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BREAKING SEED DORMANCY OF SOME ANNUAL *MEDICAGO* AND *TRIFOLIUM* SPECIES BY DIFFERENT TREATMENTS

Ersin CAN[1*], Nafiz ÇELIKTAŞ[1], Rüştü HATIPOĞLU[2], Süleyman AVCI[1]

[1]) Mustafa Kemal University, Department of Field Crops, 31034, Antakya, Hatay
[2)]Çukurova University, Department of Field Crops, 01330, Adana

*Corresponding author: ersincan69@gmail.com

ABSTRACT

The germination abilities of 14 annual legume species (six *Medicago* and eight *Trifolium)* were tested under three dormancy breaking treatments: In the first treatment, mechanical scarification plus sodium hypochlorite (MSS); in the second, chemical scarification with sulphuric acid (CSA); in the third, incubation in water bath at 90 °C (IWB) were used for breaking the dormancy. All *Medicago* species except for *Medicago polymorpha* showed improvement in germination rates using MSS. The seeds of *M. polymorpha* showed higher germination rates with CSA or IWB than MSS. For *Trifolium* species, results indicated that there was a great variability among species with regard to the treatments. Seeds of species showed a broad range of dormancy requirements for germination. The CSA treatment significantly increased the germination rates of *Trifolium lappaceum*, *T. scabrum* and *T. strictum*. On the other hand, the germination rates of *T. spumosum, T. spadieceum* and *T. angustifolium* were significantly improved by MSS. The seeds of *T. badium* showed higher germination rates with IWB than the other applications.

Key Words: Annual *Medicago, Trifolium,* seed germination, hardseededness

INTRODUCTION

Annual legumes may offer advantages in revegetation of arid rangelands where winter growth is possible. 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 (Brahim and Smith, 1993). Annual *Medicago* species have a world-wide distribution and have been used successfully for grazing in Mediterranean-type environments (Rumbaugh and Johnson, 1986). Rumbaugh and Johnson (1986) also mentioned that self-seeding annuals might be better adapted to arid rangelands than perennial legumes. Therefore, it should be better and relatively inexpensive to introduce the annuals in a combination with annual or perennial grasses and legumes. According to Ewing (1999), annual legumes have been successfully used in managed pasture systems in southern Australia in low- rainfall areas with a Mediterranean- type climate. Annual legumes can be used in a number of ways such as in rehabilitation, pasture improvement, erosion control or soil restoration programs. However, to achieve these processes there is a need of information on eco-physiological responses related to growth and development to such as seed scarification

method and growing media on germination of the related species (Vilela and Ravetta, 2001).

Annual clovers are accepted as very valuable plants in many countries, especially in mediterranean regions. However, these plants, as in medics, have hard seed coats and low germination rate, when subjected to inadequate environmental conditions (Aydin and Uzun, 2001). Therefore, seed dormancy is usually an undesirable characteristic in *Medicago* and *Trifolium* species. The dormancy of dormant seed must be broken to induce germination. Various methods are used for this, depending on on the plant species and type of dormancy (Koyuncu, 2005).

The success of rehabilitation with the annuals depends on the seed production ability and thereby producing enough amount of stand establishment in subsequent growing seasons. 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 (Bewley, 1997). 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 (Malo, 2000). Nevertheless, in many cases desired germination rates could not be attained due to the dormancy requirement that is one of the major obstacles 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 of Eastern Mediterranean region.

MATERIALS AND METHODS

The species investigated were collected from the natural pastures of Serinyol district (36.32º N and 36.20º E at 106 m. altitude) of Antakya at the east Mediterranean costal region of Turkey. The climate is a true Mediterranean-type climate with mild, wet winters and warm to hot dry summers. Annual *Medicago* species *Medicago rigidula*, *Medicago polymorpha*, *Medicago rotata*, *Medicago orbicularis*, *Medicago turbinata*, *Medicago scutellata* and *Trifolium* species *Trifolium spumosum*, *Trifolium cherleri*, *Trifolium spadiceum*, *Trifolium lappaceum*, *Trifolium scabrum*, *Trifolium angustifolium*, *Trifolium strictum*, *Trifolium badium* were selected for this study and identified at flowering stage. Pods were handpicked and the seeds were manually harvested after maturation.

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* and *Trifolium* were tested. In the first method, the seeds were shaked for a while in a bottle which of inner surface covered with sandpaper. Thus, the seed coat was mechanically scarified. Treated seeds by this way were immersed for 8 minutes in a sodium hypochlorite solution, 40 % available chlorine with two drops of Tween-80 per 100 ml solution (MSS). Secondly, unpre-treated seeds were soaked in a H_2SO_4 solution (95-97 % v/v) for 5 minutes (CSA). In the third method, non-pretreated seeds were incubated in a water bath adjusted to 90 °C for 3 minutes (IWB). After these pre-treatments, seeds were placed onto autoclaved petri dishes (100x20 mm) including watman paper wetted with sterilized distilled water before incubating in a growth chamber adjusted to 25 ± 1 °C with white fluorescent light, irradiance of 2000 lx with 16 h photoperiod. A control application is also designed

with non-pretreated seeds. The statistical method used in the present experiment was one way ANOVA with four replications. One petri dish with 25 seeds was a replication. Germinated seeds were recorded 14 days after the incubation period. The seeds with emerged from radicula and cotyledon were scored as germinated. The germination percentages were subjected to Arcsine √x transformation and analysed by ANOVA using LSD multiple-comparison test. The Software was MSTATC.

RESULTS AND DISCUSSION

Germination rates belonging to different *Medicago* species are given in Table 1. The applicationof MSS significantly improved the germination rates of seeds from all *Medicago* species except one species. The seeds of *M. polymorpha* under CSA or IWB treatments showed higher germination rates than MSS (Figure1.). The treatments of chemical scarification with H_2SO_4 or incubation in Water bath at 90 °C significantly improved the germination rate of *M. rigudula* but the germination rates with these treatments were significantly lower than that with the treatment of MSS. The treatments of chemical scarification with H_2SO_4 or incubation in Water bath at 90 °C did not improve germination of seeds from *M. rotata*, *M.orbicularis*, *M.turbinata* and *M.scutellata*.

Table 1. Mean germination rates of *Medicago* species under different treatments.

Species	Treatments			
	Control	MSS	CSA	IWB
Medicago rigidula	5.0 c*	72.5 a	22.5 b	27.5 b
Medicago polymorpha.	15 b	10.0 b	47.5 a	37.5 a
Medicago rotata	12.5 b	100 a	15.0 b	17.5 b
Medicago orbicularis	15.0 b	100 a	32.5 b	17.5 b
Medicago turbinata	10.0 b	57.5 a	27.5 ab	15.0 b
Medicago scutellata.	2.5 b	92.5 a	5.0 b	7.5 b

*Means of germination rates with different letters in the same line are significantly different at the P≤ 0.05 level as analysed by LSD test.
MSS: Mechanical scarification+sodium hypochlorite
CSA: Chemical scarification with H_2SO_4
IWB: Incubation in water bath at 90 °C

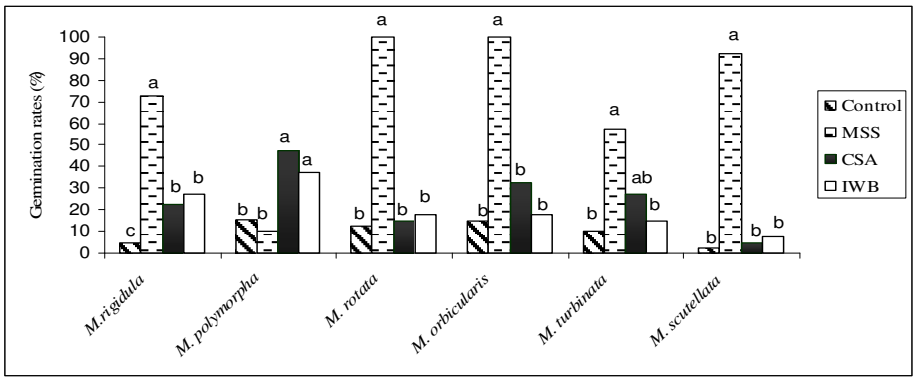

Figure1. Mean germination rates of *Medicago* species under different treatments.

It can be concluded that for all *medicago* species except for *M. polymorpha*, MSS application stimulates seed germination and has a larger effect than the CSA and IWB treatments applied in this experiment.

The effects of the treatments on the germination rates of *Trifolium* species were differed among the species (Table 2). MSS treatment significantly increased the germination rates of seeds from *Trifolium spumosum*, *T. spadiceum* and *T. angustifolium*. On the other hand, the germination rates of *T. lappaceum* , *T. scabrum* and *T. strictum* were significantly improved by the CSA application (Figure 2.). The seeds of *T. badium* incubated in water bath at 90 °C showed higher germination rate than those of the same species treated with other applications. For *T. strictum*, MSS and IWB treatments resulted in significantly decrease of germination rate. Finally, none of the treatments in this study could be improve germination rate of the seeds *Trifolium cherleri*.

Table 2. Mean germination rates of *Trifolium* species under different treatments.

	Treatments			
Species	Control	MSS	CSA	IWB
Trifolium spumosum	5.0 b*	80.0 a	0.0 b	0.0 b
Trifolium cherleri	0.0	2.5	7.5	5.0
Trifolium spadiceum	7.5 b	42.5 a	2.5 b	0.0 b
Trifolium lappaceum	0.0 b	0.0 b	90.0 a	0.0 b
Trifolium scabrum	2.0 b	0.0 b	12.5 a	0.0 b
Trifolium angustifolium	27.5 a	25.0 ab	2.5 b	12.5 ab
Trifolium strictum.	17.5 a	0.0 b	10.0 a	0.0 b
Trifolium badium	0.0 b	0.0 b	0.0 b	10.0 a

* Means of germination rates with different letters in the same line are significantly different at the P≤ 0.05 level as analysed by LSD test.
MSS: Mechanical scarification+sodium hypochlorite
CSA: Chemical scarification with H_2SO_4
IWB: Incubation in water bath at 90 °C

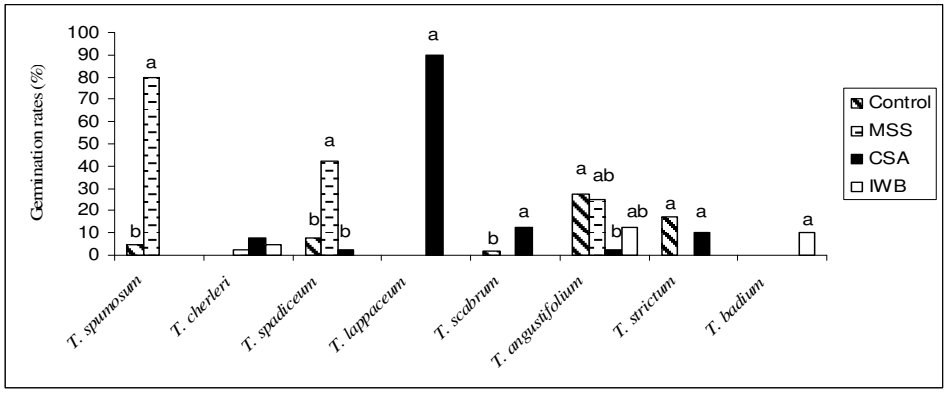

Figure 2. Mean germination rates of *Trifolium* species under different treatments.

In our experiment, MSS application stimulated the germination of *Medicago* species except for *M. polymorpha*. Similar results were also reported by Uzun and Aydin (2004) for some annual legumes, by Patene` and Gresta (2006) for *Astragalus hamosus* and *Medicago orbicularis,* by Teketay (1998) for *Acacia* species and *Pterolobium stellatum* and by Baes et al., (2002) for *Prosopis ferox.*

For *M. polymorpha* seeds, acid scarification with H2SO4 (95-97% v/v) for 5 minutes and water bath adjusted to 90 °C for 3 minutes resulted in germination rates of 47.5% and 37.5%, respectively. Similar results were reported by Nadjafi et al., (2006) for *Teucrium polium.*

From the results for *Medicago* species, it could be concluded that for all *Medicago* species studied except for *M. polymorpha*, MSS application stimulates seed germination and has a larger effect than the CSA and IWB treatments applied in this experiment.

For *Trifolium* species, results indicated a great variability among species with regard to the treatments. Seeds of each species showed a broad range of breaking seed dormancy requirements for germination.

Germination is a complex physiological process controlled by a large number of genes, which are affected by several environmental factors, such as light, temperature and the duration of seed storage (Koornneef et al., 2002). Davies (1995) mentioned that gibberellin (GA) is an essential phytohormone induces many aspects of plant development, including seed germination. The major active GA increases after seed imbibition just before radicle protrusion (Ogawa et al., 2003). However, seed imbibition and germination are blocked by some of the factors and this result is called as dormancy. Baskin and Baskin (1998) used the term of organic dormancy to describe it and categorized as a regard in seven-groups such as physiological, morphological, morphophysiological, physical, physical plus physiological, chemical and mechanical dormancy.

These classifications are derived from both endogenous (embryonic) and exogenous (structural) characteristics of the seed.

Seed coat has many functions such as regulation of imbibition and hence plays a role on germination. Species that have thick seed coat do not germinate even the ideal conditions for germination existed. This is called physical dormancy due to hard seed coat or hardseededness (De Souza and Filho, 2001). Some of the substances and

germination inhibitors may be found in seed coats at various levels in different species (Woodstock, 1988). One or few genes influenced by the environment, control hardseededness considered as heritable character (Ramsey, 1997). In the other category of dormancy, called embryo dormancy, is an inherited character induced by several genes and affected by both developmental and environmental factors (Bewley and Black 1994; Koornneef et al., 2002). The direct role of abscisic acid (ABA) in dormancy induction is not clear. However, germination was inhibited by the addition of ABA exogenously to the germination medium (Baskin and Baskin, 1998). Manipulating the expression of some genes, seed ABA content could be changed which also resulted in increased dormancy (Koornneef et al., 2002). Incubating mature seeds in ABA solution prevented the embryo radicle extension hence extending the germination (Bewley, 1997).

As a conclusion, sometimes dormancy is required to build up a seed bank in the soil (Harlan, 1998). This is useful in the ley farming system (Moneim, 1992). Weather-resistant pods and hardseededness allow natural reestablishment in succeeding years even if winter rains are insufficient in a season to permit seed production (Crawford et al., 1989). Taylor and Ewing (1992) introduced that the seeds of some species can remain impermeable for 5 years or more. However, dormancy is undesirable when a rapid germination is required. Many methods chemicals, hormones or mechanical, were improved for breaking the seed dormancy. However, the method should be convenient, easy to rich and applicable when it is needed. The methods used in the present study are practical and easily applicable for experimental studies and in the field conditions for most of the wild species.

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LITERATURE CITED

Aydin, I. and F.Uzun. 2001. The effects of some applications on germination rate of gelemen clover seeds gathered from natural vegetation in Samsun. *Pak. J. Bio. Sci*., **4**, 181-183.

Baes, P.O., M.L. De Viana and S. Suhring. 2002. Germination in *Prosopis ferox* seeds: effects of mechanical, chemical and biological scarificators. *J. Arid Environ*., **50**, 185–189.

Baskin, C.C. and J.M. Baskin. 1998. *Seeds: ecology, biogeography and evolution of dormancy and germination.* Academic Press, pp, 66.

Bewley, J.D. and M. Black. 1994. *Seeds: Physiology of development and germination.* Plenum Press New York, pp. 445.

Bewley, J.D. 1997. Seed germination and dormancy. *Plant Cell*, **9**, 1055-1066.

Brahim, K. and S.E. Smith. 1993. Annual medic establishment and the potential for stand persistence in Southern Arizona. *J. Ran. Manage*., **46(1),** 21-25.

Crawford, E.J., A.W.H. Lake and K.G. Boyce. 1989. Breeding annual *Medicago* species for semiarid conditions in Southern Australia. *Adv. Agron*., **42,** 399-437.

Davies, P.J. 1995. *Plant Hormones: Physiology, Biochemistry and Molecular Biology*. (Dordrecht, The Netherlands: Kluwer Academic Publishers).

De Souza, F.H.D. and J.M. Filho. 2001. The seed coat as a modulator of seed-environment relationships in *Fabaceae*. *Rev. Brasil. Bot.. Sao Paul*., **24(4)**, 365-375.

Ewing, MA. 1999. Annual pasture legumes: A vital component stabilizing and rehabilitating low-rainfall Mediterranean ecosystems. *Arid Soil Res. Rehabil*., **13**, 327-342.

Harlan, JR. 1998. *The Living Fields*: Our Agricultural Heritage. pp. 288. Cambridge Univ. Press, Cambridge, UK.

Koornneef, M., L. Bentsink and H. Hilhorst. 2002. Seed dormancy and germination. Curr. Opin. *Plant Biol*. **5**, 33-36.

Koyuncu, F. 2005. Breaking seed dormancy in black mulberry (*Morus nigra* L.) by cold stratification and exogenous application of gibberellic acid. *Acta Biol. Crac. Bot*., **47(2**), 23-26.

Malo, J.E. 2000. Hardseededness and the accuracy of seed bank estimates obtained through germination. *W. Ecol*., **1**, 70-75.

Moneim, A.A. 1992. Pasture, forage and livestock program. Annual report. Pp, 17, ICARDA, Aleppo, Syria.

Nadjafi, F., M. Bannayan, L. Tabrizi and M. Rastgoo. 2006. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium. J. Arid Environ*., **64**,542–547.

Ogawa, M., A. Hanada, Y. Yamuchi, A. Kuwahara, Y. Kamiya and S. Yamaguchi. 2003. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell*. **15**, 1591-1604.

Patane`, C. and F. Gresta. 2006. Germination of *Astragalus hamosus* and *Medicago orbicularis* as affected by seed-coat dormancy breaking techniques. *J. Arid Environ*., **67**, 165–173.

Ramsey, G. 1997. Inheritance and linkage of a gene for testa-imposed seed dormancy in faba bean (*Vicia faba* L.). *Plant Breed*., **116**, 287-289.

Rumbaug, M.D. and D.A. Johnson. 1986. Annual medics and related species as reseeding legumes for Northern Utah Pastures. *J. Rang. Manage*., **39(1),** 52-58.

Taylor, G.B.and M.A. Ewing. 1992. Long-term patterns of seed softening in some annual pasture legumes in a low rainfall environment. *Aust.J. Exper. Agric*., **32,** 331-337.

Teketay, D. 1998. Germination of *Acacia origena*, *A. pilispina* and *Pterolobium stellatum* in response to different pre-sowing seed treatments, temperature and light. *J. Arid Environ*., **38**, 551–560.

Uzun, F. and I. Aydin. 2004. Improving germination rate of *Medicago* and *Trifolium s*pecies. *Asian J. Plant. Sci*., **3 (6)**, 714-717.

Vilela, A.E. and D.A. Ravetta. 2001. The effect of seed scarification and soil-media on germination, growth, storage, and survival of seedlings of five species of *Prosopis* L. (*Mimosaceae*). *J. Arid Environ*., **48**, 171–184.

Woodstock, L.W. 1988. Seed Imbibition: a critical period for successful germination. *J. Seed Technol*., **12**, 1-15