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Effect of Gibberellic Acid, Prechilling, Sulfuric Acid and Potassium Nitrate on Seed Germination and Dormancy of Annual *Medics*

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Abstract: The objective of the present study was to investigate effective methods in breaking the seed dormancy for annual medics' species. Three experiments were conducted to evaluate seed germination of annual medics including *Medicago radiata, Medicago polymorpha* and *Medicago rigidula* under different prechilling, gibberellic acid and potassium nitrate concentrations and sulfuric acid concentrations in 5 and 10 min of applying time. The result showed that all methods broke seed dormancy and exhibited seed germination of annual medics, but some cases were different in cultivars. The most effective and practical method for seed dormancy breaking in *M. polymorpha* and *rigidula* was 96% sulfuric acid application for 10 min. The main advantages of this method are speed, ease of use and unaltered physical condition of the seeds following treatment and cheap. The concentration of 750 ppm gibberellic acid with prechilling in 4°C was the most effective and practical method in breaking hard seed dormancy of *M. radiata.*

Key words: Annual medic, germination, dormancy, gibberellic acid, sulfuric acid, potassium nitrate, prechilling

INTRODUCTION

Medicago species is the most valuable crop of the rangelands. These species have the ability of yielding quality forage as well as fixing at least 120 kg N ha^{-1} resulting in increased soil fertility. Annual *Medicago* species are known collectively as medics. Medics are evolved in North Africa and Middle East where they grow over a wide range of soils, temperature regimes and lengths of growing season (Uzun and Aydin, 2004). Medic pastures that produce high levels of good quality forage are well suited to grazing and are used extensively throughout dry land farming regions of the world in these regions; they are normally an integral component of cropping rotations (Walsh *et al*., 2001).

The most important germination problem of the seeds of forage legume crops that are used in over seeding, artificial rangeland and lay farming is that these plants have very hard seed coats. *Medicago* species may have hard seededness at a rate of 100% according to eco-types (Walsh *et al*., 2001; Uzun and Aydin, 2004).

Acid treatments are often used to break down especially thick impermeable seed coats. Since seeds placed in concentrated sulfuric acid (H_2SO_4) will become charcoal in time, the temperature of the acid and the length of time the seeds are soaked are very important. Also two chemicals that have proven very helpful in breaking certain types of dormancy are gibberellic acid (GA_3) and potassium nitrate. Cold stratification, or prechilling, simulates cold winter conditions for seeds with internal dormancy. The embryo of many seeds fails to germinate because oxygen does not diffuse through the seed coat. At cold temperatures, more oxygen is soluble in water, so the oxygen requirements of the embryo are better satisfied (Young and Young, 1992).

There is a lack of research on breaking dormancy of hard seededness in forage legume crops. El-Refaey and El-Dengawy (2005) reported that moist-chilling at 5°C for 1 week followed by soaking in 250 ppm GA_3 solution for 20 h significantly increased germination percentage (85%) in *Eriobotrya japonica*. Tilki (2004) shown that stratification of seeds at 4°C or treatment of seeds with 300, 600 or 900 ppm GA_3 was successfully overcome dormancy in *Arbutus unedo* seeds and Treatment of seeds with KNO_3 did not increase germination. Rehman (2000) found that the application of 100, 200 and 300 ppm gibberellic acid increased the seed germination of *Koelreateria paniculata* to 17, 18 and 15, respectively, compared with the control treatment. Prechilling of wet seeds for 60 days increased the germination rate to 44% and combination of both treatments increased the germination rates after 30 days to 60, 51 and 54%, respectively. Macchia *et al*. (2001) found that the prechilling treatment at 5°C for 7-15 days is effective on

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the rate of *Echinacea angustifolia* seed germination and significantly increases the rate of germination but GA_3 does not increase germination. Optimum temperatures for the dormancy breaking effect of chilling are generally close to 5°C (Bewley and Black, 1994). The result of Jolliff *et al.* (1994) showed that prechilling enhanced the initial germination rate of all *Limnanthes* sp. Genotypes, but had little effect on the final number of seeds germinated. These studies have been shown that chilling results in an increase in responsively to gibberellins (Hilhorst and Karssen, 1992; Derkn *et al.*, 1994). High germination percentages were obtained using concentrated sulfuric acid, followed by either a 90-min soaking procedure in a 100 ppm gibberellin (GA_{4+7}) solution, or adding 0.2% potassium nitrate to the test substrate (Sozzi and Chiesa, 1995).

Understanding alfalfa germination and growth is important to matching management decisions to alfalfa development. Thus this research is essential for increasing forage production. Therefore, the objectives of this study were to determine the effect of gibberellic acid, prechilling, sulfuric acid and potassium nitrate on seed germination and investigate effective and practical methods for breaking seed dormancy of annual medics species.

MATERIALS AND METHODS

Three experiments were conducted to evaluate seed germination of annual medics including *Medicago radiata, Medicago polymorpha* and *Medicago rigidula* at Crop Physiology laboratory of Tarbiat Modarres University, Tehran, Iran in 2006 year. The experimental design in all experiments was a factorial with treatments organized following a completely randomized design, with three replications.

Experiment 1: Factorial experiment laid out in completely randomized block design conducted in this research. Homogenous seeds of annual medics including *Medicago radiata* and *M. polymorpha* were selected. Seeds were sterilized by Mankuzeb fungicide and different gibberellic acid concentrations including 0, 250, 500 and 750 ppm were prepared. Then one hundred seeds of two annual medic cultivars were treated with each of GA_3 prepared concentration in petri dishes with filter papers in three replications. The petri dishes were placed in five similar germinators at 0, 2, 4, 6 and 20°C for one week. During the experiment the petri dishes were monitored and in case of moisture deficiency distilled water was used. After one week all petri dishes were placed in a germinator at 20°C and every 24 h the number of germinated seeds was counted.

Experiment 2: The homogenous seeds of two annual medic cultivars of *Medicago rigidula* and *M. polymorpha* were put each one in 12 petri dishes (100 seeds in each petri dishes). Three petri dishes were considered for each cultivar as control. Then potassium nitrate solutions were prepared in 0.2, 0.3 and 0.4% and 7 milliliter of each solution was added to petri dishes containing seeds. Also distilled water was applied for control petri dishes. At final, all petri dishes were placed in a germinator at 20°C and every 24 h the number of germinated seeds was counted and in case of moisture deficiency distilled water was used.

Experiment 3: The homogenous seeds of two annual medic cultivars of *Medicago rigidula* and *M. polymorpha* were selected. After preparation of sulfuric acid with 19.2, 38.4, 57.6, 76.8 and 96% concentrations, one hundred seed of each cultivar treated with each one of preparation concentrations during 5 and 10 min in three replicates. Then seeds were washed with distilled water and sterilized by Mankuzeb fungicide and each one put in one sterilized petri dishes with filter papers. Distilled water was added to each petri dish. At final, all petri dishes were placed in a germinator at 20°C and every 24 h the number of germinated seeds was counted and in case of moisture deficiency distilled water was used.

The germination percentages data were subjected to analysis of variance using the SAS statistical software package (SAS Institute Inc., 1988). When analysis of variance showed significant treatment effects, Duncan's multiple range tests was applied to compare the means at $p<0.05$ (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

There were significant differences on germination percentage affected by cultivar, temperature, gibberellic acid and the interactions between cultivar and gibberellic acid, between cultivar and temperature and between gibberellic acid and temperature ($p<0.01$) and among cultivar, gibberellic acid and temperature ($p<0.05$) (Table 1).

Table 1: Variance analysis of seed germination percent for two annual medic cultivars under gibberellic acid and prechilling treatments

SOV	df	Mean square
Cultivar	1	15526.87**
Concentration	3	703.41 **
Temperature	4	643.92**
Cultivar × Concentration	3	23.52 **
Cultivar × Temperature	4	66.00**
Concentration × Temperature	12	82.36**
Cultivar × Concentration × Temperature	12	10.95*
Experimental error	80	5.23ns

* Significant at 5%, ** Significant at 1% and ns = not significant

Table 2: Mean values (Duncan's Test, p<0.05) of seed germination percent for two annual medic cultivars under gibberellic acid concentration and prechilling treatments in first experiment

Cultivar	Temperature (°C)	Gibberellic acid concentration (ppm)	Germination (%)	Cultivars	Temperature (°C)	Gibberellic acid concentration (ppm)	Germination (%)
M. polymorpha	4	750	55a	*M. polymorpha*	0	0	23.33jk
M. polymorpha	6	750	51.67a	*M. radiata*	2	750	20.67kl
M. polymorpha	2	750	46.67b	*M. radiata*	6	750	20.67kl
M. polymorpha	0	750	45bc	*M. radiata*	0	750	19.67kl
M. polymorpha	0	500	43.33bcd	*M. radiata*	0	500	18.33lm
M. polymorpha	6	500	41.67cd	*M. radiata*	6	500	17.33lmn
M. polymorpha	4	0	40de	*M. radiata*	4	500	16.67lmno
M. polymorpha	2	500	40de	*M. radiata*	2	500	16.67lmno
M. polymorpha	4	500	40de	*M. radiata*	4	250	16.67lmno
M. polymorpha	2	250	40de	*M. radiata*	6	250	15.33mnop
M. polymorpha	4	250	40de	*M. radiata*	0	250	15.0mnop
M. polymorpha	6	0	40de	*M. radiata*	2	250	15.0mnop
M. polymorpha	2	0	36.67ef	*M. radiata*	2	0	14.0mnopq
M. polymorpha	0	250	35f	*M. radiata*	4	0	13.33nopq
M. polymorpha	6	250	33.33fg	*M. radiata*	6	0	12.33opqr
M. radiata	4	750	30gh	*M. radiata*	20	750	11.33pqrs
M. polymorpha	20	500	28.33hi	*M. radiata*	20	500	10.67qrs
M. polymorpha	20	750	28.33hi	*M. radiata*	20	250	8.67rs
M. polymorpha	20	0	25ij	*M. radiata*	20	0	7.67s
M. polymorpha	20	250	25ij	*M. radiata*	0	0	3.33t

Different letter(s) indicate significant difference between the values in the same column (Duncan's multiple comparison test, p<0.05)

Prechilling at 4°C and application of 750 ppm gibberellic acid in compared with other temperatures and gibberellic acid concentrations was the most effective on making the hard coat penetrable and also on seed germination rate of two annual medic cultivars. This temperature increased annual medic seed germination from 23.54% in control to 31.67% (Table 2). Also *M. polymorpha* cultivar in compared with *M. radiata* cultivar in reaction to prechilling treatment showed higher rates of germination that is because of less seed coat hardness or because of its bigger size and higher seed surface of this cultivar (Table 2). Moreover, prechilling at 4°C was the most effective on seed germination. Highest rate of germination was observed for *M. polymorpha* treated with 750 ppm gibberellic acid and 4°C prechilling. The most germination percentage (55%) was belonged to *M. polymorpha* cultivar that was treated with 750 ppm GA_3 at 4°C (Table 2). The least germination percent was observed in both treated varieties with no GA_3 and at 0°C.

These results supported by Macchia *et al.* (2001) whom in their research found that the prechilling treatment at 5°C for 7-15 days was effective on the rate of *Echinacea angustifolia* seed germination and significantly increased the rate of germination but GA_3 did not increase germination. Bewley and Black (1994) also reported that 5°C was generally an optimum temperature for the dormancy breaking effect of chilling. The above result was similar to other consequence that demonstrated prechilling and GA_3 were the paramount factors for seed dormancy breaking and seed germination increasing (Hilhorst and Karssen, 1992; Bewley and Black, 1994; Iglesias and Babiano, 1997; Bello *et al.*, 1998; Rehman, 2000; Macchia *et al.*, 2001). Jolliff *et al.* (1994) showed that prechilling enhanced the initial germination rate of all *Limnanthes* spp. Genotypes, but had little effect on the final number of seeds germinated. These studies have been shown that chilling results in an increase in responsively to gibberellins (Hilhorst and Karssen, 1992; Derkn *et al.*, 1994). At low temperature more oxygen dissolves in water and therefore more oxygen is prepared for embryo (Young and Young, 1992). Prechilling was resulted to in more response of seed germination to GA_3 (Hilhorst and Karssen, 1992; Derkn *et al.*, 1994). The lack of GA_3 effectiveness in stimulating seed germination might be referred to the following possibilities: a negative effect of GA_3 on the level of some enzymes activity (glutamate-oxaloacetate transaminase, pyruvate kinase and malate dehydrogenase) and consumption of nucleotides in the synthesis of nucleic acid (El-Dengawy, 1997) and/or the production of a proteinaceous germination inhibitor. On the other hand, the negative effect of GA_3 application after excess prechilling period on seed germination might be attributed to the inhibitory effect of GA_3 on the synthesis of some of the nucleosides and nucleotides (El-Dengawy, 1997). Further, GA_3 is effective in breaking the non-deep physiological dormancy, but it does not overcome the deep physiological dormancy (Baskin and Baskin, 1990). We concluded that the combination between a suitable prechilling period and an effective level of GA_3 would considerably enhance seed germination. Tilki (2004) shown that stratification of seeds at 4°C or treatment of seeds with 300, 600 or 900 ppm GA_3 was successfully overcome dormancy in *Arbutus unedo* seeds. This implies that treatment of seeds with GA_3 may substitute for cold stratification as was reported for *Arbutus andrachne*

(Karam and Al-Salem, 2001). Imbibition stimulates GA secretion from embryo. Secreted GA increases synthesis of hydrolytic enzymes located under aleuron layer. Synthesized enzymes are transported to endosperm via scutulum and are used for decomposing of stored food to supply the energy required for germination (Cirak *et al*., 2004). In the present study, GA_3 increased germination rate significantly depending on used doses.

Potassium nitrate concentration, cultivar and interaction effect among them had significant effect on germination percentage (Table 3). Seed germination of *M. rigidula* was 5.83% more than *M. polymorpha* under potassium nitrate treatment. The highest germination percent belonged to *M. rigidula* under 0.4% potassium nitrate treatment and had 23% more germination rate than control (Table 4). *M. polymorpha* had highest seed germination percent (26.67%) under 0.2% potassium nitrate treatment and had 15% more germination than control (Table 4). The lowest seed germination was belonged to two cultivars that treated with distillation water and considered as control, but sulfuric acid had the more effect on seed germination in these cultivars than potassium nitrate. Nitrate promoted germination of dormant seeds of the both cultivars but did not substitute for a stratification requirement. These results supported by Tajbakhsh (1996) and Brandel (2005) that reported seed germination could be enhanced by potassium nitrate solute on plant seeds that had seed dormancy. The presence of nitrate can be judged as a signal indicating the absence of competitors as nitrate can accumulate in the soil due to an absence of vegetation. This might be important for seed regeneration: because the absence of competition for light and nutrients can increase the chance of seedlings' survival (Brandel, 2005). Hilhorst (1998) describes a model of physiological process controlling germination in which nitrate is required for germination. Therefore, a higher amount of available nitrate would promote germination. In a study whose main objective was to evaluate seed treatments for reducing or eliminating the light requirement of seeds, KNO_3 was reported as an effective agent for reducing light requirement and enhancing germination (Pupala and Fowler, 2003). Potassium nitrate may be helpful for reactivation of metabolic process of seeds. This compound may cause biosynthesis of auxin, which ultimately triggers the growth of the embryo (Khan *et al*., 1999). The general low response to nitrate may indicate that most of these species are unresponsive to these treatments or that the concentrations tested were not optimal which was confirmed by the present study.

Sulfuric acid concentration, cultivar, treatment application period and interactions among them (sulfuric acid concentration and its application period, sulfuric acid concentration, its application period and cultivar) had high significant effect on germination percent (Table 5). Sulfuric acid with 96% concentrations had the most effect on hard coat penetrable and seed germination percent in two cultivars. Sulfuric acid with 96% concentration increased seed germination percent from 11.67% in control to 71.67% in treated seeds. *M. rigidula* had more seed germination percentage than *M. polymorpha* under different sulfuric acid concentration levels. It is probably that *M. rigidula* seeds have less hard coat than *M. polymorpha*. Treated seeds with sulfuric acid in 10 min had more germination (8.84%) than treated seeds with sulfuric acid in 5 min. It is possible that sulfuric acid application for 5 min cannot enough penetrate in hard coat of treated seeds (Table 6). Sulfuric acid application in 10 minutes in compare with 5 min increased seed germination in *M. rigidula* more than *M. polymorpha*. The highest seed germination percent in two cultivars was observed with sulfuric acid via 96% concentrations for 10 min (Table 6). Interaction among cultivar, sulfuric acid application period and its concentration levels were not significant. Acid scarification on some seeds as a

Table 3: Variance analysis of seed germination percent for two annual medic cultivars under potassium nitrate treatment

SOV	df	Mean square
Concentration	3	227.78**
Cultivar	1	204.17**
Cultivar × Concentration	3	170.83**
Experimental error	16	14.58ns

* Significant at 5%, ** Significant at 1% and ns = not significant

Table 4: Mean values (Duncan's Test, $p<0.05$) of seed germination percent for two annual medic cultivars under potassium nitrate treatment in second experiment

Cultivar	Potassium nitrate concentration (%)	Germination (%)
M. rigidula	0.4	35.00a
M. polymorpha	0.2	26.67b
M. rigidula	0.3	25.00b
M. rigidula	0.2	25.00b
M. polymorpha	0.3	23.33b
M. polymorpha	0.4	13.33c
M. rigidula	0	13.33c
M. polymorpha	0	11.67c

Different letters indicate significant difference between the values in the column (Duncan's multiple comparison test, p<0:05)

Table 5: Variance analysis of seed germination percent of two annual medic cultivars under sulfuric acid treatment

SOV	df	Mean square
Cultivar	1	220.42**
Concentration	4	3426.67 **
Time	1	1170.42**
Cultivar × Concentration	4	49.58 **
Cultivar × Time	1	20.42ns
Concentration × Time	4	49.58**
Cultivar × Concentration × Time	4	280.83**
Experimental error	40	5.23ns

* Significant at 5%, ** Significant at 1% and ns = not significant

Table 6: Mean values (Duncan's Test, p<0.05) of seed germination percent for two annual medic cultivars under sulfuric acid concentration and its application period in third experiment

Cultivar	Treatment application period (min)	Sulfuric acid concentration (%)	Germination (%)
M. rigidula	10	96.0	71.67a
M. polymorpha	10	96.0	68.33ab
M. rigidula	10	76.8	63.33b
M. polymorpha	10	76.8	53.33c
M. polymorpha	5	96.0	50.0c
M. polymorpha	5	76.8	50.0c
M. polymorpha	5	57.6	43.33d
M. rigidula	5	76.8	40.0de
M. rigidula	5	57.6	36.67ef
M. rigidula	10	57.6	36.67ef
M. polymorpha	10	57.6	36.67ef
M. rigidula	5	96.0	33.33f
M. polymorpha	10	19.2	31.67f
M. polymorpha	10	38.4	25.0g
M. rigidula	5	38.4	23.33gh
M. polymorpha	5	19.2	21.67ghi
M. rigidula	5	19.2	18.33hij
M. rigidula	10	19.2	16.67ijk
M. rigidula	10	38.4	13.33jk
M. polymorpha	5	38.4	11.67k

Different letter(s) indicate significant difference between the values in the column (Duncan's multiple comparison test, p<0:05)

pretreatment increased germination percent effectively (Sozzi and Chiesa, 1995). Acid scarification (H_2SO_4) increased germination and this has been reported for many other species with impermeable seeds. Treating *Sesbania rostrata* seeds with concentrated H_2SO_4 for 30 min increased germination from 12 to 94% (Sarker *et al*., 2000). This same result has been shown in this study. The positive responses of seeds to the pre-sowing scarification treatments (sulphuric acid) suggest that the hard testa is responsible for the low percentage germination of untreated seeds by preventing imbibition of water. Prevention of germination by hard testa of *Calligonum* species has different ecological advantages (Tao *et al*., 2000). This feature favours the accumulation of persistent seed banks in the soil, spreads germination over time, suffers the extremely environmental condition and increases the chance that some seeds will germinate, survive and establish (Morgan, 1998; Wang *et al*., 1998). Overcoming dormancy, softening of the testa and water uptake are therefore, crucial matters in the life cycle of hard seeded species (Yang *et al*., 1999).

The result of the present study showed that annual medics had low rate of germination due to hard seededness. Therefore, it seems that dormancy has to broken before seeding of these legumes in lay farming systems or rangeland improvement programmes.

In conclusions, all methods broke seed dormancy and exhibited seed germination of annual medics. But some cases were different in cultivars. The best method for seed dormancy breaking in *M. polymorpha* and *rigidula* was 96% sulfuric acid application for 10 min. This method is very simple, quick and cheap. The concentration of 750 ppm gibberellic acid with temperature of 4°C was the best method for breaking seed dormancy in *M. radiata*. The results showed that sulfuric acid application for 10 min and concentration of 750 ppm gibberellic acid with temperature of 4°C were the most effective method in breaking the dormancy of forage legumes used in this study. Effect of dormancy breaking method on germination rate depends on the plant species, thus it can be suggested that the most effective method in breaking the dormancy of forage legumes should be determined by the application of each method with different period, concentration or degree combinations to the seeds in order to obtain maximum germination rate. Supplementary studies, investigating the effective temperatures for dormancy breaking in seeds is needed to confirm these results and is required to develop seeding criteria for good seed presowing practice to ensure germination in the field. Further investigation is required into the soil environmental condition that may influence the germination of these medics' cultivars.

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