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Full Length Research Paper

The combined effect of gibberellic acid and long time osmopriming on seed germination and subsequent seedling growth of *Klussia odoratissima* Mozaff.

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***Kelussia odoratissima* Mozaff. is one of the endogenous plant species of Iran which is exposed to extinction during the recent decades. Seeds of this plant have dormancy that causes reduction of seed germination. Experiment was performed as factorial with complete randomized design with 3 factors: polyethylene glycol (PEG) priming, gibberellic acid (GA_3) treatment, GA_3 application time. This research was performed to evaluate priming effect alone and in combination with GA_3, on seed germination and seedling growth parameters. Results show that seed priming with PEG alone had negative effect and GA_3 application solely did not have any significant effect on the seed germination percentage in comparison with the control. Seedling growth responses to PEG priming was similar (in -1 MPa) or lesser (in -1.5 MPa) than control, GA_3 significantly improved seedling growth as compared to the control. Application of GA_3 after PEG was better than using of GA_3 before PEG or co-application of GA_3 and PEG. These results suggest that probably, GA_3 compensated negative effect on long time priming.**

Key words: Gibberellic acid (GA_3), germination percentage, *Kelussia odoratissima* Mozaff., priming, seedling growth.

INTRODUCTION

Kelussia odoratissima Mozaff. is one of the native plant species of Iran with economical and ecological values in Central Zagros. This plant with the local name "kluss" belongs to Umbelliferae family. Before the determination of the new scientific name of this plant, it was introduced in the resources, books and various reports with other the scientific names for example *Amirkabiria odoratissima, Apium graveolens* and *Opopanax* sp. (Mozaffarian, 2003). This plant is one of the aromatic plants and has many medical properties.

As a result of more exploitation, this plant has been exposed to extinction during the recent decades. Seeds of *K. odoratissima* often germinate poorly in the nursery, because of dormancy problems and the possible propagation and natural regeneration of this species through seed is very pool. The dormancy characteristic and optimum conditions for seed germination of this species have not been explained so far. For this reason, there has been an interest in examining all factors that affect its seed germination and seedling establishment.

Moist chilling (0 to 5°C) for 8 to 10 weeks usually releases dormancy in *Klussia* seeds (Amooaghaie, 2002), but there is little information on the effect of applied growth regulators on dormancy release and the germination response in this species. Seeds can also be primed to stimulate germination, but the exact mechanism of this response is not understood.

Endogenous factors, especially plant growth regulators, such as gibberellins (GAs) (usually GA_3) and abscissic acid (ABA) play a key role in the dormancy mechanism in many species seeds. Dormant seeds of many species contain ABA in the embryonic axis, which prevents embryo growth. However, embryo dormancy can be

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Abbreviations: GA_3, Gibberellic acid; **PEG,** polyethylene glycol.

broken by exogenous GA_3 or indirectly by applying treatments that result in the synthesis of endogenous GA, such as chilling (Finch-Savage and Leubner-Metzger, 2006). Chilling appears to change the inhibitor-promoter balance in the seeds. Although, in some species, it has been reported that the requirement for moist chilling cannot be replaced entirely by exogenous GA application but however, addition of GA_3 might reduce the chilling requirement for dormancy breaking (Amooaghaie, 2007; Kucera et al., 2005). For this reason, a combination of chilling and GA_3 treatments has been used to improve germination in a range of species (Amooaghaie, 2007; Baskin and Baskin, 1991; Schmitz et al., 2001; Thomas and Sambrooks, 1985).

Seeds can also be primed for short period of time to improve germination. This treatment may invigorate or 'prime' the metabolic activity necessary to prepare the seed for germination, but if water availability is restricted, the radicle will not emerge. However, the risk of pre-mature germination can also be reduced if moisture uptake during priming is controlled using an osmoticum, such as polyethylene glycol (PEG). Primed seeds can generally tolerate post-sowing environmental stresses better than non-primed seeds. This is usually reflected in higher germination and/or a faster rate of germination of primed when compared with non-primed seeds (Bray, 1995; McDonald, 2000).

However, the seeds of many Apiaceae species require chilling to release dormancy before they can germinate (Amooaghaie, 2007; Baskin and Baskin, 1991; Thomas and Sambrooks, 1985), and effect of priming on improvement of seed vigor for seeds that needs prechilling for dormancy breaking and seedling growth has been reported (De-Atrip and O'Reilly, 2007; Doody and O'Reilly, 2005; Wu et al., 2001). Therefore, the effect of priming on kluss germination might be expected.

However, there is a paucity of information on the effect of GA_3 and priming on the germination response of kluss seeds. The objective of this study was to determine the effect of GA_3 before chilling on dormancy release in kluss seeds. In addition, the effect of PEG priming with or without GA_3 on the germination response of seeds and subsequent seedling growth was examined.

MATERIALS AND METHODS

Seeds of kluss (shaykhalikhan population) was provided by the Natural Resources Centre of Shahrekord, Iran and the experiment was carried out in 2007. The seeds were stored at 4 ± 1°C before the treatments commenced.

Experimental design

The experiment was set up to test the effects of priming and GA_3 together with moist chilling on germination performance and subsequent seedling growth. The experimental design was a 3-way factorial with the following factors and levels:

1) Priming treatment (3 levels): not primed, primed with PEG -1.0 MPa and primed with PEG -1.5 MPa
2) GA_3 (2 levels): 0, 250 ppm
3) Time of treatments (3 levels): First GA_3 and after priming, first priming and after GA_3 and co-application of GA_3 and priming.

The experimental set up was a randomized complete design with 3 replicates. Each replicate consisted of 50 seeds.
Analysis of variance (ANOVA) was used to test the significance of treatment and interaction effects on germination and growth parameters. The multiple Duncan's test was used for the calculation of significant differences among means at $P \leq 0.05$.

Priming and GA_3 treatments

Firstly, for controls and all treatments, seeds were soaked for 1 day at room temperature (21 to 22°C) in running aerated-de-ionized H_2O, drained, sterilized with witavax 0.2% for 5 min and rinsed several times in sterilized de-ionized H_2O. The witavax treatment did not affect the subsequent germination ability of seeds (the necessity of soaking was determined in our previous research).

Prior to moist chilling, the seeds were treated according to statistical analysis. For GA_3 treatments, seeds were soaked in distilled water (0 ppm GA_3) or 250 ppm GA_3 solution for 48 h.
For priming, the seeds were placed on top of a germination paper saturated with distilled water (control) or a PEG-6000 solution at -1.0 or -1.50 MPa for 15 days. After priming, seeds were rinsed in water in order to remove PEG before being transferred to germination conditions. Seeds that were primed with PEG solution containing no GA_3 and untreated (non-primed) seeds were used as the controls.

Following 24 h surface drying at room temperature (22°C), seeds (controls and all treatments) were transferred to moist-chilling conditions (5°C, in darkness).

Germination tests

The seeds were stratified under 5°C and germinated in Petri dishes each containing one germination paper during 10 weeks in refrigerator. According to our previous experiments, short periods of chilling were not effective in seed dormancy release and without any treatment, 10 week moist chilling is essential for promoting germination.
A replicate was a Petri dish containing 50 seeds. The number of seeds that germinated was determined every3 or 4 days. A seed was considered to have germinated when the radicle protruded about 2 mm. Final germination percentage was calculated after 10 week for all treatments.
Seedling growth was assessed in all treatments after 10 week stratification period. Germinated seeds were transferred from each treatment to growth chamber with 8 h lighting (6000 lux) at 20°C and 16 h dark at 15°C. After 2 weeks, length, dry and fresh weight of seedling in all treatments was measured.

RESULTS

Germination test

Seeds given the treatments displayed large differences in the germination response to treatments. The highest germination percentage (72%) resulted from the 10-week moist-chilled seeds without any treatments. No significant difference was observed in germination percentage between solely GA_3 treated seeds and controls (Figure

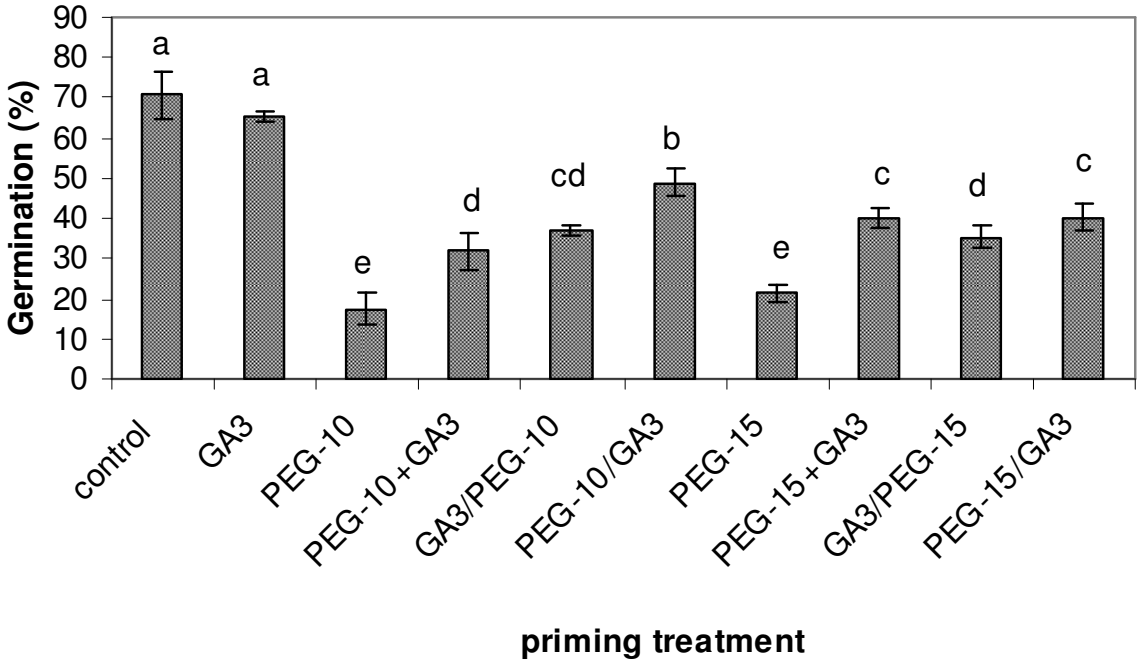

Figure 1. Effect of gibberellic acid (GA_3) and PEG priming on germination percentage of kluss seeds. The vertical lines are standard errors.

1). The differences between these treatments (control and solely GA_3) and the other tested treatments were significant (Figure 1).

Although, final germination in response to treatment with GA_3 was similar with control, GA_3 reduced the chilling requirement as compared to the control. GA_3 treated seeds germinated after 4 to 5 weeks chilling but controls germinated after 8 to 10 weeks (data not shown).

In contrast, only PEG treated seeds gave significantly lesser germination percentage than controls. Final germination in response to treatment with PEG was similar for -1.0 or -1.50 MPa.

All combined GA_3 and PEG treatments had better significant effect on the germination percentage as compared to the PEG priming alone. However, all combined GA_3 and PEG treatments had lesser significant effect on the germination percentage as compared to the control or GA_3 alone (Figure 1).

Response of primed seeds to GA_3 treatment was dependent on the treatment time. Subjecting to GA_3 treatment after priming resulted in a significantly higher germination percentage than solely priming or GA_3 treatment before priming or co-application of GA_3 and PEG (Figure 1).

Seedling growth

While shoot length response to PEG was similar (-1 MPa) or lesser (-1.50 MPa) than control, GA_3 significantly increased shoot length as compared to the control. Generally, GA_3 alone or in combination with -1 or -1.50 MPa PEG, significantly improved shoot length as compared to control or solely PEG treatments (Figure 2). Application of GA_3 after PEG was better than using GA_3 before PEG or co-application of GA_3 and PEG.

Trend of shoot length changes in response to treatments including PEG (with or without GA_3) was similar for -1.0 or -1.50 MPa. But means of shoot length in -1.50 MPa was lesser than that in -1.0 MPa.

The pattern of response to treatments for fresh and dry weight of shoot was generally consistent with that observed for shoot length (Figures 3 and 4). But application of GA_3 after -1.0 MPa significantly surpassed solely GA_3 treatment, for fresh and dry weight of shoot. However, both treatments showed higher shoot fresh and dry weight than control (Figures 3 and 4). Solely PEG priming had significantly negative effect on the fresh and dry weight of shoot. However, all combined GA_3 and PEG treatments had better significant effect on shoot fresh and dry weight than PEG alone (Figures 3 and 4). Trend of shoot fresh and dry weight in response to treatments including PEG (with or without GA_3) was similar for -1.0 or -1.50 MPa. But means of fresh and dry weight of shoot in -1.50 MPa was lesser than that in -1.0 MPa (Figures 3 and 4).

The pattern of response to treatments for length of root was generally consistent with that observed for shoot length (Figure 5). Generally, GA_3 treatments, either alone or in combination with -1 MPa PEG, had no significant effect on fresh and dry weight of root as compared to control. Solely PEG priming had significantly negative

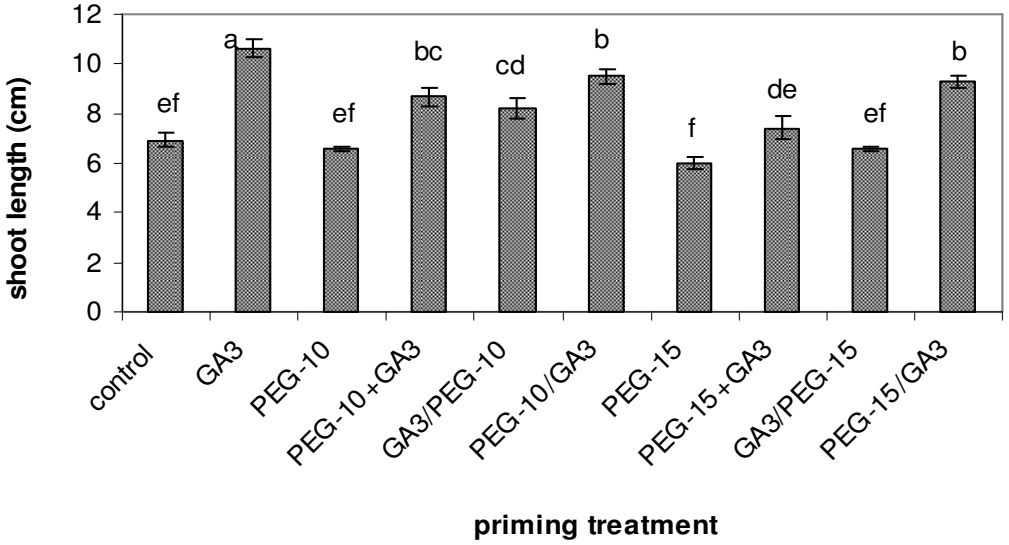

Figure 2. Effect of gibberellic acid (GA_3) and PEG priming on shoot length of kluss seeds. The vertical lines are standard errors.

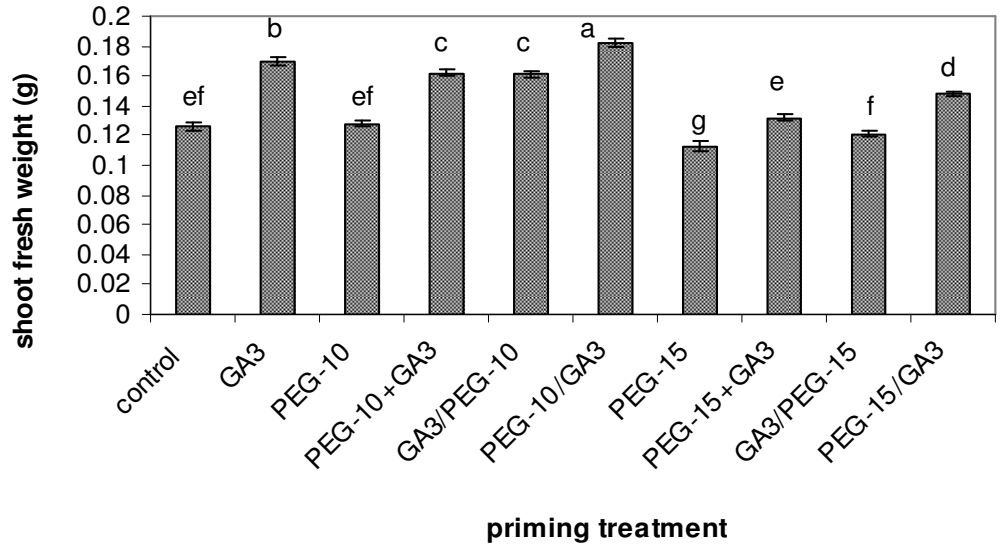

Figure 3. Effect of gibberellic acid (GA_3) and PEG priming on shoot fresh weight of kluss seeds. The vertical lines are standard errors.

effect on the fresh and dry weight of root.

Trend of root fresh and dry weight in response to treatments including PEG (with or without GA_3) was similar for -1.0 or -1.50 MPa. But means of fresh and dryweight of root in -1.50 MPa was lesser than that in -1.0 MPa (Figures 6 and 7).

DISCUSSION

The results of this research showed that kluss seeds display an endogenous dormancy that can be released by moist-chilling treatment for a certain period. The 10-week moist-chilled seeds as compared to the other tested treatments had higher germination percentage.

The effect of cold treatment on seed dormancy breaking has also been confirmed in our previous research (Amooaghaie, 2002) and for other plants of Apiaceae such as: *Osmorhiza* (Baskin and Baskin, 1991), *Ferula ovina* (Amooaghaie, 2007, 2009) and *Apium graveolens* (Thomas and Sambrooks, 1985).

Although, final germination in response to treatment

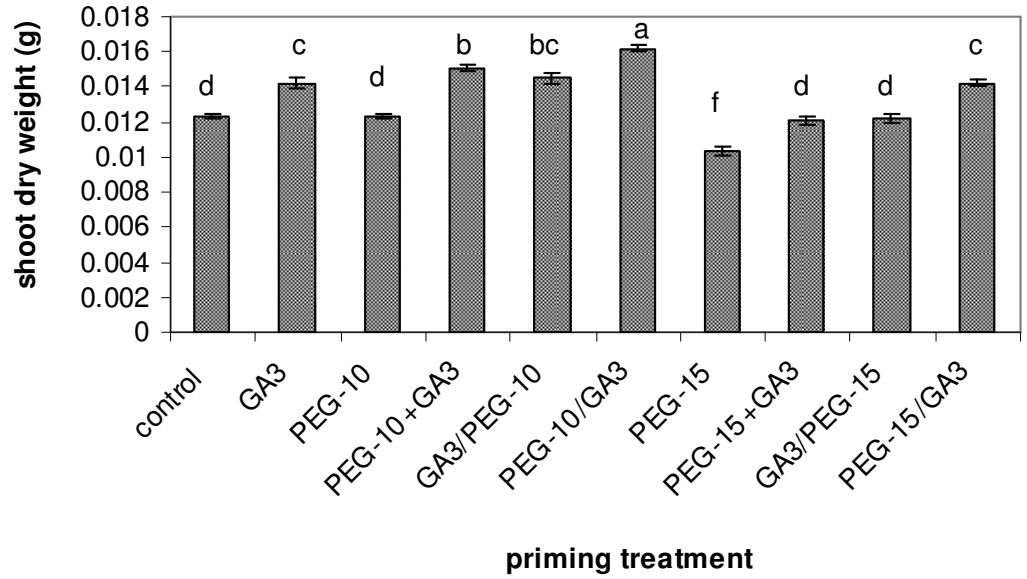

Figure 4. Effect of gibberellic acid (GA_3) and PEG priming on shoot dry weight of kluss seeds. The vertical lines are standard errors.

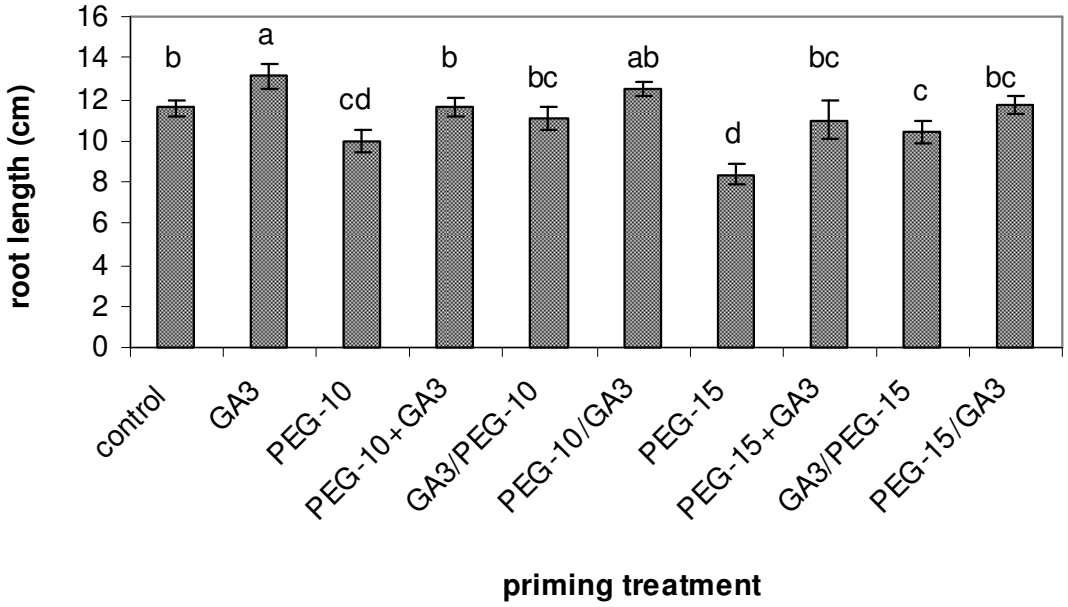

Figure 5. Effect of gibberellic acid (GA_3) and priming on root length of kluss seeds. The vertical lines are standard errors.

with GA_3 was similar with control, GA_3 reduced the chilling requirement as compared to the control. GA_3 treated seeds germinated after 4 to 5 weeks chilling but controls germinated after 8 to 10 weeks (data not shown). Baskin and Baskin (1991) found positive effect for soaking in GA_3 solution on early germination of *Osmorhiza claytonii* (apiaceae) seeds. It was reported that GA_3 is effective in breaking of non-deep physiological dormancy, but it does not overcome the deep physiological dormancy (Baskin and Baskin, 2004). Lack of GA_3 effectiveness in increasing final germination of *Kelussia odoratissima* might be seen as the possibility of *Kelussia odoratissima* seeds to have the deep physiological dormancy. But GA_3 treated seeds germinated earlier

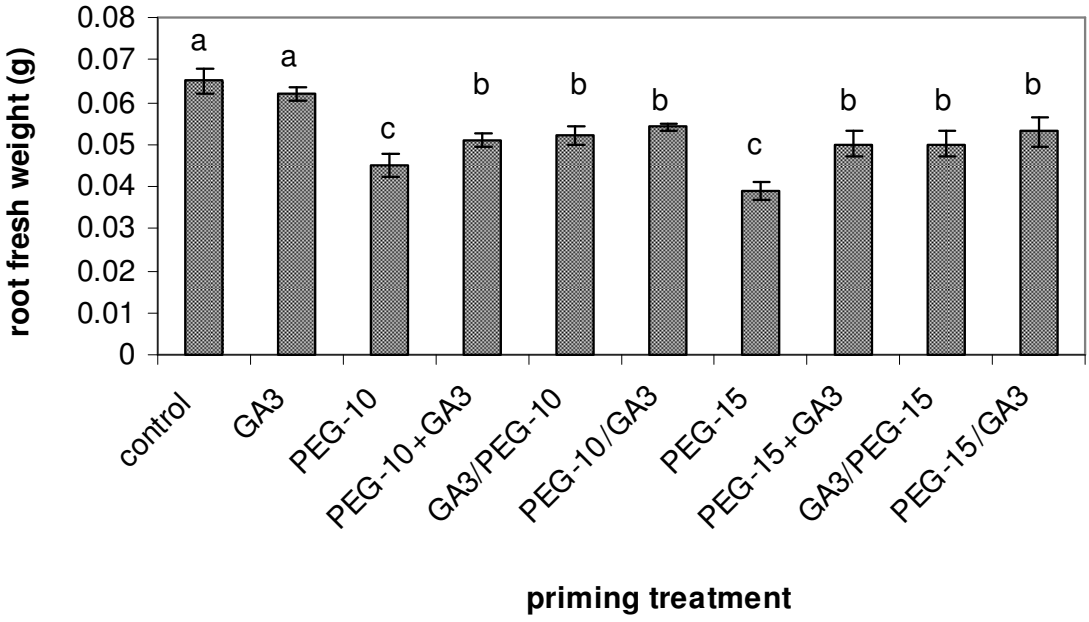

Figure 6. Effect of gibberellic acid (GA_3) and priming on root fresh weight of kluss seeds. The vertical lines are standard errors.

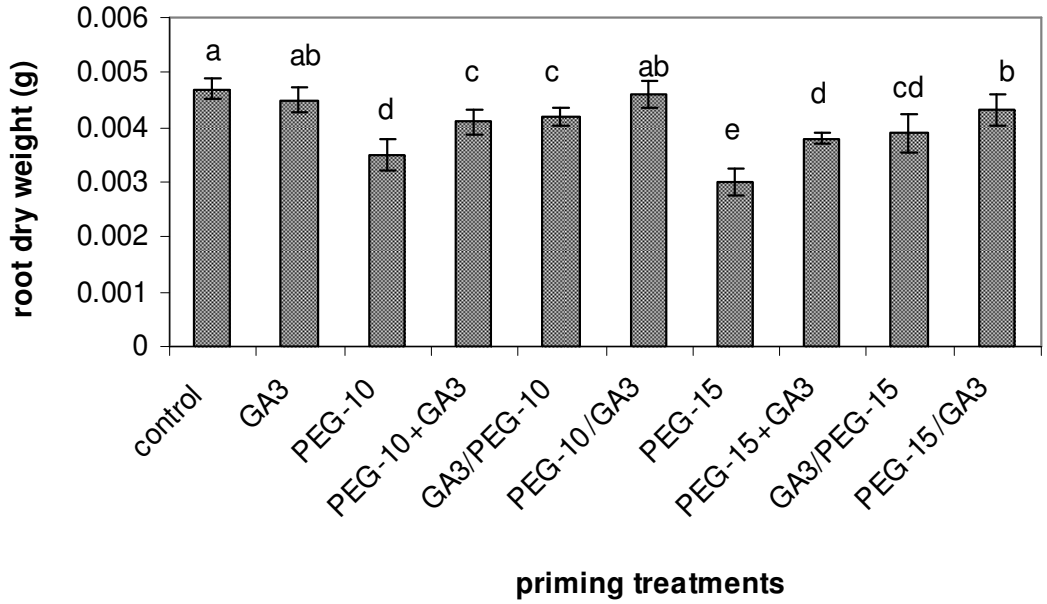

Figure 7. Effect of gibberellic acid (GA_3) and priming on root dry weight of kluss seeds. The vertical lines are standard errors.

(after 4 to 5 weeks) than control and this shows GA_3 effectiveness. Maybe, used GA_3 concentration or other conditions in this experiment was not suitable for monitoring of actual response of kluss seeds to GA_3. This GA_3 concentration may have been insufficient to weaken the seed coat and thus have not increased final germination. If the seed coat had been weakened by the applied GA_3, germination could probably have proceeded quickly thereafter. Also, GA_3 may activate the synthesis of proteins and other metabolites required by the embryo for germination (Finch-Savage and Leubner-Metzger, 2006; Kucera et al., 2005; Leubner-Metzger et al., 1996).

The results presented here showed that GA_3 could be used to shorten the chilling period. It appears that GA_3

reduced the chilling requirement to release dormancy, but it did not completely compensate for it. GA_3 might have enhanced growth promoter levels that helped overcome the effect of the inhibitors (such as ABA), leading to seed dormancy release. Thus, exogenous GA_3 may have triggered dormancy release in the fraction of seeds that had not been released from dormancy during chilling (Finch-Savage and Leubner-Metzger, 2006; Kucera et al., 2005; Leubner-Metzger et al., 1996).

GA_3 has been shown to play a key role in dormancy release in the seeds of other Apiaceae species, including *Osmorhiza* (Baskin and Baskin, 1991), *Ferula ovina* (Amooaghaie, 2007, 2009) and *Apium graveolens* (Thomas and Sambrooks, 1985).

PEG priming showed negative effect on seed germination. Similarly, the results of research on the seeds of several species have shown that PEG provided unfavorate results on seed vigor (Bennet and waters, 1987; Emmerich and Hardegrie, 1991). In contrast, PEG has been used successfully to prime the seeds of several agricultural and horticultural crop species (Amooaghaie et al., 2010; Coolbear et al., 1980; Finch-Savage et al., 1991).

Osmotic priming with PEG solution is known to improve the rate and uniformity of seed germination in several plants (Amooaghaie et al., 2010; Basra et al., 2005; Bose and Mishra, 1992; Farooq et al., 2006). Nevertheless, priming in a PEG solution could result in a detrimental effect on germination of sweet corn seeds, possibly due to the induced anoxia injury during priming (Bennet and Waters, 1987). The negative effect of osmoconditioning might also be due in part to unanticipated low water potential of PEG solution resulting from filter paper exclusion and water vapor loss (Emmerich and Hardegrie, 1991). Also, it might be that priming treatment was not in optimum condition. Perhaps, priming did not enhance germination in seeds that received 10 week chilling (Figure 1), because priming was less effective in enhancing seed germination in the seeds that received the longest chilling period. Similarly, Wu et al. (2001) reported that the priming of loblolly pine (*Pinus taeda* L.) seeds decreased as the length of chilling increased. The decrease in germination potential in the seeds over the 14 days of priming treatment was probably due to seed deterioration. Deterioration of seeds had received long period chilling as reported by De-Atrip and O'Reilly (2007) and Walters (1998), perhaps because nutrient reserves had been partially depleted during chilling. However, additional tests were not carried out to confirm this. It is unlikely that dormancy was re-introduced.

However, priming induced good effect on root and shoot growth, particularly in combination with GA. There was improvement in germination and seedling vigor by osmopriming and hardening in both coarse and fine rice as reported earlier by Farooq et al. (2006). Faster emergence rate after priming may be explained by an increased rate of cell division in the root tips as previously found for wheat (Bose and Mishra, 1992).

Increased α-amylase activity and sugar contents were also reported in the primed fine rice seeds (Basra et al., 2005). Priming of normal and naturally aged large rice seeds improved the germination and seedling vigor. Total sugar contents and α-amylase activity of normal seeds were higher than that of the aged seeds. The enhanced activity of α-amylase during the hydropriming may be attributed to hydration during treatment, resulting in increased starch hydrolysis, increased contents of total and reducing sugars and lower contents of non-reducing sugars. The benefit of increased starch hydrolysis following hydration treatments was not lost during the redrying process, as shown by the better rate and speed of germination (Amooaghaie *et al*., 2010; Lee and Kim, 2000). The combination of GA_3 and PEG treatments produced seedlings of significantly higher vegetative growth and vigor than those of GA_3 or PEG treatment alone. Similarly, several researchers reported invigorating effect of inclusion of hormones such as gibberellins and cytokinines in priming solution on the subsequent seedling growth (Korkmaz et al., 2004; Finch-Savage et al., 1991; Leubner-Metzger et al., 1996; Lorenz et al., 1988; Nayyar et al., 1995).

Priming stimulates GA metabolism leading to germination, thus inducing a similar response to that described for some GA_3 treatments. Perhaps, in undesired condition, priming had a small effect on GA metabolism and exogenous application of GA_3 after priming may help to compensate for negative effect of unsuitable priming in the seeds, leading to a faster rate of dormancy release and significantly increased seedling growth.

Conclusions

1. Appling GA_3 before chilling, reduced the chilling requirement for dormancy release in kluss seeds, but it did not increase final germination.
2. Priming for 14 days had a negative effect on germination of seeds and did not have good effect on seedling growth.
3. Application of GA_3 after PEG priming increased seedling growth. Maybe, GA_3 compensated for negative effect of long time priming.

Therefore, additional tests with other GA_3 concentrations and other osmoticums or short priming duration should be done to find out the optimal response to GA_3 and priming in kluss.

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