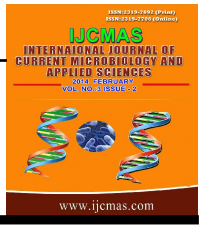
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 2 (2014) pp. 591-595**
http://www.ijcmas.com



Original Research Article

Effects of cold stress on proline and soluble carbohydrates in two chickpea cultivars

Siamak Saghfi[1*] and Ali Reza Eivazi[2]

[1]Department of plant physiology, Baku State University, Baku, Azerbaijan
[2]Agricultural Research Center, West Azerbaijan,Urmia, Iran
Corresponding author

A B S T R A C T

Keywords

Cold stress; chickpea; Proline; soluble carbohydrates.

The present study evaluates the mechanism of cold stress tolerance in two chickpea cultivars of Flip 93-174 (resistant) and Flip92-169 (susceptible) using a completely randomized factorial experiment with 3 replications. Results of variance analysis showed that Proline increased in leaves exposed to cold stress in both cultivars during the treatment period. This increase was more prominent for the resistant cultivar as compared to the susceptible cultivar .The same holds for leaf soluble carbohydrates, Glucose, Ramnose, mannose and Fructan for the resistant cultivar.

Introduction

The growing number of populations and high expenses of animal protein, together with lower level of cereal protein (9-12%), have drawn public attention towards using grains as a source of human protein (Paul et al., 1991). Grains are the most important source of nutrient protein, after cereals, in human. Grains are essential for food provision of human beings. They are planted all over the world and different crop species of them are compatible with different climates. Grains are specially favored in Africa (Navascortes et al., 1998). Grain beans with 18-32% protein play an essential role in providing human protein needs (Mcrae et al., 2005). Chickpea are especially important for human nutrition, for their high level of plant protein. Biological value of this protein is for its essential amino acid content, especially Lysine. Amino acids like Leucine, Arginine, and Methionine are found abundantly in grains, compared to cereals and even meat (Boyer, 1982). Studies indicate that a proper combination of grains protein and cereals can help eliminate malnutrition and lack of amino acids (Muehlbauer, 1993). The level of protein in gain seeds is two or three times more than starch and glandular plants. Moreover, grains are rich with calcium, iron and contain little amounts of Carotene, Riboflavin, Ascorbic acid, Niacin (Zhu, 2007). On the other hand, low temperature is a non-living factor restricting growth, production and

dispersion of plants. Most plants are exposed to temperature changes, including cyclical and seasonal changes, in their range of natural growth habits, which may restrict their respiration, photosynthesis and growth (Dubois et al., 1956). Low temperature reduces biosynthesis activity of plants and prevents their natural physiological processes and may cause permanent damages leading finally to death (Galiba et al., 1994). Therefore, effects of cold stress on plant life are studiedand attempts are directed towards increasing tolerance to cold in important crop plants. An important feature of plants in stress conditions is the increase of carbohydrate accumulation.

Carbohydrates increase inter-cellular concentration and prevent water loss due to cold stress (Mcvicar et al., 2005). Increase in freezing tolerance during cold compatibility period is due to storage of soluble sugar in plants (Miguelezfrade et al., 2005). Glucose, Fructose, Ramnose, Mannose, Raffinose, Fructanare some common soluble carbohydrates in the process of tolerance to cold in organic plants. In addition to preserving osmotic pressure inside the cells, these sugars, through binding to two-layer lipid membrane, protect cellular membrane from damages arising from water loss, freezing and phosphorylation of lipid membranes (Yuanyuan et al., 2009). In other words, any increase in accumulation of soluble sugars in the cell, promotes membrane stability against cold. Membrane stability is a prerequisite for making a cell resistant to freezing.

Another effect of soluble sugars is their acting as a nutritional substance which makes plants survive in low temperatures. The point is that, accumulation of sugars in cold conditions, unlike what occurs in normal situations, doesn't reduce photosynthetic activity. Thus, activation of compatibility path to cold decreases plant sensitivity to sugar accumulation and doesn't prevent photosynthetic activity. This prolongs the process of cellular senescence and cell death (Owies et al., 2004). On the other side, increase in Proline accumulation leads to increase tolerance to higher levels of cold stress (Maller et al., 2002). Therefore, measuring Proline and soluble carbohydrates of the leaves during cold treatment period is of high importance for identifying their roles in cold tolerance. The present study investigates physiological responses of two chickpea cultivars Flip 93-174 (resistant to cold) and Flip 92-169 (susceptible to cold) to short-term cold stress and the effectiveness of Proline and soluble carbohydrates in invoking tolerance to cold stress in chickpea.

Materials and Methods

Growth conditions and cold treatment: 10 seeds of chickpea were planted in prepared pots in a growth chamber at 22° C. In the 4-leaf stage, half of the pots were transferred to a similar growth chamber with 3° C. The samples were gathered on days 2, 4, and 7.

Measurement of Proline

0.2g leaf tissue was rubbed in 3.3% Sulfosalicylic 10 ml and the resulting homogenies were then passed through a filter paper. The extract was centrifuged for 10 min at 4° C in the 4000rpm centrifuge machine (2000*g). The upper part was removed and 2 ml reagent nine hydrine and 2 ml glacial ascetic acid were added to 2 ml extracts in capped test tubes and was kept in water bath for about an hour. 4 ml Toluene was added to each tube

and mixed completely. When two separate phases were formed, the upper phase of Toluene containing Proline amino acid was read at a wavelength of 520 nm (Ozdemir and Karadavut, 2003).

Measurement of foliar soluble carbohydrates

0.2 g of the sample was rubbed by 2 ml phosphate sodium buffer (PH=7). The resulting homogenies were centrifuged for 20 min in 10000 rpm (13000*g). Then, 10µl of the supernatant was taken and 990µl distilled water was added to it. After the color was stabilized, it was kept for 10-15 min at 27-30° C to measure Ramnose, Glucose and mannose. Sample attractions were read at 480, 485, 490 nm wavelengths (Galiba, 1994).

Measurement of Fructan

0.2 g frozen tissue was rubbed by 3 µl of phosphate sodium buffer 50 µl and the resulting homogenies were passed through a filter paper. 1ml of the solution was mixed with 5ml of Anthrone 0.02% in 70% Sulfuric acid. It was, then, kept in water bath 100° C for 7.5 min. after cooling, sample attractions were read at a wavelength 625nm (Mcvicar, et al., 2005).

Statistical methods of the analysis

Data were examined using a factorial experiment with a completely randomized plot with three replications by the use of Minitab14 software. The first factor was two cultivars of chickpea (Flip 93-174, Flip 92-169), the second factor was cold treatment at two levels (3-22° C) and the third factor was sampling time at three levels (days 2, 4 , and 7) after transference to cold growth chamber at 3° C.

Results and Discussion

Results of variance analysis showed that the two cultivars under study were significant at 1% considering Proline and foliar soluble carbohydrates, Glucose, Ramnose, Mannose and Fructan. The level of Proline during cold treatment period shows linear increase in the susceptible cultivar of chickpea (Flip 92-169). Also, increasing the period of cold treatment leads to linear increase of the level of Proline in the resistant cultivar (Flip 93-174) and the highest level of accumulation observed on day 7 of sampling was three times more than the control plants. The level of Proline in the resistant cultivar (Flip 93-174) on days 2, 4, and 7 increased to 0.8, 2.6, and 3.1 respectively. But the level of Proline accumulation on day 7 was more in Flip 93-174 cultivar than Flip 92-169 cultivar (Fig1).

Evaluation of the level of Glucose, Ramnose and Mannose in Flip 92-169 cultivar shows a sudden increase of these sugars at the beginning of the cold stress period and their high maintenance up to day 6, which decreases on day 7. It is assumed that increasing the period of stress treatment in the susceptible cultivar (Flip 92-169) and decreasing chlorophyll ad energy requirement, the plant provide sits needed energy by using the stored sugar (10). However, increasing the period of stress treatment in the resistant cultivar (Flip 93-174) leads to linear increase of Glucose, Ramnose, and Mannose.Any increase in the period leads to increase the level of Fructan in both cultivars. But, maximum level of Frucatn accumulation on day 7 of sampling in the susceptible cultivar was 62% and maximum level of Fructan accumulation on day 4 of sampling in the resistant cultivar was 43%, compared to control plants (Fig2).

Fig.1 Effect of cold stress on proline levels in both tolerant and Sensitive cultivars of Chickpea

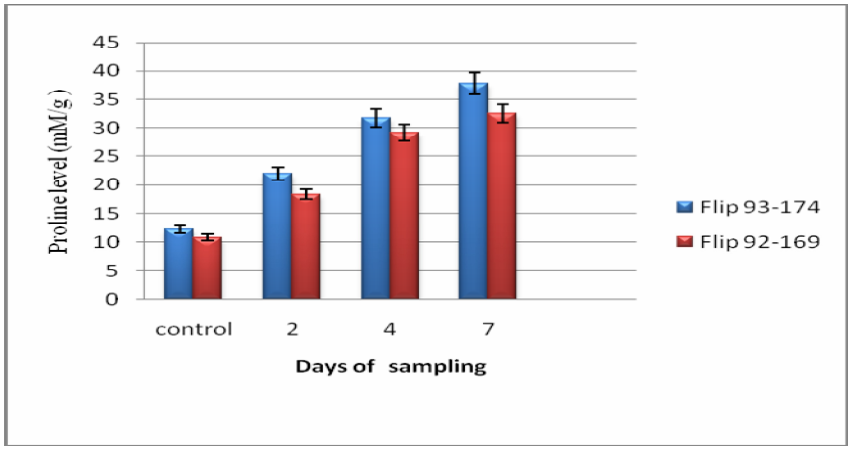
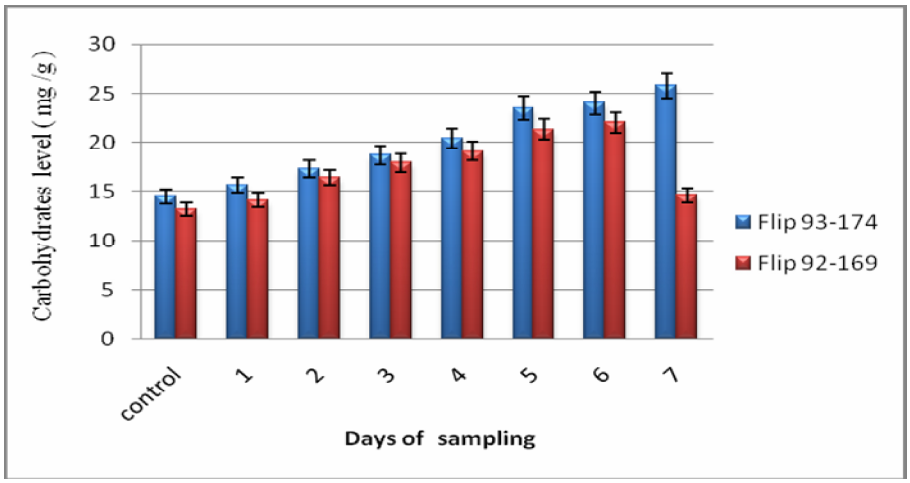


Fig.2 Effect of cold stress on soluble carbohydrates levels in both tolerant and sensitive cultivars of chickpea



References

Boyer, J. S, Plant productivity and environment //Science. 218. 1982.p .443- 448.

Dubois, M.,Hamilton, J. K., Rebes, P. A. and Smith, F. Colorimetric method for determination of sugars and related substrates//Analytical Chemistry, 28.1956.p.350-356.

Galiba, G. In vitro adaptation for drought and cold hardiness in wheat// Plant Breeding Reviews, 1994.p.115- 162.

Mcrae, F. J., D. W. Mccaffry and P. W. Matthews. Wintre crop variety sowing guide. State of south wales. NSW Department of Primary Industries. 2005.p.84-86.

Mcvicar, R.,S. Hartley and D. Goodwillie. Chickpea in Saskachewan. Saskachewan Agriculture and food. http:// www. Agr. Gov. Sk. Ca/ docs/ crops/ pulses/ production- information/ chickpea. 2005.

Miguelezfrade, M. M and J. B. Valenciano. Effect of sowing density

on the yield and yield components of spring-sown irrigated chickpea (*Cicer arietinum L.*) grown in Spain. New Zealand Journal of crop and Horticultural Science. 2005.p.367-371.

Maller, P. R., K. N. Mckay, and B. A. Jenks. Growing chickpea in the northern great plains. Montana State University. 2002.p.134-137.

Muehlbauer. F. J. Food and grain legumes.. In Janick. J. E. Simon (eds.). New crops Wiley. New York. 1993.p.256-265.

Navascortes, J. A., B. Hau, and R. M. Jimenezdiaz. Effect of sowing date, host cultivar, and race of *Fusarum oxysporum* F. sp. Ciceris on development of Fusarium wilt of chickpea. Phytopathology. 1998.p.1338-1346.

Owies, T., A. Hachum and M. Pala. Water use efficiency of winter-sown chickpea under supplement irrigation in a Mediterranean environment. Agricultural water Management. 2004.p.163-179.

Ozdemir, S. and U. K. Karadavut. Comparison of the performance of autumn and spring sowing of chickpea in a temperate region. Turkey Journal of Agriculture. 2003.p. 345-352.

Paul, M. J., Driscoll, S.P. and Lawlor, D. W. The effect of cooling on photosynthesis, amounts of carbohydrate and assimilate export in sunflower// Journal of Experimental Botany, 42. 1991.p.845- 852.

Yuanyuan, M., Yali, Z., Jiang, L. and Hongbo, S. Roles of plant soluble sugars and their responses to plant cold stress// African Journal of Biotechnology, 8. 2009.p.145-153.

Zhu, J. Dong, C. H. and Zhu, J. K. Interplay between cold-responsive gene regulation, metabolism and RNA processing during plant cold acclimation// Current Opinion in Plant Biology, 10. 2007.p. 290-295.