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Priming in the Presence of Plant Growth Regulators Hastens Germination and Seedling Emergence of Dormant Annual Ryegrass (*Lolium multiflorum* Lam.) Seeds

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Abstract: The effects of various priming solutions on germination of freshly harvested *Lolium multiflorum* Lam. seeds were investigated. The results revealed that priming seeds in various concentrations of KNO_3, KH_2PO_4 or polyethylene glycol for five days at 25°C with a 12 h photoperiod had no effect on dormancy release mechanism of annual ryegrass seeds although 1% KNO_3 gave slightly increased germination percentage compared to untreated control. Inclusion of various concentrations of methyl jasmonate (MeJA), 1-aminocyclopropane-1-carboxylic acid (ACC) or 6-benzylamino-purine (BAP) into priming solution indicated that 100 μM BAP was the only treatment that improved germination rate while 1 μM ACC along with 500 μM BAP enhanced seedling emergence rate only. The possible reasons of the lack of reaction to the other plant growth regulators were also discussed.

Key words: Dormancy loss, priming, plant growth regulator, germination, emergence

INTRODUCTION

Annual ryegrass (*Lolium multiflorum* Lam.) is a cool season bunchgrass native to Southern Europe and widely grown all around the world. Like many other winter annuals, annual ryegrass seeds are dormant at maturity and a period of time is required to lose dormancy after ripening[1]. Seed dormancy is the main cause of the wide range in germination and emergence timing of this plant[2]. It has been shown that temperature influences the level of dormancy in seeds, as it alters the range of conditions in which germination will occur and sensitivity to germination stimulants such as light, nitrate, ethylene and smoke[3]. There are several reports indicating that priming seeds in various salts could also stimulate dormant seed germination of different crops[4,5].

Plant growth regulators (PGRs), on the other hand, affect several aspects of plant growth and development, including seed germination. Germination of many dormant seeds of various plants was stimulated by cytokinins, ethylene and methyl jasmonate (MeJA)[6-8] and inhibited by abscisic acid and MeJA[6,9].

The objectives of this study are to test various priming agents on the freshly harvested seeds of annual ryegrass and investigate whether priming per se can stimulate dormant seed germination and inclusion of plant growth regulators (PGRs) into priming solution would further improve germination and emergence of *Lolium multiflorum* Lam. seeds.

MATERIALS AND METHODS

Plant material: Experiments were carried out with *Lolium multiflorum* Lam. seeds harvested in early January 2004, in Kahramanmaras, Turkey. Seeds were stored in paper bags at room temperature until the start of the experiment in mid February 2004.

Determination of the priming agent: To determine the best priming agent for dormant *Lolium multiflorum* Lam. seeds, three different priming agents were used. Seeds were primed in KNO_3 (1, 2 and 3%), KH_2PO_4 (1, 2 and 3%) and polyethylene glycol [(PEG-8000), 50, 100 and 200 g kg^{-1} water] for five days at 25°C with a 12 h photoperiod[2]. Seeds were placed in covered transparent polystyrene germination boxes (10x10x4 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, the priming solutions were changed tree days after priming started. Following the priming, seeds were rinsed under running tap water for one minute and surface dried for 2 h under room conditions on paper towels and then subjected to germination test subsequently. Untreated (non-primed) seeds were used as the control.

Germination tests were carried out with a 12 h photoperiod in a temperature-controlled incubator held at 25±05°C[2]. Seeds were placed on two layers of filter paper moistened with 2 mL of deionized water in covered 5.5 cm

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petri dishes. Four replications of 50 seeds were arranged in a Completely Randomized Design. We recorded the number of germinated seeds (radicle visible) daily until the numbers stabilized (for 14 days). From the total number of seeds germinated, final germination percentage (FGP) and its angular transformation (arcsine√FGP), days to 50% of FGP and days between 10 and 90% of FGP were calculated[10]. 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.

Inclusion of PGRs into the priming solution: To explore whether inclusion of PGRs into priming solution improves dormant *Lolium multiflorum* Lam. seed germination and emergence, three different PGRs were used. Based on the results of the previous experiment, 1% KNO_3 was chosen as the priming agent. Seeds were primed for five days at 25°C with a 12-h photoperiod in 1% KNO_3 solution containing methyl jasmonate (MeJA, 1, 3, 5, or 10 μM) or 1-aminocyclopropane-1-carboxylic acid (ACC, 1, 3, 5, or 10 μM) or 6-benzylamino-purine (BAP, 50, 100, 200, or 500 μM). Following priming germination test was conducted as described previously. Seeds primed in 1% KNO_3 solution containing no PGRs and untreated (non-primed) seeds were used as the controls. Data collection was as described previously.

For emergence test, 25 seeds in four replications from each treatment were sown into 1 cm depth in 7x3 (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 25±05°C with a 12 h photoperiod. Seedling emergence (coleoptile visible) was recorded daily until stabilized. From the total number of seedlings emerged, final seedling emergence percentage of seedlings (FEP) and its angular transformation (arcsine√FEP), days to 50% of the FEP (E_{50}), days between 10 and 90% of FEP (E_{10-90}), 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 LSD test if F test was significant at p=0.05.

RESULTS

Determination of the priming agent: Germination of dormant annual ryegrass seeds was not promoted by priming regardless of the priming agent used. Although not significantly different, the highest FGP was, however, obtained from priming seeds in 1% KNO_3 (33%) compared to untreated seeds which had an FGP of 29% (Table 1). The results also showed that priming freshly harvested *Lolium multiflorum* Lam. seeds in higher concentrations (>1%) of KNO_3 and KH_2PO_4 had an adverse effect on seed germination and resulted in lower FGP compared to untreated seeds while PEG had no effect on seed germination at any concentrations tested (Table 1).

All priming treatments, on the other hand, worsened germination rate and synchrony (higher G_{50} and G_{10-90})

Table 1: Final germination percentage (FGP) and angular transformation of FGP [FGP], days to 50% of FGP (G_{50}) and days between 10 and 90% germination (G_{10-90}) of *Lolium multiflorum* Lam. seed germination with a 12 h photoperiod at 25°C following priming for 5 days at 25°C in various concentrations of priming solutions

	FGP			
Treatments	%	[FG]	G_{50} (Days)	G_{10-90} (Days)
KNO_3 (%)				
1	33	[36]	4.3	8.0
2	29	[32]	5.4	9.7
3	26	[31]	5.0	8.5
KH_2PO_4(%)				
1	31	[34]	4.5	8.5
2	29	[33]	4.8	9.7
3	23	[29]	6.5	9.7
PEG (g kg^{-1} water)				
50	27	[31]	4.6	8.3
100	28	[32]	5.3	9.5
200	28	[32]	4.6	8.0
Untreated seeds	29	[32]	4.6	5.7
$LSD_{0.05}$		[4]	1.2	1.9
Significance		NS	*	***

***, *, NS Significant at p≤0.001, p≤0.05, or non significant, respectively

Table 2: Final germination percentage (FGP) and angular transformation of FGP [FGP], days to 50% of FGP (G_{50}) and days between 10 and 90% germination (G_{10-90}) of *Lolium multiflorum* Lam. seed germination with a 12 h photoperiod at 25°C following priming for 5 days at 25°C in 1% KNO_3 combined with different plant hormones at various concentrations

	FGP			
Treatments	%	[FGP]	G_{50} (Days)	G_{10-90} (Days)
MeJA(μM)¶				
1	36	[37]	2.3	4.6
3	33	[36]	2.6	4.7
5	35	[36]	2.2	3.7
10	35	[36]	2.4	5.9
ACC(μM)¶				
1	27	[31]	2.6	5.2
3	37	[38]	2.0	4.5
5	34	[36]	2.4	3.9
10	38	[38]	2.3	3.8
BAP(μM)¶				
50	38	[38]	2.2	3.9
100	41	[40]	1.8	4.1
200	32	[35]	2.2	4.2
500	35	[36]	2.4	3.9
1% KNO_3 only	37	[37]	2.3	3.9
Untreated seeds	30	[33]	3.9	4.7
$LSD_{0.05}$		[4]	0.4	1.5
Significance		*	***	NS

¶, KNO_3 + related plant hormone at given concentrations

***, *, NS Significant at p≤0.001, p≤0.05, or non significant, respectively

Table 3: Final emergence percentage (FEP) and angular transformation of FEP [FEP], days to 50% of FEP (E_{50}) and days between 10 and 90% emergence (E_{10-90}) of *Lolium multiflorum* Lam. seedling emergence at 25°C following priming for 5 days at 25°C in 1% KNO_3 combined with different plant hormones at various concentrations

Treatments	FEP %	FEP [FEP]	E_{50} (Days)	E_{10-90} (Days)
MeJA(μM)¶				
1	17	[24]	3.9	1.1
3	20	[27]	3.9	1.3
5	20	[26]	3.7	1.3
10	19	[26]	3.7	1.2
ACC(μM)¶				
1	22	[28]	3.4	1.6
3	19	[26]	3.8	1.3
5	20	[26]	3.6	1.0
10	20	[27]	3.9	1.4
BAP(μM)¶				
50	19	[26]	3.8	1.2
100	19	[26]	3.7	1.3
200	19	[26]	3.8	1.2
500	20	[27]	3.4	1.3
1% KNO_3 only	18	[26]	3.7	1.1
Untreated seeds	20	[27]	3.9	1.3
$LSD_{0.05}$		[24]	0.2	0.2
Significance		NS	**	**

¶,KNO_3+ related plant hormone at given concentrations
**, NS, Significant at $p \leq 0.01$ or not significant, respectively

except that priming in 1% KNO_3 (G_{50}=4.3 days) or 1% KH_2PO_4 (G_{50}=4.5 days) resulted in slightly increased germination rate compared to the rest of the treatments including untreated seeds (G_{50}=4.6 days); however, this improvement was not significant (Table 1).

Effects of inclusion of PGRs into the priming solution: Priming annual ryegrass seeds in the presence of PGRs did not further improve FGP of dormant seeds compared to seeds primed in KNO_3 only (Table 2). Inclusion of 100 μM BAP into priming solution (G_{50}=1.8 days) was the only treatment that improved germination rate of annual ryegrass compared to seeds that were primed in KNO_3 only (G_{50}=2.3 days) (Table 2). In contrast, all concentrations of the other PGRs resulted in germination rates similar to that of seeds primed in KNO_3 only. Priming annual ryegrass seeds in the presence of PGRs had no significant effect on the germination synchrony (Table 2).

Inclusion of PGRs into the priming solution had no beneficial effect on FEP of annual ryegrass seeds compared to seeds primed in KNO_3 only (Table 3). Seeds primed in the presence of 1 μM ACC and 500 μM BAP (E_{50}=3.4 days) were the only treatments that improved seedling emergence rate compared to seeds primed in KNO_3 only (E_{50}=3.7 days). Inclusion of 1μM ACC into priming solution also resulted in a less synchronous seedling emergence (higher E_{10-90}) than seeds primed in KNO_3 only.

DISCUSSION

Seed dormancy is an important developmental program allowing plants to withstand especially adverse environmental conditions, such as low temperature or drought and present in seeds of several plants, including *Lolium* spp.[11]. There are several factors or events that reduce or overcome seed dormancy and that vary among species[12]. Of those, priming seeds in a proper salt solution promotes seed germination and seedling emergence as well as it improves germination rate and synchrony[13]. A recent study indicated that dark-stratification could be an alternative, but not equivalent dormancy release mechanism to dry after-ripening present in annual ryegrass seeds[14]. Although seeds primed in 1% KNO_3 was slightly better than the other priming agents tested, the results of this study revealed that priming dormant seeds of annual ryegrass had no effect on dormancy release mechanism of annual ryegrass. The results also suggested priming in 1% KNO_3 could be tested under such dormancy release conditions reported previously[2,14].

Jasmonic acid or its methyl ester form methyl jasmonate (MeJA) affects several important biological activities of plants, including seed germination[15]. There are several reports indicating that germination of dormant seeds of various plants was stimulated by JA or MeJA=[16,17] whereas the germination of nondormant seeds of some other plants was inhibited[6,18]. This study showed that MeJA neither stimulates nor inhibits the germination and emergence of dormant annual ryegrass seeds at any concentration of MeJA tested. The lack of reaction to MeJA may be related to lack of response mechanism or it may mean that the level of endogenous MeJA is suitable for germination of dormant of *Lolium multiflorum* Lam seeds.

Germination of dormant seeds of several plants was also stimulated by cytokinins and ethylene[8,19]. Of these hormones, ethylene or its precursor 1-aminocyclopropane-1-carboxylic acid (ACC), is the simplest unsaturated hydrocarbon that regulates many diverse metabolic and developmental processes in plants, including seed dormancy[20]. Lack of germination or low germination caused by dormancy is related to insufficient ethylene production[21]. The highest ethylene production is, on the other hand, correlated with radicle protrusion[21]. However, it is not clear whether ethylene produced during seed imbibition is responsible for the induction of seed germination[21]. The results of this study suggested that the level of endogenous ACC in dormant *Lolium multiflorum* Lam. seeds is sufficient and ACC oxidase activity *in vivo* is insufficient to convert more

ACC to ethylene than the seed contains since there was no stimulatory effect of ACC on seed germination compared to primed seeds in 1% KNO_3 only (Table 2). One micro mole ACC along with 500 μM BAP were the only treatments that significantly hastened seedling emergence compared to seeds primed in 1% KNO_3 only. Higher concentrations of ACC, however, gave results similar to seeds primed in 1% KNO_3 only, suggesting that ethylene does not involve in the seedling emergence advancement effect of dormant seeds of *Lolium multiflorum* Lam. which is in agreement with the germination results.

Cytokinins are one of the mysterious groups of PGRs that control several important physiological activities in plants, such as photomorphogenesis[22]. Previous report indicated that the addition of cytokinin (6-Benzyladenine) into priming solution of lettuce seeds further increased germination percentage compared to seeds primed in 1% K_3PO_4 only[23]. In this study, BAP was the only PGR that increased germination rate when 100 μM BAP was included into 1% KNO_3 priming solution. It was previously reported that dormancy release of *Lolium rigidum* seeds was faster when the germination test was performed in darkness than when germination was tested in light/ dark conditions[2], suggesting that the light could be involved in the effect of cytokinins on primed seeds of annual ryegrass.

In conclusion, the results of this study revealed that dormancy present in freshly harvested *Lolium multiflorum* Lam. seeds could not be eliminated by priming but inclusion of 100 μM BAP and 1 μM ACC along with 500 μM BAP could hasten germination and seedling emergence, respectively. However, a further investigation is needed since the same PGRs did not improve FGP and FEP of annual ryegrass seeds.

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