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Incorporation of Plant Growth Regulators into the Priming Solution Improves Sugar Beet Germination, Emergence and Seedling Growth at Low-Temperature

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Abstract: In a series of experiments, impact of inclusion of plant growth regulators into the KNO_3 priming solution on low temperature seed germination, emergence percentage and seedling growth of sugar beet was investigated. Seeds were primed in 3% KNO_3 solution for 6 days at 25°C in darkness containing one of the following: 0, 0.05, 0.1, 0.5, or 1 mM acetyl salicylic acid (ASA) or 1, 3, 5 or 10 μM methyl jasmonated (MeJA). A non-primed treatment was also included in the experiment. Priming seeds in the presence or absence of plant growth regulators in general improved final germination percentage (FGP), germination rate (G_{50}) and germination synchrony (G_{10-90}) at 15°C compared with non-primed seeds which had an FGP of 42%, G_{50} of 11.3 days and G_{10-90} of 11.7 days. Priming seeds in KNO_3 solution containing 0.05 mM of ASA resulted in the highest germination percentage (89%), fastest germination rate (G_{50} = 5.3 days) and the most synchronous germination (G_{10-90} = 10.7 days). Emergence percentages were the highest for the seeds primed in the presence of 0.05 mM ASA (83%) and 3 μM MeJA (81%) while non-primed seeds had an emergence percentage of 40%. Fastest emergence rate (E_{50}) were also obtained from seeds primed in KNO_3 supplemented with 3 μM MeJA (E_{50} = 14.4 days) and 0.05 mM ASA (E_{50} = 14.4 days). Shoot fresh and dry weight of seedlings were significantly affected by treatments and priming in the presence of 0.05 mM ASA resulted in highest seedling shoot fresh and dry weight. These results indicate that priming seeds in 0.05 mM of ASA or 3 μM MeJA incorporated into the KNO_3 solution can be more effective than KNO_3 alone to improve low temperature germination performance of seeds and subsequent seedling growth.

Key words: ASA, *Beta vulgaris*, chilling stress, KNO_3, MeJA, priming

INTRODUCTION

In the Iranian prairies, sugar beet is cultivated on approximately 178355 ha. Its production is often limited by poor seed germination and stand establishment due to low temperature at seeding in most regions as sugar beet is very vulnerable to a wide range of abiotic stresses (i.e., moisture, temperature and oxygen) during the critical first three weeks after planting (Reyes and McGrath, 2000). Optimum temperature range for sugar beet seed germination is between 20 and 30°C; seeds are sometimes sown in early spring in the field when the temperatures are suboptimal for germination. Preplant low water potential seed treatments permit partial seed hydration so that pregerminative metabolic activities proceed but germination is prevented. Such treatments affect the speed, synchrony and percentage of germination especially under such adverse seed bed conditions as low temperature, high temperature, salinity and reduced water availability (Khan, 1992). This technique which is called priming consists of the incubation of seed in an osmoticum, usually a salt or polyethylene glycol solution, in order to control their water uptake and prevent radicle protrusion (Pill, 1995).

KNO_3 has been widely used as an osmoticum in many crop seeds to increase the germination rate, total germination and seedlings uniformity, mainly under unfavorable environmental conditions. This salt has been used to enhance seed germination in low temperature in watermelon (Sachs, 1977; Demir and Oztokat, 2003; Demir and Mavi, 2004), Sugar beet (Durrant *et al*., 1983), sweet pepper by Korkmaz (2005) and Muskmelon by Korkmaz *et al*. (2005).

The approaches taken to develop stress tolerant plant include genetic engineering, breeding, *in vitro* selection and the use of plant growth regulators. Many

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molecules, for example, jasmonic acid, abscisic acid, salicylic acid, calcium and polyamines have been suggested as signal transducers and messengers (Klessig and Malamy, 1994).

Increasing evidence indicates that plants with high levels of these molecules have increased tolerance to low temperatures and an increase in endogenous concentrations of these molecules before low temperature exposure might be an essential step to activate a protection mechanism against chilling.. For example, Soaking tomato and bean seeds in aspirin or acetyl salicylic acid (ASA) solution increased seedling survival during subsequent chilling, high temperature and drought stresses (Senaratna *et al*., 2000) while inclution of methyl jasmonate (MeJA) into the KNO_3 priming solution improved germination and emergence at low temperatures in watermelon (Korkmaz *et al*., 2004) and sweet pepper (Korkmaz, 2005) more effectively than KNO_3 alone.

We hypothesized that treating the seeds which plant growth regulators that are known to enhanced plant's cold tolerance would improve performance of sugar beet seed at low temperature. Therefore, this study was undertaken to investigate if incorporation of ASA or MeJA into KNO_3 priming solution would more effectively enhance sugar beet seed germination and subsequent seedling emergence and growth at low temperature.

MATERIALS AND METHODS

Sugar beet (*Beta vulgaris* L.) cv. Karaji seeds used in the present study were obtained from the Agricultural Research Center of Kerman. All experiments reported in this study were carried out in the beginning of 2006 at College of Agriculture, Shahid Bahonar University of Kerman, Iran.

Seeds were disinfested in 5% (active ingredient) sodium hypochlorite for 5 min to eliminate seed borne microorganisms. Following disinfestations, they were rinsed under running tap water for 1 min and surface dried by placing them between paper towels for 30 min at room temperature.

Seed germination at low temperature: Priming was accomplished by imbibing 20 g of seeds for 6 days at 25°C in darkness in 3% KNO_3 containing one of the following: 0, 0.05, 0.1, 0.5, or 1 mM ASA or 1, 3, 5, or 10 μM MeJA into the priming solution, they were first dissolved in a few drops of 99% ethanol and distilled water was added to make a stock solution. A non-primed treatment was also used in the experiment as the control.

Seeds were placed in covered transparent polystyrene germination boxes (10×10×4 cm) on double layers of filter paper (Whatman No. 1) saturated with 10 mL priming solution supplement with one of the growth regulators. The priming solutions were changed every other day to maintain a constant osmotic potential. Following priming, the seeds from each box were washed in a sieve and rinsed under running tap water for 1 min and left to surface dry on paper towels under room conditions (22°C and 60% relative humidity) for 2 h to make the singulation of the seed easier. Seeds primed in 3% KNO_3 solution containing no plant growth regulators and non-primed seed were also included in the germination test.

Germination test was carried out in darkness in a temperature controlled incubator held at 15±0.5°C. Seeds were placed on two layer of filter paper moistened with 3 mL of distilled water in covered 5.5 cm petri dishes. Treatments were arranged in completely randomized design with three replications of 50 seeds. Radicle protrusion to 1 mm was scored as germination was recorded daily until the numbers stabilized (for 17 days) and germinated seeds were removed from the petri dishes. From the total number of seed germinated final germination percentage (FGP) and its angular transformation (arcsinev√FGP), days to 50% of FGP were calculated. Time to 50% of FGP (G_{50}) is an inverse measure of germination rate, while time between 10 and 90% of FGP (G_{10-90}) is considered to be an estimate of the spread of germination, the inverse of germination synchrony.

Seedling emergence at low temperature: Seed were primed as described above and 15 seeds from each treatment were planted into 2 cm depth in 15×4 cm (diameter×height) round plastic pots filled with growth medium consisting of peat and perlite in the ratio of 4:1. The pots were watered and placed in a growth chamber (model FOC 225I; Welp Scientifica, Usmate, Italy) at 15±0.5°C and under cool florescent lamps providing a photosynthetic photon flux density of 40 μmol m^{-2} sec^{-1} for 14 h $days^{-1}$ at the seedling level. The treatments were replicated four times and all the pots were arranged in a randomized complete block design in the growth chamber. Emergence counts (hypocotyls are visible) were made daily and final emergence percentage (FEP) and its angular transformation (arcsinev√FEP), days to 50% of FEP (E_{50}) and days between 10 and 90% of FEP (E_{10-90}) were calculated 30 days after planting, when the percentage of emergence had stabilized in all treatments, the number of seedlings that emerged and lived (survival ratio), shoot fresh (cut at medium surface) and dry weights (dried at 65°C for 48 h) were recorded.

Statistical analysis: Data from all experiments were subjected to analysis of variance and mean separation was performed by Fisher's least significant difference (LSD) test if F-test was significant at p = 0.05.

RESULTS

Seed germination at low temperature: Priming seeds regardless of plant growth regulators added to the priming solution significantly improved germination and G_{50} at 15°C compared with non-primed seeds; however the effects of KNO_3 + ASA or KNO_3 + MeJA was more pronounced than KNO_3 alone, for both parameters recorded (Table 1). Inclusion of 0.05 or 0.1 mM ASA into the priming solution significantly increased FGP (89 and 81%) compared with non-primed (42%) and KNO_3 treated seeds (64%). However, 0.5 mM ASA significantly increased FGP compared with non-primed seeds but not with KNO_3 treatment. Addition of 1 or 3 μM MeJA to the priming solution also caused significant improvement in seed germination performance (75 and 83%) compared with KNO_3 alone (64%) or non-primed (42%). However, as with the ASA, increasing MeJA concentration added to the priming solution from 5 to 10 μM decreased FGP of sugar beet seeds from 61 to 55%. All treatments (KNO_3 and KNO_3 + ASA or KNO_3 + MeJA) significantly improved G_{50} compared with non-primed or KNO_3 alone and the effect of KNO_3+0.05 mM ASA was more pronounced. Among the treatments, priming in KNO_3+0.05 mM ASA was the only treatment that improved the germination synchrony compared with non-primed seeds (10.7 vs. 11.7 days, respectively).

Seedling emergence at low temperature: Priming seeds regardless of plant growth regulators added to the priming solution significantly improved FEP, E50, shoot fresh weight and shoot dry weight (Table 2). However the effects of KNO_3 in the presence of ASA or MeJA were generally more effective than KNO_3 alone for some traits recorded. Furthermore, the effects of KNO_3+ASA (0.05 and 0.1 mM) or KNO_3+MeJA (1, 3 and 5 μM) treatments were significantly better than KNO_3 alone for FEP. Compared with KNO_3 alone, addition of plant growth regulators had no improving effect on E_{50}, though significantly improvement was observed when compared with non-primed seeds. KNO_3 in the presence of 0.05 mM ASA was the only treatment that significantly improved the emergence synchrony compared with KNO_3 alone treatment (7.4 vs. 9.2 days, respectively). All treatments had > 91% survival and there were no significant differences among them. Compared with KNO_3 alone, KNO_3 in the presence of 0.05 or 0.1 mM ASA or 1, 3 or 5 μM MeJA, significantly increased shoot fresh wt but in terms of shoot dry wt, only the effect of KNO_3 in the presence of 0.05 mM ASA was highly significant (25% more).

Table 1: Final germination percentage (FGP) and its angular transformation (data in parenthesis), days to 50% of FGP (G_{50}), days between 10 and 90% FGP (G_{10-90}) of sugar beet seed germination in darkness at 15°C following priming for 6 days at 25°C in 3% KNO_3 with various concentration of methyl jasmonate (MeJA) and acetyl salicylic acid (ASA)

Treatments	FGP (%)	G_{50} (days)	G_{10-90} (days)
Non-primed seeds	42 (40)f	11.30a	11.7c
KNO_3	64 (53)de	9.70b	11.0cd
KNO_3 + 0.05 mM ASA	89 (71)a	5.30g	10.7d
KNO_3 + 0.1 mM ASA	81 (64)b	7.30def	13.0b
KNO_3 + 0.5 mM ASA	67 (55)cd	8.00cd	14.0a
KNO_3 + 1 mM ASA	53 (47)e	8.30c	13.3ab
KNO_3 + 1 μM MeJA	75 (60)bc	6.70f	11.0cd
KNO_3 + 3 μM MeJA	83 (66)ab	7.3def	11.0cd
KNO_3 + 5 μM MeJA	61 (51)de	7.00ef	11.7c
KNO_3 + 10 μM MeJA	55(48)e	7.70cde	13.7ab
$LSD_{0.05}$	6.64	0.88	0.880
Significance	***	***	***

***: Significant at p≤0.001. Means with similar letter(s) are not significantly different

Table 2: Final emergence percentage (FEP) and its angular transformation (data in parenthesis), days to 50% of FEP (E_{50}), days between 10 and 90% FEP (E_{10-90}), survival ratio and shoot fresh and dry weights of sugar beet seed germination in darkness at 15°C following priming for 6 days at 25°C in 3% KNO_3 with various concentration of methyl jasmonate (MeJA) and acetyl salicylic acid (ASA)

Treatments	FEP (%)	E_{50} (days)	E_{10-90} (days)	Survival (%)	Shoot fresh wt. (g plant^{-1})	Shoot dry wt. (g plant^{-1})
Non-primed seeds	40 (39)e	18.2a	8.4d	97a	0.79g	0.065e
KNO_3	54 (47)d	14.8d	9.2cd	91a	1.36d	0.102bcd
KNO_3 + 0.05 mM ASA	83 (66)a	14.4d	7.4e	97a	1.75a	0.127a
KNO_3 + 0.1 mM ASA	74 (59)b	14.8d	9.6bc	97a	1.52bc	0.114b
KNO_3 + 0.5 mM ASA	63 (52)c	15.8cd	10.4b	96a	1.02f	0.100cd
KNO_3 + 1 mM ASA	46 (43)de	17.4ab	10.2b	95a	1.04f	0.099d
KNO_3 + 1 μM MeJA	73 (58)b	14.8d	11.4a	96a	1.51c	0.102bcd
KNO_3 + 3 μM MeJA	81 (65)a	14.4d	8.6d	98a	1.57b	0.112bc
KNO_3 + 5 μM MeJA	63 (52)c	16.4bc	9.6bc	98a	1.12e	0.106bcd
KNO_3 + 10 μM MeJA	48 (44)d	17.2abc	9.8bc	92a	1.10e	0.098d
$LSD_{0.05}$	5.993	1.452	0.828	---	0.057	0.013
Significance	***	***	***	NS	***	***

NS: Non Significant; ***: No significant or significant at p≤0.001, respectively. Means with similar letter(s) are not significantly different

DISCUSSION

The beneficial effects of priming with a KNO_3 solution on low temperature germination of sugar beet found in this study for most parameters recorded confirm the findings of Durrant *et al.* (1983). Similar results have also been obtained with other species, such as watermelon (Sachs, 1977; Demer and Oztakat, 2003), Barley, corn, sorghum, soy bean and wheat (Bodsworth and Bewley, 1981). A remaining obstacle to commercial application of seed priming by osmoticum or salt is the variability of results among species, cultivar and even seed lots (Bradford, 1986) however using combination of KNO_3 with ASA, MeJA or spermine as priming solution has yielded very consistent and promising results at low temperature in many crop species including sweet pepper (Korkmaz, 2005), watermelon (Korkmaz *et al.*, 2004) and muskmelon (Korkmaz *et al.*, 2005) compared with KNO_3 alone. The results of this study on sugar beet are also very consistent with these studies and indicated that priming sugar beet seeds in KNO_3 supplemented with 0.05 mM ASA and 3 μM MeJA resulted for better germination and emergence at 15°C when compared with non-primed and seeds primed in KNO_3 only. KNO_3 in the presence of 0.05 mM ASA was also most effective in increasing shoot fresh and dry wt.

Increasing evidence suggests that benzoic acid derivatives such as salicylic acid (SA) or ASA regulate stress tolerance in plants (Lopez-Delgado *et al.*, 1998). These molecules trigger the expression of the potentioal to tolerate stress rather than having any direct effect as a protectant (Senaratna *et al.*, 2000). Mendoza *et al.* (2002) reported that imbibing pepper seeds in 0.1 mM SA prevented seedlings from subsequent chilling-induced damage. Senaranta *et al.* (2003) documented that soaking bean and tomato seeds in 0.1 mM ASA for 24 h caused 100% seedling survival following chilling and stresses while none of the control plants survived. The fact that seed imbibition with ASA provided multiple stress tolerance in tomato and bean plants is more consistent with a signaling role for the expression of tolerance rather than a direct effect.

Reduction in germination and emergence percentage caused by ASA concentrations higher than 0.5 mM reported in this study also confirmed the results of Mendoza *et al.* (2002) and Senaratna *et al.* (2000) who noted that treating pepper and tomato seeds with 1 mM ASA had an adverse effect on seedling survival following a chilling stress treatment, respectively.

Jasmonates including jasmonic acid and its methyl ester form, methyl jasmonate have been regarded as endogenous plant growth regulators because of their ubiquitous occurrence in the plant kingdom and their effects on plant growth and development. They are known to induce genes encoding proteins inhibitors, enzymes involved in flavonoid biosynthesis and lipoxygenas all of which are involved in plant response to stressful conditions and plant defense mechanism (Creelman and Mullet, 1997). When applied exogenously to plants, they seem to have important effects on numerous biological activities one of which is inhibition of seed germination (Sembdner and Parthier, 1993). Inhibition of seed germination by jasmonates was reported in such species as lettuce (Yamane *et al.*, 1981) and amaranth (Kepczynski and Bialecka, 1994). However, the concentration of jasmonates as germination inhibitors cannot be assumed from these results due to high concentrations. On the other hand, it was reported that MeJA applied exogenously at the concentration of 10^{-6} M stimulated seed germination in apple (Ranjan and Lewak, 1995) and that priming seed in KNO_3 supplemented with 1 μM MeJA improved low temperature germination and emergence of watermelon (Korkmaz *et al.*, 2004). Therefore, in studying the potencies, of plant growth regulators including jasmonates and ASA, a clear discrimination between physiological and supraoptimal concentrations should be made as suggested by sembdner and parthier (1993). Korkmaz (2005) and Korkmaz *et al.* (2004 and 2005) reported that priming seeds in 1 or 3 μM of MeJA and 0.05 or 0.1 mM ASA incorporation into the KNO_3 solution were most effective in germination and emergence at low temperature and higher levels (5 or 10 μM MeJA and 0.5 or 1 mM ASA) were less effective. These results are very consistent with our results, as in this study the most effective concentrations were 1 and 3 μM MeJA and 0.05 or 0.1 mM ASA) were less effective.

In summary, the result of the present study revealed that inclusion of lower concentrations of ASA (0.1 mM) or MeJA (3 μM) significantly improved sugar beet germination and emergence compared with seed priming in KNO_3 only, which is very consistent with the reported results so far. Priming with the addition of these plant growth regulators may be an effective way to shorten the time of emergence and increase stand establishment in sugar beet at low temperatures. The fact that aspirin, available everywhere in the world, could be used to prevent crop losses due to stress may have a significant practical application.

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