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Effects of New Strategies for Breaking Dormancy of Two Annual Medics (*Medicago scutellata* and *Medicago polymorpha*)

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Abstract: *Medicago scutellata* and *Medicago polymorpha* are two species of annual medics. These are two annual forage legumes very interesting for grazing of live stocks. The germination abilities of these annual legume species were tested under three dormancy breaking treatments: mechanical treatment was performed by hand scarification with sandpaper. For physical treatment used hot water, precooling and surface run off. Chemical treatment by sodium hypochlorite was used for breaking the dormancy. The complete and sharp germination achieved by mechanical scarification with sandpaper that determined dormancy originating by seed coat (hardseededness). Soaking in hot water was very useful physical method, but *M. scutellata* seed coat was thicker than to remove by this method. Low temperature by keeping in refrigerator was also effective in final germination and germination rate in seeds of *M. scutellata* and *M. polymorpha*. After 10 days freezing the germination rate increasing by 90%. Keeping seeds on surface run off also has applied result. After 7days germination rate in seed of *M. scutellata* become 90%. Chemical scarification with sodium hypochlorite was not well to breaking hardseededness.

Key words: *Medicago scutellata* · *Medicago polymorpha* · Dormancy and germination

INTRODUCTION

Many seeds fail to germinate after processing and placement in favorable growing conditions such seeds are said to be dormant [1]. Seed dormancy and germination are complex adaptive traits of higher plants that are influenced by a large number of genes and environmental factors. In general, there are two types of seed dormancy: seed coat dormancy and internal dormancy. Seeds with seed coat dormancy usually have a seed coat that is impermeable to oxygen and/or water. The seed coat is impermeable to water in seeds with a hard seed coat. Heat and scarification release these seeds from dormancy by breaking the impermeable seed coat, allowing imbibitions and germination to proceed. *Medicago* species are the most valuable crop of the rangeland and are known collectively as medics. In addition to the N fixation and forage quality attributes common to legumes, many annual legumes have characteristics that make them especially well adapted to arid environments [2]. Annual *Medicago* species have a world-wide distribution and have been used successfully for grazing in Mediterranean-type environments [3]. According to Ewing [4], annual legumes have been successfully used in managed pasture systems in low-rainfall areas with a Mediterranean-type climate. Annual legumes can be used in a number of ways such as pasture improvement, erosion control or soil restoration programs. These plants, as in medics, have hard seed coats and low germination rate, when subjected to inadequate environmental conditions [5]. The dormancy of dormant seed must be broken to induce germination. Various methods are used for this, depending on the plant species and type of dormancy [6]. As a starting generation, seed is structurally and physiologically equipped as a dispersal unit and providing food for growing seedlings until it establishes itself as an autotrophic organism [7]. To do this the mature seed should germinate first. This physiological reaction begins with water uptake by the dry seeds and terminates with the initial elongation of the embryonic axis. Germination tests give some information about seed constituents [8]. Nevertheless, in many cases desired germination rates could not be attained due to the dormancy requirement that is one of the major obstacles

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especially for most legume forage species. Therefore, the objective of the present study was to find more efficient ways to break seed dormancy and hardseededness of important genetic resources of natural pastures.

MATERIALS AND METHODS

Medicago scutellata and *Medicago polymorpha* were investigated and collected from the natural pasture of Iran. The climate is a true Mediterranean-type climate, wet winters and warm to hot dry summers. 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. A hundred seeds that are well shaped and vigorous in appearance from each species were selected for the germination test. The effects of three different methods on the germination rates of selected species of annual *Medicago* were tested. Mechanical treatment was performed by a bottle which of inner surface covered with sandpaper. Thus the seed coat was mechanically scarified. Physical treatments were performed through soaking the seeds in hot distilled water at 80 and 90 and 100 °C for 20, 15, 5 and 2min. In a second treatment, precooled at -5 °C in refrigerator for 4,7,10 days, then germinated in distilled water. In a third Physical treatment, seeds put in pockets of cloth and then pick them in surface run off with EC 250 ìmhos and SAR 10 then germinated in petri dishes.

The chemical treatment seeds were fluting in 30 ml of 2%, 5%or 10% concentrated sodium hypo-chloride solution for 2, 5 or 10 min. After these pre-treatments,seeds were placed onto autoclaved petri dishes including watman paper wetted with sterilized distilled water before incubating in a growth chamber adjusted to 25 with fluorescent light. [9]. Water was replenished as needed. Germinated seeds were recorded 12 days after the incubation period the seeds with emerged from radical and cotyledon were scored as germinated. At the end of the experiment the germinated seeds and mean germination time (MGT) were calculated, the last according to the following formula [10]:

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

Where Ti is the number of days after beginning of experiment, Ni the number of seeds germinated on day i, ÓNi the total number of seeds germinated. A control application is also designed with non-pretreated seeds. The statistical method used in the present experiment was one-way analysis of variance performed by using MSTATC. One petri dish with 25 seeds was a replication Differences between mean values were evaluated for significance by Duncan's Multiple Range Tests at p = 0.01. [11].

RESULTS

The application of hand scarification with sandpaper significantly improved germination in two species (Table 1). Seeds germinated rate significantly became high after hand mechanical scarification (MGT 5 day in three species without pods) (Figure1). The seeds were not died. Soaking in hot water partially effective on seed coat dormancy in M. polymorpha but not in *M. scutellata* (Table 2). In the M. polymorpha, the thermal increase from 80 to 100°C in hot water from 15 to 2 min, significantly improved germination from 30% to 65 %.the percentage of hard seeds declined from 80% to 20% and seeds that injured was obtained. The germination speed improved as well as germination percentage, the MGT ranging from 10 to 7 days. As temperature increased, the germination percentage declined but the seeds showed injured. Low temperature by keeping in refrigerator for 4,7,10 days has effective on final germination of *M. scutellata* and *M. polymorpha* (Table 3). However, germination was not as high as for the hand-scarification treatment (Figure 3). Keeping seeds on surface run off (Figure 4) also has a great result. After 7days germination rate in seed of *M. scutellata* become 90% (Table 4).

Sodium hypochlorite was not effective in reducing hardseededness in two species (Table 5). Seeds apparently damaged were observed at the two highest concentrations (Table 5).The germination speed did not improve with the chemical treatment in sodium hypochlorite. The greatest percentage of seeds was damaged in 10% treatment. MGT was not affected by sodium hypochlorite concentration (Figure5).

Keeping seeds on surface run off has a great result. after 7days germination rate in seed of *M. scutellata* become 90%. Also M.polymorpha germination rate become 80%. Seeds germinated rapidly after hand mechanical scarification with sandpaper (Figure 6). The treatment in hot water and sodium hypochlorite did not succeed with seeds of *M. scutellata*, whatever the high temperature poorly germinated as well as the control, because the seeds was injured (Figure 6).

Germination was high for the hand-scarification with sandpaper. Therefore this technique is very suitable way for breaking seed coat dormancy. With keeping seeds on surface run off also has high germination rate. This technique also is useful for large seeds as *M. scutellata* (Figure 6).

Table 1: Mean Germination percentage, germination rate (MGT), seeds with pods, seeds without pods in *M. scutellata* and *M. polymorpha,* under different mechanical treatments

	Traits			
Treatments	Germination mean(%)	MGT (days)	Seeds with pods%	Seeds without pods %
M. scutellata				
Sandpaper	64.2a	5b	30.8a	97.6a
Control	40.8b	7a	30.8a	50.8b
M. polymorpha				
Sandpaper	72.0a	5b	65.2a	78.9a
Control	54.4	10a	43.2a	65.6b

Values of germination percentage followed by different letters significantly differ at p<0.01

Table 2: Mean Germination percentage, germination rate (MGT), seeds with pods, seeds without pods in *M. scutellata* and *M. polymorpha,* under different physical treatments

	Traits			
Treatments	Germination mean (%)	MGT (days)	Seeds with pods%	Seeds without pods %
Hot water				
M. scutellata				
80 °C×15 min	12.0c	10	4.5c	8.5c
90° C× 5 min	12.1c	10	4.6c	8.5c
100° C× 2 min	13.1b	10	4.4b	8.7b
Control	22.6a	10	6.9a	15.7a
M. polymorpha				
80 °C×15 min	56.3a	7	51.7a	60.9a
90° C× 5 min	65.9a	7	59.0a	72.8a
100° C× 2 min	44.7b	7	40.5b	48.9b
Control	28.4c	10	26.7c	30.1c

Values of germination percentage followed by different letters, when present, significantly differ at p<0.01.

Table 3: Mean Germination percentage, germination rate (MGT), seeds with pods, seeds without pods in *M. scutellata* and *M. polymorpha,* under different Precooling treatments

	Traits			
Treatments	Germination mean (%)	MGT (days)	Seeds with pods%	Seeds without pods %
M. scutellata	precooling			
4 days	39.9b	7	38.6b	41.2b
7 days	35.3b	5	32.4b	38.2b
10 days	48.3a	5	42.9a	53.7a
Control	30.9c	7	27.4c	34.4c
M. polymorpha				
4 days	57.6b	7	51.6b	63.6a
7 days	58.3b	5	51.8b	64.8a
10 days	61.9a	5	58.4a	65.4a
Control	54.4c	10	43.2c	65.6a

Values of germination percentage followed by different letters, when present, significantly differ at p<0.01.

Table 4: Mean Germination percentage, germination rate (MGT),seeds with pods, seeds without pods in *M. scutellata* and *M. polymorpha,* on surface run off treatments

	Traits			
Treatments	Germination mean (%)	MGT (days)	Seeds with pods%	Seeds without pods %
Surface run off				
M. scutellata	90.1a	7	89.1a	91.1a
Control	56.4b	10	54.1b	58.7b
M. polymorpha	76.4a	7	74.3a	78.5a
Control	54.4b	10	49.7b	59.1b

Values of germination percentage followed by different letters, when present, significantly differ at p<0.01

Table 5: Mean Germination percentage, germination rate (MGT), seeds with pods, seeds without pods in *M. scutellata* and *M. polymorpha*, in sodium hypochlorite treatments

	Traits			
Treatments	Germination mean (%)	MGT (days)	Seeds with pods %	Seeds without pods %
M. scutellata	Sodium hypochlorite			
10%×2 min	10.9c	10	9.4c	12.4c
5%× 5 min	20.7c	10	19.1c	22.3c
2 %× 2 min	30.5b	10	27.7b	33.3b
Control	56.4a	10	54.1a	58.7a
M. polymorpha				
10%×2 min	15.3c	10	11.1c	19.5c
5%× 5 min	24.5c	10	22.2c	26.8c
2%× 2 min	32.4b	10	29.2b	35.6b
Control	54.4a	10	49.7a	59.1a

Values of germination percentage followed by different letters, when present, significantly differ at p<0.01

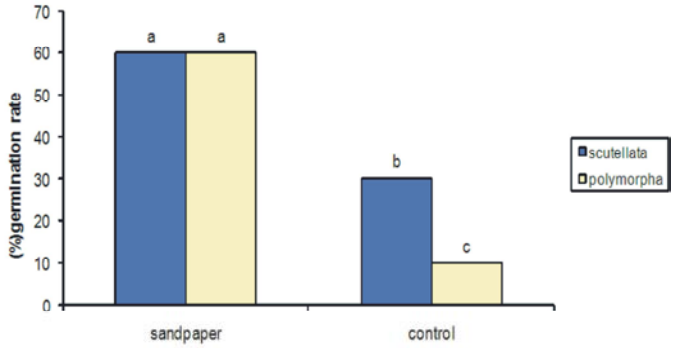

Fig. 1: (%) germination rates of *Medicago* species under scarification with sandpaper
Values of germination rate percentage followed by different letters, when present, significantly differ at p<0.01.

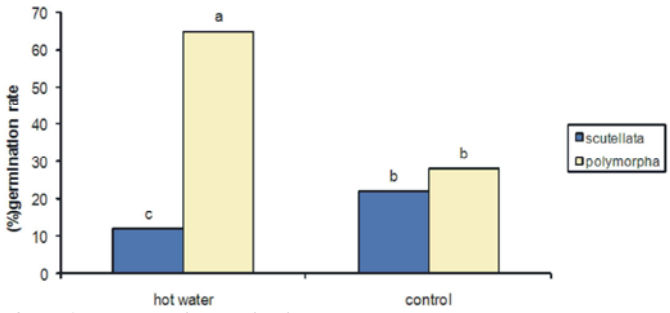

Fig. 2: (%) germination rates of *Medicago* species under hot water
Values of germination rate percentage followed by different letters, when present, significantly differ at p<0.01.

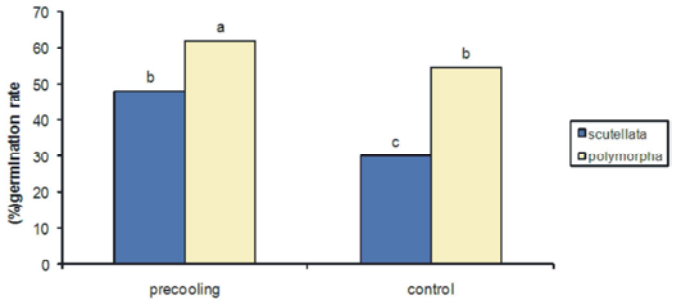

Fig. 3: (%) germination rates of *Medicago* species under precooling
Values of germination rate percentage followed by different letters, when present, significantly differ at p<0.01.

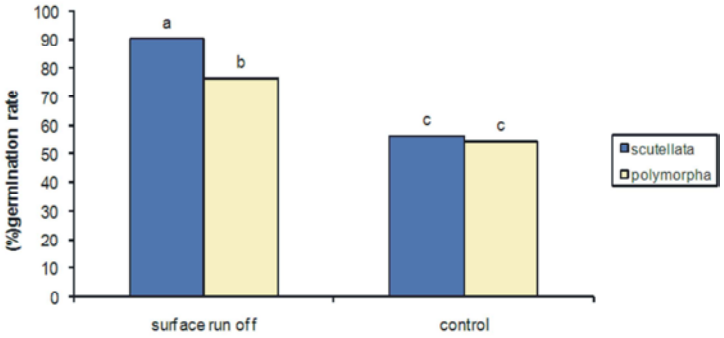

Fig. 4: (%) germination rates of *Medicago* species under surface run off
Values of germination rate percentage followed by different letters, when present, significantly differ at p<0.01.

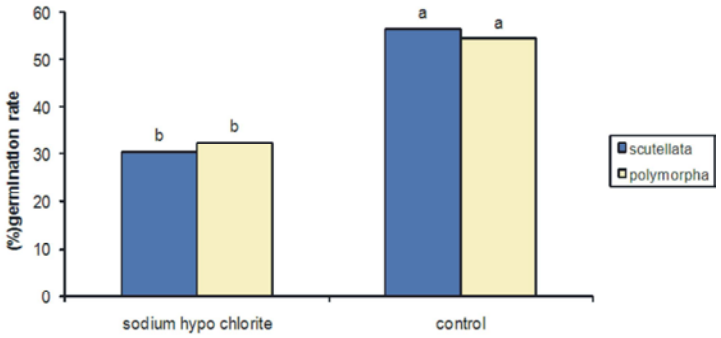

Fig. 5: (%) germination rates of *Medicago* species under sodium hypo chlorite
Values of germination rate percentage followed by different letters, when present, significantly differ at p<0.01.

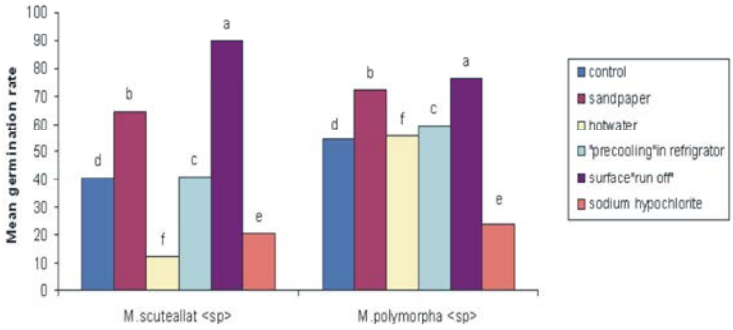

Fig. 6: Mean germination rates of *Medicago* species under different treatment.
Values of germination rate percentage followed by different letters, when present, significantly differ at p<0.01.

DISCUSSION

In some species of leguminosae that produce indehiscent fruits, the seed coat is well developed, in which case the seed coat mechanically protect the embryo. The main seed coat is the endocarp [12]. Removal or mechanical scarification of the endocarp promotes rapid germination of the seed. However, according to Rizzini [12], the endocarp is impermeable to water and dormancy is caused by it. These results suggest that a survey is needed to determine whether the endocarp of drupeproducing taxa of leguminosae is impermeable to water [13]. In *Medicago scutellata* and *Medicago polymorpha*, the seed coat is very thick and has a water impermeable layer (hardseededness). This layer is believed to be responsible for impermeability to water [14]. Hand scarification with sandpaper was more benefit to improve germination; a rapid and almost full germination was obtained (Table 1) similar results were reported in *Panicum maximum* by Previero ogha [15] and

in tropical grass *Cenchrus* by Geetha and Van staden [16]. Germination was improved when the seeds were soaked in hot water.

Hot water soaking might have leached out some of the water soluble inhibitors present in the glumes resulting in germination improvement of seeds. The results were inconformity with Geetha and Van staden [16] in *Cenchrus* sp. Further improvement of germination through sodium hypochlorite was tried but again it was not a complete success. Seeds scarified for 2 min recorded a moderate increase in germination with high vigor index (Table 2). The results were in conformity with Usberti *et al*. [17] in many plant species, viz, *Brachiaria brizantha, B. decombuns* and *Panicum antidotale*. The reason may be removal or disruption of lemma and palea, which improves the permeability of seed coat and allows water into the seed for partial destruction or removal of specific germination inhibitor present in freshly harvested seed, thereby improving the germination percentage. Though scarification of seed with commercial sodium hypochlorite improves germination to some extent, the failure of germination of scarified seed may be due to presence of some other type of dormancy in addition to physical dormancy. The seeds treated by precooling improved germination (Table 3), but again it was not a complete success. Cooling is well documented as a compound, which increases the germination of photo-dormant seeds. According to Bewley and Black [18], cooling raises the ambient oxygen levels by making less oxygen available for citric acid cycle. Again a combination of treatments by surface run off with EC 250 ìmhos and SAR 10 improved germination,especially in *scutellata.* So very useful to obtained rapid germination in field and that was very applied way for Medics. The success of combined seed treatment (hand scarification and surface run off) showed that the seed possessed physical and none deep physiological dormancy.

CONCLUSION

The integument breaking or softening, for instance, is needed to remove dormancy imposed by seed coat hardness or impermeability. However, it is very difficult to use mechanical scarification to break the hard seed coat of *Medicago*; therefore, chemical scarification with concentrated NaOCl was used to remove exogenous dormancy. Our results indicated that seed coat dormancy is clearly present in third species of *Medicago* tested, since removal of the seed coat resulted in complete germination or partial germination. Thus the extent to which the seed coat restricted germination in intact seeds differed between the three species. There was no evidence that the seed coat contained chemical inhibitors that were broken down by heat. There was no evidence that any dormancy resided in the embryo. Seeds germinated rapidly after hand scarification with sandpaper and surface run off. This shows that seed coat is believed to be responsible for impermeability to water and oxygen.

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