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Full Length Research Paper

Effect of cold stress on cell membrane stability, chlorophyll *a* and *b* contain and proline accumulation in wheat *(Triticum aiestivum* L.*)* variety

Habibi, F[1,5], Normahamadi, GH[2], Heidary Sharif Abad, H[2], Eivazi, A[3] and Majidi Heravan E[4]

[1]Department of Agronomy, Science and Research Branch, Islamic Azad University,Tehran, Iran.
[2]Department of Agronomy, Science and Research Branch, Islamic Azad University,Tehran, Iran.
[3]Research Center for Agriculture and Natural Resources Research Station in West Azerbaijan province, Iran.
[4]Biotechnology Center, Karaj – Iran.
[5]Second floor, no 5, koye 36, nobovat street, Oromie, Iran.

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The aim of this research was to determine the effect of cold stress on chlorophyll content, proline accumulation and the cell membrane stability of 15 bread wheat (*Triticum aestivum* L.) at two stage of growth (2 leaf stage and 4 leaf stage). Experiment was carried out in complete block design with tree replications at year 2009 in west Azerbaijan Agriculture science and research center laboratory. 15 cultivar (five genotypes with types winters names Sayson, Martin, Gaskoghen, C-82-12, C-81-14, 5 genotype with type intermediate including Alvand, Mahdavi, Zarrin, Marvdasht, Toos and five genotypes with type spring names Shiraz, Pishtaz, M-79-7, M-81-13 and Kavir) were sown in lab condition . According to the results, cold stress had a significant effect on chlorophyll *a* and *b*, total chlorophyll, prolin content and cell membrane stability. At the other hand variety and growing stage have significant effect on treats (P>0.01). The result showed that the M-81-13 with 53.05 mΩ EC has the highest cell membrane stability and the lowest EC was obtaining from gaskoghen with 18.987 mΩ. Also M-79-7 with 2.315 mg chlorophyll *a* per 1 g leaf fresh weight has highest chlorophyll content. It was determining the ratio of maximal chlorophyll content after cold stress is reliable and a method to determine the frost hardness of wheat variety. The proline content of the leaf however increased in both growth stages in all variety of wheat. Gaskoghen with 0.340 (µg/0.5 g dwt) in 2 leaf growing stage had the highest rate of proline amino acid and Marvdasht variety from inter mediate type in 4 leaf growing stage with 0.003 (µg/0.5 g dwt) had the lowest rate of proline

Key words: Wheat variety, chlorophyll content and proline.

INTRODUCTION

Low temperature constitutes one of the major hazards to agriculture and is an important factor that limits the survival, productivity and geographical distribution of plants in large area of the world (Boyer, 1982). Exposure to low non- lethal temperature usually induct a variety of biochemical, physiological and enzymatic change in plant, which can result in an acclimation response that is characterized by a greater ability to resist injury or survive an otherwise lethal low temperature stress (Howarth et al., 1993; Hughes et al., 1996). The major malicious effect of freezing is that it induces severe membrane damage (Steponkus et al., 1984, 1993). This damage is largely due to the acute dehydration associated with freezing (Steponkus et al., 1993). Overall, cold acclimation results in protection and stabilization of the integrity of cellular membrane, enhancement of the anti oxidative mechanisms, increase intercellular sugar levels,

*Corresponding author. E-mail: f.h1356@gmail.com. Tel: +984413460246, +989143468362.

Table 1. Growth type of common wheat in Iran.

	Growth type		
	Winter type	**Inter mediate type**	**Spring type**
Wheat variety	Sayson	Alvand	Shiraz
	MV17	Mahdavi	Pishtaz
	Gaskogen	Zarrin	M-79-7
	C-81-14	Marvdasht	M-81-13
	C-81-12	Tous	Kavir

as well as accumulation of other cryoprotectants including polyamine that protect the inter-cellular proteins by inducting the genes encoding molecular chaperones (Jones et al., 1989). Chlorophyll a and b contained in leaves of higher plants are the main pigments of photosynthesis in the chloroplast, and have important function in the absorption and exploitation of the light energy, thereby influence photosynthetic efficiency (Pan and dong, 1995). Some study has demonstrated that chlorophyll content is positively correlated with photosynthetic rate (Thomas et al., 2005). Increasing the chlorophyll content in crops may be an effective way to increase biomass production and grain yield (wang et al., 2008). Mutagenesis study result in the identification of a gene, *Eskimo 1* (*esk 1*), which has a major effect on freezing tolerance. These plants were more freeze tolerance than the wild type plants without cold acclimation. The concentration of free proline (Xin et al., 1998) in the esk 1 mutant was found to be 30-fold higher than in the wild – type plants. Proline has been shown to be an effective cryoprotectant and this is also on of the major factors impairing freezing tolerance. This study has been designed to examine the role of proline, cell membrane stability and chlorophyll content in cold tolerance of wheat *(Triticum aiestivum* L.*)*.

MATERIALS AND METHODS

The experiment was carried out with 15 wheat cultivar and genotype and two growing stage (2 and 4 leaf stage), the first five genotypes of wheat were winter type (Sayson, Martin, Gaskoghen, C-82-12, C-81-14), second five genotype were intermediate type (Alvand, Mahdavi, Zarrin, Marvdasht, Tous) and the last five genotypes were spring type (Shiraz, Pishtaz, M-79-7, M-81-13 and Kavir) (Table 1).

Seed of these varieties were obtained from the west Azerbaijan Agriculture and Nature research center gene bank. Seed of the wheat variety were sown in 1 m^2 woody plot and growth in controlled condition at two growing stage (2 and 4 leaf stage and -25℃ and 10 hours photo period). The temperature was gradually reduced with a cooling rate of 5℃ per day to -25 and held for 24 h. Chlorophyll were measured in the laboratory. Photosynthetic pigment (chlorophyll *a* and *b)* were measured in fresh leaf sample after they were obtained from the growth chamber. One plant per replication was used for chlorophyll determination. Prior extraction, fresh leaf sample were cleaned with dionized water to remove any surface contamination. Leaf sample (1 g) were homogenized with acetone (80% v/v), filtered and make up to final volume of 5 ml. Then the solution was centrifuged for 10 min at 3000 rpm. Pigment concentration were calculated from the absorbance of extract at 663 and 645 nm using the formula (Ashraf et al., 1994; Arnon, 19775):

$$[Chl\ b]= 22.90.E^{645} -4.68.E^{663} \quad (1)$$
$$[Chl\ a]=12.70.E^{663}-2.69.E^{645} \quad (2)$$

Free proline accumulation was determine using the method of Bates et al. (1973): 0.5 g dry weight of leaves was homogenized with 3% sulfosalsylic acid and after 72 h, the praline was released, the homogenous sample was centrifuged at 3000 rpm for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 h and then absorbance was determined at 520 nm by UV-Visible spectrophotometer (jenway 6105). A conductivity test was determined using the method of Dexter (1932) and Blume (1982). Statistical analysis of the data was done using randomized complete block design. The average of attendance was calculated base on Duncan method at 1% probability level.

RESULTS AND DISCUSSION

In the present study, results of the analysis of variance show that there was significant difference between the threat on growing stage, ion leakage (membrane stability), *chlorophyll la*, and *b*, total chlorophyll and proline content. Interactions of growing stage and variety level were also significant (p<0.01) (Table 2).Comparison figures for the trait showed that the highest ion leakage (low membrane stability) among the threat groups was obtained from M - 81-13 (53.05 mΩ) on the spring type followed by zarrin, Marvdasht and kavir, respectively with 43.90, 42.20 and 40.06 mΩ of ion leakage with the intermediate type. The lowest ion leakage (high membrane stability) was observed for gaskogens (18.97 mΩ) among the cultivar and genotype, indicating minimal damage to cell membranes due to cold stress and it has been the most resistant variety (Figure 1).

It was observed that the low temperature (-25℃) inducted a significant decreases in the content of pigment fractions (chlorophyll a and b) as a result of the total chlorophyll content of the leaves. There were significant differences among interaction of growing stage with variety (p< 0.01) (Table 2). The highest chlorophyll *a* were obtained from Marvdasht variety with 2.315 mg chlorophyll *a* \ 1 g leaf fresh weight in 2 leaf growing stage, m-79-7, and toos variety with 2.409 and 2.304

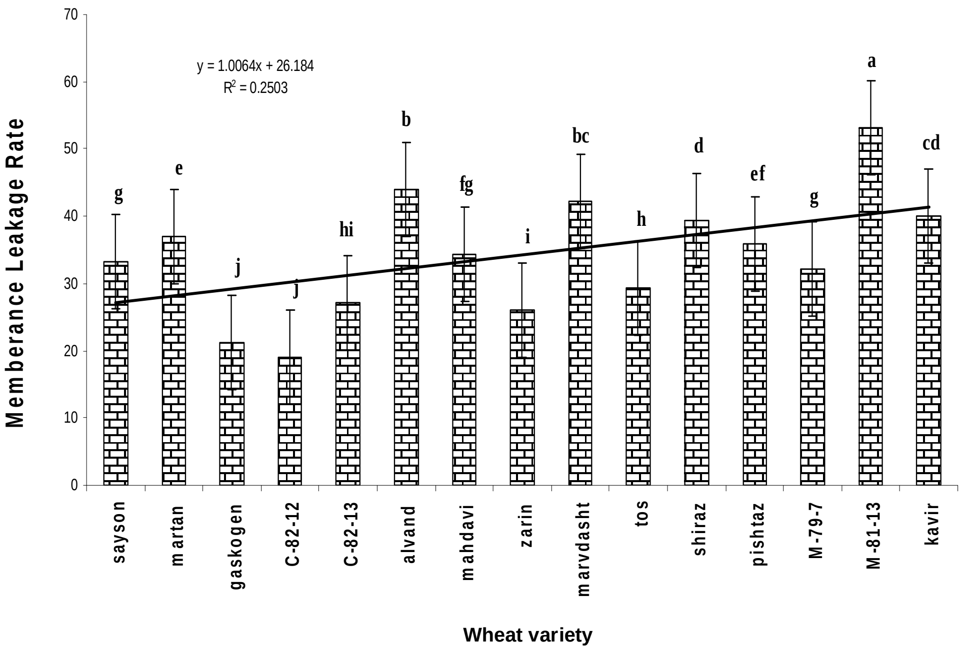

Figure 1. Means of cultivar membrane leakage (cell wall stability) 15 wheat cultivar.

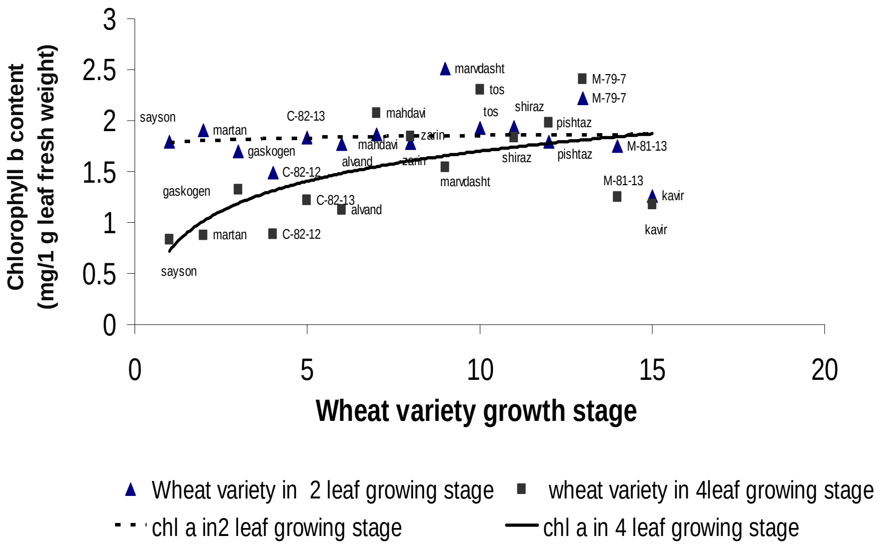

Figure 2. Means of chlorophyll a content in 15 wheat cultivar at two growing stage under lab.

(mg\1 g leaf fresh weight) respectively, in the middle category at the two leaf growing stage, and as such, sayson variety in 4 leaf growing stage had the lowest chlorophyll *a* content (Figure 2). When the averages of the results were compared, a direct linear correlation between chlorophyll *a* content of cultivars and their growth type in different growing stage was observed.

Winter type's wheat (Sayson, Martin, Gaskoghen, C-82-12, C-81-13) at 4 leaf growing stage showed a less chlorophyll *a* content and when compared with two leaf growing stage, showed increase in the amount of chlorophyll *a* (Figure 2).

From the result, it is estimated that the interactive effect of variety and growing stage on chlorophyll *b* content were significant ($p<0.01$)(Table 2).Toos variety as the intermediate type had the highest chlorophyll *b* content in 4 leaf growing stage with 3.477 (mg \1 g leaf fresh weight), the lowest chlorophyll *b* content was related to

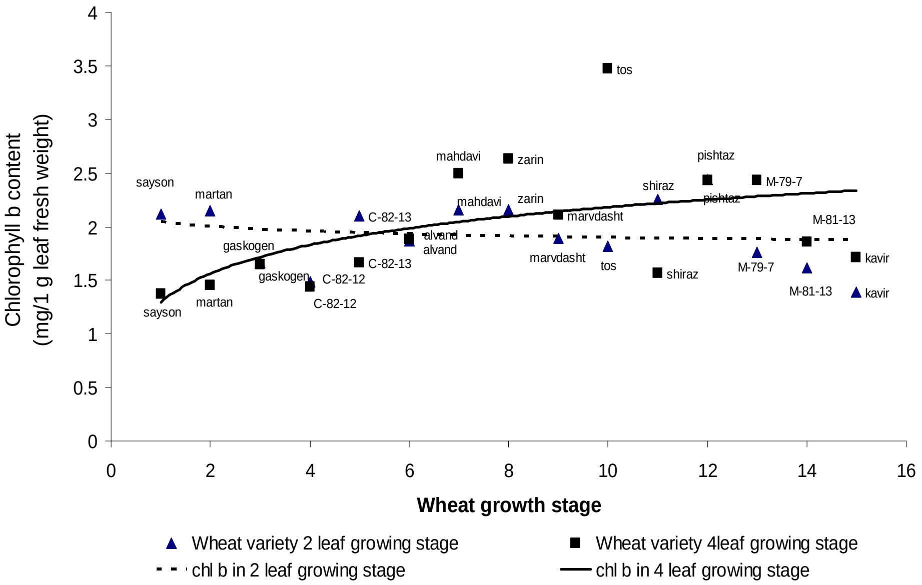

Figure 3. Means of chlorophyll b content in 15 wheat cultivar at two growing stage under lab conditions.

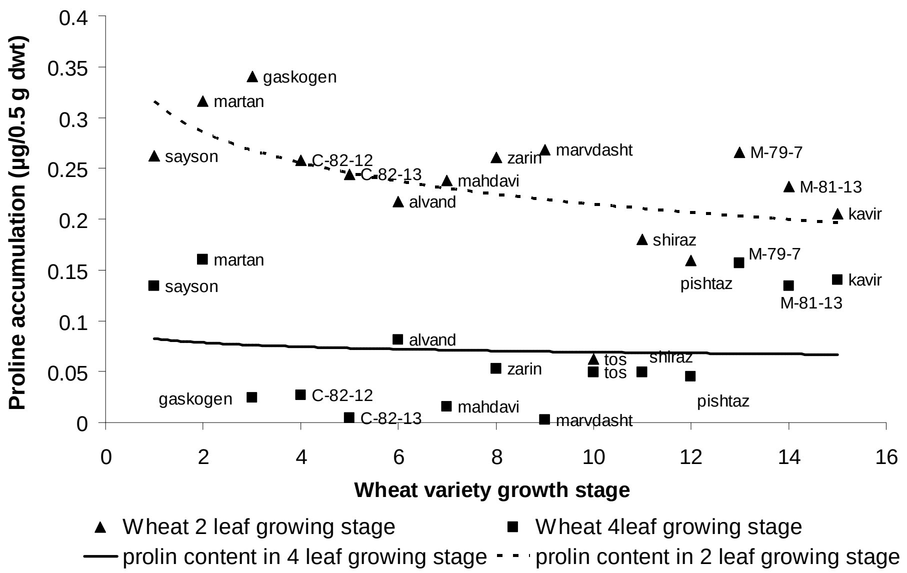

Figure 4. Means of proline amino acid content in 15 wheat cultivar at two growing stage under lab conditions.

the sayson and C-82-12, respectively, with 1.377 and 1.392 (mg\1 g leaf fresh weight) (Figure 3).

The proline content of the leaf however increased at both growth stages in all variety of wheat. The increase in proline content due to cold stress was observed more at 2 leaf growing stage. Gaskoghen with 0.340 (µg/0.5 g dwt) in 2 leaf growing stage had the highest rate of proline amino acid and Marvdasht variety from intermediate type in 4 leaf growing stage with 0.003 (µgr/0.5 g dwt) had the lowest rate of proline (Figure 4).

Conclusion

Cell membrane stability

Most varieties of spring type showed the height degree of ion leakage than the other types and appear to be sensitive to cold stress (Table 1). Yamada et al. (2004) worked on Rye grass plants in order to measure the ion leakage under cold stress. The result showed that the genotypes with high ion leakage were sensitive to the

Table 2. Analysis of variance for traits of Wheat in cold stress under controlled condition.

S.O.V	MS					
	df	Chl a	Chl b	EC	Total Chl	Proline content
Replication	2	0.189	0.080	133.467	0.04	0.003
Growth stage (A)	1	2.349**	0.167*	4032.251**	0.797**	0.695**
Variety (B)	14	0.733**	0.722**	485.551**	0.462**	0.011**
A*B	14	0.341**	0.573**	644.577**	0.708**	0.011**
Error	58	0.026	0.030	2.341	0.091	0.02
Total E	89					
CV%		9.58	8.79	4.47	16.69	27.14

n, * and **: No significant and significant at the 5 and 1% levels of probability, respectively.

cold stress and results were quite consistent. In other investigation on bean seedlings (Chanq et al., 1994), it was reported that the leakage observed in less tolerant cultivars was more than that observed in tolerant cultivar and in minerals like potassium and ion which dripped from cell membrane under cold stress in spring type varieties. This is due to the electrical conductivity increases in the destruction of cell wall contents as a result of the environmental stress.

Chlorophyll content

Low temperature is one of the most important factors that may limit photosynthetic activity. Decrease of photosynthesis inducted by low temperature is a well known response of chilling- sensitive plants (Yadegari et al., 2007). It has been reported that chlorophyll *a* and *b* content decreased in plant when subjected to cold stress (Yadegari et al., 2007). These observed decline of chlorophyll content inducted by low tempreture confirm the study's result. Relative chlorophyll content of the cultivars decreased in 4 leaf growing stage under cold stress condition compared to 2 leaf growing stage. The decrease of chlorophyll *a* and *b* ratio at first in developmental stage in wheat after freezing indicated a change of photosystem II reaction center from functional to down regulated nonfunctional ones (Krause, 1988). It represents a disturbance of photosynthetic performance, since photochemical capacity was empirically correlated with quantum yield of photosystem II (Adams et al., 1990). However, the decrease of photochemical capacity does not necessary imply a direct rupture of the thylakoid membrane after freezing. As shown earlier, thylakoid membrane is not the primary site of freezing injury (Sundbum et al., 1982; Adam and perkins, 1993). Cold acclimation is a complex process involving a number biochemical and physiological change associated with the accumulation of sugars, several types of proteins and amino acids and other products of altered metabolism (Pienedo et al., 2000; Nagao et al., 2005).

Proline accumulation

The accumulation of prolin increased in young leaf at 2 leaf growing stage and was down regulated with the increase in growing stage (from 2 leaves to 4 leaves) (Figure 4). In those variety which had high level of proline for example Gaskoghen and Martan, proline amino acid was found in young leaves in most environmental stresses. Like other productive units of the plant, protein (in the course of stress) converts sugar in the strength of plant tissues. This increases the maintenance of these tissues and reduces the stress damages. The accumulation of proline seems to be associated with adaptation to temperature stress (Figure 4). Free proline concentration increased under low temperatures (Wang et al., 2008; Tamizi et al., 1995) and proline could serve as the stress indicator in plant exposed to these unfavorable growth conditions. It was found that the generative part of plants contained considerably lower concentration of proline than the vegetative parts. The level of amino acids, especially proline, increased during cold acclimation at the beginning of growing stage, this is associated with frost tolerance in several species (Hommo, 1994). These observations are in line with the result of this study. This fact offers a possibility for improving frost tolerance in common wheat by selecting "high proline plant" by means of *in vitro* technique, as successfully demonstrated (Tantu and Dorffling, 1991; Dorfling et al., 1993, 1994). Prolin accumulation may also be a part of the stress signal influencing adaptive response (Maggio et al., 2002)

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