According to statistics, only about 10% of the world's cultivated land may be tense. About two-thirds of the world's lands are subjected to temperatures below the freezing point each year. In other words, about 180 million hectares (cultivated in the world at 1200 × 10³) annually are affected by cold stress (Ghassemi et al., 1995). Today, plants exposure at low temperatures and freezing has become a global and ecological problem (Pallioti and Bongi, 1996; Adya et al., 2013). Research showed that environmental stresses reduced the quality and quantity of crop production (up to 71%), so this decrease was observed in drought stress (17%), salinity (20%), heat (40%), cold (15%) and other factors (8%) (Ashraf et al., 2008). Cold and frost damage in the United States is reported to be between 3% and 4% annually, causing $ 50 million in cotton cultivation in 1895 (Jouyban et al., 2013). In Iran, more than 2.5 million hectares of farms in cold regions are exposed to winter. Given that about 50% of the cultivated areas of clover are in the western and mountainous regions of the Iran, about 6 - 7 thousand hectares of clover will be affected by cold stress annually. Freezing is one the most important abiotic stresses limiting the growth and production of plants in temperate regions. Cold stress, similar to drought and salinity, affects the water...
relations of plants at the cellular level, as well as the whole-plant level, resulting in plant injury and adaptation reactions (Beck et al., 2007). According to Beck et al. (2007), cold stress imposes a considerable negative effect on crop productivity, quality, and survival of plants. Low-temperature stress can cause serious damage at the physiological, cellular, and molecular levels, which is expressed by various plant phenotypic symptoms (Fig.-1).

Plants in conditions of environmental stresses, due to the impossibility of movement and escape, must have mechanisms of compromise and tolerance for survival. Cold acclimation is one of the important mechanisms of cold tolerance in plants. In this method, the plant is exposed to cool temperatures before being exposed to freezing temperatures and to some extent compatible with freezing temperatures (Mahfoozi et al., 2000). In a study on Arabidopsis, it was found that all the plants that did not eat were lost at -6°C, but many of the plants that were eaten at 4°C for 8 days at a temperature of -8 °C survived (Ruelland et al., 2009).

Cold stress is one of the most important limiting factors for autumn plant crops (Hasani Fard et al., 2016). Crop yield in autumn crop due to the proper use of plants from precipitation, having a longer growing period, non-stresses of heat and drought is often more than spring crop. Therefore, planting tolerant plants in this season of the year can lead to increased production and yield (Singh et al., 1997). Persian clover (Trifolium resupinatum L.) is one of the most important crops in the legume family (Fabaceae = Leguminosae), which in Iran is cultivated in cold regions (Zamanian, 2002). In recent years, due to climate change and water-resource constraints in most regions, autumn planting of Persian clover for optimum use of precipitation will be delayed. Also, raining will be delayed due to climate change. Consequently, seed sowing and subsequent germination and seedling emergence will be delayed. As such, it is necessary to identify cold-tolerant ecotypes of Persian clover and determine the range of low temperatures that Persian clover may withstand without any considerable damage. Using a technique that can lead to successful germination of Persian clover seeds under cold conditions will result in the increasing cultivation of this plant and greater levels of forage production in Iran (Zamanian et al., 2012).

**Review of literature**

In the United States, Kendall and Stringer (1985) reported that cold resistant in clover increases during the shortening of the days and lower temperatures in the fall, and the short days will result in better winter survival. This is while continuous light causes loss of cold tolerance in the crop. Usually, the resistance to cold starts from the month of October and reaches its maximum in mid-December. William (2002) from the selection of Sacromet cultivar were able to produce the Bigbee cultivar, which could survive from -15 to -18°C. Crimson clover have suitable growth in late fall and winter, and it is well tolerated against freezing conditions (Brandsater et al., 2000). Volence and Nelson (1995) argue that if red clover grows before the winter begins, it will improve its resistance to frost
and the presence of high nitrogen reserves in the roots of red clover during the winter will help the plant to resist colds. Calder et al. (1965) reported that legume seedlings are more sensitive to freezing temperatures during vegetative growth (48 days after culture) compared to other growth stages. Larcher (1991) reported that seedling stage in clover is the most sensitive growth stage to low-temperature stress. Levitt (1980) states that Cold Acclimation or Hardening can increase the resistance to frost resistance in plants. Zamanian (2005) from an effect of planting season on a yield of clover species and reported that forage yield of clover species in the autumn crop was 15% higher than spring crop. Research showed that use of cold resistant cultivars in lentil sowing autumn (Lens culinaris Medik.) Increased the crop area, production and harvest index compared to spring crop (Khomdi et al., 2006). Bagheri et al. (1999) showed that autumn sowing of canola (Brassica nupus L.) had about 15% more grain yield than spring crop. Identifying and introducing crops that can tolerate the early autumn and late spring frosts are suitable methods to reduce the damage to the cold and increase the level of cultivation in the autumn (Mousavi et al., 2006). The selection of low-temperature tolerant crops is very important for the sustainability of agriculture (Yadav, 2010).

Zamanian et al. (2012) showed that low-temperature stress leads to significant reduction in chlorophyll content of all Trifolium species, with the lowest value for Trifolium pratense cv. ‘Nassim’. Low-temperature stress results in high levels of reduction in chlorophyll a content than chlorophyll a+b and chlorophyll b. The quantum efficiencies of photosystem II (Fv/Fm) over all the species were 0.734, 0.788, and 0.555 for S1, S2, and S3, respectively. This indicates the effects of low-temperature stress on the Fv/Fm parameter, especially for temperature observed at S3. Moreover, the results indicated that under low-temperature stress, the Nassim cultivar had the low efficiency of photosystem II (Fv/Fm). In general, Fv/Fm index, chlorophyll, and carotenoid content are proposed for evaluating and field screening of clover species under low-temperature stress. Environmental stresses that affect PSII efficiency leads to a characteristic decrease in the Fv/Fm ratio (Krause and Weis, 1991).

Cold tolerance has been defined as the ability of a plant to survive exposure to low temperature with little or no injury (Castonguay and Guckert, 1996). Waldron et al. (1998) concluded that controlled freezing as an indirect selection method has great potential for improving winter hardiness of turf-type perennial rye-grass (Lolium perenne L.). They observed that successive freezing stresses at two temperatures (-6 and -9°C) resulted in greater additive genetic correlations with field winter hardiness than a single exposure to -6°C. Nezami et al. (2010) showed that freezing temperatures increased electrolyte leakage from leaf and crown tissues of Lolium perenne, Festuca arundinacea, Poa pratensis, and Cynodon dactylon. The effects of freezing temperatures on electrolyte leakage from leaf tissue were higher than the crown. The maximum level of electrolyte leakage from leaf tissue (at -16.5°C) was 90%, while the maximum leakage from crown tissue (at -18 °C) was 76%. Based on leaf LT50, the ‘Mahalat’ ecotype of Lolium perenne and Cynodon dactylon were the coldest sensitive and the ‘Starlet’ ecotype of Festuca arundinacea and the ‘Yarandi’ ecotype of Lolium perenne were the coldest tolerant grasses. The crown electrolyte leakage and LT50 were different between the grasses. Accordingly, the crown electrolyte leakage percent was minimum for the ‘Starlet’ ecotype and maximum for the ‘Mahalat’ ecotype. The crown LT50 index was minimum for ‘Mahalat’ and maximum for ‘Yarandi’. Freezing stress caused a significant reduction in the survival rate of the grass species. Most lawn grass cannot tolerate -15°C.
Indeed, with the increasing amount of cold, their survival rate decreased. At -16°C, plants of all grass species experienced 100% mortality. In general, the stability of the crown cell membrane was greater than that of the leaves. At a temperature of -13°C, the stability of the membrane of the crown was more than 60 percent. However, at temperatures exceeding -15°C, the index hit zero for both. There was a significant correlation (r = 0.98) between a lethal temperature of 50% (LT50) of plants with survival rate. Based on the LT50 index, Bermuda grasses and Poe proteins Meadgrass had the lowest cold tolerance.

Nezami et al. (2016) reported that by decreasing temperature, electrolyte leakage increased in rhizomes and stolons. At -20°C, electrolyte leakage was 50% more than the control (0°C) treatment. Moreover, at -12°C, leakage from stolons was 8% less than rhizomes. Studies showed that cold-sensitive plants or organs showed further amount of ion leakage from their cells. Additional leakage of material from rhizomes should be interpreted as this organ being more sensitive to freezing temperatures in comparison to stolons. The lowest and highest electrolyte leakage was observed in January and April, respectively. And the lowest and highest LT50 values occurred in February and April, respectively. It seems that due to the occurrence of cold hardening in both organs during the colder period of the year, the stability of membranes increased. Consequently, electrolyte leakage decreased. Stabilization of membranes in response to cold stress damage is a key role of cold hardening.

In addition, due to the occurrence of dehardening in samples during the warmer months of the year, freeze-tolerance levels of organs declined, which was based on LT50 values. LT50 values for stolons, which ranged from -8.4 to -14.5 ºC, were influenced by sampling date. For rhizomes, LT50 values ranged between -8.8 to -13.9 ºC. The three-way interaction of organs, temperature, and sampling date on electrolyte leakage was significant. The highest electrolyte leakage value occurred in stolons in April at -20°C. The lowest electrolyte leakage value was observed in this organ in December at -4°C. The highest electrolyte leakage occurred in April at -20 ºC. The lowest electrolyte leakage happened at 0°C in February. Measuring the cytoplasm electrolyte leakage (EL) or electrical conductivity (EC) is one of the most common methods to assess the level of cell damage by low temperature (Mirzai-Asl et al., 2002). Coursolle et al. (2000) used electrolyte leakage method for assessment of root-freezing damage of 2-year-old white spruce, black spruce, and jack pine seedlings. Tamura (2000), using this method to evaluate the freezing tolerance of komatsuna (Brassica campestris L.) and spinach (Spinacia oleracea L.), found that the freezing tolerance correlated well with electrolyte leakage from leaf tissues, especially with the leaf area that was damaged by freezing during cold acclimation. Mean comparison of quantitative and qualitative forage yield of superior lines compared with a total mean in cold region (Karaj) showed that were higher 50.66%, 52% and 51.33% of dry matter, protein yield and digestible protein yield respectively (Zamanian, 2006). In relation with Role of Persian clover early lines and Crimson clover in forage production in cold and water deficiency regions, reported that the five superior lines (from seed lots Hamedan, Arak and FAO), with a yield of about 57.5 - 71.25 ton ha-1 forage yield and 6.41-8.85 ton ha-1 dry matter yield had the highest forage production and it is recommended for regions of with cold and water deficiency (Zamanian, 2013).

Eco-physiological needs of clover

Clover adapts to a wide range of climatic conditions in the rainforest and has the highest distribution and cultivation in the north of 30 to 40ºC. The optimum temperature range of clover is 12 - 25ºC,
but temperatures range from 0 to 35°C. Clover cannot tolerate temperatures of less than 5 – 8°C and more than 40 degrees Centigrade. The base temperature (Tb) clover is 0-5 degrees (in some sources 10°C, Fig.-2) and its maximum temperature is 30°C (Taylor, 1985). Considering the importance and position of clover in forage production in Iran, it is essential to accurately determine its ecological needs in order to succeed in cultivating and minimizing the damage of abiotic stress. The occurrence of phonological stages in annual legumes depends on factors such as photoperiod, ambient temperature, weather conditions, and light. The growth day, photothermal index and thermal unit efficiency are the most important thermal indicators that are calculated based on temperature and daytime and they are used to predict the phonological stages and plant performance and determine the suitable areas for cultivation and agronomic operations (Bernier and Périlleuxm, 2005). Research shows that the thermal requirements of the growth stages and the production of forage and seed of Berseem clover and produce two cut forage are needed 93 days with 1563 GDD, and 120 days for seed production of 2000 GDD. Growth average of GDD indicated that in the Karaj-Iran region, for the production of three cut forage, 2437 GDD is needed. The highest heat requirement with 612.7 GDD is from the emergence stage to vegetative growth stage and the minimum requirement with 82.1 GDD from the emergence stage until the first simple leaf emerges. Comparison of clover species showed that the Red clover with 2999.9 GDD the highest, and Crimson clover with 1107.7 GDD the lowest had the heat requirement for forage production (Zamanian et al., 2012).

**Cold stress injury to clover agronomy**

Cold stress in the following ways causes injury to the clover agronomy:

1) Temperature fluctuations with freezing and melting ice, causing severe injury to the buds on the clover's crown in the field. This injury is higher in farms with lower drainage.
2) Reduced temperature and late consumption of chemical fertilizers, especially nitrate, increase the cold injury in clover cultivars non-tolerance.
3) The ice layers on the soil surface, soils saturated with water and not drained will cause injury to the clover.
4) Dew freezing at the beginning of the spring, especially in low-lying areas where air does not flow, will cause bud death that start re-growth.

**Indicators for selecting clover germplasm under cold stress conditions**

Cold stress is one of the slow-growing factors and stopping the growth of clover species in the spring and early spring. Also, factors such as plant species, growth stage, duration of freezing temperatures, soil moisture, soil type, freezing and successive melting, the presence of pathogens and pests complicate the determination of frost tolerance of plants (Brandsaeter et al., 2000). Generally, for selection of cold tolerance in clover germplasm can be used from one or characters combination of the following:

![Fig.- 2. Base temperature for some crops and pasture plants (T_b =10 C for Clover)](image)
1. Morphological traits

Fowler et al. (1981) presented a series of morphological traits related to cold tolerance at field condition, including leaf area, fresh and dry weight of leaves, fresh and dry weight of the crown, fresh and dry weight of seedling, water content of leaf and crown, relative growth rate, plant height, mesocotyl and hypocotyls length, root length, erect planting and shooting stage. Asghari et al. (2008) also have characteristics such as the number of leaves per plant in the rosette stage, the fresh and dry weight of the crown, the relative water content of crown, winter survival as indicators of cold stress tolerance. Roy and Basu (2009) reported that different plant organs have different reactions to low temperatures, so that the roots, rhizomes, and bulbs show a higher sensitivity to cold stress than plant aerial parts. Simon (1979) stated that low temperatures, in addition to reducing the germination of seedlings of crops, reduce seedling growth and accumulate dry matter in them. Mirmohammadi and Turkesh (2004) showed that decreasing root growth due to the decrease in temperature, caused by decreases in the capacity of absorption of water and minerals by the root, followed by the emergence of secondary effects due to food shortages and plant growth disturbance. The most important symptoms of cold injury were germination rate reduction, seedling growth stopping, leaf yellowing (Fig.-3), decreased leaf development, plant wilt, tissue death (necrosis), and increased chlorophyll fluorescence, and eventually decreased photosynthesis (Deborah et al., 2005). In the Berseem clover, the temperature of less than 0°C causes the color change of leaves and growth decreases, and extreme freezing will cause frostbite of the leaves. If before winter, a cut forage is harvested, its resistance to cold increases and cold injury to the plant decreases. In this case, the leaves are frozen and die, but freezing does not damage the root and grows rapidly with warming (Arakeri and Schmid, 1949).

2. The temperature that causes the loss of 50% of the plant (LT50)

The criterion of LT50 or temperature that causes the loss of 50% of plants under cold stress is reported as an index of cold and frost tolerance in crops and clover (Philley et al., 1998; Sofalian et al., 2006). The LT50 index is different in different organs of the forage crops, and this index is the highest in the crown and least in the root. As shown in Fig.4 the maximum LT50 index in the forage crops crown occurs around three days and in the leaf occurs around 5 days after the cold stress and has the same trend in the roots and it is not exposed to cold days.
Azizi et al. (2007) found a positive correlation between leaf area and seedling dry weight with the LT$_{50}$ method. The electrolyte leakage technique is one of these methods, which is simple, repeatable, inexpensive, relatively quick, and determines the degree of tissue membrane damage caused by the stress effect (Arvin and Donnelly, 2008). Freezing tolerance of whole seedlings was determined as reported previously (Perras and Sarhan, 1984) and expressed as the temperature required to kill 50% of the seedlings (LT$_{50}$ 2°C), as indicated after a 2-week re-growth period.

3. Electrolyte leakage (EL)

Roy and Basu (2009) increased in leakage of cellular solutions as a result of changes in membrane permeability was the first cold injury to the cell membrane. Therefore, membrane stability was used as an indicator for evaluating cold stress tolerance. Electrolyte leakage test from plant tissues has been used as a suitable method for evaluating membrane permeability in relation to the effects of environmental stresses such as cold on crops (Eugenia et al., 2003). Eugenia et al. (2003) reported that for determining the electrolyte leakage in young leaves of rose clover, two days after the freezing stress with -14°C is suitable. A study in Pennsylvania, USA, showed that the highest electrolyte leakage occurred in red clover at -8 ºC (Fig.- 5).

In this regard, researchers have reported that cold stress increases electrolyte leakage. Accordingly, this trait has been used as a criterion for the evaluation of freezing tolerance (Campos et al., 2003 and Uosofi, 2008). Also, the temperature causing 50% electrolyte leakage from plant cells is used as the 50% degradation temperature and the threshold of damage (Xuan et al., 2009 and Vanae et al., 2011). Acclimation capacity was determined for whole plant and electrolyte leakage assays using three different plant organs, stem, crown, and rhizome (Pietsch et al., 2009). Freezing tolerance of leaves, crowns, and roots were estimated by the electrolyte leakage method. Measuring the cytoplasm electrolyte leakage (EL) or electrical conductivity (EC) is one of the most common methods to assess the level of cell damage by low temperature (Mirzai-Asl et al., 2002). Tamura (2000), using this method to evaluate the freezing tolerance of komatsuna (Brassica campestris L.) and spinach (Spinacia oleracea L.), found that the freezing tolerance correlated well with electrolyte leakage from leaf tissues, especially with the leaf area that was damaged by freezing during cold acclimation. Yamada (1983) reported that electrical conductivity is an effective method for measuring cold tolerance in white clover seedlings.

4. Fat and fatty acids

Simon (1979) reported that cold stress reduces the unsaturated fatty acids of the membrane and loses fluidity and permeability of the membrane to water and, as a result, reduces the tolerance of the plant to cold. So, the higher the proportion of unsaturated fatty acids to saturated fatty acids, the lateral and lower cell membrane changes. Cold stress causes a change in the lipid composition of the membrane of the dual layer.
cells. Steinshamn et al. (2010) reported on the factors affecting the concentration of fatty acids in forage that growth stage, nutrition, planting method, growth period, cultivar had an effect on the amount of forage fatty acids, so that in Timothy grass the elongation stage of the stem before flowering reduced the amount of palmitic acid, linoleic acid, linolenic acid and total fatty acids by 15, 16, 31 and 23%, respectively. The results showed that consumption of 120 kg of nitrogen fertilizer increased 18% palmitic acid, 12 linoleic acids, 40 linolenic acid and 26% total fatty acids, while consuming 45 kg/ha of phosphorus fertilizer compared to control (non-consuming). With control (no consumption), the percentage of forage fat had no significant effect (Mirmohammadi and Turkesh, 2004). Comparison of harvest season showed that the concentration of fatty acids in Red clover and Alfalfa in the summer was 14.7% and 2.5% higher than spring season. Comparison of White clover and Alfalfa cultivars showed that White clover cultivars had the highest amount of linoleic acid (16 mg/g dry matter) and Alfalfa cultivars had the lowest amount (6 mg/g dry matter). The results showed that forage harvest in the pre-flowering stage was increased total fatty acids in the forage. The fat content in the leaf dry matter of forage crops to be 8% was reported. Most of the unsaturated fatty acids in forage freshly are Linoleic acid, Linolenic acid (18:3 n6), Linoleic acid (18:2n6) and Oleic acid (18:1 n9). A number of fatty acids in the stem of the forage plants are one-third to one-second of the fatty acids in the leaves, and therefore, the percentage and amount of leaf in forage crop has a great influence on a number of fatty acids in livestock products (Steinshamn et al., 2010). Comparison of pure and mixed cropping clover species showed that a number of fatty acids in the mix-cropping were higher than pure crop. The results showed that increasing the ratio of clover in mixed crop cultivation with Grass

Fig.- 6. Comparison of fat percentage, saturated and unsaturated fatty acids in dry matter yield of clover cultivars in spring and autumn (Zamanian, 2012)

caused decreases a number of fatty acids (Steinshamn et al., 2010). The results of autumn and spring studies showed that there is a significant difference in the percentage of fatty acids in clover cultivars, and the amount of these acids in the spring is due to cold more than spring (Zamanian et al., 2012; Fig.-6).

5. Cellular Pigment

Increasing the level of tocoferols in the leaves or increasing the levels of lesser pigments such as carotenoids, which are preferably oxidized at cold temperatures and lead to a decrease in chlorophyll a and b losses, may cause the plant to tolerate cold and prevent cold injury (Abbasi et al., 2007). Cold stress has an irreversible effect on chlorophyll and causes destruction and degradation. Cold causes disturbance in the production of chlorophyll and decomposition of chloroplast and chlorophyll degradation due to optical oxidation in sensitive plants. The cold causes the disturbance in the Thylakoid membranes and decreases the production of chlorophyll and yellowish green leaves (Peterson et al., 1993). Ma et al. (1995) stated that between photosynthesis leaf intensity and chlorophyll content have the relations, so measurement of chlorophyll can be a criterion for measuring the intensity of photosynthesis. Zamanian (2017) from study relation-
ship between the reduction of ambient temperature with chlorophyll content and Fv/Fm, reported that Low-temperature stress induced a significant reduction in chlorophyll content in clover species. Among clover species, the species of Red clover (Nassim variety) had the least amount of chlorophyll. Also, low-temperature stress in the field caused more decrease in the amount of chlorophyll a to chlorophyll a+b, a/b, and chlorophyll b.

6. Sugar content in the root and crown

In most studies, plant survival and re-growth after cold and frost stress can be one of the important indicators in evaluating cold resistance in plants. According to many researchers, the amount of carbohydrate storage in the root and crown plays a key role in the regrowth of forage crops (Fig.-7), and the amount of carbohydrate storage can be affected by cold stress and low temperatures (Volence et al., 1991; Henderson and Volence, 1993). Matsumoto and Sato (1983), Shibata and Shimada (1986) and Tronsmo (1993) reported the relationship between WSC (Water Soluble carbohydrate) and cold and frost tolerance in grasses. Several studies have shown that high levels of NSC in winter increase the survival and survival of forage crops such as clover at low temperatures (Fry et al., 1993; Ball et al., 2002; Shabba et al., 2003). Guadet et al. (1999) reported that soluble sugars, especially the amount of sucrose and raffinose in the root, are among the determinants of cold tolerance and winter survival for alfalfa.

Zamanian et al. (2012) showed that between clover cultivars at growth stages were significant differences and the amount of carbohydrate storage in the roots of clover has greatly on the winter cold tolerance of cultivars. The amount of carbohydrate in the root with grows to the maximum, with the harvesting of plants and the beginning of re-growth, the level of carbohydrates to decrease (Fig.-8). Sanada et al. (2007) reported that cold resistance in Cocksfoot ecotypes depends largely on the amount of fructose sugar in these plants in autumn, Sanada et al. (2010) reported that the amount of water soluble carbohydrate (WSC) in the Orchardgrass (Dactylis glomerata L.) crown is one of the important criteria for resistance to cold.

7. Fluorescence of chlorophyll leaf

In clover of fluorescence, the rate is varied in different levels of radiation and nutrition. The study of the use of chlorophyll fluorescence components in the selection of some tolerant clover species in Karaj, Iran showed that there is a significant difference between leaf chlorophyll fluorescence components (0.597-0.755) and physiological traits at low temperatures in clover species. The Fv/Fm component showed a low-temperature stress on clover cultivars has been effective, because caused injury to the electron transport chain and increased heat dissipation and decreased photochemical absorption of electrons (Fig.- 9). The maximum amount of Fv/Fm was related to Persian clover and at least it was related to Red clover and in from those of view, the most tolerant and sensitive species were evaluated. The results showed that chlorophyll content had the most significant correlation with leaf chlorophyll fluorescence components in dark conditions. Among the components of chlorophyll fluorescence, the maximum quantum function of the
photosystem II (Fv/Fm) and the maximum physiological traits, electrolyte leakage trait (EL) and chlorophyll number (CCI) are the best criteria for selection of low tolerant clover cultivars (Table -1, Zamanian et al., 2012).

8. Scoring of cold injury in field conditions
To determine the cold injury, we can use the plant injury caused by the appearance of the plant and the level of surface coverage of the plant in end of October to mid-November and mid-April to mid-May. To estimate the amount of cold injury in field conditions, a scoring method is usually used, so that score 1 indicates non injury to the cold and the score of 9 indicates the maximum injury to the cold and the

Table – 1. Mean of chlorophyll fluorescence attributes, electrolyte leakage (EL) and SPAD value (CCI) in clover genotypes under low temperature conditions in Karaj-IRAN (Zamanian et al., 2012)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Code of genotype</th>
<th>Physiological characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F₀</td>
</tr>
<tr>
<td><em>Trifolium resupinatum</em> L.</td>
<td>KPC-PL1</td>
<td>88.3a</td>
</tr>
<tr>
<td><em>Trifolium resupinatum</em> L.</td>
<td>KPC-PM</td>
<td>87.5a</td>
</tr>
<tr>
<td><em>Trifolium resupinatum</em> L.</td>
<td>KPC-PE1</td>
<td>84.4a</td>
</tr>
<tr>
<td><em>Trifolium resupinatum</em> L.</td>
<td>KPC-PE2</td>
<td>79.8a</td>
</tr>
<tr>
<td><em>Trifolium resupinatum</em> L.</td>
<td>KPC-L13</td>
<td>82.1a</td>
</tr>
<tr>
<td><em>Trifolium resupinatum</em> L.</td>
<td>KPC-L17</td>
<td>82.6a</td>
</tr>
<tr>
<td><em>Trifolium resupinatum</em> L.</td>
<td>KPC-PL2</td>
<td>78.5ab</td>
</tr>
<tr>
<td><em>Trifolium alexandrinum</em> L.</td>
<td>KBC-Toli.K</td>
<td>69.2b</td>
</tr>
<tr>
<td><em>Trifolium pratense</em> L.</td>
<td>Nasim</td>
<td>88.3a</td>
</tr>
<tr>
<td><em>Trifolium incarnatum</em> L.</td>
<td>Alborz 1</td>
<td>80.2ab</td>
</tr>
</tbody>
</table>

In each column, means followed by same letter are not significantly different at the 5% of probability level- using Duncan s Multiple Range Test.
plant's death. The most important symptoms of cold injury were germination reduction, seedling stall cessation, leaves yellowing (chlorosis), reduced leaf development, plant wilt, tissue death (necrosis), and increased chlorophyll leaf fluorescence, and eventually reduced photosynthesis (Deborah et al., 2005). Eugenia et al. (2003) the Survival Rate of Clover plants of after cold stress in field condition is the best method for selecting clover germplasm for cold tolerance (Fig.-10).

Conclusions and future strategies

In general, clover cultivation in the autumn is limited by cold stresses, which include low temperatures and freezing temperatures. Therefore, identification and investigation of crop and wild germplasm of clover under cold stress conditions can be effective in the advancement of breeding goals and their selection for cultivation in cold regions. To achieve these goals, there are fast, low-cost, and repeatable methods that, in addition to being precise, do not have restrictions on field cultivation. In order to evaluate clover germplasm under cold stress conditions, in addition to field studies, the use of indicators such as quantum function of photosystem II (Fm/Fv) and chlorophyll content in the growth stage of the first stage of emergence of the first three leaflets to the feeding, electrolyte leak test (EL), chlorophyll content, root and brown sugar in autumn (cold before) and early spring (cold after), survival rate and re-growth in March are recommended, and these indices can be used in field screen of tolerant clover cultivars.

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References


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