced stress tolerance in Lemon Balm seedlings. Generally, a decreasing trend in all traits was observed for increasing concentrations of NaCl. Pre-treatment with salicylic acid by the improved percentage and rate germination make to increase radicle and hypocotyl length and finally make increasing in the radicle and hypocotyl dry weight in stress and non-stress condition.

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