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Improving Germination Rate of *Medicago* and *Trifolium* Species

Ferat Uzun and Ibrahim Aydin
Department of Agronomy, Faculty of Agriculture, The University of Ondokuz Mayis,
55139, Kurupelit, Samsun, Turkey

Abstract: The objective of the present study was to investigate effective methods in breaking the seed dormancy for forage legume species. Seeds of legume species were collected from a natural rangeland in Samsun in Turkey. Pre-cooling, pre-heating, hot water, sulfuric acid gibberellic acid and mechanic disruption treatments were used to improve germination rate of forage legume seeds. The results obtained from this study indicated that the seeds of legumes had a hard seededness of 56.7 to 89.0%. Pre-cooling, pre-heating, sulfuric acid and gibberellic acid treatment had a slight effect on the germination rate of the studied species. Hot water treatment increased the germination rate of *M. meneghinianum* seeds from 11.0% in control to 82.8%. Mechanic scarification with sandpaper effectively increased the germination rates of seeds. For, *M. scutellata* and *T. striatum* seeds were 52.3 and 70.8%, respectively. This treatment increased the rate of germination of seeds in other species from 82.3 to 97.0%. The results obtained from this study indicated that the most effective treatment in breaking hard seed dormancy of legume seeds was mechanic disruption.

Key words: *Medicago*, *Trifolium*, seed treatments, dormancy, germination

INTRODUCTION

Medicago and *Trifolium* species are the most valuable crops of the rangelands. These species have the ability of yielding quality forage as well as fixing at least 120 kg N per ha resulting in increased soil fertility[1].

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[2]. Most medic species are winter annuals adapted to Mediterranean climates[3]. Self seeding annual legumes may be better adapted than perennial legumes to arid rangelands. Kebe and Smith indicated that the medics are reasonable rangeland plants even in the arid regions where annual rainfall is about 110 mm[4]. The medics and related species as reseeding legumes are spread over very wide range of areas in the world owing to a great adaptation capability[5-7].

Medic pastures that produce high levels of good quality forage are well suited to grazing and are used extensively throughout dryland farming regions of the world. In these regions, they are normally an integral component of cropping rotations [7,8].

Annual clovers are accepted as very valuable forage 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[9,10].

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. *Trifolium* and *Medicago* species may have hard seededness at a rate of 100% according to eco-types[7,11]. Many legumes produce indehiscent fruit (pods) that break down slowly and prevent moisture from being absorbed by rapidly the seeds[12]. This trait, along with hard seededness mechanism, allows a proportion of a seed population to survive even in extended drought and promotes germination[13]. Quinlivan showed that medics could survive for many years of drought due to the hard seed mechanism[14].

There is a lack of research on breaking dormancy of hard seededness in forage legume crops. Muir and Pitman stated that the most effective methods of breaking dormancy of hard seed of legumes were sulfuric acid and mechanic scarification[15]. Horowitz and Taylorson[16], Mujica and Rumi[17] indicated that one of the most effective methods in breaking hard seededness dormancy in the seeds of medic species was mechanical disruption of the seeds. Cabrales and Bernal[18] proposed a hot water treatment in breaking hard seed mechanism for legumes. Therefore, the objective of the present study was to investigate effective methods of overcoming hard

Corresponding Author: Ferat Uzun, Ondokuz Mayis Üniversitesi, Ziraat Fakültesi, Tarla Bitkileri Bölümü, 55139, Kurupelit, Samsun, Türkiye
Tel: +90 362 4576020 x 1368 Fax: +90 362 4576034 E-mail: fuzun@omu.edu.tr

seededness dormancy for forage legume species and to determine the rate of hard seededness in forage legume species collected from natural rangeland.

MATERIALS AND METHODS

Seeds of forage legumes were handpicked from natural rangeland in the province of Samsun (41°21' N, 36°15' W, elevation 120 m) during July of 2002. Since flowering occurred over an extended period of time, all pods, which were either full or dry, were collected. After 6 months, under laboratory conditions, germination rates of the legumes were determined.

In this study, the seeds of 5 annual clovers (*T. resupinatum, T. subterraneum, T. alexandrinum, T. meneghinianum* and *T. striatum*), 3 medics (*M. hispida, M. arabica* and *M. scutellata*) and 2 perennial *Medicago* (*M. lupulina* and *M. falcata*) species were used.

Seed treatments consisted of the following: (1) mechanical disruption of seed coat by sandpaper (2) immersion in 0.18 mol L^{-1} sulfuric acid for 30 sec., (3) immersion in 75°C water for 5 min, (4) immersion in 25 mg L^{-1} gibberellic acid, (5) pre-heating at 40°C for 30 min, (6) pre-cooling at 3°C for 7 days and (7) an untreated control germinated in distilled water.

The treated seeds were placed in individual, sterilised petri dishes containing moisture-retaining paper liners. Paper liners in the petri dishes were kept moist throughout the germination period. Germination trials were conducted in a controlled environment germinator with 12 h light and dark cycles at a constant temperature of 25°C. Germinated seeds were counted at day 21 after the initiation of the germination trial. Germination was defined as the development of both a functional radical and at least 1 cotyledon. Germination percentages were transformed for statistical analysis (arcsine of square root of percent germination x 0.01) with the original data. The transformed data numbers were analysed according to design of randomised plots. Differences among each application were assessed according to LSD test (Level of significance $p<0.01$).

RESULTS

Germination rate of un-treated seeds of clover and medic species were between 11.0-43.3% as shown in Fig. 1. The highest germination rates in un-treated were found to be 43.3, 30.8 and 28.8% for *T. resupinatum, T. subterraneum* and *T. striatum,* respectively (Fig. 1). But, germination rates of other legumes tested in this study were 20.0% or lower.

Pre-cooling increased germination rates of *M. lupulina* slightly when compared to control. Pre-heating had a negative effect on germination

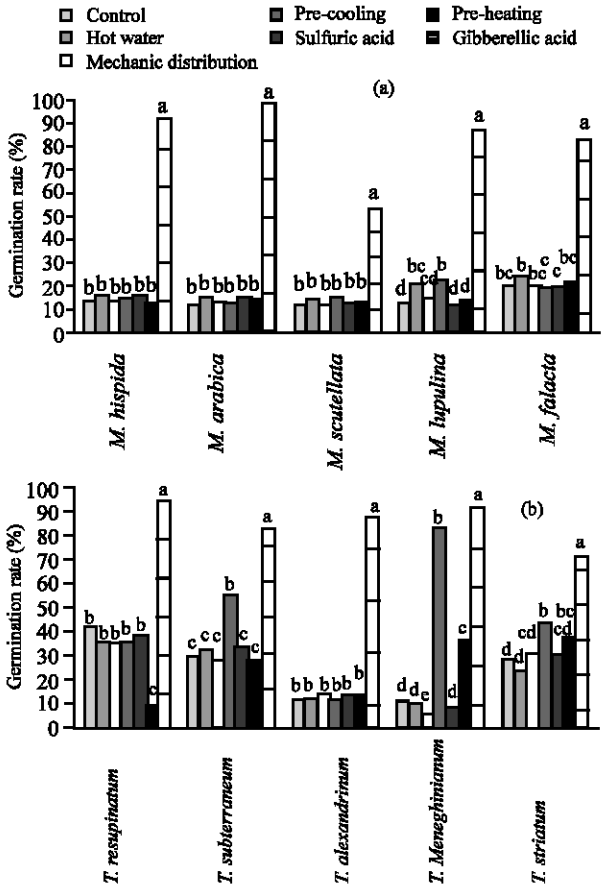

Fig. 1: The germination rate of legumes seeds with different treatments: a) *Medicago* species, b) *Trifolium* species (Values with different letters within columns differ significantly at the level of $p<0.01$)

rate of *T. meneghinianum.* Hot water treatment enhanced germination rate of *T. subterraneum, T. meneghinianum, T. striatum* and *M. lupulina* seeds. The effect of hot water treatment on germination rate of *T. meneghinianum* seeds was very highs such as the germination rate was 11.0% in control, while it increased up to 82.8%.

The effect of gibberellic acid treatment on germination rates of *T. resupinatum* seeds was negative, while it increased the germination rates of *T. meneghinianum* and *T. striatum* seeds. It was determined that sulfuric acid treatments had no effect on germination rates of legumes seeds.

Mechanic disruption was found to be very effective way to eliminate seed dormancy in legumes. But the positive effect was changeable among species. This treatment increased the germination rates of *M. scutellata* seeds to 52.3%, while it was 11.3% in control. Other seeds treated with mechanic disruption germinated higher than

70.8% and the highest germination rate of scarified seeds was obtained from *M. arabica* with 97.0%.

DISCUSSION

The results of the present study showed that self-seeding forage legumes used in this study have a low rate of germination due to hard seededness. Therefore, it seems that dormancy has to be broken before seeding of these legumes in lay farming systems or rangeland improvement programmes.

Cabrales and Bernal[16] Horowitz and Taylorson[18] and Tomer and Maguire[19] suggested the use of sulfuric acid in breaking dormancy of different forage legume seeds. However, it is interesting that none of *Medicago* and *Trifolium* species used in this study has showed increase in germination rate in response to sulfuric acid application to the seeds. The application of treatments, in breaking seeds dormancy had varying effects on germination rates depending on plants species, except disruption with sandpaper. For example, gibberellic acid application decreased significantly germination rate of *T. resupinatum* seeds, while it increased germination rate of *T. meneghinianum* seeds. Additionally, hot water application increased germination rate of *T. meneghinianum* seeds to 82.8% when compared to control group (11.0%) while it did not have a marked effect on the germination rate of some of seeds tested in this study. There are studies suggesting the use of hot water application in effectively breaking seed dormancy in forage legume[20,21].

Disruption with sandpaper increased dramatically, germination rate in seeds of all species (52.3% *M. scutellata*, 70.8% *T. striatum* and over 82.3% in other species). The reason why germination rate was lower in some species following disruption with sandpaper of seeds may be due to inadequacy of the 10 min disruption period with sandpaper. Indeed, Aydin and Uzun showed that germination rate differed between 5 and 10 min disruption with sandpaper period in *T. meneghinianum* (34 and 93%, respectively)[10].

In conclusion, self-seeding legumes have a low germination rate due to their hard seededness and mechanic and chemical treatments of seeds can break dormancy. The results of the present study showed that disruption with sandpaper was the most effective method in breaking the dormancy of forage legumes used in this study. Because the effect of dormancy breaking method on germination rate depends on the plant species, it can be suggested that the most effective method in breaking 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.

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