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Full Length Research Paper

Effect of pretreatments on seed germination rate of red clover (*Trifolium pratense* L.) populations

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The aim of the present study was to investigate effective methods in breaking the seed dormancy for red clover populations collected from Central-Black Sea Region of Turkey. To increase germination percentage of red clover, precooling, preheating, hot water, potassium nitrate and mechanical scarification were used. The results pointed out that there was a significant difference among the populations and treatments regarding germination rates. While average germination vigor of seeds was 29.4% in control treatments, it increased to 54.2% in mechanical scarification. Additionally preheating and precooling treatments were recommended.

Key words: Germination, hardseedness, red clover.

INTRODUCTION

Red clover (*Trifolium pratense* L.) is one of the most cultivated perennial forage legumes in the world (Bowley et al., 1984). Like many common plant species, it has a broad range of adaptation due to the existence of a high number of local adapted genotypes rather than a single ubiquitous genotype (Joshi et al., 2001; Taylor and Smith, 1995). Indeed, Taylor and Smith (1995) showed that most cultivars are not adapted to areas far from where they were developed.

Inter and intra species diversity is mostly seen in the gene centre for plants. Turkey is the cross-point of Mediterranean and close (near) East gene centres, and also has five sub-gene centres (phyto-geographic region) for plants (Demir, 1990). Therefore, Turkey has a rich genetic source and high diversity for a great many genus and species. Moreover, Central-Black Sea Region has a rich inter and intra-genus/species diversity, especially for legumes (Kılınc and Ozkanca, 1991; Kılınc and Karakaya, 1992; Acar et al., 2001). But, this diversity has been damaged by over-grazing in this region and Turkey (Acar et al., 2001). For this reason, collection and conservation of red clover seeds in Black Sea Region is essential for sustainability. Many new cultivars could also be improved by using this germplasm. Seed germination is a critical stage in the life of each crop plant, affecting both the vigor and stand of the crop and the final crop yield (Desai et al., 1997). Seed dormancy is defined as a state in which seeds are not able to germinate under favourable environmental conditions (Copeland and McDonald, 1995). The understanding of the complexities of the mechanism of seed dormancy is of great biological significance. An understanding of the phenomenon will lead to control of seed germination, which is of enormous practical importance (Desai et al., 1997). Two types of dormancy mechanisms are known: (1) coat-imposed dormancy and (2) embryo dormancy (Basu, 1995; Desai et al., 1997). In legume seeds, the testa is the main barrier to the entry of water, and the outermost region of the testa, particularly the waxy cuticle is the major impermeable layer (Desai et al., 1997). *Trifolium* and *Medicago* species may have hardseededness at a rate of 100% according to eco-types (James et al., 1970; Crawford et al., 1989; Aydin and Uzun, 2001; Uzun and Aydin, 2004; Can et al., 2009). Additionally, Colgecen et al. (2008) determined that germination rate of natural red clover seed was 13.5 % due to hard seed coat.

Hard seeds can be softened artificially by different

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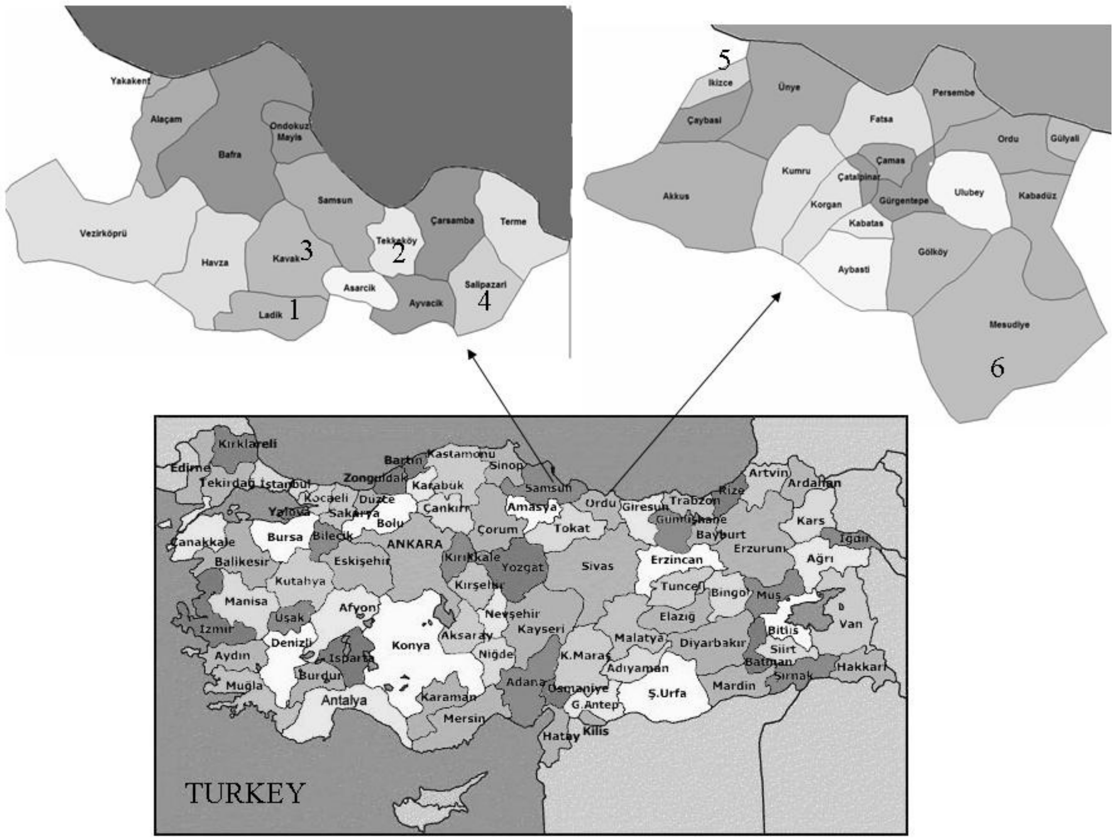

Figure 1. Geographic distribution of naturalized populations of red clover (*T. pratense* L.) in Central-Black Sea Region, Turkey.

treatments. Three common scarification techniques are used to soften or break the seed coat. These are mechanical, chemical and heat scarification (Tarawali et al., 1995). The objective of the present study was to investigate effective methods of overcoming hardseedness dormancy for red clover.

MATERIALS AND METHODS

Six native red clover (*T. pratense* L.) populations obtained from native pastures were used in the study. Red clover seeds were collected in the period of June-September 2008 from Samsun and Ordu Provinces located at Central-Black Sea Region of Turkey (Figure 1). The collected seeds were sown in seed trays and then seedlings were transplanted into field :(70 cm row spacing and 50 cm plant spacing) at the end of the March 2009 in Samsun (41°21´ N, 36°15´ E, 195 m). The experimental area had the typical Mediterranean climatic conditions during the study period. Harvest for seed was performed by hand picking ripe inflorescences when inflorescence colours turned to brown (Manga et al., 2003) in July 2009. The harvested red clover seeds had been stored in polyethylene bags for 9 months at the temperature of 20 ± 2°C. Different methods were taken under test conditions to figure out germination rates of red clover seeds. Mechanical scarification (MS) of seed coat by sandpaper (Aydin and Uzun, 2001), hot water (HW): seeds were soaked in boiling water (1:10 v/v) until water reached room temperature, preheating (PH): Seeds were incubated in water at 60°C for 45 min, precooling (PC) at 4°C for 5 days (Acar et al., 2005), being wetted with 0.01% KNO_3 solution (KNO_3) (Cirak et al., 2004), an untreated control germinated in distilled water (C).

For each test, twenty treated seeds individually were placed in sterilized Petri dishes containing moisture-retaining filter papers at room temperature (20 ± 2°C) for the process of germination. Seeds were considered to have germinated when radicles reached at 5 mm length (Nichols et al., 2009). Germinated seeds were counted on 4 and 10th day of treatments to observe germination speed and germination vigor, respectively (Sehirali, 1997). All tests were performed in triplicate. Germination percentages were transformed (arcsin ($\sqrt{x}/100$)) before analysis (Gulumser et al., 2006) in order to stabilize variances. The transformed data were analyzed according to split plot design with three replicates. Red clover populations were in the main plots, and germination treatments were in the subplots. The means were ranked according to DUNCAN Multiple Range Test. Analysis of variance of data was done by SPSS 10.0 V (SPSS 10.0 V. 1999).

RESULTS

There were significant differences (P≤0.01) among the red clover populations regarding germination speed. As

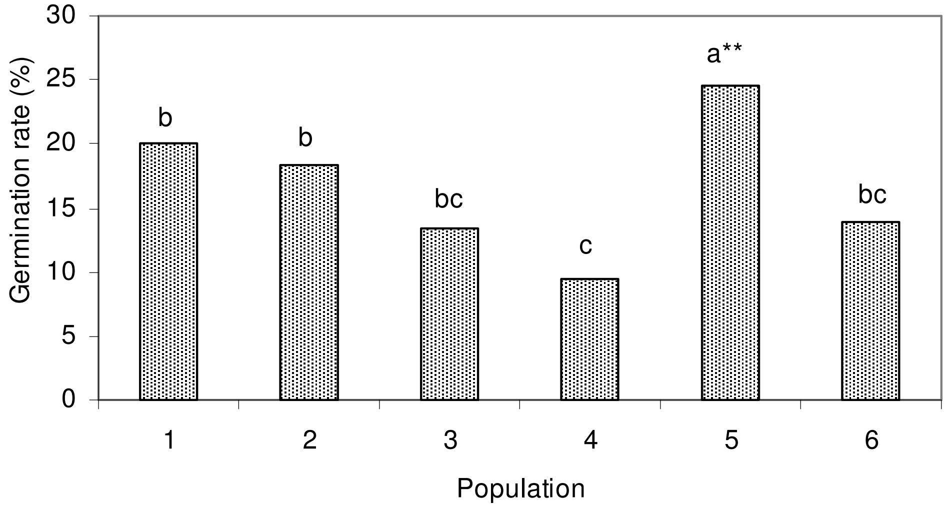

Figure 2. Germination speed of red clover populations seeds.

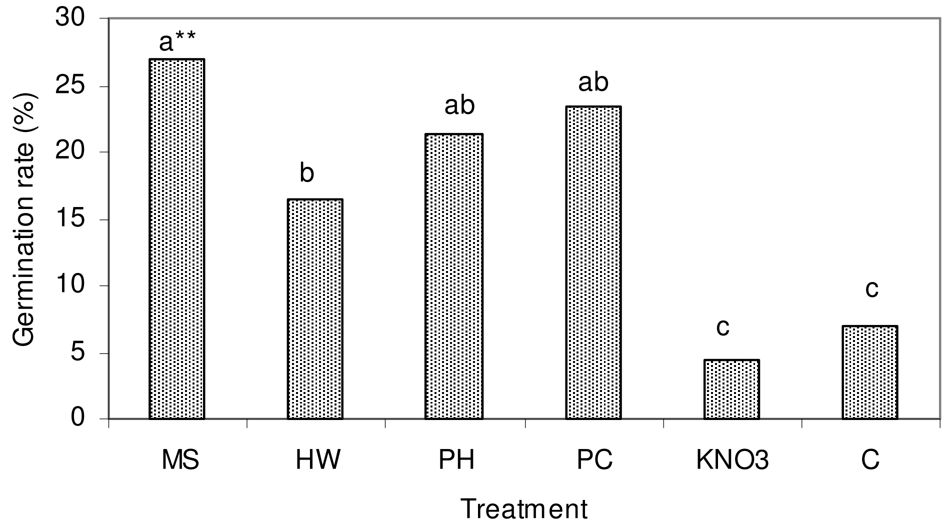

Figure 3. The effects of different treatments on the germination speed of red clover seeds.

mean of the treatments, germination speed of the populations varied from 9.4 to 24.4% (Figure 2). The maximum germination speed was determined in population 5 collected from Ikizce District.

The germination speeds of red clover seeds taken under different treatments were presented in Figure 3. Significant differences ($P \leq 0.01$) were figured out among treatments. Germination speed of red clover's seeds was between 4.4% (KNO_3) and 26.9% (MS). Except KNO_3 treatments, germination speed in seeds was markedly increased by other treatments. While the highest germination rate obtained from MS treatment, PH and PC treatments had the similar effect like MS treatment on germination speed of red clover.

The six red clover populations were significantly differed from each others ($P \leq 0.05$) in term of germination vigor. The highest germination vigor, just like germination speed, was determined at population 5 (39.7%) (Figure 4). There was a statistically significant difference ($P \leq 0.05$) among populations considering germination vigor. It ranged from 21.9 to 54.2%. However, HW and KNO_3 treatments affected negatively germination vigor of red clover seeds, the other treatments increased germination vigor of seeds compared to control (Figure 5). As in the

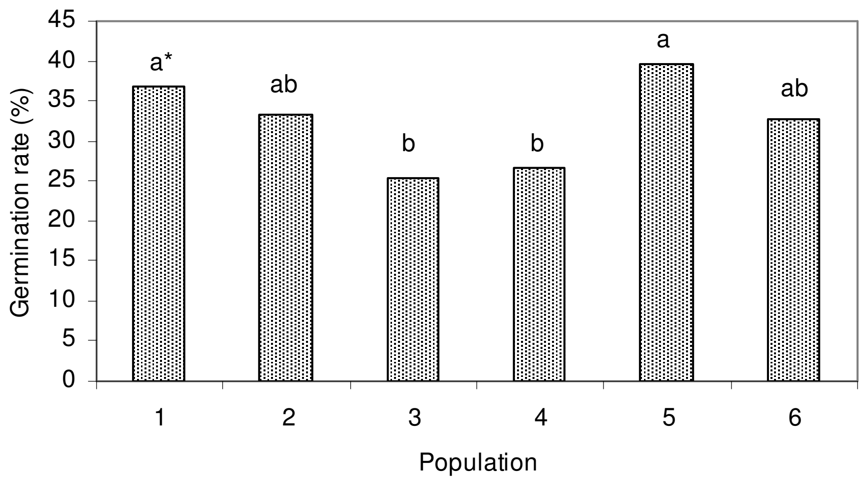

Figure 4. Germination vigor of red clover populations seeds.

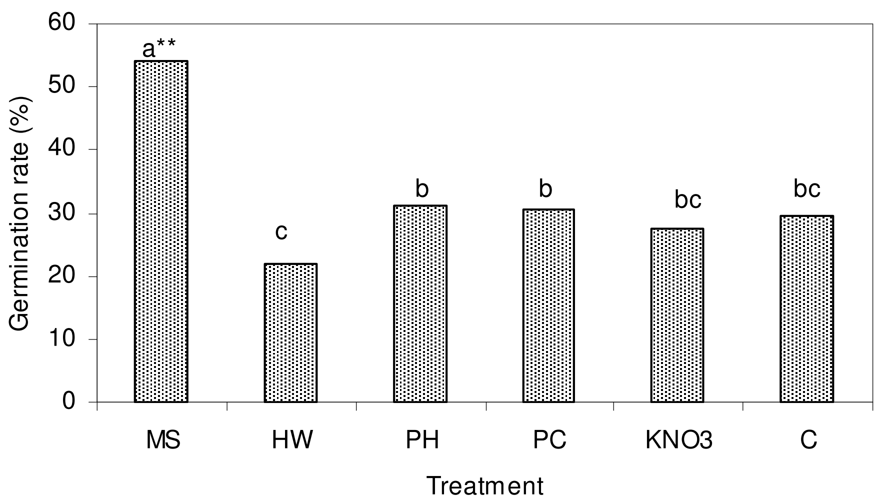

Figure 5. The effects of different treatment on the germination vigor of red clover seeds.

germination speed, MS was the best treatment and resulted in highest germination vigor value (54.2%).

DISCUSSION

Protection of natural habitats, gene pools and meeting the growing demand for plant-based products is now only possible by cultivating natural plant species. For this purpose, it is necessary to ascertain seed germination character (Yucel and Yılmaz, 2009). Results of this study showed that germination speed and vigor were significantly different among red clover populations (Figures 2 and 4). Genetic factors (Tekeli and Ates, 2007) and environmental conditions during plant growth period (Onal, 2002) determine the proportion of hard seeds. Acharya et al. (1999) reported that cultivar had significant effect on hard seed content in alfalfa. It is generally accepted that environmental effects particularly during the period of seed ripening can strongly influence seed quality. The percentage of hard seeds is governed by edaphic and climatic factors during and after seed maturation. The same cultivar grown in different environments may exhibit wide variations in hard seed content. Severe moisture stress increases the hard seed percentage (Simon, 1997).

Rapid and synchronized germination is necessary when there is a need to subject a uniform set of seedlings to a treatment. In addition, rapid germination is advantageous when seed is used in reclamation (Greipson, 2001). Germination speed and vigor of red clover seeds in control treatment were very low due to hardseedness. Colgecen et al. (2008) pointed out similar results in naturally tetraploid red clover seeds. Also they reported that although in the testa of normal imbibed seeds (mostly, followed by germination) has some macrosclereid cells with wide lumens extending to seed surface where they are probably the weak zones facilitating imbibitions, such cells do not present in the hard seed.

It is known that a precooling treatment applied to seeds has an effect on germination. Its effect can change according to plant species (Aydin and Uzun, 2001; Uzun and Aydin, 2004). Precooling increased germination rates of red clover. These results are similar to findings of Balouchi and Modarres, 2006). Precooling 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). Although hot water treatment enhanced germination speed of red clover, similarly Al-Sherif's (2007) results, germination vigor of seeds in this treatment was less than in control treatment. Also Sakhanokho (2009) found out that germination rate of *Hibiscus* sp. seeds decreased with increasing the immersion time of seed in 99°C tap water. After hot water treatment, it was observed that almost all seeds imbibed, and testa discoloured. When imbibition is too fast seed viability and vigor could be reduced through injury inflicted on the cotyledon cells (Powell, 1989).

According to the results of the study, treatment of seeds with KNO_3 did not increase the germination rate similar to Tilki's (2004) findings. Additionally, some researchers reported that the effect of KNO_3 applied to break seed dormancy was inadequate for some annual medics (Balouchi and Modarres, 2006) and *T. meneghinianum* Clem (Aydin and Uzun, 2001). In contrast to all of these researchs, Tian (2009) determined that the germination rates of red clover seeds were increased by 0.2% KNO_3 solution soaking for 12 or 6 h. "Solution concentration or soaking period of KNO_3 in this study is probably not appropriate for red clover."

Disruption with a sandpaper was the most effective method in breaking the dormancy of *Medicago* and *Trifolium* species which naturally grow (Uzun and Aydin, 2004). Same results were found out in this study. Much as it was the best dormancy breaking method in red clover, germination percentage was low. The effect of this method might show variation among *Trifolium* species (Can et al., 2009). Therefore, the effect of treatments on germination rate should be determined for each species. Application period could also influence the efficiency of the method (Aydin and Uzun, 2001). Mechanical scarification is intended to cause minor damage to the seed coat so that the seed imbibes water. However, the process can damage other parts of the seed, including the embryo, and reduce seed viability. Seed damage may also allow pathogenic and saprophytic organisms to gain entry into the seed and thus reduce the shelf life of the seed (Acharya et al., 1999). Different mechanical scarification period should also be tested in order to determine standard time for practical usage.

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