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Seed Treatment to Overcome Autotoxicity of Forage Rape

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ABSTRACT

In order to evaluate the effects of different priming treatments on reducing autotoxicity potential of forage rape, an experiment was conducted with a factorial arrangement based on completely randomized design with four replications at Islamic Azad University of Karaj during 2014. Factors of experiment consisted of forage rape extract in three levels (aerial parts, underground tissues, and combination of different parts) and various seed priming methods in six levels (control, hydro-priming, osmo-priming with PEG, hormonal priming with gibberellic acid, potassium nitrate priming, and priming by salicylic acid. The studied traits according to ISTA (International Seed Testing Association) instruction were: rate and percentage of germination, length of shoot and root, fresh and dry weight of seedling. The results showed that the extracts of various parts of forage rape inhibits the seedling growth in different ways by affecting its germination traits. The lowest and highest percentage of seed germination was observed in extracts of underground parts and aerial organs, respectively. Different priming treatments also have various inhibiting role for reducing autotoxicity potential of forage rape. As regards, hydro-priming and osmo-priming with PEG were the most influential methods of priming. Germination rate of seeds primed with salicylic acid was zero. It seems that salicylic acid has inhibitory effects on seed germination of forage rape. Germination rate of seeds primed with gibberellic acid (1.29 day^{-1}) and potassium nitrate (1.57 day^{-1}) was less than control treatment (2.19 day^{-1}). According to results, hydro-priming or osmo-priming with PEG could be a suitable tool for reducing autotoxicity potential of forage rape and improving germination characteristics, particularly for monoculture systems.

1. Introduction

Autotoxicity is a form of allelopathy which inhibits germination and growth of a species of a same type by self-produced toxins. In other words, autotoxicity refers to adverse effects of one species by self-produced toxins (Waller et al., 1987). These toxins are secondary metabolites with different biochemical reactions depended on plant species (Chung et al., 2001). The chemical compounds are found in different parts of some plants such as root, leaf, stem, flower and fruits (Golzardi et al., 2009; Golzardi et al., 2014). Allelopathic components spread over an environment in certain conditions to affect germination and growth rate of root and shoot of target plants as well as soil microorganisms (Golzardi et al., 2014; Golzardi et al., 2015). Researches have shown that the family Brassicaceae has a kind of potential which inhibits other plants' germination and growth (Golzardi et al., 2009; Oleszek et al., 1996). Forage rape is of this family of plants which, added to its nutritional and medical use, is known as a soil refiner and reduces soil-borne diseases and creates a perfect environment for growth by controlling insect popula-

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tion, soil microorganisms, and weed growth (Bruce et al., 1990; Bones and Rossiter, 1996). Forage rape defensive system is glucosinolate-myrosinase which is an active allelopathic system. Glucosinolates are secondary metabolites released from vacuoles, in certain circumstances such as mechanical damages, wounds, insect attacks or cell damage, to change into an active inhibitor (Bones and Rossiter, 1996). Studies have shown that residues of cultivated rape leave adverse effects on the future crops. The observed effects are reduction in plants' dry weight, height, number of tillers per plant, and grain yield (Horricks, 1969). Researches on extracts of rapeseed residues proved the existence of toxic compounds (Mson-Sedun and Jessop, 1989; Mondani et al., 2010).

Priming is one of the most significant treatment methods increasing seeds' germination potential (Farooq et al., 2006; Hesabi et al., 2014). Seed priming is a planting preparation provided to improve seedling growth, germination rate, and plant resistance against various stresses (Taylor and Harman, 1990). Among different priming treatments we can name osmo-priming (having seeds soaked in osmotic solutions such as poly ethylene glycol (PEG)), halo priming (salt treated seeds), hydro-priming (seeds soaked in water). After cultivation, primed seeds germinate sooner which leads to faster, better and more uniform plant adaptation. Fulfillment of such conditions, ecologically and biologically, specialize plants borne out of primed seeds (Cheng and Bradford, 1999).

Therefore, regarding forage rape's autotoxicity potential and the significance of this strategic plant, it seems necessary to find ways of reducing the inhibiting factors. Since it is proved that priming treatments can affect various stresses, the methods may be helpful in reducing forage rape's autotoxicity potential. Hence, this experiment was carried out to assess this possibility by implementing different priming techniques.

2. Materials and Methods

2.1. Experimental Design

In order to evaluate the effects of different priming treatments on reducing autotoxicity potential of forage rape, an experiment was conducted with a factorial arrangement based on completely randomized design with four replications at Islamic Azad University of Karaj during 2014. Factors of experiment consisted of forage rape extract in three levels (aerial organs, underground tissues, and combination of different organs) and various seed priming methods in six levels (control, hydro-priming, osmo-priming with PEG, hormonal priming with gibberellic acid (GA), potassium nitrate priming, and priming by salicylic acid (SA). The studied traits according to ISTA instruction (ISTA, 1996) were: rate and percentage of germination, length of shoot and root, fresh and dry weight of seedling.

2.2. Preparation of Forage Rape Aqueous Extract

In order to prepare aqueous extraction of forage rape, we gathered aerial and underground parts of forage rape from randomly selected farms around Karaj at the end of the growing season (harvest stage). Each organ, after division and purification, was separately completely dried in the shade for 72 hr. In the next step, different parts of forage rape were grinded to finer pieces to go through a 0.5 mm sieve. To prepare the extract, added 100 ml distilled water per 5 gram residues of plant (Mondani et al., 2010) and placed it on stirrer set as 200 rpm for 24 hours at laboratory temperature. The obtained liquid extract, after passing through four Whatman filter paper No.1 centrifuged for about 30 minutes at 3000 rpm, passed through a filter paper No.1, and kept in a refrigerator to be used (Golzardi et al., 2009; Mondani et al., 2010; Golzardi et al., 2014; Golzardi et al., 2015).

2.3. Seed Priming Methods

For hydro-priming, we used distilled water to soak seeds for 12 hours at 25℃; then, the seeds should be removed from water. In priming with poly ethylene glycol (PEG), seeds should be placed in PEG solution for 24 hours at 25℃; then, the seeds should be removed from the solution (Mondani et al., 2010). Priming by salicylic acid (SA) requires seeds to be soaked in 0.1% SA solution for 24 hours. In order to prime seeds with gibberellic acid (GA), we placed them in GA solution (0.2%) for 24 hours. In order to prime by KNO_3, we put the seeds in 500 ppm NO_3 for 12 hours at 25℃. After priming, we washed the seeds with distilled water and exposed them to air for 24 hours for its humidity returns to initial state, before priming (Demir et al., 1994).

2.4. Germination Experiment

A petri dish (8 cm diameter) was selected for the experiment on which 2 paper filters were placed for seed cultivation. In each dish 25 seeds (forage rape cultivar Pac101) are placed in which 6 ml extract was added. As a control experiment, petri dishes containing distilled water are also prepared. To prevent evaporation of the extract, petri dishes were closed by Parafilm and were transferred to a germinator set between 18-25℃ (day and night) with 12 hour running cycle. Seedling count and aeration was conducted on a daily basis according to ISTA instruction. The final counting and measurement of root and shoot length, fresh and dry weight, was also performed separately for each species again according to ISTA instruction (ISTA, 1996). To obtain the dry weight of seedlings, the samples were dried and weighed after 4 hours at 60℃ (Cheng and Bradford, 1999). To perform statistical operation, we used SAS (version 9.1), Excel, and Duncan's multiple range tests at the 1% level to analyze data, draw graphs, and determine differences among mean values, respectively.

3. Results and Discussion

3.1. Germination Percentage

The results showed that forage rape extract and different priming treatments and their interactions significantly affected on its germination (Table 1). The highest germination rate obtained through hydro-priming and osmo-priming by PEG with extract of aerial organs of forage rape which was 80 and 75 percent, respectively (Fig. 1). Demir et al. (1994) reported that osmo-priming by PEG significantly increased germination rate and speed of aged seeds of eggplant (*Solanum melongena*) compared to control seeds. The lowest germination rate was observed in SA priming which resulted in zero germination. It seems that salicylic acid has inhibitory effects on seed germination of forage rape. Regardless of SA priming treatment, the lowest germinate rate (17.8%) belongs to priming by KNO_3 with extract of forage rape's underground parts. To reduce the effects of autotoxicity potential of forage rape's underground parts, the most influential priming treatments seems to be hydro-priming and osmo-priming by PEG which resulted in 32.7% and 31.2%, respectively (Fig. 2). Generally, it appears that SA, KNO_3, and GA priming are not proper to reduce the effects of autotoxicity potential of forage rape. Extract derived from different parts of forage rape showed various autotoxicity effects so that root extract had the most and aerial extracts have the least inhibiting effects on germination rate (Fig. 1). Mondani et al. (2010) conducted a research on the autotoxicity effects on canola and concluded that the level of damage depends on canola's organs. They reported that the maximum inhibitory effect on germination was observed in canola's root extract.

Table 1. Analysis of variance for the effect of priming methods and forage rape extract on seed germination and seedling growth of forage rape.

Source of Variation	df	Mean Square					
		Germination Percentage	Root Length	Shoot Length	Seedling Fresh Weight	Seedling Dry Weight	Germination Rate
Priming Methods	5	46102.133**	324.9991**	355.7189**	12.9232**	0.0772 **	109.5907**
Forage Rape Extract	2	66722.4667**	425.7847**	710.4280**	21.7535**	0.1350 **	151.9734**
Interaction	10	3514.2000**	24.2214**	36.0146**	1.2768**	0.0076 **	9.3767**
Error	54	127.615	2.2382	1.066	0.052	0.0005	0.5533
CV		12.55	13.13	12.40	11.60	14.04	14.74

3.2. Root Length

The results showed that the effect of different priming treatments and forage rape extracts and their influence on root length is significant at 1% probability (Table 1). The highest root length of forage rape was observed in seeds placed in aerial extract with osmo-priming by PEG (6.4 cm), and hydro-priming (6.2 cm) (Fig. 2).

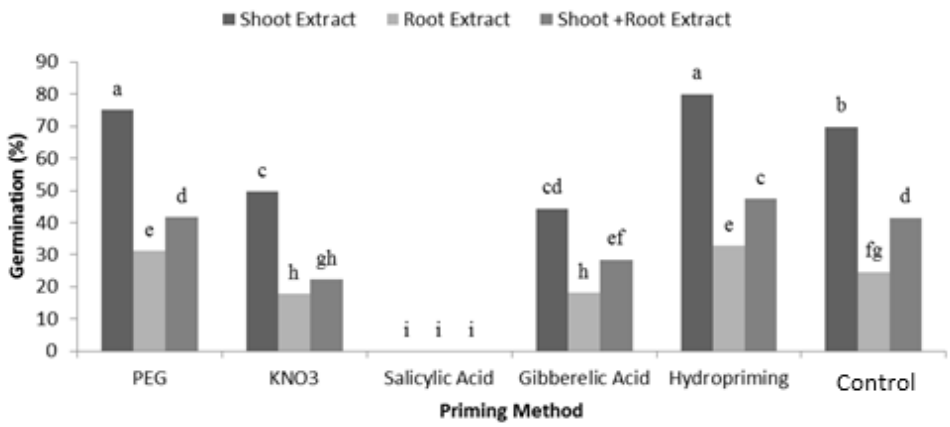

Fig. 1. The effect of different priming treatment and forage rape extract on seed germination percentage of forage rape. Means denoted by the same letter did not significantly differ at P < 0.01 according to Duncan's multiple range test.

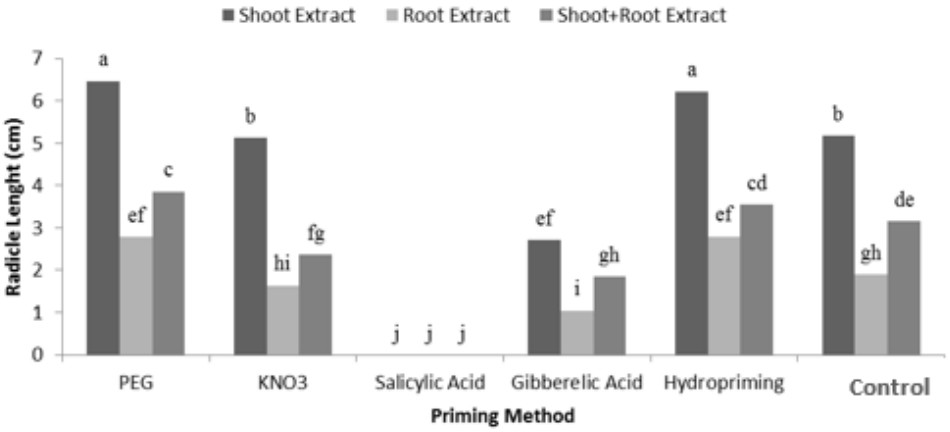

Fig. 2. The effect of different priming treatments and forage rape extract on root length of forage rape. Means denoted by the same letter did not significantly differ at P < 0.01 according to Duncan's multiple range test.

Sanchez et al. (2001) argued that hydro-priming adds up to the root length of cucumber (*Cucumis sativus*) and pepper (*Capsicum annuum*) seeds. The lowest root length belonged to seeds primed with SA (0 cm). Regardless of SA priming, it can be said that the lowest root length for the seeds in underground extract resulted from GA priming (1.04 cm) and KNO_3 priming (1.6 cm). Hydro-priming treatment showed the highest resistance against inhibiting effects on underground organs (2.8 cm) (Fig. 2). Masiunas and Eastman (1991) have also suggested that, since extract derived from underground organs have higher allelopathic material than aerial organs, canola's root length goes higher when exposed to the former extract.

3.3. Shoot Length

The results showed that the effect of different priming treatment and forage rape extracts and their influence on shoot length was significant at 1% probability (Table 1). The highest shoot length of forage rape was obtained from seeds placed in aerial part extract with osmo-priming by PEG (6.79 cm), and KNO_3 priming (6.70 cm) (Fig. 3). The lowest shoot length belongs to seeds primed with SA (0 cm). In an experiment it was shown that, under salinity stress, primed seeds of oat would have higher germination rate and shoot length (Rashid et al., 2006). Regardless of SA priming, it can be said that the lowest shoot length (1.1 cm) for the seeds in underground extract resulted from GA priming (Fig. 3). Mondani et al. (2010) reported that the lowest and highest shoot length of canola brought about by extract derived from underground and aerial organs of forage rape, respectively.

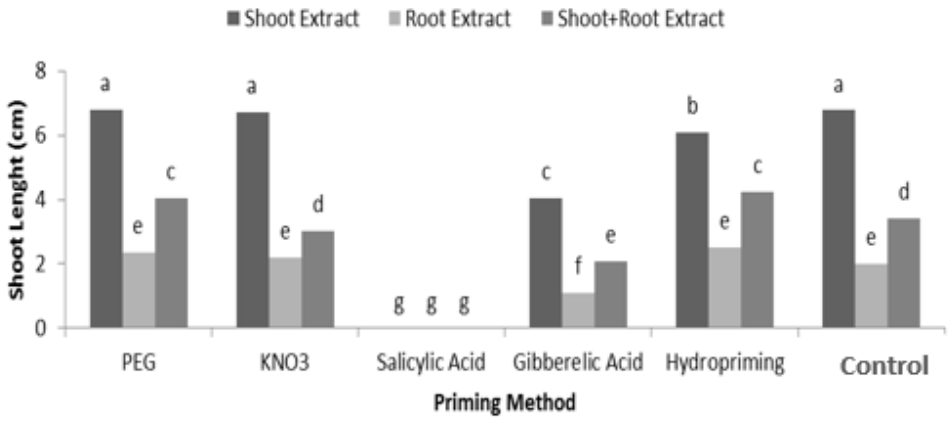

Fig. 3. The effect of different priming treatment and forage rape extract on shoot length of forage rape. Means denoted by the same letter did not significantly differ at P < 0.01 according to Duncan's multiple range test.

3.4. Seedling Fresh Weight

The effect of priming treatment and extracts of forage rape and their interaction on seedling fresh weight of forage rape was significant at 1% probability (Table 1). The highest Rape fresh weight (1.40 g) was observed in seeds placed in aerial extract with osmo-priming by PEG (Fig. 4). In addition, hydro-primed seeds weighed more than control seeds when placed in aerial extract (1.26 g). Jyotsana and Srivastava (1998) reported that hydro-priming treatment had positive effects on seedling fresh weight of pigeon pea (*Cajanus cajan*). The lowest fresh weight of forage rape belonged to seeds primed with SA (0 g). Regardless of SA priming, it can be said that the lowest (0.20 g) and highest (0.51 g) fresh weight of forage rape for the seeds in underground extract resulted from GA priming and hydro-priming, respectively (Fig. 4).

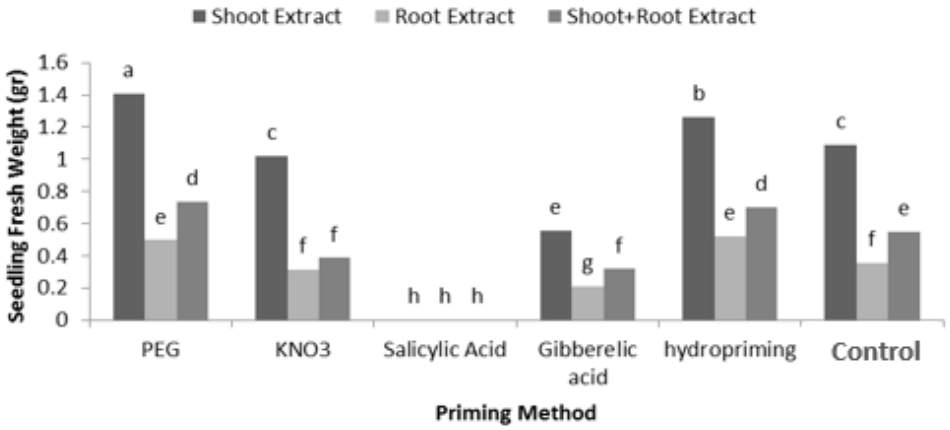

Fig. 4. The effect of different priming treatment and rape extract on seedling's fresh weight of forage rape. Means denoted by the same letter did not significantly differ at P < 0.01 according to Duncan's multiple range test.

Also, Levanon et al. (1982) showed in an experiment that Saponins[1] in alfalfa (*Medicago sativa*) roots negatively affects seedling growth by self-produced toxins which results from reduced population of Nitrogen-fixing microorganisms. Existence of aqueous extract of corn residue in soil can significantly reduce the plant's seedling (Ben-Hanunoud et al., 2001).

3.5. Seedling Dry Weight

The results showed that the effect of different priming treatment and forage rape extracts and their influence on dry weight of forage rape seedling is significant at 1% probability (Table 1). The highest forage rape seedling dry weight (0.1078 g) was observed in seeds placed in aerial extract with osmo-priming by PEG (Fig. 5). Kaya et al. (2006), after an experiment on sunflower seeds under salinity stress, observed that priming treatment improves germination rate, seedling appearance, and seedling dry weight while it reduces abnormal seedlings. In addition, hydro-primed seeds weighed more control seeds when placed in aerial extract (0.0988 g) (Fig. 5).

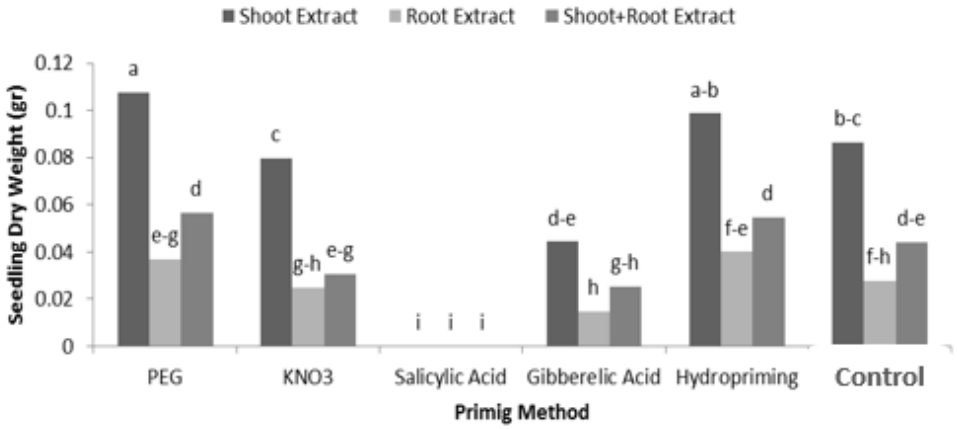

Fig. 5. The effect of different priming treatment and forage rape extract on seedling's dry weight of forage rape. Means denoted by the same letter did not significantly differ at $P < 0.01$ according to Duncan's multiple range test.

In an experiment it was shown that, under salinity stress, primed seeds of oat would have higher germination rate, seedling appearance, and biomass (Rashid et al., 2006). At all levels of experiment, the lowest dry weight of forage rape belongs to seeds primed with SA (0 g). Regardless of SA priming, it can be said that the lowest (0.0147 g) dry weight of forage rape for the seeds in underground extract resulted from GA priming (Fig. 5). Mondani et al. (2010) also reported canola's extract of different organs can significantly affect seedling dry weight so that the lowest and highest reduction in the seedling dry weight brought about by extract derived from aerial and underground organs of canola, respectively.

3.6. Germination Rate

The results showed that the effect of different priming treatment and forage rape extracts and their influence on germination rate of forage rape seeds is significant at 1% probability (Table 1). The highest germination rate (4.07) was observed in hydro-primed seeds placed in aerial. Osmo-priming treatment with PEG in aerial extract increased germination rate (3.39) to more than that of control treatment (Fig. 6). Afzal et al. (2006) believed that priming treatment can increase germination rate of forage rape seeds. Seed priming improves germination rate and uniformity while decreasing seeds sensitivity to environmental factors (Mson-Sedun and Jessop, 1989). Regardless of SA priming, it can be said that the lowest germination rate (0.77) forage rape seeds in underground extract

[1]Saponins are a class of chemical compounds found in particular abundance in various plant species

resulted from GA priming (Fig. 6). Pedersen et al. (2001) also found out that low concentration of compounds in forage rape extracts can either control or inhibit weeds germination yet the seeds remain alive and continue their lives; therefore, increased concentration inhibits germination rate of the seeds.

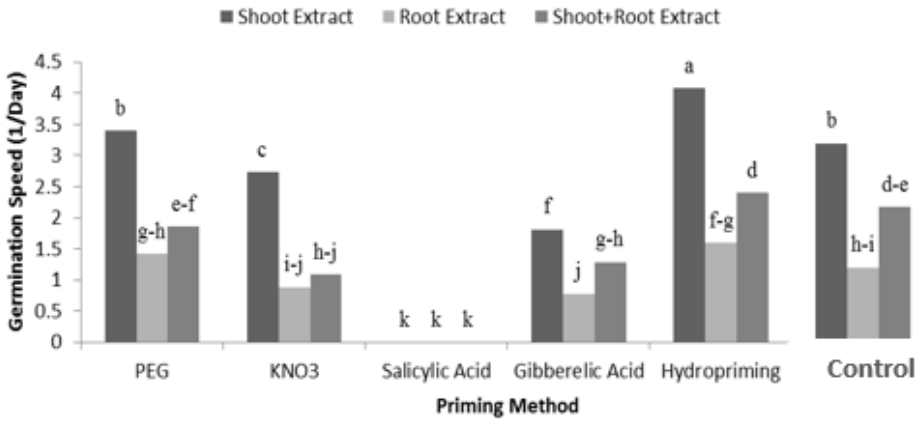

Fig. 6. The effect of different priming treatment and forage rape extract on germination rate of forage rape seeds. Means denoted by the same letter did not significantly differ at $P < 0.01$ according to Duncan's multiple range test.

4. Conclusion

Results were obtained from investigating the effects of forage rape's autotoxicity potential indicates that various levels of damages may occur which depends on Rape organs. Among different organs of forage rape, the highest and lowest inhibitory effects were observed in underground and aerial organs, respectively (Fig. 1-6). Osmo-priming by PEG and hydro-priming showed the most while GA and KNO_3 priming showed the least germination trait. SA priming resulted in zero germination. Generally, SA, KNO_3, and GA priming methods are not proper treatment for reducing the effects of forage rape's autotoxicity potential; while, hydro-priming and priming by PEG can play a significant role in this regard. According to obtained results, in order to reduce adverse effects of autotoxicity potential of forage rape, particularly for monoculture systems, it is recommended to farmers to treat seeds of forage rape by hydro-priming or osmo-priming with PEG before planting.

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