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EFFECTIVE METHODS FOR IMPROVING SEED GERMINATION OF *MEDICAGO SCUTELLATA* AND *MEDICAGO RIGIDULA*

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ABSTRACT

Four experiments were conducted to evaluate seed germination of annual medics including *Medicago scutellata, Medicago rigidula,* under different gibberellic acid, sulfuric acid concentration, potassium nitrate and polyethylene glycol osmotic potential treatments. The experimental design in all experiments was factorial with treatments organized as a completely randomised block design, with four replications. Pods were handpicked and half of them were hand separated from the pods and stored in paper bags at room temperature (25 °C) until germination tests were performed. The result showed that all methods broke seed dormancy and exhibited seed germination of two annual medics species, but in some cases the effect was different according to species. The most effective and practical method for seed dormancy breaking in *Medicago rigidula* was 98% sulphuric acid application for 2 min. The concentration of 600 ppm gibberellic acid was the most effective and practical method in breaking hard seed dormancy of medics. Potassium nitrate was also effective in *M. rigidula* at moderate concentration. Polyethylene glycol (PEG) had significant effect on germination percentage only in *M. rigidula* the highest germination percent, 10.8% higher than the control, being recorded under 0.7 bar Polyethylene glycol treatment.

Key words: seed germination, *Medicago scutellata* and *Medicago rigidula*.

INTRODUCTION

When a mature viable seed is placed under favourable conditions and fails to germinate, it is said to be dormant. Seed dormancy is referred to as embryo dormancy or internal dormancy and is caused by endogenous characteristics of the embryo that prevent germination (Black et al., 1987). Embryo dormancy should not be confused with seed coat dormancy, external dormancy, or hardseededness, which is caused by the presence of a hard seed covering or seed coat that prevents water and oxygen from reaching and activating the embryo. It is a physical barrier to germination, not a true form of dormancy (Quinliven and Nichol, 1971).

Seeds may contain chemicals inhibitors that often retard embryo growth to the point where it is not strong enough to break through the seed coat or other tissues. Physiological dormancy is indicated when an increase in germination rate occurs after an application of gibberellic acid (GA3) or after dry after-ripening or dry storage. Physiological dormancy is broken when inhibiting chemicals are broken down or are no longer produced by the seed; often by a period of cool moist conditions, normally below (+4ºC), or in the case of many species in *Ranunculaceae* and a few others, (-5ºC). Abscisic acid is usually the growth inhibitor in seeds and its production can be affected by light. Some plants like Peony species have multiple types of physiological dormancy, one affects radicle (root) growth while the other affects plumule (shoot) growth. Seeds with physiological dormancy most often do not germinate even after the seed coat or other structures that interfere with embryo growth are removed (Bewley et al., 1994).

The three early phases of seed germination are: (1) imbibition, (2) lag phase, and (3) protrusion of radicle through the testa (Simon, 1984). Priming is a procedure that partially hydrates seed, followed by drying of seed, so that germination processes begin, but radicle emergence does not occur. These priming treatments which enhance seed germination include hydropriming and

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osmopriming and hormonal priming (Afzal et al., 2002; Afzal and Brandel, 2004). Control of seeds water content by osmotic solutions or other methods can be used to prevent seeds from initiating radicle emergence and becoming susceptible to injury. Primed seeds can be dehydrated, stored and when rehydrated, they will germinate more rapidly and completely than these untreated seeds, particularly under stress conditions. Data from several studies indicated that seed priming shortens the time to germinate without lowering the potential that promote the radicle growth, that is, the primed seeds germinated faster but were not able to germinate at water potential significantly lower than that required for control seeds.

The objective of the present study was to find more efficient ways to break seed dormancy and improving germination of two annual medics including *Medicago scutellata* and *Medicago rigidula.*

MATERIAL AND METHODS

Four experiments were conducted to evaluate seed germination of two annual medics species (*Medicago scutellata* and *Medicago rigidula*) at biotechnology laboratory of Agriculture University, Shiraz, Iran, in 2010. The experimental design in all experiments was factorial with treatments organized as a completely randomised block design, with four replications. Pods were handpicked and half of them were hand separated from the pods and stored in paper bags at room temperature (25°C) until germination tests were performed.

Experiment 1:

This research was conducted as factorial experiment carried out in completely randomised block. Homogenous seeds of two annual medics species (*Medicago scutellata* and *Medicago rigidula*) were selected. Different gibberellic acid concentrations including 0, 200, 400 and 600 ppm were prepared. Then seeds of two annual medic species were treated with each of GA prepared concentration in Petri dishes with filter paper in four replications. The Petri dishes were placed in incubator at 20-25°C for one week. During the experiment the Petri dishes were monitored and in case of moisture deficiency distilled water was added. Every 24 h the number of germinated seeds was counted.

Experiment 2:

The homogenous seeds of two annual medic species of *Medicago scutellata* and *Medicago rigidula* were selected. After preparation of sulphuric acid with 50, 80 and 98% concentration, in each Petri dishes twenty seeds treated with each one of preparation concentration during 2 and 5 min. in four replicates.

Then seeds were washed with distilled water 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 an incubator at 25^{o}C and every 24 h the number of germinated seeds was counted. In case of moisture deficiency, distilled water was added.

Experiment 3:

The homogenous seeds of two annual medic species of *Medicago scutellata* and *Medicago rigidula* were put each one in 30 Petri dishes (25 seeds in each Petri dish). Six Petri dishes were considered for each cultivar as control. Then potassium nitrate solutions were prepared in 0.1, 0.3, 0.5, 0.7, 0.9 bar and 5 millilitre of each solution was added to Petri dishes containing seeds. Also distilled water was applied for control Petri dishes. Then, all Petri dishes were placed in an incubator at 25^{o}C and every 24 h the number of germinated seeds was counted. In case of moisture deficiency, distilled water was added.

Experiment 4:

Homogenous seeds of two annual medics species including *Medicago scutellata* and *Medicago rigidula* were selected. Six Petri dishes were considered for each cultivar as control. Polyethylene glycol (PEG 4000) solutions were prepared in 0.1, 0.3, 0.5, 0.7, 0.9 bar and 5 millilitre of each solution was added to Petri dishes containing seeds. Distilled water was applied for control Petri dishes. Then, all Petri dishes were placed in an incubator at 25^{o}C and every 24 h the

number of germinated seeds was counted. In case of moisture deficiency, distilled water was added.

At the end of the experiment the germinated seeds and mean germination time (MGT) were calculated, the last one according to the following formula (Scott et al., 1984):

$$MGT(days) = \frac{\sum Ti\ Ni}{\sum Ni}$$

where: Ti is the number of days after beginning of experiment, Ni - the number of seeds germinated on day i, ΣNi - the total number of seeds germinated. The statistical method used in the present experiment was one-way analysis of variance performed by using MSTATC. Differences between mean values were evaluated for significance by Duncan's Multiple Range Tests at p≤ 0.01.

RESULTS

There were significant differences on germination percentage and germination rate affected by gibberellic acid. 600 ppm gibberellic acid was the most effective in making the hard coat penetrable and improving seed germination rate of both *M. scutellata* and *M. rigidula*, as compared with other gibberellic acid concentrations. This hormone increased germination percentage of two annual medics from 55% in control to 87% and 73% respectively. The lowest germination percentage was observed in control (Table 1).

Sulphuric acid was also effective in reducing hardseededness, but only at the highest concentration (98%) and the shortest time (2 min.) in seeds of *M. rigidula* (Table 2). Sulphuric acid with 98% concentration increased seed germination percent from 54% in control to 81.65% in treated seeds of *M. rigidula*, but decreased seed germination percentage in *M. scutellata* under different sulphuric acid concentration levels. Seeds apparently damaged were observed at the two lowest concentrations. Germination rate was not affected by sulphuric acid concentration (Table 2).

Table 1. Mean germination percentage, germination rate (MGT), seeds with pods and seeds without pods in *M. scutellata* and *M. rigidula* under gibberellic acid concentration

Treatments	Germination mean (%)	MGT (days)	Seeds with Pods (%)	Seeds without pods (%)
M. scutellata				
200 ppm	66 b	7 b	62 b	63 b
400 ppm	70 b	7 b	65 b	67 b
600 ppm	86. 5 a	5 b	82 a	84 a
Control	5 c	10 a	51 c	53 c
M. rigidula				
200 ppm	59 c	10 a	55 c	57 c
400 ppm	62 b	10 a	59 b	61 b
600 ppm	73 a	10 a	70 a	72 a
Control	55 c	10 a	51 c	53 c

Means at each column followed by similar letters are not significantly different at p≤ 0.01.

Table 2. Mean germination percentage, germination rate (MGT), seeds with pods and seeds without pods in *M. scutellata* and, *M. rigidula* under different sulphuric acid concentrations

Treatments	Germination mean (%)	MGT (days)	Seeds with pods (%)	Seeds without pods (%)
M. scutellata				
50% ×5 min	23 d	10 a	18 d	20 d
80% × 2 min	37 c	10 a	30 c	34 c
98% × 2 min	46 b	10 a	41 b	43 b
Control	55 a	10 a	51 a	53 a
M. rigidula				
50% × 5 min	64 c	10 a	60 c	63 c
80% × 2 min	73 b	10 a	69 b	72 b
98% × 2 min	81.65 a	10 a	76 a	80 a
Control	54 d	10 a	49 d	51 d

Means at each column followed by similar letters are not significantly different at p≤ 0.01.

Potassium nitrate had significant effect on germination percentage (Table 3). The highest germination percent was recorded in *M. rigidula* under 0.5 bar potassium nitrate treatment, with 27% higher germination than

the control, while in *M. scutellata* the highest germination, with 9% higher than the control was recorded under 0.1 bar nitrate treatment. The lowest seed germination was recorded in both species in the control, treated with distilled water. Germination rate was also improved by nitrate treatment (Table 3).

Table 3. Mean germination percentage, germination rate (MGT), seeds with pods and seeds without pods in *M. scutellata* and *M. rigidula* under potassium nitrate osmotic potential

Treatments	Germination mean (%)	MGT (days)	Seeds with pods (%)	Seeds without pods (%)
M. scutellata				
0.1 bar	79 a	7 b	73 a	75 a
0.3 bar	77 a	7 b	71 a	74 a
0.5 bar	75 a	7 b	72 a	73 a
0.7 bar	74 a	7 b	70 a	74 a
0.9 bar	71 a	7 b	68 a	70 a
Control	70a	10 a	65 a	68 a
M. rigidula				
0.1 bar	80 b	5 b	75 b	79 b
0.3 bar	83 a	5 b	78 a	82 a
0.5 bar	87 a	5 b	82 a	85 a
0.7 bar	78 c	5 b	71 c	76 c
0.9 bar	73 c	5 b	67 c	72 c
Control	60 d	10 a	53 d	58 d

Means at each column followed by similar letters are not significantly different at $p \leq 0.01$.

Table 4. Mean germination percentage, germination rate (MGT), seeds with pod and seeds without pods in *M. scutellata* and *M. rigidula* under polyethylene glycol osmotic potential

Treatments	Germination mean (%)	MGT (days)	Seeds with pods (%)	Seeds without pods (%)
M. scutellata				
0.1 bar	73 a	5 b	71 a	72 a
0.3 bar	72 a	5 b	67 a	71 a
0.5 bar	78 a	5 b	73.5 a	74 a
0.7 bar	76 a	5 b	70.67 a	74 a
0.9 bar	77 a	5 b	69.54 a	74 a
Control	70 a	10 a	68.45 a	70 a
M. rigidula				
0.1 bar	60 c	5 b	58.56 c	59 c
0.3 bar	63 b	5 b	60.76 b	61 b
0.5 bar	66 b	5 b	61.98 b	63 b
0.7 bar	70 a	5 b	69.5 a	70 a
0.9 bar	69 a	5 b	67 a	68.78 a
Control	60 c	10 a	53 c	58 c

Means at each column followed by similar letters are not significantly different at $p \leq 0.01$.

Polyethylene glycol (PEG 4000) had significant effect on germination percentage only in *M. rigidula* the highest germination percent, 10.8% higher than the control, being recorded under 0.7 bar Polyethylene glycol treatment (Table 4). The 8% percent improvement of germination percentage over the control observed in *M. scutellata* under 0.5 bar Polyethylene glycol treatment was not significant. The lowest seed germination was recorded in the dishes treated with distilled water, considered as control (Table 4).

DISCUSSION

Highest rate of germination was observed for *M. scutellata* treated with 600 ppm gibberellic acid. The least germination percent was observed in control with no GA and only distilled water. These results are supported by Macchia et al. (2001), who found that GA significantly increased the rate of germination in *Echinacea angustifolia*. Bewley and Black (1994) also reported that GA was generally optimum for dormancy breaking of *Chrysarztemum* spp.

The lack of GA effectiveness in stimulating seed germination, reported by some researchers might be due to: a negative effect of GA on the level of some enzymes activity and consumption of nucleotides in the synthesis of nucleic acid or the production of proteinaceous germination inhibitors. Further, it was reported that GA is effective in breaking non deep dormancy (Baskin and Baskin, 1990).

Sulphuric acid had significant effect on *M. rigidula* when used with 98% concentration during 2 minutes. But *M. scutellata* had lower germination percentage with different concentrations of sulphuric acid, because the seed coat of these species is thin and the embryo was injured by the sulphuric acid (Windauer et al., 2007). Potassium nitrate had significant effect on germination percentage of *M. rigidula*, seed germination in -0.5 bar osmotic potential being 27% more than in distilled water. These results are supported by Tajbakhsh (1996) reported that seed germination could be enhanced by potassium nitrate solutions in plants that had seed dormancy. 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.

Polyethylene glycol (PEG) also had significant effect on germination percentage of *M. rigidula*, promoting germination of dormant seeds at relatively low osmotic potentials. Drought stress induced by low concentration of PEG was reported to have had positive effect on germination percentage of dormant seeds, because it reduced synthesis of some amino acids that inhibit germination (Omidi et al., 2005), However at high concentration of PEG the rate of protein synthesis and transportation of material become low and inhibiting germination (Donuvan and Doud, 1987).

CONCLUSIONS

Gibberellic acid, sulfuric acid, potassium nitrate and polyethylene glycol were useful for breaking dormancy of two species. According this study gibberellic acid was very effective in two annual medics species. High concentration of GA increased germination rate, seedling length and seedling dry weight of seeds with hardseedness to some extend. In practice however, this method is not useful because of high cost. Sulphuric acid was effective in M. rigidula at high concentration with shortest time. However with this treatment the embryo may be injured. Potassium nitrate was also effective in *M. rigidula* at moderate concentration. This method was the best method in field because the low price of potassium nitrate.

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