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Combining Priming and Plant Growth Regulators Improves Muskmelon Germination and Emergence at Low Temperatures

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Summary

The effects of incorporating plant growth regulators into the priming solution on low temperature germination and emergence performance of muskmelon [*Cucumis melo* L. (Reticulatus Group) cv. 'Athena'] seeds were investigated. Seeds were primed for 6 days at 25 °C in darkness in 3.5 % (0.35 M) KNO_3 solution containing one of the following: 1, 3, 5 or 10 µM methyl jasmonate (MeJA), or 1, 3, 5 or 10 mM spermine. Following priming, seeds were subjected to germination and emergence tests at 15±0.5 °C. Priming muskmelon seeds in the presence or absence of plant growth regulators significantly improved germination percentage, rate and synchrony at 15 °C compared to untreated seeds. Inclusion of both MeJA and spermine at all concentrations except 10 µM MeJA resulted in higher germination percentages compared to seeds primed in KNO_3 only. The highest germination percentages were obtained from seeds that were primed in KNO_3 solution supplemented with 1 µM MeJA (96 %) and 1 mM (94 %) spermine. Moreover, the presence of both growth regulators in the priming solution improved low temperature emergence of muskmelon seeds compared to untreated seeds and seeds primed in KNO_3 solution alone. Inclusion of 1 µM MeJA and 3 mM spermine into the priming solution led to the highest emergence percentages with 90 % and 85 %, respectively, while priming in KNO_3 solution in the absence of plant growth regulators resulted in significantly lower emergence percentage (31 %). Therefore, priming in 1 µM of MeJA or 3 mM spermine incorporated into the KNO_3 solution can be used as an effective method to improve low temperature performance of muskmelon seeds.

Zusammenfassung

Die Kombination von Saatgutvorbehandlung und Wachstumsregulatoren verbessern die Keimung und das Auflaufen von Netzmelonen bei niedrigen Temperaturen. Untersucht wurde die Wirkung der Beimengung von Wachstumsregulatoren zur Vorbehandlungslösung von Saatgut der Netzmelonen- [*Cucumis melo* L. (Reticulatus Group)] Sorte 'Athena' auf die Keimung und das Auflaufverhalten bei niedrigen Temperaturen. Saatgut wurde 6 Tage bei 25 °C und Dunkelheit in 3.5 % (0.35 M) KNO_3-Lösung unter Zugabe von 1, 3, 5 oder 10 µM Methyljasmonat (MeJA) bzw. 1, 3, 5 oder 10 mM Spermin vorbehandelt. Daran anschließend wurden die Samen zur Keimung und für die Auflauftestungen bei 15±0.5 °C gehalten. Die Vorbehandlung von Netzmelonensaatgut mit KNO_3 verbesserte die Keimprozente, die Keimrate und die Gleichmäßigkeit der Keimung bei 15 °C im Vergleich mit unbehandelten Samen. Im Vergleich zur Vorbehandlung nur mit KNO_3 führte der Zusatz von beiden, MeJA und Spermin, in allen untersuchten Konzentrationen mit Ausnahme von 10 µM MeJA zu höheren Keimprozenten. Die höchsten Keimprozente wurden bei Samen erzielt, die mit KNO_3-Lösungs unter Zugabe von 1 µM MeJA (96 %) und 1 mM (94 %) Spermin vorbehandelt wurden. Außerdem verbesserte die Zugabe beider Wachstumsregulatoren in die Vorbehandlungslösung das Auflaufen der Netzmelonensamen bei niedrigen Temperaturen sowohl im Vergleich mit unbehandelten als auch mit nur KNO_3 behandelten Samen. Die Zugaben von 1 µM MeJA bzw. 3 mM Spermin zur Vorbehandlungslösung führten mit 90 bzw. 85 % zu den höchsten Keimprozenten, wohingegen die Vorbehandlung mit KNO_3-Lösung ohne Wachstumsregulatoren mit 31 % zu signifikant geringeren Keimprozenten führte. Deshalb kann die Zugabe von 1 µM MeJA oder 3 mM Spermin zur KNO_3-Lösung als wirkungsvolle Methode zur Leistungsverbesserung von Netzmelonensamen bei niedrigen Temperaturen angewendet werden.

Key words. *Cucumis melo* – germination – low temperature – methyl jasmonate – muskmelon – polyamines – priming.

Introduction

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 seedbed conditions as low temperature, high temperature, salinity, and reduced water availability (KHAN 1992). One technique to increase emergence percentage and to hasten emergence is seed priming which consists of the incubation of seeds in an osmoticum, usually a salt or polyethylene glycol solution, in order to control their water uptake and prevent radicle protrusion (PILL 1995).

The approaches taken to develop stress tolerant plants include genetic engineering, breeding, in vitro selection, and the use of plant growth regulators. Many 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 acetylsalicylic acid solution improved seedling survival during subsequent chilling, high temperature, and drought stresses (SENARATNA et al. 2000). Exogenous applications of abscisic acid (YAMAZAKI et al. 1995) and polyamines (SHEN et al. 2000) at warm temperatures have been reported to reduce injury from subsequent low temperature exposure in cucumber seedlings.

Muskmelon is classified as a very tender warm season crop and is native to the Old World tropics and subtropics (tropical and southern Africa) (BATES and ROBINSON 1995). Optimum temperature range for muskmelon seed germination is between 25 and 35 °C; however, seeds are sometimes sown in early spring in the field when the temperatures are suboptimal for germination. Muskmelon seed germination is slowed or reduced at temperatures below 20 ºC resulting in poor stand establishment and is prevented totally at temperatures lower than 15 °C (LORENZ and MAYNARD 1988). Several studies reported the benefits of priming seeds of Cucurbit species on germination at low temperatures. For example, SACHS (1977) reported that priming watermelon seeds in 2 % KNO_3 for 6 days resulted in 80 % germination at 15 °C while none of the untreated seeds germinated. DEMIR and OZTOKAT (2003) reported that watermelon seeds primed in KNO_3 germinated 66 % at 14 °C; however, germinated seeds were unable to grow shoots at this temperature. NASCIMENTO (2003) also found that priming muskmelon seeds in different salt solutions improved germination rate and seedling growth at 17 °C.

Based on the results mentioned above, we hypothesized that treating the seeds with plant growth regulators that are known to enhance plant's cold tolerance would improve performance of muskmelon seeds at low temperatures. Therefore, this study was undertaken to investigate if incorporation of methyl jasmonate and spermine into priming solution would enhance muskmelon seed germination and subsequent seedling emergence at low temperatures.

Materials and Methods

Seed germination at low temperature

Seeds of 'Athena' muskmelon [*Cucumis melo* L. (Reticulatus Group)], product of Rogers Brothers Seed Co., Salinas, CA, USA, were acquired from Dr. Robert J. Dufault of Clemson University, Charleston, SC, USA. All the experiments reported in this study were carried out in the fall of 2003 at Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey.

Muskmelon seeds were primed for 6 days at 25 °C in darkness in 3.5 % (0.35 M) KNO_3 solution (NASCIMENTO 2003) containing one of the following: 1, 3, 5 or 10 μM methyl jasmonate (MeJA) (Sigma Aldrich, St. Louis, MO, USA), or 1, 3, 5 or 10 mM spermine (Sigma Aldrich, St. Louis, MO, USA). Seeds were placed in covered transparent polystyrene germination boxes (10 x 10 x 4 cm) (Ater Plastik, Kocaeli, Turkey) on double layers of filter paper saturated with 8 ml of one of the priming agents. The priming solutions were changed every other day to maintain a constant osmotic potential of the solutions. Following priming, the seeds from each box were washed in a sieve and rinsed under running tap water for 1 minute and subjected to germination test at 15 °C. Seeds that were primed in 3.5 % KNO_3 solution containing no plant growth regulators and untreated (non-primed) seeds were also included in the germination test.

Germination tests were carried out in darkness in a temperature-controlled incubator held at 15±0.5 °C. Seeds were placed on two layers of filter paper moistened with 3 ml of distilled water in covered 5.5 cm petri dishes. Treatments were arranged in completely randomised design with four replications of 25 seeds. Radicle protrusion to 1 mm was scored as germination. Seed germination was recorded at 24 h intervals until the numbers stabilized (for 14 days) and germinated seeds were removed from the petri dishes. From the total number of seeds germinated, final germination percentage (FGP) and its angular transformation (arcsine$\sqrt{\text{FGP}}$), days to 50 % of FGP and days between 10 % and 90 % of FGP were calculated (MURRAY et al. 1993). 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

Seeds were primed as described above; however, since 5 μM and 10 μM MeJA and 5 mM and 10 mM spermine added to the priming solution significantly decreased germination of muskmelon seeds at 15 °C (see Table 1), these concentrations were not used in seedling emergence test.

For emergence test, twenty seeds from each treatment were planted horizontally (NASCIMENTO and WEST 1998) into 1.5 cm depth in 5.5 cm deep flat cells (75 cm^3) filled with growth medium consisting of peat

Table 1. Final germination percentage (FGP) and its angular transformation [FGP], days to 50 % of FGP (G_{50}) and days between 10 % and 90 % germination ($G_{10–90}$) of 'Athena' muskmelon seed germination in darkness at 15 °C following priming for 6 days at 25 °C in 3.5 % KNO_3 with various concentrations of methyl jasmonate (MeJA) and spermine.

Treatments	FGP		G_{50}	$G_{10–90}$
	%	[FGP]	(Days)	(Days)
KNO_3 + 1 μM MeJA	96	[80]	1.66	1.43
KNO_3 + 3 μM MeJA	93	[77]	1.53	1.09
KNO_3 + 5 μM MeJA	79	[63]	1.76	1.69
KNO_3 + 10 μM MeJA	54	[47]	1.85	1.71
KNO_3 + 1 mM Spermine	94	[78]	1.59	1.59
KNO_3 + 3 mM Spermine	88	[71]	1.55	1.47
KNO_3 + 5 mM Spermine	74	[60]	1.67	1.49
KNO_3 + 10 mM Spermine	73	[59]	1.97	2.17
KNO_3	61	[52]	1.76	2.51
Untreated seeds	36	[37]	4.88	3.44
$LSD_{0.05}$		[6]	0.15	0.31
Significance		***	***	***

*** Significant at $P \leq 0.001$.

and perlite in the ratio of 3:1. The flats were watered and placed in a growth chamber (Welp Scientifica, Model No: FOC 225I, Usmate, Italy) at continuous 15±0.5 °C and under cool fluorescent lamps providing a photosynthetic photon flux density of 40 μmol m^{-2} s^{-1} for 14 h day^{-1} at the seedling level. The treatments were replicated four times and all the flats were arranged in a randomised complete block design in the growth chamber. Emergence counts (hypocotyl arc visible) were made daily and final emergence percentage (FEP) and days to 50 % of FEP (E_{50}) were calculated. Twenty four days after planting, when the percentage of emergence had stabilized in all treatments, the following growth variables were recorded: The number of seedlings that emerged and survived (survival ratio), shoot and washed root fresh weight (cut at the medium surface) and dry weight (dried at 65 °C for 48 h).

Emergence and growth data were tested by analysis of variance (ANOVA) by using SAS statistical software (SAS INST. 1997) 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 muskmelon seeds in the presence or absence of plant growth regulators significantly improved

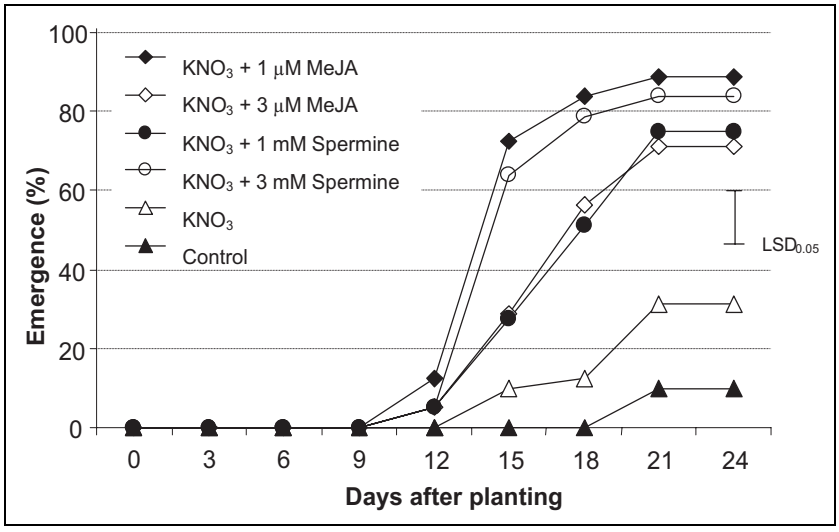


Fig. 1. The effects of priming 'Athena' muskmelon seeds for 6 days at 25 °C in 3.5 % KNO_3 supplemented with 1 μM and 3 μM methyl jasmonate (MeJA) and 1 mM and 3 mM spermine on subsequent seedling emergence at 15 °C.

muskmelon seed germination at 15 °C compared to untreated seeds which had FGP of 36 % (Table 1). Seeds primed in KNO_3 with lower concentrations (1 μM and 3 μM) of MeJA had significantly higher germination percentages, 96 % and 93 %, respectively, than seeds primed in KNO_3 only (61 %). However, the addition of 5 μM and 10 μM of MeJA reduced germination percentage to 79 % and 54 % respectively. In addition, the inclusion of spermine at all four concentrations into the priming solution improved significantly FGP and germination rate of muskmelon seeds compared to seeds that were primed in KNO_3 only. However, as with the MeJA, increasing spermine concentration added to the priming solution from 1 mM to 10 mM decreased FGP of muskmelon seeds from 94 % to 73 %. Priming in the presence of 3 μM MeJA (G_{50}=1.53 days), 1 mM spermine (G_{50}=1.59 days) and 3 mM spermine (G_{50}=1.55 days) were the only treatments that improved the low temperature germination rate of muskmelon seeds compared to seeds that were primed in KNO_3 only (G_{50}=1.76 days) (Table 1). Even though the inclusion of all concentrations of both growth regulators into the priming solution improved the germination synchrony of muskmelon seeds compared to primed in KNO_3 only ($G_{10–90}$=2.51 days), the most synchronous germination was obtained when the seeds were primed in the presence of 3 μM MeJA ($G_{10–90}$=1.09 days).

Seedling emergence at low temperature

Priming also improved low temperature emergence of muskmelon seedlings regardless of plant growth regulators added to the priming solution compared to seeds primed in KNO_3 only which had FEP of 31 % (Fig. 1). The highest FEP was obtained when 1 μM MeJA (90 %) and 3 mM spermine (85 %) were added into the priming solution. The same treatments also resulted in the fastest emergence rates since 50 % of FEP was reached by the 14th day after planting while emergence rate of seeds primed in KNO_3 only was considerably slower (50 % of FEP reached by the 18th day). Emergence of untreated seeds started 19 days after planting and FEP of these seeds was only 10 %.

All treatments had greater than 90 % survival ratio and there were no significant differences among the treatments (Table 2). Both shoot and root fresh weights of muskmelon seedlings were significantly affected by priming treatments. The heaviest shoots were obtained when the seeds were primed in the presence of 1 μM of MeJA while priming seeds in KNO_3 only resulted in the lowest shoot fresh weight. Moreover, priming improved root fresh weight of muskmelon seedlings regardless of plant growth regulators added to the priming solution compared to seeds primed in KNO_3 only. However, neither shoot nor root dry weight of muskmelon seedlings was significantly affected by any of the priming treatments.

Discussion

The beneficial effects of priming with a KNO_3 solution on low temperature germination of muskmelon seeds found in this study confirm the findings of NERSON and GOVERS (1986) and NASCIMENTO (2003). Similar results have also been obtained with other species of the same family, such as watermelon (SACHS 1977; DEMIR and OZTOKAT 2003). However, in these studies, very little information about soil emergence performance of primed muskmelon seeds at low temperatures has been reported. Although NASCIMENTO (2003) reported improved germination rate and seedling development at 17 °C by priming, it is inappropriate to compare our findings with those of that study in which seed germination and seedling evaluation were conducted in the petri dishes not in growth medium. We report in this study that we obtained 96 % germination and 90 % emergence at 15 °C by using seeds primed in 1 μM MeJA added to the priming solution which are significantly higher than the germination and emergence of seeds primed in KNO_3 only, the most com-

Table 2. Shoot and root fresh and dry weights and survival ratio of 'Athena' muskmelon seedlings grown at 15 °C for 24 days following priming for 6 days at 25 °C in 3.5 % KNO_3 supplemented with 1 μM and 3 μM methyl jasmonate (MeJA) and 1 mM and 3 mM spermine.

Treatments	Survival (%)	Fresh weight (mg plant^{-1})		Dry weight (mg plant^{-1})	
		Shoot	Root	Shoot	Root
KNO_3 + 1 μM MeJA	98	96.7	70.3	12.5	4.5
KNO_3 + 3 μM MeJA	93	70.3	68.9	12.0	4.8
KNO_3 + 1 mM Spermine	99	65.0	65.0	11.5	4.4
KNO_3 + 3 mM Spermine	97	86.8	74.8	12.8	5.0
KNO_3	100	63.3	55.6	11.1	4.0
Untreated seeds	–[z]	–	–	–	–
$LSD_{0.05}$	–	9.2	7.5	–	–
Significance	NS	***	***	NS	NS

[z] Not included in the statistical analysis due to insufficient data.
***, NS Significant at $P \leq 0.001$ or not significant, respectively.

monly used priming treatment for Cucurbit species. Moreover, all priming treatments in the presence of MeJA and spermine at all concentrations improved the final emergence percentage when compared to seeds primed in KNO_3 only. These results confirm the results of our previous study in which we found that priming watermelon seeds in KNO_3 supplemented with1 μM MeJA resulted in 96 % germination and 75 % emergence at 15 °C which were significantly higher than the germination and emergence of seeds primed in KNO_3 only (KORKMAZ et al. 2004).

Increasing the initial moisture content of dry seeds is known to protect the seeds against chilling injury during low temperature imbibition (HERNER 1986). Rapid movement of water into dry seeds interferes with membrane reorganization and, therefore, the high rate of leakage is sustained and injury results. Increasing seed moisture content by priming prior to low temperature exposure enables membrane reorganization, which, in turn, results in less leakage and injury (HERNER 1986). In seed priming studies, seeds usually are transferred directly from the priming solution to the germination media, generally resulting in very rapid germination compared to non-primed seeds (PILL 1995). Since primed seeds have completed phase I (hydration) and II (lag phase) of germination, they only require a favourable water potential gradient for water uptake in order to begin radicle growth. Early events in the initiation of muskmelon seed germination are apparently limited by low temperature, with successive fractions of seed population becoming unable to initiate radicle growth as the temperature is lowered (BRADFORD et al. 1988). Therefore, seed priming, by allowing all seeds to complete the temperature sensitive phases of germination metabolism at an optimum temperature, results in much more rapid and uniform radicle growth and emergence.

Jasmonates especially 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 proteinase inhibitors, enzymes involved in flavonoid biosynthesis and lipoxygenase all of which are involved in plant response to stressful conditions and plant defence mechanism (CREELMAN and MULLET 1997). When applied exogenously to plants, they seem to have important effects on numerous biological activities including 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 (BIALECKA and KEPCZYNSKI 2003). However, the concentration of jasmonates used in these studies were in the range of 10^{-4} to 10^{-3} M; thus, a specific role 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 $\leq 10^{-6}$ M stimulated seed germination in apple (RANJAN and LEWAK 1995) and microtuber germination in yams (BAZABAKANA et al. 1999) by breaking dormancy. Therefore, in studying the potencies of jasmonates, a clear discrimination between physiological and supraoptimal concentrations should be made as suggested by SEMBDNER and PARTHIER (1993). In our study, we found that the lowest concentration of MeJA (1 μM) used was the most effective concentration to improve the low temperature germination and emergence of muskmelon seeds, and that increasing MeJA concentration significantly reduced germination and emergence.

Polyamines (putrescine, spermidine and spermine) are involved in the regulation of plant growth and development including cell division, morphogenesis, flower initiation, pollen tube growth and senescence, and stresses such as chilling, oxygen deficiency, low pH, and K^+ and Mg^{2+} deficiencies increase polyamine biosynthesis (BOUCHEREAU et al. 1999). It has also been found that chilling-tolerant plants increase polyamine levels in response to chilling to a much greater extent than chilling-sensitive plants (LEE 1997). Exogenously applied polyamines are also known to protect plants against low temperature injury. SHEN at al. (2000) reported that chilling-tolerant cucumber cultivars elevated endogenous polyamine levels in response to chilling to a much greater extend than chilling-sensitive cultivars, and that pre-treatment with spermidine prevented chill-induced damage. It is suggested that the primary function of polyamines is to protect membranes probably by the inhibition of chill-induced oxidize enzymes and consequential regeneration of reactive oxygen species (TADOLINI 1988). Our results demonstrated that seeds primed in the presence of spermine showed significantly improved germination and emergence performance, which may be the result of these protective effects of spermine during imbibition and germination process.

In summary, the result of the present study revealed that inclusion of 1 μM and 3 μM MeJA or 1 mM or 3 mM spermine significantly improved muskmelon germination and emergence compared to seeds primed in KNO_3 only. Therefore, these plant growth regulators can be added to KNO_3 priming solution, the most commonly used priming treatment for Cucurbit species, in order to further increase germination and emergence at low temperatures.

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