ISSN 2320-5407 International Journal of Advanced Research (2014), Volume 2, Issue 10, 301-309

ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of drought stress on proline accumulation in peanut genotypes

Jayant K Solanki and S K Sarangi
Department of Microbiology and Biotechnology, Bangalore University, Bangalore – 560 056, India.

Manuscript Info

Manuscript History:

Received: 12 August 2014
Final Accepted: 26 September 2014
Published Online: October 2014

Key words:

peanut, drought stress, relative water content, protein, amino acids, proline

***Corresponding Author**
Jayant K Solanki

Abstract

The changes in the concentration of total protein, total free amino acids and free proline in the leaves of drought susceptible and drought tolerant varieties of peanut was studied under various durations of drought stress. In addition, the relative water content in the leaves was also analyzed. Under drought stress, the concentration of total protein was found to decrease in both drought susceptible and drought tolerant varieties and the degree of decrease was found to be higher in drought susceptible variety compared to drought tolerant variety. Whereas, the content of free amino acids was found to be higher in both the varieties and the increase in drought tolerant variety was found to be more significant than drought susceptible variety. The concentration of proline (a major osmolyte) was found to be significantly higher in the drought tolerant variety compared to drought susceptible variety. Further, the increase in the proline content under increasing drought stress (21 days) was found to be more than 2 folds in the tolerant variety when compared with susceptible variety. The relative water content was found to be inversely proportional to the proline accumulation. The results are discussed in the light of proline, acting as an osmolyte, facilitating the development of drought tolerance.

Introduction

Peanut (*Arachis hypogaea* L.) is an important oilseed crop with 44–56% oil and 22–30% protein on a dry seed basis (Savage & Keenam, 1994). It is grown on 19.3 million hc. of land area in about 82 countries and more than half of the production area is in arid and semi-arid regions, where it is frequently exposed to drought stress of different durations and intensities (Reddy et al., 2003). The major peanut producing countries such as China, India, Nigeria and USA are all facing severe water shortage for crop irrigation, which would definitely affect the world peanut production in the future (Long et al., 2010).

To cope with adverse effects of drought stress, plants have evolved morphological, anatomical, physiological, biochemical, and molecular strategies that enable them to survive under stress conditions (Chaves et al., 2003). Among the physiological strategies, accumulation of osmolyte is one of the most efficient method that assist plants to combat the detrimental effects of water deficit. Osmolytes are compatible solutes that accumulate at high intracellular concentrations without hindering normal cellular metabolism and thus facilitate the maintenance of favorable osmolality during water stress and several osmolytes such as proline are known to provide tolerance at both vegetative and reproductive phases of the crop development (Ramanjulu & Bartels, 2002). Besides osmotic adjustment and maintaining turgor, these osmolytes are involved in signaling and regulating plant responses to multiple stresses (Yancey et al., 1982).

Proline, an imino acid with an exceptional conformational rigidity, zwitterionic and high hydrophilic characteristics acts as one of the most common compatible osmolyte in plants under drought stress (McCue & Hansen, 1990). Among all the amino acids, proline has the highest water solubility and exists in a zwitterionic state. Proline shares this property with other compounds and is collectively referred as "compatible solutes" that are accumulated in the wide range of organisms to adjust cellular osmolarity (Yancey, 2005). Therefore, a comprehensive study is undertaken to elucidate the adaptive mechanisms involving proline accumulation in drought susceptible and drought tolerant genotypes of peanut, in order to gain further insight into the development of stress tolerance in peanut plant.

Materials and Methods

Sample collection

Drought susceptible (JL-24) and drought tolerant (K-1375) peanut varieties were collected from Acharya N.G. Ranga Agri-University, ARS, Kadiri, Andhra Pradesh, India. The collected seeds were sown in different pots filled with soil and sand (2:1). Plants were maintained for 60 days with watering every day for a minimum growth stage under natural photoperiod of about 10-12 h with a temperature of about 26 ± 4^0C. After 60 days, the pots were segregated as control and experimental sets. The experimental sets were subjected to 3 different durations of drought such as 7, 14 and 21 days. The leaves from the experimental sets along with their respective control were harvested, surface sterilized with 0.2% $HgCl_2$ solution for 5 min, then thoroughly washed with distilled water and taken for measurement of relative water content and analysis of total protein, total free amino acids and free proline contents.

Measurement of relative water content (RWC)

For measurement of relative water content (RWC), the fresh weight (FW) of harvested leaves was recorded. Then, the leaves were dipped in deionized water and kept at 4^oC for 12 h to allow the leaves to rehydrate. The leaves were blotted dry on a blotting paper and the weight was recorded as the turgid weight (TW). The leaves were then dried in a hot-air oven at 80^oC for 12 h and the dry weight (DW) was recorded. The RWC was then measured and calculated as per the method described by Sharp et al. (1990).

Estimation of total protein

A 10% homogenate of the fresh leaf samples was prepared with extraction buffer (50 mM Tris buffer pH 8.0, 50 mM NaCl, 2 mM EDTA, 50 mM DTT, 1 mM PMSF and 0.5% PVP) in pre-chilled mortar and pestle using liquid nitrogen.. The homogenate was centrifuged (Remi-24BL) at 11,500 rpm for 10 min at 4^oC, the supernatant was collected and used for protein estimation. The total protein was estimated by the method described by Lowry et al. (1951).

Estimation of total free amino acids

The concentration of total free amino acids was estimated following the method described by Yemm & Cocking (1955). A 10% homogenate of the fresh leaf samples was prepared with absolute alcohol and centrifuged at 3000 rpm for 15 min at room temperature. The supernatant was collected and used as sample for the estimation of total free amino acids. To 50µl of the sample, distilled water was added to make up the volume to 1.0 ml. One milliliter of ninhydrin reagent was added and kept in water bath for 10 minutes. After cooling, 3.0 ml of propanol-water mixture (1:1) was added. The absorbance was measured at 570 nm using Spectrophotometer (UV-1800, Shimadzu) and total free amino acid content was estimated using glycine standard.

Estimation of free proline content

Free proline content was determined according to the method described by Bates et al., (1973) with slight modifications. A 2% homogenate of the fresh leaf was prepared with 3% aq. sulfosalicylic acid and centrifuged at 11500 rpm at 4^oC for 15 min. Two milliliter of supernatant was taken and 2 ml of glacial acetic acid and acid ninhydrin reagent was added. The reaction mixture was boiled in water bath for 60 min and then cooled on ice. Then 4 ml of toluene was added and incubated at room temperature for 30 min. Tubes were then shaken for 15 sec and allowed to stand for 10 min for phase separation. The upper phase was separated and absorbance was measured using spectrophotometer and the concentration of free proline was calculated using proline standard.

*All the experiments were carried out in triplicates (n=3) and results are presented with mean and standard deviation (S.D.).

Results

Changes in the relative water content, total protein, total free amino acids and free proline in the leaves of drought susceptible (JL-24) and drought tolerant (K-1375) varieties of peanut plants exposed to different durations of drought stress are presented in the figures.

Relative water content

In control plants of both JL-24 and K-1375 varieties, there was a steady decrease in RWC with increase in the age of the plant. A significant decrease in RWC was observed with increase in the intensity of drought stress in both the varieties. However, the decrease in RWC in JL-24 (susceptible) variety was found to be more significant than K-1375 (tolerant) variety. In JL-24 variety, the RWC was found to be 61.03 % and 37.60% on 7th and 21st day of drought stress whereas, in K-1375 variety, RWC was found to be 78.87% and 54.53% on 7th and 21st day respectively (Fig. 1).

Concentration of total protein

The total protein content was found to increase with increase in the age of the plant in both drought susceptible (JL-24) and drought tolerant (K-1375) varieties. Whereas, a significant decrease in the total protein content was observed in both the varieties under different durations of drought. However, the decrease in the total protein content in drought susceptible (JL-24) variety was found to be more significant than drought tolerant (K-1375) variety. In JL-24 variety, the concentration of total protein was found to be the maximum (83.22 mg/g) on 7th day and the minimum (64.78 mg/g) on 21st day and 69.45 mg/g on 14th day of drought stress (Fig. 2). In K-1375 variety, the total protein was found to be the maximum of (133.56 mg/g) on 7th day and the minimum (116.45 mg/g) on 21st day and 127.44 mg/g on 14th day of drought stress (Fig. 2).

Concentration of total free amino acids

In general, there was a steady increase in total free amino acid content with increase in the age of the plant in both drought susceptible (JL-24) and drought tolerant (K-1375) varieties. A significant increase in the total free amino acids was observed in both the varieties at different durations of drought. Further, the increase in the total free amino acids in drought tolerant (K-1375) variety was found to be more significant than drought susceptible (JL-24) variety. In JL-24 variety, the concentration of total free amino acids was found to be the highest of 4.261 mg/g on 21st day followed by 3.108 mg/g on 14th day and 2.028 mg/g on 7th day of drought stress (Fig. 2). In K-1375 variety, the total free amino acid content was found to be the maximum of 6.124 mg/g on 21st day, the minimum on of 3.192 mg/g on 7th day of drought and 4.784 mg/g on 14th day of drought stress (Fig. 3).

Free proline content

The free proline content did not show any change with increase in the age of the plant in both drought susceptible (JL-24) and drought tolerant (K-1375) varieties. Whereas, it increased significantly under drought stress in both the varieties (Fig. 4). However, the increase in the content of free proline in K-1375 variety was found to be more significant than JL-24 variety. In JL-24, the concentration of free proline was found to be the maximum (1.776 µmole/g) on 21st day, the minimum on (0.934 µmole/g) on 7th day and 1.667 µmole/g on 14th day of drought stress (Fig. 3). In K-1375 variety, the free proline content was found to be the maximum of 6.751 µmole/g on 21st day followed by 5.104 µmole/g on 14th day and 2.533 µmole/g on 7th day of drought (Fig. 4).

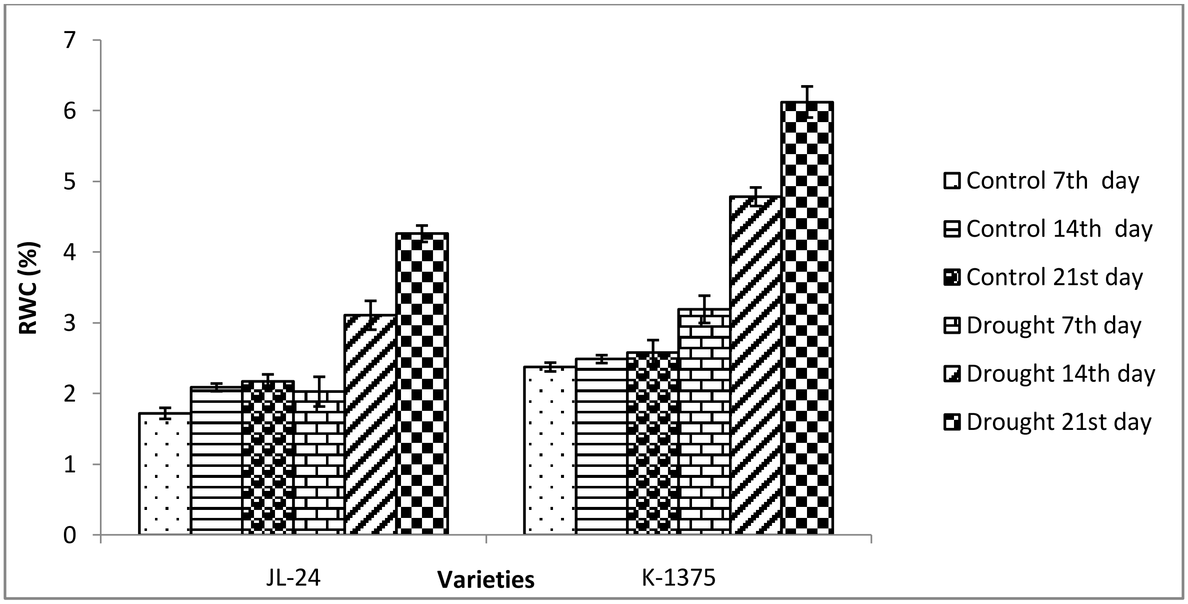

Fig 1: Relative water content (%) in the leaves of drought susceptible (JL-24) and drought tolerant (K-1375) varieties of peanut under different durations of drought.

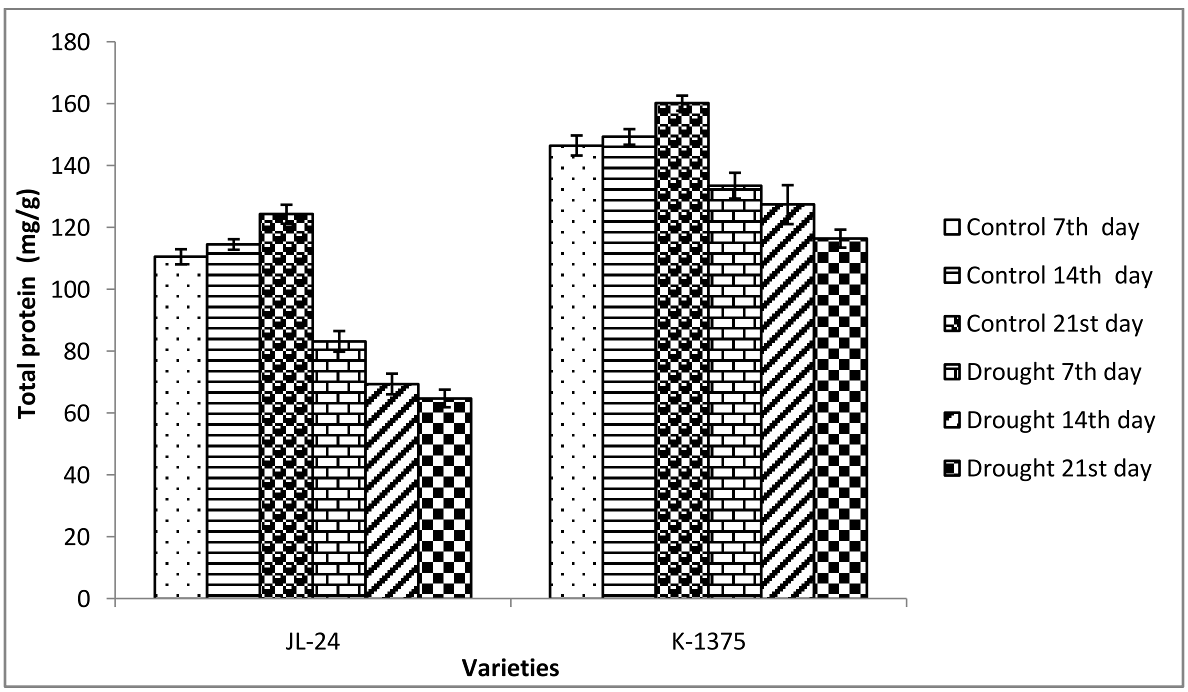

Fig 2: Concentration of total protein (mg/g) in the leaves of drought susceptible (JL-24) and drought tolerant (K-1375) varieties of peanut under different durations of drought.

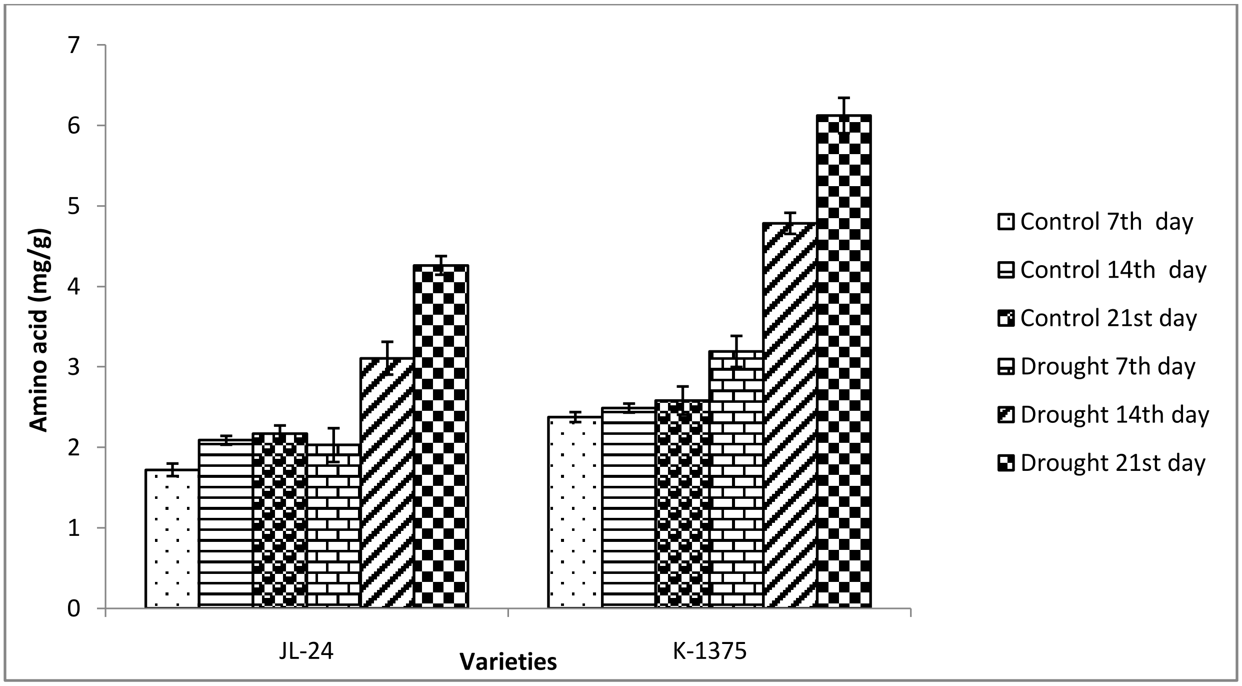

Fig 3: Concentration of free amino acids (mg/g) in the leaves of drought susceptible (JL-24) and drought tolerant (K-1375) varieties of peanut under different durations of drought.

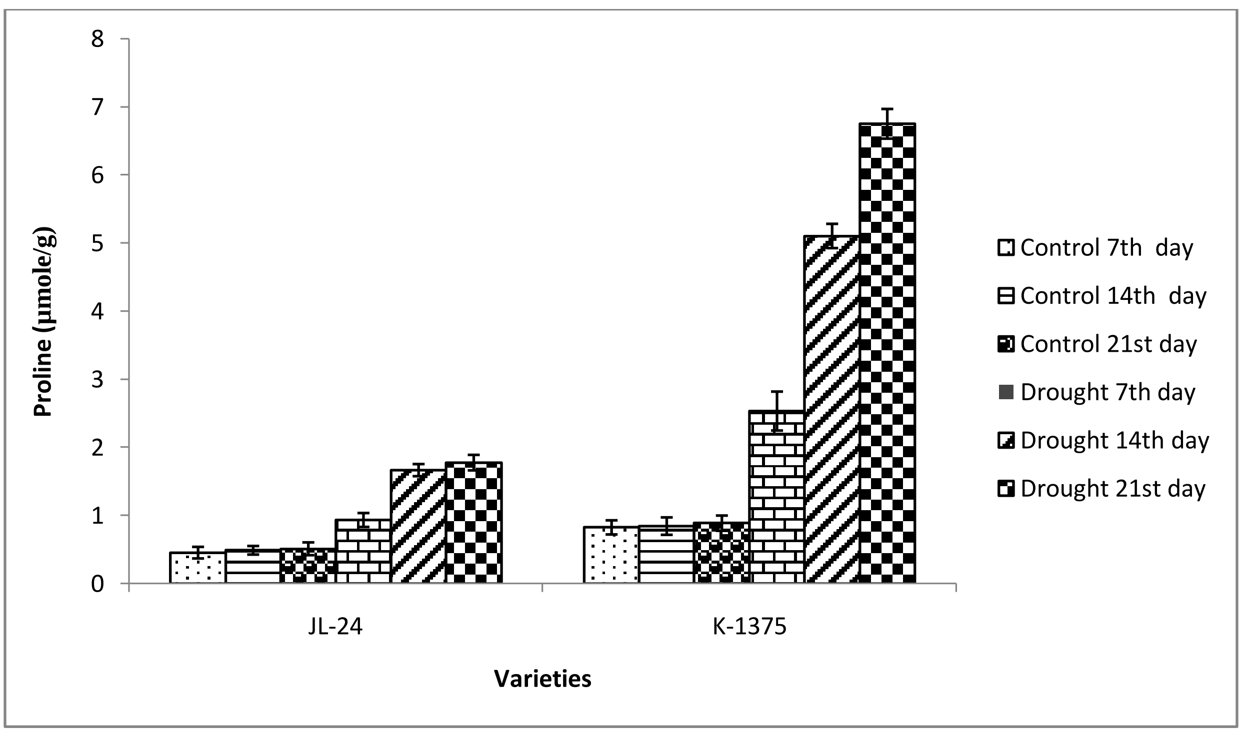

Fig 4: Concentration of free proline (µmole/g) in the leaves of drought susceptible (JL-24) and drought tolerant (K-1375) varieties of peanut under different durations of drought.

Discussion

Peanut is a relatively drought tolerant crop having improved water-use efficiency mechanisms that allow the plant to withstand water stress for certain period of time (Nautiyal et al., 2002). One of the early responses of drought stress is the decrease of RWC, which is considered to be the best physiological measure of plant water status, representing water potential, turgor potential and osmotic adjustment in the plant (Rampino et al., 2006 and Sanchez et al., 2010). In the present study, drought susceptible (JL-24) and drought tolerant (K-1375) varieties were evaluated for their drought tolerance based on reduction in RWC. The ability of K-1375 variety to maintain high RWC is a resistant mechanism which may be a result of efficient osmotic regulation due to favorable osmolarity maintained by various osmolytes. On the contrary, drought susceptible variety (JL-24) showed reduction of RWC to a greater extent attributing its inability to accumulate osmolytes over longer periods leading to decreased osmotic potential. RWC is reported to be an important indicator of plant water status in tomato, maize, wheat etc. for screening tolerant and sensitive cultivars (Khanna-Chopra & Selote, 2007 and Sanchez et al., 2010).

The total protein content in the plant decreases due to abiotic stress (Baruah et al., 1998). A class of proteins, called late embryogenesis abundant globular protein known as osmotin or dehydrin (Singh, 2003), are shown to accumulate in dry seeds, which play an important role in the regulation of dehydration in seeds. Water stress condition caused a marked change in protein synthesis mechanism in plants (Genkel, 1967) with a decrease in the rate of protein synthesis (Hsiao, 1970). In the present study, the total protein content in the leaf of both the varieties was found to be lower under all stress regime compared to the control. However, the higher protein content in drought tolerant variety (K-1375) compared to drought susceptible variety (JL-24) is in agreement with the earlier findings (Chinoy et al., 1974). The alteration in protein synthesis is one of the fundamental metabolic processes that influence water stress tolerance (Jiang & Huang, 2002 and Ouvrard et al., 1996). Thus it is presumed that under drought stress, a large set of genes get activated leading to the synthesis of new proteins that might contribute to osmotic adjustments thus conferring tolerance to drought stress (Chandler & Robertson, 1994).

The significant increase in total free amino acid content was observed in both the varieties under increasing drought stress. However, the increase in the content of total free amino acids in drought tolerant (K-1375) variety was found to be more significant than drought susceptible (JL-24) variety. A significant increase in the free amino acid content under drought stress in both the varieties may be due to the increased protein degradation that might contribute to the increase in the osmotic potential which leads to the development of tolerance in plants against water stress. An increase in the level of free amino acids under drought stress may suggest enhanced metabolite level for cellular homeostasis. In addition, free amino acids have been shown to promote K^+ uptake and Ca^{2+} uptake contributing to osmoregulation through inorganic solutes (Navari-Izzo *et al.*, 1990).

Proline, a proteinogenic amino acid, acts as one of the most common non-toxic and compatible osmolyte in drought-stressed plants (McCue & Hanson, 1992). The accumulation of proline in plant tissues is a clear marker of environmental stress, particularly the drought stress (Routley, 1966). In the present investigation, an increased accumulation of proline was observed with increase in the intensity of drought stress in both the varieties. The increase in the accumulation of proline under increasing drought stress (21 days) was found to more than 2 times in drought tolerant variety (7.6 folds) when compared with the susceptible variety (3.5 folds). The change in proline content was found to be inversely proportional to the relative water content. The higher concentration of proline observed in water stressed plants indicates an efficient mechanism for osmotic regulation, stabilization of sub-cellular structures and cellular adaptation to water stress which is in agreement with the earlier reports (Gunes et al., 2008). Thus accumulation of proline is believed to play an adaptive role in plants during drought stress. Variations in the accumulation of proline during drought stress have been reported in different genotypes and a positive correlation between magnitude of proline accumulation and drought tolerance has been suggested as an index for determining drought tolerance potential of cultivars (Blum & Ebercon, 1976). The present study shows a strong positive correlation between accumulation of proline and drought tolerance in peanut genotypes. The significant increase in the concentration of proline in drought tolerant variety (K-1375) shows its possible role to overcome prolonged drought through osmotic adjustments. Whereas, in JL 24, the increase in the accumulation of proline was not to the extent as observed in tolerant variety, thus might prove the plant's inability to overcome drought stress. Proline accumulation has been reported under drought stress in several crops like rice, pea and maize (Alexieva et al., 2001; Ayerb & Tenori, 1998 and Choudhary et al., 2005). Proline level thus could be used as a reliable index to screen plants tolerant to drought stress.

Conclusion

From the present study, it can be surmised that the drought tolerant variety maintained high relative water content during drought stress which may be due to efficient osmotic regulation maintained by accumulation of proline. In addition, increase in the level of free amino acids and relatively higher protein content in drought tolerant variety compared to drought susceptible variety during all drought stress regimes suggest an enhanced intracellular metabolite level for maintaining cellular homeostasis. Hence, the present study throws light in the understanding of the physiology of drought tolerance in peanut plant, which might act as an indicator for the breeders to identify the suitable genotypes for drought prone areas.

Acknowledgements

Authors thank CSIR, New Delhi, India for the award of JRF (CSIR Award No. 09/039(0091)/2009-EMR-1) to Mr. Jayant K Solanki and the Department of Microbiology and Biotechnology, Bangalore University for providing facilities.

References

Alexieva, V., Sergiev, I., Mapelli, S. and Karanov, E. (2001): The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ., 24: 1337-1344.

Ayerbe, L. and Tenorio, J. L. (1998): Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. Field Crop Res., 59: 225-235.

Baruahand, K. K., Singh, K. and Kumar, A. (1998): Evaluation of Drought Tolerance in Groundnut Stress and Environmental Plant Physiology. Pointer Publishers, 145-152.

Bates, L. S., Waldren, R. P. and Teare, I. D. (1973): Rapid determination of free proline for water stress studies. Plant Soil, 39: 205-207.

Blum, A. and Ebercon, A. (1976): Genotypic responses in sorghum to drought stress. Crop Sci., 16: 428-431.

Chandler, P. M. and Robertson, M. (1994): Gene expression regulated by abscisic acid and its relation to stress tolerance. Annu. Rev. Plant Physiol., 45: 113-141.

Chaves, M. M., Maroco, J. P. and Pereira, J. S. (2003): Understanding plant response to drought. Functional Plant Biology, 30: 239-64.

Chinoy, J. J., Singh, Y. D. and Gurumurti, K. (1974): Biosynthesis of Ascorbic Acid and Mobilization Patterns of Macromolecules during Water Stressing Germinating Ciser Seedling. Biology Plant, 16: 301-307.

Choudhary, N. L., (2005): Expression of delta-1-pyrroline-5-carboxylate synthetase gene during drought in rice (*Oryza sativa* L.). Ind. J. Biochem. Biophys., 42: 366–370.

Genkel, P. A., Satarova, N. A. and Tvorus, E. K. (1967): Effect of Drought on Protein Synthesis and the State of Ribosomed in Plants. Fiziol. Rast., 14: 898-907.

Gunes, A., Inal, A., Adak, M. S., Bagci, E. G., Cicek, N. and Eraslan, F. (2008): Effect of drought stress implemented at pre- or post-anthesis stage some physiological as screening criteria in chickpea cultivars. Rus. J. Plant Physiol., 55: 59-67.

Hsiao, T. C. (1970): Rapid Changes in the Levels of Polyribosomes in Zea mays in Reponse to Water Stress. Plant Physiology, 46, 2: 281-285.

Jiang, Y. and Huang, B. (2002): Protein alternations in tall fescue in response to drought stress and abscisic acid. Crop Sci., 42: 202-207.

Khanna-Chopra, R. and Selote, D. S. (2007): Acclimation to drought stress generates oxidative stress tolerance in drought resistant than susceptible wheat cultivar under field conditions. Environ. Exp. Bot., 60: 276-283.

Long, S. P. and Ort, D. R. (2010): More than Taking the Heat: Crops and Global Change. Current Opinion in Plant Biology, 13(3): 241-248.

Lowry, O. L., Rosebrough, N. J., Farr, A. L. and Randall, R. S. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265–275.

McCue, K. F. and Hanson, A. D. (1990): Drought and salt tolerance towards understanding and application. Trends Biotech, 8: 358-362.

McCue, K. F. and Hanson A. D. (1992): Salt-inducible betaine aldehyde dehydrogenase from sugar beet: cDNA cloning and expression. Plant Mol. Biol., 18: 1-11.

Nautiyal, P. C., Nageswara Rao, R. C. and Joshi, Y.C. (2002): Moisture deficit- induced changes in leaf-water content. Field Crops Res., 74: 67-79.

Navari-Izzo, F., Quartacci, M. F. and Izzo, R. (1990): Water-stress induced changes in protein and free amino acids in field grown maize and sunflower. Plant Physiology and Biochemistry, 28: 531-537.

Ouvrard, O., Cellier, F., Ferrare, K., Tousch, D., Lamaze, T., Dupuis, J. M. and Casse-Delbart, F. (1996): Identification and expression of water stress and abscisic acid regulated genes in a drought-tolerant sunflower genotype. Plant Mol. Biol., 31: 819-829.

Rai, V.K. (2009): Role of amino acids in plant responses to stresses. Biol. Plant, 45: 481-487.

Ramanjulu, S. and Bartels, D. (2002): Drought- and desiccation- induced modulation of gene expression in plants. Plant Cell and Environment, 25: 141–151.

Rampino, P., Pataleo, S., Gerardi, C., Mita, G. and Perrotta, C. (2006): Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. Plant Cell Environ., 29: 2143-2152.

Reddy, T. Y., Reddy, V. R. and Anbumozhi, V. (2003): Physiological responses of groundnut (*Arachis hypogaea* L.) to drought stress and its amelioration: a critical review. Plant Growth Regulation, 41: 75–88.

Routley, D. G. (1966): Proline accumulation in wilted ladino clover leaves. Crop Sci., 6: 358-361.

Sanchez-Rodriguez, E., Rubio-Wilhelmi, M., Cervilla, L. M., Blasco, B., Rios, J. J., Rosales, M. A., Romero, L. and Ruiz, J. M. (2010): Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci., 178: 30-40.

Savage, G. P. and Keenan, J. I. (1994): The Groundnut Crop: A Scientific Basis for Improvement. Smart J., 173-213.

Sharma, K. K. and Lavanya, M. (2003): Recent Developments in Transgenic for Abiotic Stress in Legumes of the Semi-Arid Tropics. Genetic Engineering of Crop Plants for Abiotic Stress, 23: 61-73.

Sharp, R. E., Hsiao, T. C. and Silk, W. K. (1990): Growth of the maize primary root at low water potentials. Plant Physiol, 93: 1337-1346.

Singh, D. P. (2003): Water Deficit Stress in Stress Physiology. New Age Publishers, 64-79.

Yancey, P. H., Clark, M. E., Bowlus, R. D. and Somero, G. N. (1982): Living with water stress: Evolution of osmolyte systems. Science, 217: 1214-1222.

Yancey, P. H. (2005): Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J. Exp. Biol., 208: 2819-2830.