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INFLUENCE OF SOME HARDSEEDEDNESS-BREAKING TREATMENTS ON GERMINATION IN PERSIAN CLOVER (*TRIFOLIUM RESUPINATUM* SSP. *TYPICUM* FIORI ET PAOL.) SEEDS

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ABSTRACT

The objective of this investigation was to determine the effect of some hardseededness-breaking treatments on germination in Persian clover seeds of different colour. The investigation was carried out in the Analyses Laboratory of Field Crops Department at Agriculture Faculty, University of Namik Kemal, Turkey. Seeds were manually harvested after maturation from a permanent meadow of Persian clover growing in a xeralf soil in Gazioglu village (41.0° N, 27.5° E) of Tekirdag province (Turkey), during 2006 and 2007. Treatments of collected seeds included mechanical scarification, deep freeze, and chemical scarification using concentrated sulfuric acid for various durations. The non-viable seed ratio (% non-viable seeds after germination), and germination ratio (% germinated seeds after seven days), hardseededness ratio (%, not germinated seeds) in yellow, green, red and mixed colour Persian clover seeds was determined. Hardseededness ratios of seeds were reduced by all treatments. The applications of deep-freeze for 15 days, mechanical scarification for 5 minutes and chemical scarification for 15 minutes significantly improved the germination ratios of different colours seeds, up to 68.4-91.2%, 70.3-90.4%, and 67.4-90.1%, respectively. Red colour seed had the highest non-viable seed ratios in all treatments.

Key words: germination, hardseededness, Persian clover, seed.

INTRODUCTION

The forage crops are the main sources of protein, mineral and energy for herbivores and omnivores. Clovers (*Trifolium* sp.) are among the most known forage legumes in the world. These are probably the first legumes that were cultivated in the ancient times to produce forage. The clovers are among the most important forage crops native from the temperate regions cultivated in this regions to produce seeds. Because of the presence of hard seed coat, which is the main reason for dormancy of the seeds, the germination and therefore the seedling establishment in clover, do not take place easily.

The Persian clover (*T. resupinatum* L.) is a native of Anatolia, Iran, Greece, Bulgaria, Portugal, Iraq and Afghanistan. It was cultivated for forage during the 19th century. Persian clover varieties have been used as hay, green manure, self-regenerating grasslands and seed production in subtropical climates (Ates and Servet, 2004). Tekeli and Ates (2008) have outlined the high feeding value and various roles of Persian clover in Mediterranean countries, southern Australia and America. However, like all annual grassland species, its persistence is dependent on time of flowering, the level of seed production, seed survival over summer, and subsequent germination in autumn when climatic conditions are favourable (Evers et al., 1988; Nair et al., 2004). Seed survival over summer is greatly affected by the regulation of germination. In Mediterranean environment, annual forage legumes survive as seed over the dry hot summer, representing an important resource for pasture and meadow improvement and soil erosion preservation (Patanè et al., 2008). Hardseededness is considered more significant in continuous grassland systems (Evans, 1993).

High quality seed is the basis of higher agricultural productivity. Quality in seeds embraces all the physical, biological, pathological, and genetic characteristics,

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which contribute to the final yield of a forage crops. Seeds should be specifically bred and genetically pure; free from diseases, vigorous, and high in germination percentage. The inability of a viable seed to germinate under conditions normally considered favorable for the purpose, including suitable water, temperature, and oxygen availability, is termed dormancy. Two important mechanisms regulate germination: embryo dormancy and hardseededness. Relevant reviews (Doran, 1997; Souza and Marcos-Filho, 2001) cite fifteen angiosperm families with species producing hardseededness. Among these families is the *Fabaceae*, of which most species produce hard seeds. Most *Fabaceae* seeds present hardseededness, because of impermeability to water [for example, alfalfa (*Medicago* sp.) and clover species (*Trifolium* sp.)] and gas [for example, coffee (*Gymnocladus* sp.) and ash tree (*Fraxinus* sp.) species], due to the thickness and biochemical composition of their testa (Soya and Geren, 1999; Tekeli and Ates, 2006a).

Variations in hardseededness in annual clovers are affected by both genetic and environmental conditions during plant growth, seed development and maturation (Taylor et al., 1996). Hardseededness varies inter- and intraspecifically, depending on the testa's degree of development and the generated impermeability strategies. It also depends on other factors such as the geographical location of the populations, the earliness of the seeds, the ecological differences in relation to temperature and relative humidity, the physico-chemical properties of the soil, the action of the photoperiod, and so forth (López et al., 1999). For example, the ratio of hardseededness (water-impermeable) in berseem clover (*T. alexandrinum* L.) and broad bean (*Vicia faba* L.) changed with fertilization regime (El Bagoury and Niyazi, 1973; El Bagoury, 1975). Degree of hardseededness in subterranean clover (*T. subterraneum* L.) and in annual *Medicago* species differed with level of moisture stress (Collins, 1981; Taylor, 1996). The rate of hardseededness breakdown in Persian clover depended on the climatic conditions. The highest hardseededness ratio (94.3%) was found from yellow seeds after harvest (Tekeli and Ates, 2008).

Variation in hardseededness occurs within a clover species, between varieties of the same species (Tekeli and Ates, 2008).

There are three main subspecies of *T. resupinatum*: *majus*, *typicum* and *resupinatum*. The Persian clover subspecies show considerable variation in seed coat characteristics. The objective of this investigation was to determine the effect of some hardseededness-breaking treatments on germination in seeds of different colour in Persian clover.

MATERIAL AND METHODS

This investigation was carried out in the Analyses Laboratory of Field Crops Department at Agriculture Faculty, University of Namik Kemal, Turkey. Seeds were manually harvested after maturation from a permanent meadow of Persian clover growing in a xeralf soil in Gazioglu village (41.0° N, 27.5° E) of Tekirdag province (Turkey), during 2006 and 2007. Persian clover was identified at flowering stage. The climate is a half-humid subtropical, with cold, wet winters and warm to hot dry summers. Immediately after harvest, seeds were stored in the dark in natural conditions of pressure, temperature and relative humidity.

The collected seeds were subjected to treatments including mechanical scarification, deep freeze, and chemical scarification using concentrated sulfuric acid for various durations (Samarah and Abu-Zanat, 2005). In the first treatment, the seeds were scarified for a while on a plank which had the surface covered with sandpaper (average particle diameter of grit = 35 μm) for 5, 10 and 15 minutes. This produced a mechanical scarification of the seed coat. In the second treatment, the seeds were soaked in a concentrated sulfuric acid (H_2SO_4) (98% v/v) for 5, 10 and 15 minutes. In the third treatment, non-pretreated seeds were incubated in a deep freezer adjusted to -5°C for 5, 10 and 15 days. In all treatments seeds were surface sterilized for 2 min in a 5 g L^{-1} sodium hypochlorite (NaHClO) solution

(Clua and Gimenez, 2003). A hundred Persian clover seeds, well shaped and vigorous in appearance, were selected for the germination test. Germination trials were conducted in 14x21 cm sterile plastic germination dishes lined with two Whatman No. 1 filter papers and moistened with 50 mL sterile distilled water. The seeds were incubated in the darkness at 20±1ºC. Each germination experiment ended after seven days of incubation (Ates and Tekeli, 2007). In the experiments, the non-viable seed ratio (%, non-viable seeds after germination), and germination ratio (%, germinated seeds after seven days), hardseededness ratio (%, not germinated seeds) was determined in Persian clover seeds of different colours (yellow, green, red and mixed) (Tekeli and Ates, 2008).

The statistical method used in the present experiment was one way ANOVA with four replicates. The germination percentages were subjected to $\arcsin\sqrt{x}$ transformation and analyzed by ANOVA using LSD (Fisher's Least Significant Difference) multiple comparison test. The software was MSTAT-C.

RESULTS AND DISCUSSION

The germination, hardseededness and non-viable seed rates in Persian clover seeds of different colours are given in figures 1 to 3. Hardseededness ratios were reduced by all treatments. The application of deep-freeze (68.4-91.2%, 15 days), mechanical (70.3-90.4%, 5 min) and chemical (67.4-90.1%, 15 min) scarification significantly improved the germination ratios of seeds for different colours. The highest non-viable seed ratios were counted from red colour seed in all treatments.

There was difference in germination percentages between 0, 5, 10 and 15 minutes treatments of mechanical scarification (Figure 1). The highest germination rates were obtained from mechanical scarification for 5 min (70.3-90.4%). The non-viable seed ratios were found to increase significantly at mechanical scarification for 15 min treatment ($P \leq 0.05$). However, the lowest hardseededness ratio (63.6%) was counted in red colour seeds, while the maximum hardseededness ratio (92.4 %) was determined in the yellow seeds of Persian clover in the control application. Sandpaper, dehulling and abrasive elements (i.e. sand, percussion and hammer-milling) are mechanical methods commonly used to break down hardseededness in legumes (Ramamoorthy and Rai, 1990). Their utility, however, depends on the species and the type of seed used (Argel and Paton, 1999). These results were in accordance with Tekeli and Ates (2006b, 2008). They found a hardseededness ratio of 94.3% for yellow seeds in Persian clover. Seed coat has many functions such as regulation of imbibitions and hence plays a role on germination. Species that have thick seed coat do not germinate even when ideal conditions for germination exist. This is called physical dormancy due to hard seed coat or hardseededness (Souza and Marcos-Filho, 2001; Can et al., 2009). The hardseededness of many pasture legumes is reduced during the summer and autumn under natural conditions by a combination of high and fluctuating temperatures (Norman et al., 2002). High quality seed is the basis of higher forage crops productivity. For this reason, hardseededness should be eliminated.

The breaking seed dormancy of some annual *Medicago* and *Trifolium* species by different treatments was investigated by Can et al. (2009); they reported that the mechanical scarifications + NaHClO treatment significantly increased the germination ratios of seeds from Mediterranean clover (*T. spumosum* L.), brown moor clover (*T. spadiceum* L.) and narrow-leaved clover (*T. angustifolium* L.). The germination rates ranged 80.0%, 42.5% and 25.0% respectively, in these clover species. Samarah and Abu-Zanat (2005) reported that mechanical scarification and soaking seeds in H_2SO_4 for 24 min improved seed germination up to 98% and 76% for seeds harvested at full-seed pod stage and 100% and 93% for seeds harvested at brown pod stage respectively, in barn vetch (*Vicia monantha* Retz.).

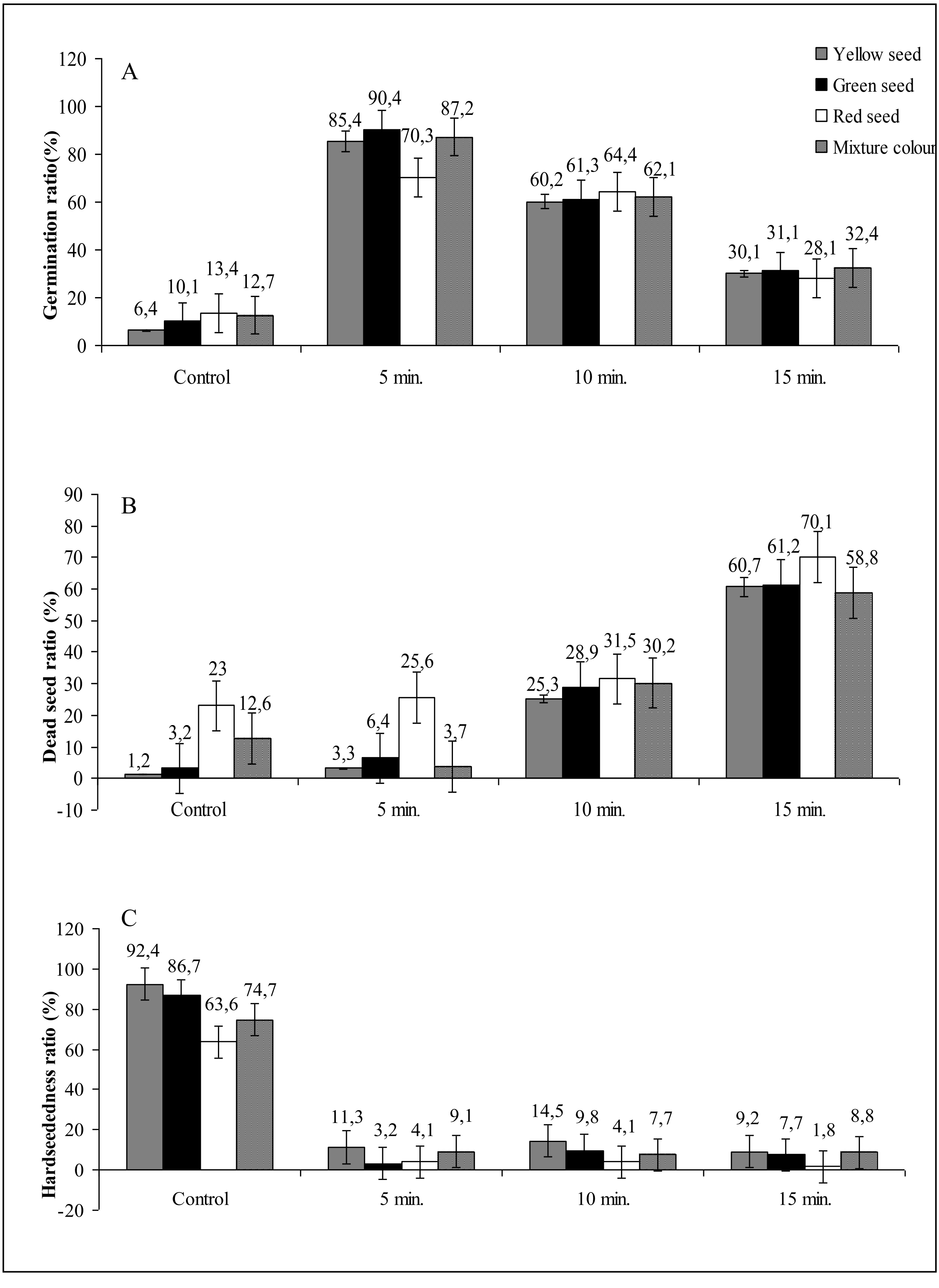

Figure 1. Germination (A), dead seed (B) and hardseededness (C) ratios of Persian clover seeds under different durations of mechanical scarification (All ratios are significantly different at the P≤0.05 level as analyzed by LSD test. Interactions are not significantly different at the same level)

The hardseededness, germination and dead seed rates in treatments with concentrated H_2SO_4 (98% v/v) are shown in figure 2. Soaking seeds in H_2SO_4 for 15 min improved seed germination up to 67.4-90.1% for seeds and this treatment decreased hardseededness ratio up to 2.5-7.6% for Persian clover seeds ($P \leq 0.05$). The highest dead (not-viable) seed ratios (25.7-45.2%) were determined from the red seeds of Persian clover in all treatments. H_2SO_4 scarification is commonly used in laboratory seed germination tests, but particular care is required, since it may cause skin irritation if not handled properly. It is also highly corrosive to metal containers, implying the utilization of special equipment to scarify large volumes of seeds (Argel and Paton, 1999).

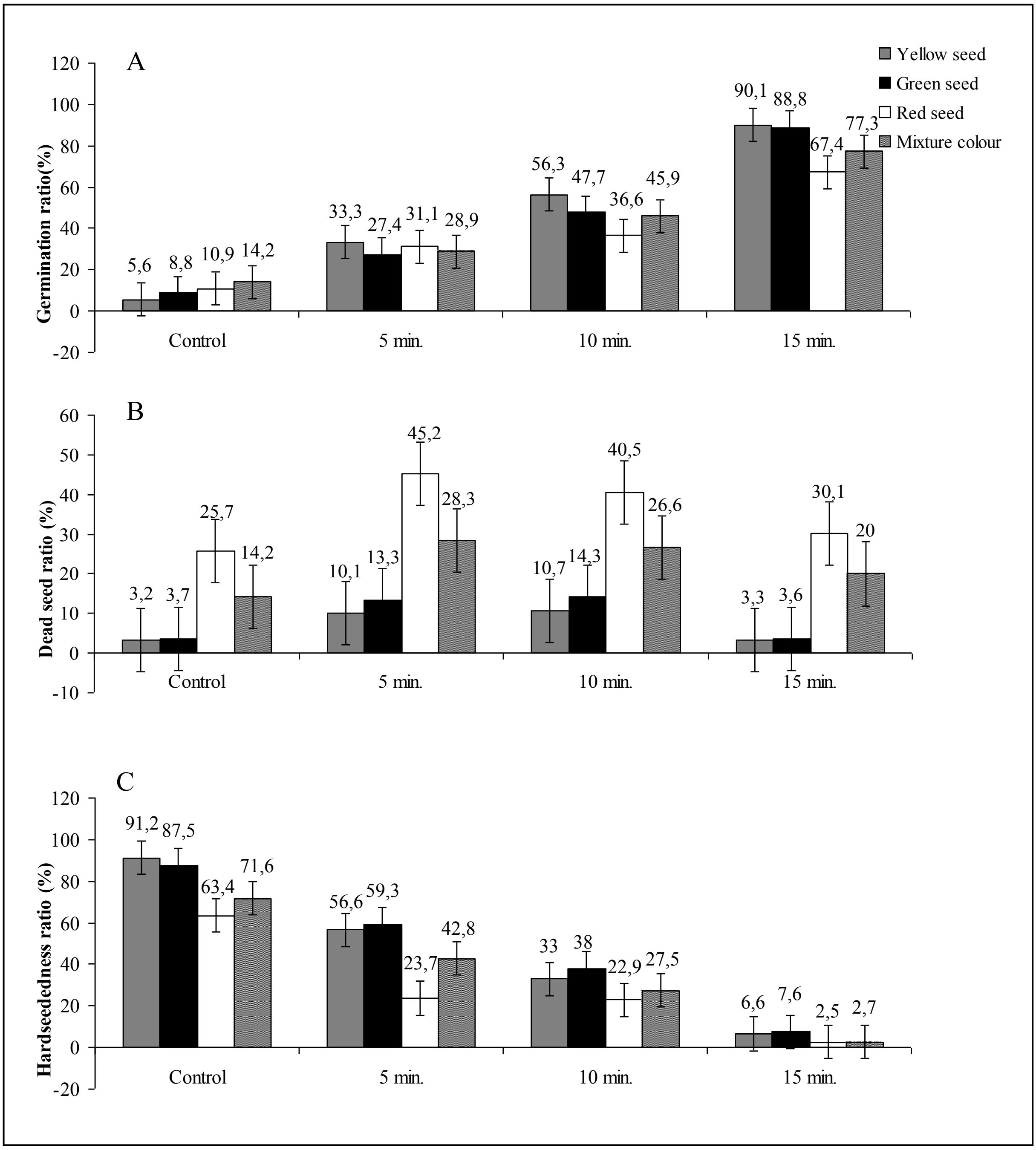

Figure 2. Germination (A), dead seed (B) and hardseededness (C) ratios of Persian clover seeds under different durations of chemical (H_2SO_4) scarification (All ratios are significantly different at the $P \leq 0.05$ level as analyzed by LSD test. Interactions are not significantly different at the same level)

Significant increases in seed germination using acid scarification have been reported for butterfly pea (*Centrosema pubescens* Benth.) (Aragao and Da Costa, 1983), tropical kudzu (*Pueraria phaseoloides* (Roxb.) Benth.) (Cabrales and Bernal, 1983) and petit mimosa (*Desmanthus virgatus* (L.) Willd.) (Ramamoorthy and Rai, 1990). Similar results were also reported by Baes et al. (2002) for mesquite (*Prosopis ferox* Griseb.), by Uzun and Aydin (2004) for annual legumes, by Samarah and Abu-Zanat (2005) for barn vetch, by Can et al. (2009) for some annual *Medicago* and *Trifolium* species. However, Barker and Abdi (1987) determined that germination of true indigo (*Indigofera tinctoria* L.) was drastically reduced by as little as 3 min scarification with 95% H_2SO_4.

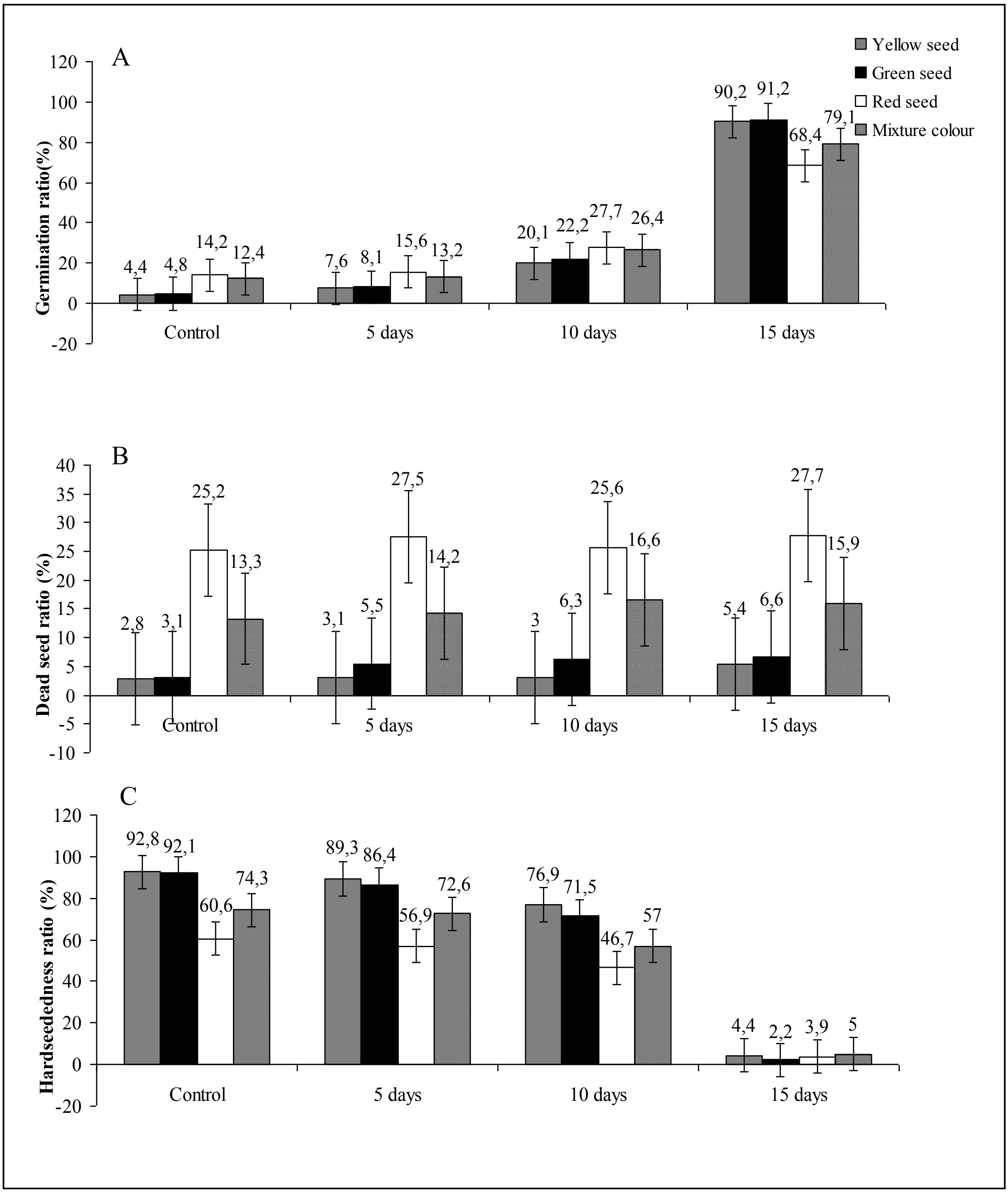

Figure 3. Germination (A), dead seed (B) and hardseededness (C) ratios of Persian clover seeds under different periods of deepfreeze treatment (All ratios are significantly different at the P≤0.05 level as analyzed by LSD test. Interactions are not significantly different at the same level)

For all seeds, deep-freeze adjusted to -5°C for 15 days resulted in germination ratios of 68.4-91.2% (Figure 3). The lowest hard-seededness ratios (46.7-60.6%), but also the highest dead seed ratios (25.2-27.7%) were found in red colour seeds. High constant temperatures and temperature fluctuations are major factors in overcoming hardseededness (Evers, 1991). However, temperature treatments capable of causing significant rates of hard-seed breakdown for any particular species do not occur until the amplitude of the temperature fluctuation, or the maximum temperature, reaches a certain level (Quinlivan, 1966; Argel and Paton, 1999). Similar results were obtained in *Stylosanthes hamata* cv. Verano for changes of hard-seededness (Argel and Humphreys, 1983). Nair et al. (2004) found a hardseededness ratio of 37.4% (five months after storage) for balansa clover (*T. michalianum* Savi.). Balkaya (2004) reported that the effect of temperature on germination was much more important than the other possible effective parameters, since 83 to 99% of the variations in germination were explained by temperature depending on the plant species and varieties.

In conclusion, hardseededness is an important aspect of seed quality in relation to Persian clover establishment. Mechanical, chemical and deep-freeze treatments can be used to overcome hardseededness for Persian clover seeds of different colours. Further research is required on treatments that gave variable proportions of scarified seeds.

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