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Possible effects of priming on germination performance of white clover (*Trifolium repens* L.) seeds in hypothermia condition

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Abstract

The aim of the study is investigate the effects of hidropriming on germination performance of white clover (*Trifolium repens*) seedsunder cold stress, optimum temperature and dark condition. The seeds were germinated applying hidropriming with dH_2O in different periods (2, 4, 6, 8, 16, 18, 20, 22, 24 and 48 hours) in dark at 4 °C. The study was applied in according to randomized controlled trials as 4 replications to measure radicular lengths, germination rate and homogeneity parameter in the seeds. The results of the study was indicated that radicle length, germination rate, rate and the homogeneity parameter were positive and statistically significant ($P<0.001$) comparing with control group in short period (2 and 4 hours). On the other hand radicle length, germination rate, the homogeneity parameter were found negative and statistically significant when compare with control group in the other periods ($P<0.001$). In addition, this study was discussed possible physiological effects to the priming process implemented with dH_2O in hypothermia condition at white clover seeds.

Keywords: Cold stress, Germination, Hidropriming, White clover

Introduction

White clover (*Trifolium repens*) which widely cultivated in the Marmara, Black Sea and transition zones in Turkey is quite valuable for animals. This plant which is short, abundant leaf, thin handle, soil surface covering, and high quality production is a high quality meadow pasture and forage crop (Manga et al. 1995). Germination of plants varies between varieties but it is highly influenced by environmental factors such as light, water, temperature, oxygen etc. Water and temperature factors are the most commonly used environmental factors investigated to start germination of the seeds. Theoretically, it is possible to stimulate the germination of the seeds with these applications to get fast and high germinate percentage (Karakurt et al. 2010). For this purpose, humidification / hydropriming applications on plants have demonstrated beneficial effects on the rate of germination such as Basu and Pal (1980) in rice seeds, Rao et al. (1987) in lettuce seeds, Sivritepe and Dourado (1995) in pea seeds, Sivritepe and Demirkaya (2002) in onion seeds, Demirkaya (2006) in Çetinel-150 pepper seeds (Karakurt et al. 2010). Özdemir (2006) observed a positive effect on the germination rate of the kiwi (Hayward varieties) seed with low germination rate by applying hdropriming with pure water at different temperatures and different durations.

The damage of plants due to low temperature has been seen varies between species and variety. According to Oquist (1983), low temperature is a relative expression. Plants adapted

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to cold climates may photosynthesize at temperatures below 0 °C and may develop normally, while the photosynthesis mechanism may undergo irreversible damage at temperatures below 10 °C in tropical and semi-tropical climatic plants. According to Raison and Lyons (1986), the term cold damage defines the physiological damage that occurs when most tropical and semi-tropical plants are exposed to a low but not freezing temperature (usually up to 15 - 0 °C). Inhibition of photosynthesis is a precursor reaction to low temperature. The effect of cold damage photosynthesis is increases with medium or high light intensity. The damage to the cold is partly increased by free oxygen radicals that cause secondary damage to membranes and photosystems (McKersie and Leshem 1994). The increase in electrolyte output with membrane damage in tissues exposed to low temperature is the most commonly known effect of low temperature (Belous and Bondarenko 1982; Zauralov and Lukatkin 1985). Rylski (1973) in his study on pepper has been reported the flowering time and growing and development time of vegetative organs in plants increased with the decrease in air and soil temperature. Polowick and Sawhney (1985) conducted a study on Vinedale type. According to results, the low temperature (18 °C day / 15 °C at night) cause functional male infertility by abnormal such as petal leaf, male organ and female organ formation, male organ deterioration, in some cases partially carpel-like male organs have the ability to abnormal flower powder production. Researchers who determine that the ovary is larger than normal and the style extension is prevented were reported plants that grow at low temperatures produce small fruits, which are seedless, but are pollinated with flower dust from plants growing at normal and high temperatures. Aloni et al. (2001) reported that low temperature affected the carbohydrate mechanism negatively. It negatively affects the characteristics of the pollen and the germination rate and also accordingly fruit shape, shape and development are affected (Shaked et al. 2004). Farrell et al. (2006) reported that low temperature application on rice decrease the amount of flower powder and starch it accumulates. In the study on chickpea of Nayyar et al. (2005), low temperatures on the reproductive organ causes abortion and flowering and fruit attitude were negatively affected. During cold stress, due to disruption of the membrane function (cytoplasmic, mitonodrial and chloroplast membrane), photosynthesis is reduced, carbohydrate transport slows, respiration slows, protein synthesis is inhibited, the rate of disintegration of existing proteins increases, the dissolved substance exudes from the cell. Low temperature affects the activity of integral membrane proteins that regulate the transport of H+-ATPaz' carriers, enzymes, ions and other dissolved contents of required for metabolism (Taiz and Zieger 2008). In cold sensitive plants, the double layer oils have a high proportion of saturated fatty acid chains. This type of membrane reaches the semi-crystalline phase at temperatures above 0 °C. As the membrane loses its fluid, the protein components are no longer working. The membrane lipids of cold-resistant plants have generally higher unsaturated fatty acids than cold-sensitive plants (Kaçar et al. 2006). A gene was transferred from Escherichia coli to Arabidopsis in order to increase the high-melting (saturated) membrane lipids. This gene has greatly increased the resistance of gene-modified plants to low temperature (Taiz and Zieger 2008).Moran et al. (1989) have studied that effect of the study of growth and root growth of lentil (*Lens culinaris Medik*. Cv. Castellana) plant under water and alternative temperature (4-20 °C, 16/8 hours). According to this study, they determined that the osmotic potential was initially high but it was lower controls than in the alternative temperature regimen. The cellulose content of the stem cell wall was similar in all applications on the 3rd day of development, but lower than the controls. In all cases during the study period, natural non-cellulosic sugars in the cell wall decreased but in stressful roots were higher than controls. While uronic acids and protein content were higher in roots at control groups, the amount of uronic acids increased and differences of protein content was not found significant.

In this study, the application of hydropriming and the times of this application were investigated of the effects of rate and speed of the seed germination besides the development of the plant under the cold stress.

Materials and Methods

In the study, white clover (*Trifolium repens*) seedswere used as trial material. The study was carried out in the Department of Field Crops, Faculty of Agriculture, Kahramanmaras Sutcu Imam University.

Hydro-Priming Application

White clover seeds used as test material were soaked with dH2O at different times (0, 2, 4, 6, 8, 16, 18, 20, 22, 24 and 48 hours) and 4 ± 1 ºC in dark condition. Then, all seeds taken to the drying paper in the room conditions. The main aim in there is to remove the water from the seed surface. The dried seeds were placed in glass petri dishes (5x5.5 cm) in which two layers of drying paper.

Petri dishes with seeds was added 3 ml of distilled water and then these were germinated in a climatic cabinet at 15 ± 1 ºC temperature and in the dark condition. Also, untreated

seeds were used as control seeds. The study was carried out with 4 replications according to the experimental design of randomized plots.

In this study, germinated seeds were counted daily and removed from petri dishes and then their radicle length was measured. This process continued until the number of seeds germinated in all petri dish was zero for 3 consecutive days (9 days). In the experiment, 1-2 mm root outlet was accepted as germinated seed.

This study was examined traits such as percentage of germination, angular transformation of germination percentage, time to germinate 50% of seeds (day), time to germinate 90% of seeds (day), time for germination of 10% of seeds (day), span value (the number of days required for seeds to reach 90% germination by 10%), total radicle length, average radicular length.

Statistical Analysis

The last germination percentage (GerY) and its angular transformation (arcsine√GerY) and also the root length of germinated seeds were determined in germinated seeds. Then, the data of obtained were analysed by SAS (SAS 1997) statistical package program. The differences between the means were tested by Fisher's smallest significant difference (LSD) test at grade of $p < 0.05$.

Results and Discussion

The results of the research have shown Table 1 and Figure 1-2 belong to white clover seeds which are germinated after hydropriming application at different times under cold stress. When the results were examined, it was observed statistically significant differences between the seeds applied of hydropriming at different times under cold stress. Accordingly, it has been observed a small but continuous decrease due to increased hydropriming time at the germination rates. It was determined that the highest germination rate with 95% was obtained from the seeds which were applied hydropriming for 0 and 2 hours while the lowest germination rate was obtained from the seeds applied hydropriming for 48 hours with 37%. The germination rates of the seeds which were applied hydropriming for other periods (4, 6, 8, 16, 18, 20, 22 and 24 hours) were 82%, 78.5%, 68.5%, 68%, 56%, 54.5%, 49.5% and 57.5%, respectively (Fig. I).

The highest root length value was obtained from seeds which were applied of hydropriming for 2 hours with 176 mm. Total root length of the seeds of control (0 hour) was obtained 149.5 mm. In the other periods (4, 6, 8, 16, 18, 20, 22, 24 and 48 hours), the total root length values from seeds which were applied of hydropriming were determined as70.75, 48.50, 44.50, 40.75, 33.50, 30.50, 32.25, 31.75 and 20.00mm, respectively (Fig. 1). At the application of hydropriming for 4 hours and more time, there have been significant decreases in the proportions total root lengths. According to obtained data, the ratio of the total root length to the total number of germinated seeds and the average root length per seed germinated (ARL) were calculated. When the ARL data were compared between the applications, the maximum root length was determined as 1.85 mm and 1.73 mm values from seeds which were applied of hydropriming for 2 hours and 4 hours , respectively, while this value was determined as 1.57 mm in the control seeds. The ARL data for other applications (6, 8, 16, 18, 20, 22, 24 and 48 hours) were determined as 1.24, 1.30, 120, 1.20, 1.12, 1.30, 1.10 and 1.08 mm, respectively (Figure 2).

At the seeds which were hydropriming applicated at different times under cold stress, germination parameters such as speed of germination, TRL, ARL and germination rate of seeds were found different ($p<0.01$). The most rapid germination (G50 = 1.55 days) in seeds germinated depending on the duration of the application was obtained from the seeds the applied of hydropriming for 2 hours. In addition, this value was determined as 1.67 days in any untreated control seeds. In the seeds germinated depending on the application time, the slowest germination was determined as 2.52 (for 22 hours), 2.50 (for 18 hours) and 2.47 days (for 20 hours), respectively. In other applications, the speed of germination which 4, 48, 16, 8, 6 and 24-hours applications was determined to 1.74, 1.85, 2.09, 2.13, 2.14 and 2.20 days, respectively. According to these results, the other times except for 24-hour application showed slower germination than control and the 2-hour application, it was determined that the germination rate was slowed continuously with little difference in increasing time. But the 24-hour application showed faster germination than 18, 20 and 22 hours application.

In the light of this data, the highest values of all germination parameters were obtained from the seeds which were subjected to hydropriming for 2 hours, and then were obtained from the seeds which were subjected to hydropriming for 4 hours. When compared with the control group, it was determined that these applications were higher than the control group. It was determined that the other applications had less but gradually decreased values in the control group.

The purpose of the application of hydropriming was to provide water transition to inside of the seed to start germination. In this study, we used hidropriming of applications under cold

stress (4 ºC) and applicated different time periods in order to determine how it promote germination. According to these results, the 2-hour hydropriming application caused an increase in speed of germination, germination rate, TRL and ARL values compared to control. However, we found that the other applications decreased continuously. The germination and development in seeds is slowing due to O_2 deficiency in excess water stress. However, it changed death in plants according to the duration of exposure to excessive water stress. On the other hand, seed and plants under cold stress are getting slowing water intake and damage due to O_2 shortage usually occurs in plants after 24 hours depending on the variety and species (Taiz and Zieger 2008). This situation made us think that this hydropriming application under cold stress did not cause any damage due to O2 scarcity in seeds. The white clover seeds which the most suitable germination temperature of 20-25 ºC were determined that didn't adversely affect the seeds up to 4 hours in temperature of 4 ºC.

Even, it has been observed that hydropriming positively promotes germination but the negative effects of the 4 ºC temperature on the germination. And root growth in the white clover seeds were found to be lower gradually after 4 hours. In the light of previous studies by many researchers, this situation has been disclosed as increase in electrolyte output by membrane destruction in tissues exposed to low temperatures (Belous and Bondarenko 1982; Zauralov and Lukatkin 1985), negative effects on carbohydrate metabolism (Aloni et al. 2001), decreased photosynthesis, slow down transport of carbohydrates, slowing of respiration, inhibition of protein synthesis, increasing the rate of fragmentation of existing proteins, leakage of dissolved substances from cells, the physical properties of the lipids involved in the membrane structure affect the activity of H+-ATPaz' carriers, integral membrane proteins that regulate the transport of enzymes, ions and other dissolved substances necessary for metabolism (Taiz and Zieger 2008), osmotic potential during root growth and reduction of cellulose content of root cell wall (Moran et al. 1989).

Table 1. Data on germination performance of white clover seeds of cold stress pre-treatment at different times

Application	Germination Rate (GerY)							
	%	[GerY]	G_{50}	G_{90}	G_{10}	Span	TRL	ARL
0 hour	95.00	61.22^{A}	1.67^{CD}	2.94^{DE}	1.10^{AB}	1.84^{E}	74.75^{B}	1.57^{B}
2 hours	95.00	62.97^{A}	1.55^{D}	2.62^{E}	1.00^{B}	1.62^{E}	88.00^{A}	1.85^{A}
4 hours	82.00	53.43^{B}	1.74^{BCD}	3.60^{CD}	1.09^{AB}	2.51^{DE}	70.75^{B}	1.73^{AB}
6 hours	78.50	51.80^{B}	2.14^{ABC}	4.11^{BC}	1.14^{AB}	2.97^{CD}	48.50^{C}	1.24^{CDE}
8 hours	68.50	48.17^{BC}	2.13^{ABC}	4.48^{ABC}	1.03^{AB}	3.44^{BCD}	44.50^{C}	1.30^{C}
16 hours	68.00	48.03^{BC}	2.09^{ABC}	4.12^{BC}	0.91^{B}	3.22^{BCD}	40.75^{CD}	1.20^{CDE}
18 hours	56.00	43.85^{CD}	2.50^{A}	4.99^{AB}	1.19^{AB}	3.80^{ABC}	33.50^{D}	1.19^{CDE}
20 hours	54.50	43.40^{CD}	2.47^{A}	4.78^{AB}	1.31^{A}	3.48^{BCD}	30.50^{D}	1.12^{CDE}
22 hours	49.50	41.83^{DE}	2.52^{A}	5.13^{AB}	1.09^{AB}	4.03^{AB}	32.25^{D}	1.28^{CD}
24 hours	57.50	44.28^{CD}	2.20^{AB}	4.85^{AB}	1.06^{AB}	3.79^{ABC}	31.75^{D}	1.11^{DE}
48 hours	37.00	37.44^{E}	1.85^{BCD}	5.19^{A}	0.46^{C}	4.73^{A}	20.00^{E}	1.09^{E}
p>0.001		**	**	**	**	**	**	**

**: significant at p <0.01; §, GerY: Percentage of germination; [GerY]: Angular transformation of germination percentage; G50: Time to germinate 50% of germinated seeds (day); G90: Time to germinate 90% of germinated seeds (day); G10: Time for germination of 10% of germinated seeds (day); Span: The number of days required for germinating seeds to reach 90% germination by 10%; TRL: Total radicle length of germinated seeds; ARL: Average radicular length of germinated seeds

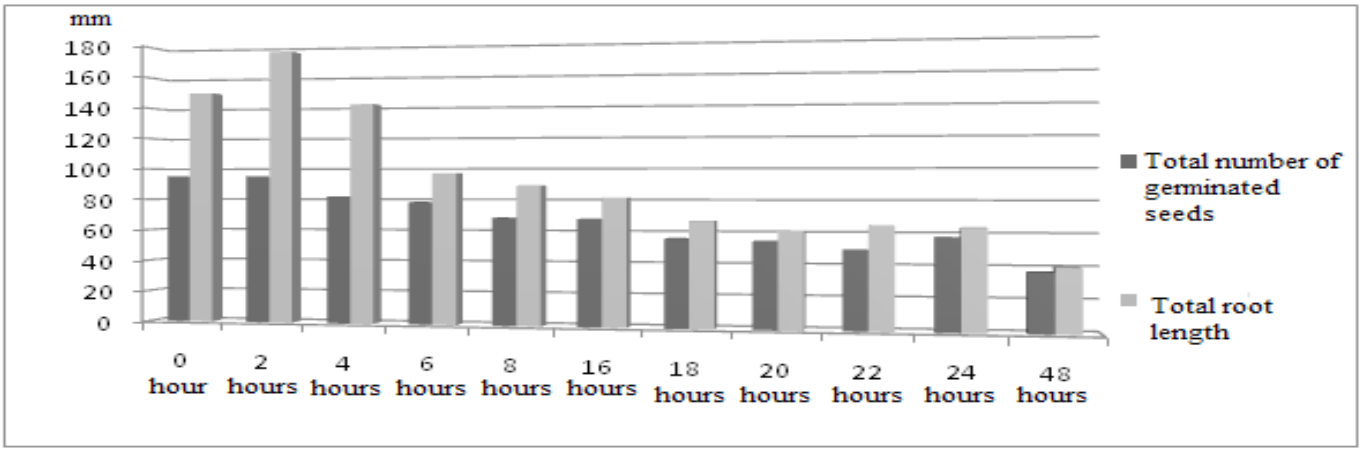

Figure 1. The means values of total germinated seeds and total root length data of germinated seeds

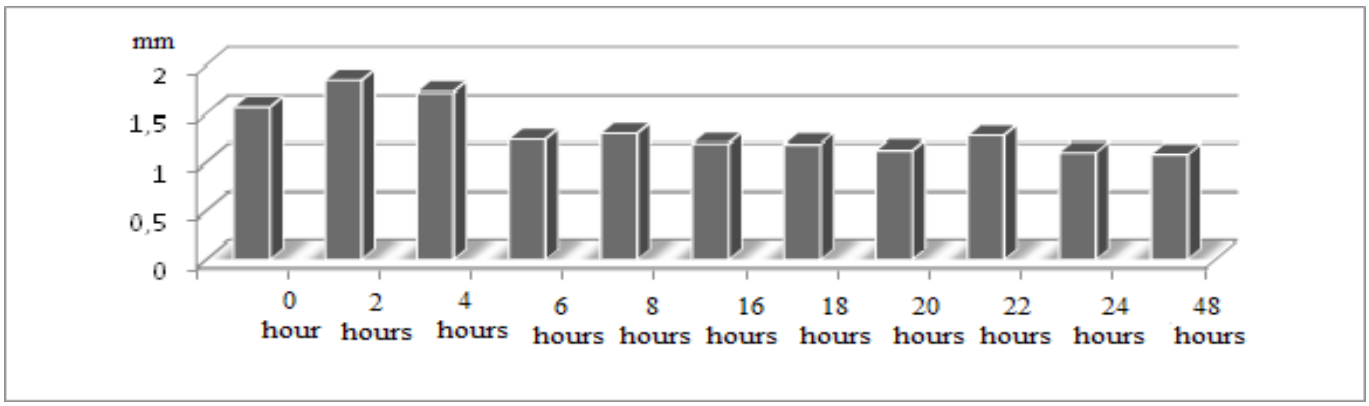

Figure 2. The means of average root length data per seed germination

Compliance with Ethical Standards

Conflict of interest
The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution
The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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