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## Inclusion of benzyladenine into priming solution promotes germination of Kentucky bluegrass (*Poa pratensis* L.) seeds

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### Abstract

The objective of this work is to study whether inclusion of various stress related plant growth regulators into priming solution would improve germination and emergence of Kentucky bluegrass (*Poa pratensis* L.) seeds. Seeds were primed in 1, 2 or 3% KH<sub>2</sub>PO<sub>4</sub> for six days at 20 degrees C. Four replications of 100 seeds for germination and 50 seeds for emergence were arranged in a completely randomized design. Priming seeds in 1%KH<sub>2</sub>PO<sub>4</sub> significantly increased germination percentage compared to untreated seeds, although germination rate and synchrony were reduced. Inclusion of methyljasmonate (MeJA, 1, 3, 5, or 10 mu M), 1-aminocyclopropane-1-carboxylic acid (ACC, 1, 3, 5, or 10 mu M) or benzyladenine (BA, 50, 100, 200, or 500 mu M) into priming solution revealed that germination characteristics (germination percentage, rate, and synchrony) could be further improved by supplementing plant growth regulators (PGRs) into priming solution. The highest final germination percentage was obtained from the seeds primed in the presence of 500 mu M BA (79%) compared to seeds primed in 1% KH<sub>2</sub>PO<sub>4</sub> (65%) only. The PGRs improved final emergence percentage and the highest BA concentration Was more effective compared to other PGRs tested. The results indicated that 1%KH<sub>2</sub>PO<sub>4</sub> supplemented with 500 mu M BA could be used to promote germination of *Poa pratensis* L. seeds to some extent while 3 mu M MeJA can be used to enhance seedling emergence rate of Kentucky bluegrass seeds.

### Keywords

**Author Keywords:** [Kentucky bluegrass](#); [germination](#); [seed priming](#); [plant growth regulators](#)

**KeyWords Plus:** [PLANT-GROWTH REGULATORS](#); [LOW-TEMPERATURE](#); [ETHYLENE](#); [ESTABLISHMENT](#); [BIOSYNTHESIS](#); [PERFORMANCE](#); [EMERGENCE](#); [STORAGE](#)

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## Inclusion of benzyladenine into priming solution promotes germination of Kentucky bluegrass (*Poa pratensis* L.) seeds

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The objective of this work is to study whether inclusion of various stress related plant growth regulators into priming solution would improve germination and emergence of Kentucky bluegrass (*Poa pratensis* L.) seeds. Seeds were primed in 1, 2 or 3%  $\text{KH}_2\text{PO}_4$  for six days at 20 °C. Four replications of 100 seeds for germination and 50 seeds for emergence were arranged in a completely randomized design. Priming seeds in 1%  $\text{KH}_2\text{PO}_4$  significantly increased germination percentage compared to untreated seeds, although germination rate and synchrony were reduced. Inclusion of methyl jasmonate (MeJA, 1, 3, 5, or 10  $\mu\text{M}$ ), 1-aminocyclopropane-1- carboxylic acid (ACC, 1, 3, 5, or 10  $\mu\text{M}$ ) or benzyladenine (BA, 50, 100, 200, or 500  $\mu\text{M}$ ) into priming solution revealed that germination characteristics (germination percentage, rate, and synchrony) could be further improved by supplementing plant growth regulators (PGRs) into priming solution. The highest final germination percentage was obtained from the seeds primed in the presence of 500  $\mu\text{M}$  BA (79 %) compared to seeds primed in 1 %  $\text{KH}_2\text{PO}_4$  (65 %) only. The PGRs improved final emergence percentage and the highest BA concentration was more effective compared to other PGRs tested. The results indicated that 1%  $\text{KH}_2\text{PO}_4$  supplemented with 500  $\mu\text{M}$  BA could be used to promote germination of *Poa pratensis* L. seeds to some extent while 3  $\mu\text{M}$  MeJA can be used to enhance seedling emergence rate of Kentucky bluegrass seeds.

Key words: *Kentucky bluegrass, germination, seed priming, plant growth regulators.*

Germination of Kentucky bluegrass (*Poa pratensis* L.) seeds takes 10 to 28 d under optimal germination conditions (Pill and Necker 2001). There are various seed treatments including osmotic priming of seeds that would advance Kentucky bluegrass seed germination and subsequent plant establishment (ISTA 1999). Osmotic priming is a pre-germinative treatment, in which seeds are incubated in an osmoticum, usually a salt (i.e.,  $\text{K}_3\text{PO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{Mg SO}_4$ ,  $\text{NaCl}$ ) or polyethylene glycol (PEG) solution, in order to control their water uptake, but prevents radicle extension (Bradford 1986). Primed seeds may exhibit a higher germination percentage and rate than nonprimed seeds, particularly under such adverse seedbed conditions as low or high temperature and decreased water availability (Pill and Finch-Savage

1988). Such advantages of seed priming were also reported for Kentucky bluegrass (Lush and Birkenhead 1987 and Pill and Necker 2001).

Kentucky bluegrass seeds have been primed either matrixally in fine vermiculite or osmotically in  $\text{KNO}_3$  or PEG-8000 and priming increased germination rate compared to non-primed seeds, but germination synchrony and percentage were not enhanced (Adegbuyi *et al.* 1981). There are a number of other osmotica, including  $\text{KH}_2\text{PO}_4$  that can be used (Pill 1995) to enhance the germination of Kentucky bluegrass seeds; however, to the best of our knowledge such osmotica have not been employed in priming of Kentucky bluegrass seeds. Korkmaz and Pill (2003) reported that priming of lettuce (*Lactuca sativa* L.) seeds in  $\text{KH}_2\text{PO}_4$  resulted in improved germination

parameters (i.e. increased germination percentage, rate, and synchrony) compared to seeds primed in  $K_3PO_4$ ,  $NH_4H_2PO_4$  or PEG.

Plant growth regulators (PGRs), on the other hand, affect several aspects of plant growth and development including seed germination. Increasing evidence indicates that inclusion of stress related PGRs such as jasmonic acid, ethylene and spermine in seed treatments helps to improve germination and emergence of several species, particularly under such adverse seedbed conditions as low temperatures (Tiryaki 2006). The accelerated germination treatment, soaking the seeds at 30 °C for 24 h in a solution of  $GA_3$ , kinetin (6-furfurylaminopurine) and 1 mM  $KNO_3$  increased the rate and percentage of seedling emergence of Bermudagrass (*Cynodon dactylon* (L.) Pers.) (Young *et al.* 1977) but had no effect on germination percentage and rate of Kentucky bluegrass (Pill and Necker 2001). It was also reported that inclusion of PGRs into priming solution could further enhance the priming effect and could be more effective than applying as a prepriming soak (Pill and Finch-Savage 1988). Therefore, a further study is needed to examine the effect of different combination and concentrations of PGRs combined into priming solution on Kentucky bluegrass seed germination and seedling emergence (Pill and Necker 2001).

The present study was undertaken to test  $KH_2PO_4$  as a priming agent and investigate whether inclusion of PGRs into priming solution would further improve germination and emergence of Kentucky bluegrass seeds.

### Materials and Methods

Experiments were carried out with *Poa pratensis* L. seeds that were harvested in 2000 and stored at 4 °C until used. All the experiments reported in this study were carried out at Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey.

To explore whether inclusion of PGRs into priming solution improves germination and emergence of *Poa pratensis* L., three PGRs were tested. Based on results of the preliminary ex-

periments (unpublished data) conducted to determine which concentration of  $KH_2PO_4$  to be used 1 %  $KH_2PO_4$  was chosen as the priming agent. Seeds were primed for six days at 20 °C in darkness in 1 %  $KH_2PO_4$  solution containing methyl jasmonate (MeJA, 1, 3, 5, or 10  $\mu$ M, Sigma Aldrich, St. Louis, MO, USA) or 1-aminocyclopropane-1-carboxylic acid (ACC, 1, 3, 5, or 10  $\mu$ M, Sigma Aldrich, St. Louis, MO, USA) or benzyladenine (BA, 50, 100, 200, or 500  $\mu$ M, Sigma Aldrich, St. Louis, MO, USA). Single layers of 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 10 mL of one of the priming agents. To maintain a constant osmotic potential of the solutions, 5 mL of the priming solutions were replaced three days after priming started. Following the priming, seeds were rinsed under running tap water for one minute and surface dried for 3 h under room conditions on paper towels, and then subjected to germination test subsequently. Seeds primed in 1 %  $KH_2PO_4$  solution containing no PGRs and untreated (non-primed) seeds were used as the controls.

Following priming, germination tests were carried out in darkness in a temperature controlled incubator held at  $20 \pm 0.5$  °C (Pill and Korengel 1997). Seeds were placed on two layers of filter paper moistened with 2 mL of distilled water in covered 5.5 cm petri dishes. Four replications of 100 seeds were arranged in a completely randomized design. The number of germinated seeds (visible coleorhiza or radicle) were recorded and removed from petri dishes daily until the numbers stabilized (for 20 d). From the total number of seeds germinated, final germination percentage (FGP) and its angular transformation ( $\arcsine \sqrt{FGP}$ ), 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.

For emergence test, 50 seeds in four replications from each treatment were sown into 0.5-cm depth in 7 x 3 (diameter and height) cm round plastic cups filled with peat-based growth medium. The treatments were arranged in a completely randomized design. Cups were watered with tap water as needed and placed in a growth chamber at continuous temperature of  $20 \pm 0.5$  °C and under cool fluorescent lamps providing a photosynthetic photon flux density of  $40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for  $12 \text{ h d}^{-1}$  at seedling level. Seedling emergence (hypocotyl visible) was recorded daily until the numbers stabilized. From the total number of seedlings emerged, final seedling emergence percentage (FEP) and its angular transformation, days to 50 % of the FEP ( $E_{50}$ , an inverse measure of emergence rate), days between 10 and 90 % of FEP ( $E_{10-90}$ , an inverse measure of emergence synchrony), were calculated.

Germination and emergence data were tested by analysis of variance by using SAS statistical software and mean separation was performed by Fisher's least significant difference (LSD) test if F test was significant at  $P < 0.05$ .

### Results and Discussion

Priming seeds in the presence of various PGRs indicated that germination characteristics of Kentucky bluegrass can be further improved by applying PGRs into priming solution at various concentrations (table 1). However, inclusion of BA provided a better improvement for all the parameters measured at any given concentration tested than the seeds primed in 1 %  $\text{KH}_2\text{PO}_4$  only. The highest FGP was obtained from the seeds primed in the presence of 500  $\mu\text{M}$  BA (79 %). The same BA concentration also gave the fastest germination rate ( $G_{50} = 7.5$  d) and the best synchronous germination ( $G_{10}-G_{90} = 7.5$  d) when compared to seeds primed in  $\text{KH}_2\text{PO}_4$  ( $G_{50} = 9.0$  d and  $G_{10}-G_{90} = 11.5$  d) only. Earlier work showed that PGRs had no effect on germination percentage and rate of Kentucky bluegrass seeds (Pill and Necker 2001). Our results, however, revealed that inclusion of 500  $\mu\text{M}$  BA into  $\text{KH}_2\text{PO}_4$

improved all germination characteristics of Kentucky bluegrass seeds (germination percentage, rate and synchrony) (table 1). These results also suggested that higher concentrations of BA (higher than 500  $\mu\text{M}$ ) should be tested since the best germination performance of Kentucky bluegrass seeds was obtained from the highest BA concentration tested.

There are several reports indicating that seed germination was either inhibited (Kepczynski *et al.* 1999) or promoted (Daletskaya and Sembdner 1989) by MeJA. However, MeJA has not been tested on Kentucky bluegrass seed germination. Inclusion of 3  $\mu\text{M}$  MeJA into the priming solution had the highest FGP (76 %) while the fastest and the most synchronous germination were obtained from the seeds primed in the presence of 1  $\mu\text{M}$  MeJA ( $G_{50} = 8.5$  d and  $G_{10}-G_{90} = 8.0$  d) in comparison to the other MeJA concentrations tested. Previous studies indicated that MeJA could be incorporated into priming solution to improve germination characteristics of various crop seeds (Korkmaz *et al.* 2004 and Tiryaki *et al.* 2005). The result of this study suggested that the effect of MeJA on Kentucky bluegrass seed germination could be tested under adverse conditions such as low temperature since MeJA had little or no effect on Kentucky bluegrass seed germination at any concentration tested relative to that of seeds primed in  $\text{KH}_2\text{PO}_4$  only (tables 1 and 2).

On the other hand, exogenous application of ethylene or its immediate precursor ACC stimulates the germination of dormant and non-dormant seeds although they inhibit or do not affect seed germination in some cases (Kepczynski *et al.* 1999 and Locke *et al.* 2000). The level of ethylene in dormant seeds of many plant species is below the threshold necessary for germination (Kepczynski and Kepczynska 1997). It was reported that inclusion of 3  $\mu\text{M}$  ACC into priming solution significantly improved the rate and percentage of *Amaranthus cruentus* seeds at low temperature (Tiryaki 2006). This study revealed that inclusion of the lowest concentration of ACC (1  $\mu\text{M}$ ) into

Table 1. Final germination percentage (FGP) and the angular transformation of FGP (degree), days to 50 % of FGP ( $G_{50}$ ) and days between 10 and 90 % germination ( $G_{10-90}$ ) of *Poa pratensis* L. seed germination in darkness at 20 °C following priming for 6 d at 20 °C in 1 %  $KH_2PO_4$  combined with different plant growth regulators at various concentrations

Treatments	FGP		$G_{50}$ (Days $\pm$ SE)	$G_{10-90}$ (Days $\pm$ SE)
	%	(Degree $\pm$ SE)		
MeJA ( $\mu$ M) <sup>1</sup>				
1	75	(60 $\pm$ 1.5) <sup>abc</sup>	8.5 $\pm$ 0.1 <sup>bc</sup>	8.0 $\pm$ 0.4 <sup>ef</sup>
3	76	(61 $\pm$ 0.9) <sup>abc</sup>	9.3 $\pm$ 0.2 <sup>a</sup>	9.3 $\pm$ 0.5 <sup>bcde</sup>
5	74	(60 $\pm$ 1.7) <sup>abc</sup>	9.0 $\pm$ 0.1 <sup>ab</sup>	9.0 $\pm$ 0.1 <sup>cdef</sup>
10	72	(58 $\pm$ 2.0) <sup>bcd</sup>	9.0 $\pm$ 0.1 <sup>a</sup>	9.0 $\pm$ 0.4 <sup>bcde</sup>
ACC ( $\mu$ M) <sup>1</sup>				
1	74	(60 $\pm$ 0.5) <sup>abc</sup>	9.0 $\pm$ 0.1 <sup>a</sup>	10.3 $\pm$ 0.4 <sup>abc</sup>
3	69	(56 $\pm$ 1.2) <sup>f</sup>	9.3 $\pm$ 0.2 <sup>a</sup>	10.5 $\pm$ 0.5 <sup>ab</sup>
5	67	(55 $\pm$ 2.3) <sup>def</sup>	8.3 $\pm$ 0.2 <sup>cd</sup>	10.3 $\pm$ 0.7 <sup>abcd</sup>
10	63	(53 $\pm$ 1.6) <sup>cdef</sup>	8.3 $\pm$ 0.1 <sup>c</sup>	9.8 $\pm$ 0.6 <sup>abcde</sup>
BA ( $\mu$ M) <sup>1</sup>				
50	74	(60 $\pm$ 1.8) <sup>abc</sup>	8.0 $\pm$ 0.1 <sup>de</sup>	9.9 $\pm$ 0.6 <sup>abcd</sup>
100	77	(61 $\pm$ 1.2) <sup>ab</sup>	8.5 $\pm$ 0.2 <sup>c</sup>	10.3 $\pm$ 0.5 <sup>abcd</sup>
200	77	(61 $\pm$ 0.8) <sup>ab</sup>	8.5 $\pm$ 0.1 <sup>c</sup>	8.8 $\pm$ 0.5 <sup>def</sup>
500	79	(63 $\pm$ 1.4) <sup>a</sup>	7.5 $\pm$ 0.2 <sup>e</sup>	7.5 $\pm$ 0.5 <sup>f</sup>
1% $KH_2PO_4$ only	70	(57 $\pm$ 0.7) <sup>cde</sup>	9.0 $\pm$ 0.1 <sup>a</sup>	11.5 $\pm$ 0.4 <sup>a</sup>
Untreated seeds	65	(54 $\pm$ 0.2) <sup>ef</sup>	9.3 $\pm$ 0.2 <sup>a</sup>	8.8 $\pm$ 1.3 <sup>bcde</sup>

<sup>1</sup> $KH_2PO_4$  + related plant growth regulator at given concentrations; SE, standard error of the means (n = 4). Means that differ significantly (5 % level) between treatments are indicated by different letters

the priming solution gave the highest FGP (74 %) among all the other ACC concentrations tested. The results also showed that increasing the concentration of ACC from 1 to 10  $\mu$ M decreased the germination percentage from 74 to 63 % while germination rate and synchrony were improved when compared to seeds primed in  $KH_2PO_4$  only (table 1). This study revealed that ACC had no or little effect on germination characteristics of Kentucky bluegrass seeds, suggesting that the level of endogenous ethylene is in balance for germination of Kentucky bluegrass seeds. The results may also suggest that inclusion of ACC into priming of Kentucky bluegrass seed germination could

be tested under low temperature seedbed conditions since ACC supplemented into priming solution significantly improved rate and percentage of some other crop seeds at low temperature (Tiryaki 2006).

The PGRs improved FEP and 500  $\mu$ M BA along with 3  $\mu$ M MeJA was more effective than the other PGRs tested (table 2). The FEP was 45 % and 41 % for the seeds primed in the presence of 500  $\mu$ M BA and 3  $\mu$ M MeJA, respectively. The fastest emergence rate was obtained from the seeds primed in 3  $\mu$ M MeJA ( $G_{50}$  = 5.8 d) while untreated seeds gave the most synchronous seedling emergence ( $G_{10}$ - $G_{90}$  = 5.3 d). Ethylene precursor ACC, on

Table 2. Final emergence percentage (FEP) and the angular transformation of FEP (degree), days to 50 % of FEP ( $E_{50}$ ) and days between 10 and 90 % of FEP ( $E_{10-90}$ ) of *Poa pratensis* L. at 20 °C following priming for 6 d at 20 °C in 1 %  $\text{KH}_2\text{PO}_4$  combined with different plant growth regulators at various concentrations

Treatments	FEP		$G_{50}$ (Days $\pm$ SE)	$G_{10-90}$ (Days $\pm$ SE)
	%	(Degree $\pm$ SE)		
MeJA ( $\mu\text{M}$ ) <sup>1</sup>				
1	39	(38 $\pm$ 2.3) <sup>abcd</sup>	7.5 $\pm$ 0.5 <sup>abcd</sup>	11.8 $\pm$ 1.3 <sup>a</sup>
3	41	(40 $\pm$ 0.8) <sup>abc</sup>	5.8 $\pm$ 0.3 <sup>cd</sup>	7.0 $\pm$ 1.2 <sup>bc</sup>
5	38	(38 $\pm$ 4.2) <sup>cd</sup>	7.8 $\pm$ 0.5 <sup>abcd</sup>	5.8 $\pm$ 1.0 <sup>bc</sup>
0	45	(42 $\pm$ 0.3) <sup>a</sup>	7.5 $\pm$ 0.3 <sup>abcd</sup>	7.8 $\pm$ 0.6 <sup>bc</sup>
ACC( $\mu\text{M}$ ) z				
1	39	11.8 $\pm$ 1.3 <sup>a</sup>	7.8 $\pm$ 0.5 <sup>abcd</sup>	8.3 $\pm$ 1.8 <sup>bc</sup>
3	36	7.0 $\pm$ 1.2 <sup>bc</sup>	9.0 $\pm$ 1.4 <sup>a</sup>	8.3 $\pm$ 1.3 <sup>bc</sup>
5	36	5.8 $\pm$ 1.0 <sup>bc</sup>	6.5 $\pm$ 0.6 <sup>abc</sup>	7.8 $\pm$ 1.5 <sup>bc</sup>
10	35	7.8 $\pm$ 0.6 <sup>bc</sup>	7.3 $\pm$ 1.9 <sup>d</sup>	9.0 $\pm$ 1.1 <sup>ab</sup>
BA ( $\mu\text{M}$ ) z				
50	35	(36 $\pm$ 1.6) <sup>bcd</sup>	7.0 $\pm$ 0.2 <sup>abcd</sup>	6.8 $\pm$ 0.8 <sup>bc</sup>
100	37	(37 $\pm$ 1.1) <sup>abcd</sup>	8.3 $\pm$ 0.6 <sup>ab</sup>	8.5 $\pm$ 1.4 <sup>abc</sup>
200	39	(39 $\pm$ 1.2) <sup>abcd</sup>	7.5 $\pm$ 0.5 <sup>abcd</sup>	6.8 $\pm$ 0.7 <sup>bc</sup>
500	45	(42 $\pm$ 2.3) <sup>ab</sup>	8.0 $\pm$ 0.1 <sup>abc</sup>	7.5 $\pm$ 0.6 <sup>bc</sup>
1% $\text{KH}_2\text{PO}_4$ only	34	(35 $\pm$ 1.0) <sup>cd</sup>	7.0 $\pm$ 1.3 <sup>abcd</sup>	6.3 $\pm$ 1.2 <sup>bc</sup>
Untreated seeds	20	(26 $\pm$ 3.6) <sup>e</sup>	8.8 $\pm$ 0.4 <sup>ab</sup>	5.3 $\pm$ 0.7 <sup>c</sup>

<sup>1</sup> $\text{KH}_2\text{PO}_4$  + related plant growth regulator at given concentrations; SE, standard error of the means (n = 4). Means that differ significantly (5 % level) between treatments are indicated by different letters

the other hand, did not show any significant effect on seedling emergence performance (table 2). Pill and Necker (2001) also reported similar results for the effect of PGRs on seedling emergence of Kentucky bluegrass, except seedling emergence rate.

In conclusion, this study indicated that 1 %  $\text{KH}_2\text{PO}_4$  can be used to improve germination characteristics of Kentucky bluegrass seeds and that inclusion of 500  $\mu\text{M}$  BA may further increase germination performance, but higher concentrations of BA should be tested to determine the optimum concentration. The results also revealed that 3  $\mu\text{M}$  MeJA can be used to improve seedling emergence rate or synchrony while ACC had little or no effect on

germination and seedling emergence performance of Kentucky bluegrass seeds.

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