http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (5): 797-801, 2007
ISSN 1028-8880

NThe Study of Desiccation-Tolerance in Drying Leaves of the Desiccation-Tolerant Grass *Sporobolus elongatus* and the Desiccation-Sensitive Grass *Sporobolus pyramidalis*

Hamid Reza Ghasempour and Jahanbakheshe Kianian
Department of Biology, Faculty of Sciences, Razi University, Kermanshah, 67149, Iran

Abstract: Hydrated leaves of the resurrection grass *Sporobolus elongatus* are not desiccation tolerant (DT), but moderate to severe drought stress can induce their DT with the leaves remain attach to drying intact plants. *In vivo* protein synthesis was studied with SDS-page of extracts of leaves of intact drying plants of *S. elongatus* (a desiccation-Tolerant grass (DT)) and *S. pyramidalis* (a desiccation-sensitive species (DS)). Free proline increased in drying leaves. Soluble sugar contents also increased with drying but were less than fully hydrated leaves at 8% RWC. Total protein also showed an increase with an exception at 8% RWC which showed a decrease. SDS-page of extracts of drying leaves of both DT and DS plants were studied as relative water contents (RWC) decreased. In first phase, DT species at 58% RWC (80-51% RWC range), two proteins increased in contents. In the second phase, at 8% (35-4% RWC range) two new bands increased and two bands decreased. In leaves of DS species some bands decreased as drying progressed. Also, as drying advanced free proline increased in DT species. Total protein increased as drying increased but at 8% RWC decreased. All data of results are consistent with current views about studied factors and their roles during drying and induction of desiccation tolerance in DT plants.

Key words: Drought tolerance, desiccation-sensitive, relative water contents

INTRODUCTION

Water availability is an important factor affecting plant growth and yield, mainly in arid and semi-arid regions, where plants are often subjected to periods of drought. The occurrence of morphological and physiological responses, which may lead to some adaptation to drought stress, may vary considerably among species. In general, strategies of drought-avoidance or drought-tolerance can be recognized, both involving diverse plant mechanisms that provide the plants the ability to respond and survive drought (Levitt, 1980). Desiccation tolerance is common in lower plants and in mature seeds, but is less common in vegetative tissues of higher plants (Gaff, 1977).

The maintenance of high cell water potentials in species with a drought-avoidance strategy avoids direct metabolic injuries (Levitt, 1980) and minimizes the necessity of metabolic adjustments, which are usually found in drought-tolerant species (Levitt, 1980; Shackel and Hall, 1983). When leaves of the desiccation tolerant grass *sporobolus elongatus* are dehydrated to air-dryness and then rehydrated in water they recover and resume growth, as long as the leaves have dried while attached to intact drying plants, fully hydrated leaves that dehydrate after being removed from the plant die during drying (Gaff, 1980; Gaff and Loveys, 1984). One hypothesis for this distinction between the survival of attached and detached leaves is that hormone signal passes from drought-stressed roots and triggers the induction of desiccation tolerance in the protoplasm of the leaves (Ghasempour *et al*., 1998a, 2001; Smith-Espinoza *et al*., 2005).

There was no correlation between growth and cell viability during the stress period while a significant correlation was found during the recovery period suggest that the relationships between these two parameters are not the same during exposure to stress on the one hand and during recovery after the stress relief on the other hand (Stanley *et al*., 2004).

Also, for induction of desiccation tolerance in resurrection plants such as *Sporobulolus stapfianus* sugar contents changes gradually as drying progress (Ghasempour *et al*., 1998b). In drought tolerant grass *S. stapfianus* Gandoger *in vitro* and *in vivo* proteins synthesized after induction of full desiccation tolerance, involved both Novel proteins and proteins of greater abundance (Gaff and Loveys, 1993; Kuang *et al*., 1995; Gianello *et al*., 2000; Ghasempour *et al*., 2001).

Corresponding Author: Hamid Reza Ghasempour, Department of Biology, Faculty of Sciences, Razi University, Kermanshah, 67149, Iran Fax 0098-831-4274555

We investigated total protein, protein profile, soluble sugar content and Free proline contents *in vivo* to study (a) The extent to which these internal constituents changes in their amounts during drying and influence on induction of desiccation tolerance at progressive levels of water stress and (b) How changes of proteins and other factors correlates with the onset of induction of desiccation tolerance in *S. elongatus*.

MATERIALS AND METHODS

Plant material: *Sprobolus elongatus* and *Sprobolus pyramidalis* plants (original provevavee of seeds was Transvaal, south Africa; 7) were grown in 15 cm pots containing a coarse sand-loam-peat mixture (3:1:1) in a green house at 20-25°C under natural light. Plants were watered and fertilized regularly with a half-concentration modified Hoglands nutrient solution (Hoagland, 1937). A few weeks before commencement of experimental treatment, plants were transferred to a controlled environment chamber (CEC) maintained at 28°C and 260 μmol m^2 sec^{-1} photosynthesis photon fluxes with photoperiod of 16 h.

Relative water content: Plants were allowed to dry intact to different stress levels by with holding water and then leaves were sampled at each stress level. Relative Water Contents (RWC) was calculated from: (a) the initial fresh weight of a leaf sample (W_F), (b) the fresh weight at full turgor (W_{FT}), measured by immersing a leaf sample in distilled water in the dark for 2-3 h for moderate stress and 8-10 h for severely stressed leaves and (c) the dry weight after oven-drying at 70°C for 48 h (W_D), i.e, $RWC = (W_{FT}-W_F)/W_D$.

Soluble sugar extraction: Soluble sugar content was determined by the modified phenol sulphoric acid method (Dubois *et al*., 1956; Kennedy, 1987). Data were measured at 485 nm by Bausch and Lomb spectrophotometer 70. A standard curve; 0, 5, 10, 1 5, 20, 25, 30 and 40 mg of glucose were prepared. Glucose content of treated and untreated extracts was calculated by using the standard curve and recorded.

Assessment of proline in leaves: Proline of leaves was determined by Bates (1973) method. Data were measured at 520 nm by Bausch and Lomb spectrophotometer 70. A standard curve; 0, 1.9, 7.8, 15.62, 31.25, 62.5 and 125 μg of proline were prepared. Proline content of treated and untreated extracts was calculated by using the standard curve and recorded.

Measuring the total protein: In order to quantify the total protein of leaves in each sample, a 0.05 g of dry weight of leaves was assessed by Lowry *et al.* (1951) method. The total protein was determined with Folin reagent and the color compared with Bovine Serum Albumin (BSA), serving as the standard for determining protein content, read at A660 (OD) and recorded.

Protein SDS-PAGE and gel electrophoresis analysis: Peterson (1977) method was used to determine the protein concentration of fresh leaves of treated and untreated plants. For one-dimensional SDS-PAGE the supernatant of samples was diluted with UKS-buffer (9.5 M Urea, 5 mM K_2CO_3, 1.25% (W/V) SDS) (1:1). For each well 20 μL was applied, totally 6 wells. A Hoeffer SE 600 vertical unit was employed and coomassie blue used for staining.

In order to analyze molecular weight and mobility of proteins bands the UV. Doc program was used and molecular weight of marker bands entered to the program. The program gives the molecular weight of the bands on the stained gel.

RESULTS

Total sugar contents (mg g^{-1}, Fig. 1) were higher in fully hydrated dry leaves of desiccation-sensitive leaves than in desiccation tolerant plant. At 78% RWC, total sugar content (mg g^{-1}) in dry leaves of desiccation tolerant plants showed a decrease comparing with fully hydrated leaves but rose gradually as drying progressed. In desiccation-sensitive grass, after a decrease did rose at 58% RWC but due to drying injury decreased and declined later at lower RWC as drying progressed (Fig. 1).

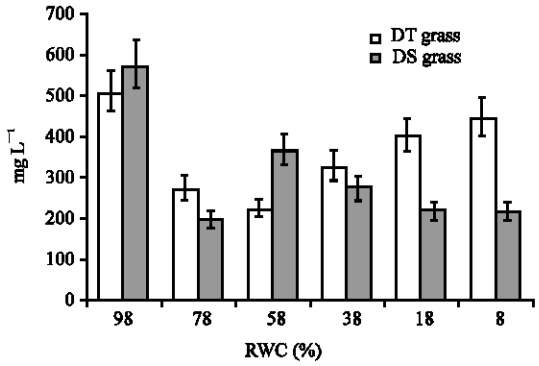

Fig. 1: Total soluble sugar contents in leaves of Desiccation Tolerant (DT) grass *S. elongatus* and Desiccation-Sensitive (DS) grass *S. pyramidalis* at different stages of drying (Tukey and one-way ANOVA test meaningful at 0.05%)

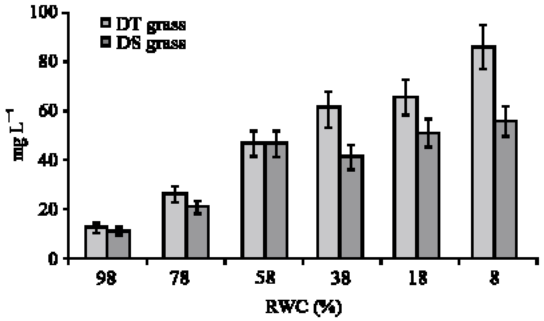

Fig. 2: Proline contents in leaves of desiccation tolerant (DT) grass *S. elongatus* and desiccation-sensitive (DS) grass *S. pyramidalis* at different stages of drying (Tukey and one-way ANOVA test meaningful at 0.05%)

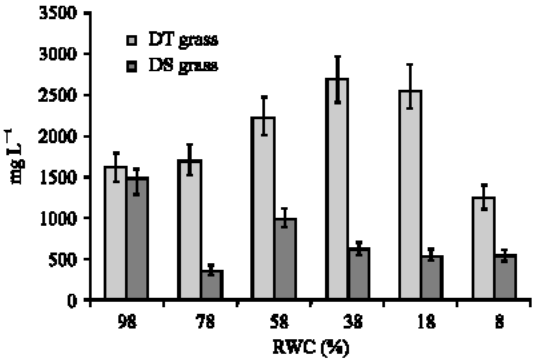

Fig. 3: Total protein contents in leaves of Desiccation Tolerant (DT) grass *S. elongatus* and Desiccation-sensitive (DS) grass *S. pyrammidallis* at different stages of drying (Tukey and one-way ANOVA test meaningful at 0.05%

In our experiment, both plants presented foliar proline accumulation in response to water stress (Fig. 2). Water stress induced proline accumulation in both desiccation tolerant and desiccation-sensitive plants but it was higher in desiccation tolerant plants as drought increased.

Total protein did increase in both desiccation-tolerant and desiccation-sensitive plants up to 38% RWC but decreased as drying progressed (Fig. 3). As Fig. 3 clearly indicates, it was higher in desiccation-tolerant plants in all levels of drying.

Studies of *in vivo* protein synthesis for induction of desiccation tolerance in *S. elongatus* showed two distinct protein synthesis phases (85-58% RWC and 58-8% RWC phase). Study of width and number of protein bands in SDS-page indicates that in the early phase of drying new band No. 1 appeared. In the late phase of drying another new band No. 15 appeared. At 8% RWC,

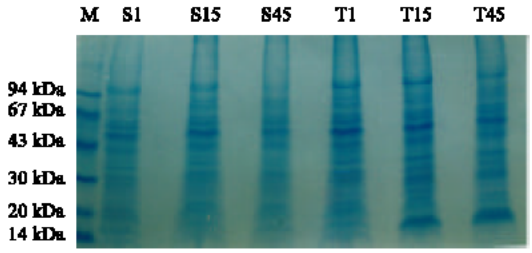

Fig. 4: Protein profile of desiccation tolerant grass *S. elongatus* and desiccation-sensitive grass *S. pyramidalis* leaves at different stages of drying

in desiccation-tolerant species bands No. 3, 8, 9, 13 and 14 increased in width but in desiccation-sensitive plants bands No. 1, 2, 8, 11, 12 and 13 disappeared (Fig. 4).

DISCUSSION

The higher total sugar contents in desiccation tolerant plants than in the desiccation-sensitive grass is consistent with earlier investigations (Kaiser *et al*., 1985; Shwab Gaff, 1986). Sucrose is clearly the major protectant sugar accumulated in the late stage of drying in angiosporm desiccation tolerant plants (Ghasempour *et al*., 1998b). Drought induced accumulation of raffinose and stachyose probably assisted the role of sucrose by reducing the tendency of sucrose to crystallize at high concentrations (Caffrey *et al*., 1988). The disparity between relative changes in total sugar content in desiccation to learnt as opposed to-sensitive species is greater in this study (plants dried in artificial light) than in earlier studies in which plants were dried in full sunlight (Gaff and Loveys, 1993).

Proline accumulates in plants under drought and salinity stress in a number of species and it is thought to play important role in plant cells for adaptation to water stress (Delauney *et al*., 1993). In plants, proline is synthesizes from glutamate, the P5CS activity being key in this type of synthesis in plants (KaviKishor *et al*., 1995). In this study, increase in proline contents might be the adjustment of plants in response to progressive levels of water stress as an osmoregulators.

Total protein decrease in sensitive plants as drying progressed (Crowe *et al*., 1984) which might be due to protein hydrolysis because of increase in protease activity (Bray 1988). Drought stress did increase amount of proteins in desiccation tolerant plants compared to sensitive plants (Delauney *et al*., 1993). Previous studies of *in vivo* protein synthesis for induction of desiccation tolerance in *S. stapfianus* and its survival showed two

distinct protein synthesis phase (85-51% RWC and 37-3.5% RWC phase) (Gaff and Loveys, 1984; Kuang *et al*., 1995). Increase in total protein up to 38% RWC is probably due to increase in enzyme activity mainly enzymes relating to sucrose, raffinose and stachyose production for membrane protection as earlier studies also indicated (Ghasempour *et al*., 1998b). Also, hormones such as ABA induces most of the genes that are induced by dehydration in the whole plants which plays major role in synthesis of new proteins related to induction of desiccation tolerance (Smith-Espinoza *et al*., 2005).

In this experiment, shifts in protein bands of SDS-page is probably due to enzyme changes due to hormones such as ABA for induction of desiccation tolerance in desiccation tolerant plants. The main thrust of the data from majority of above applied experiments proline accumulation, total sugar content, total protein and protein profile agree with hypothesis that sugars acts as agents for induction of desiccation tolerance. Also, in response to progressive levels of water stress, proline accumulates as an osmoregulators.

ACKNOWLEDGMENTS

This research was supported by grants from the research council of the Razi University, Kermanshah, Iran.

REFERENCES

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

Bray, E.A., 1988. Drought-and ABA-induced changes in polypeptide and mRNA accumulated in tomato leaves. Plant Physiol., 86: 754-758.

Caffrey, M., V. Fonesca and A.C. Leopold, 1988. Lipid-sugar interactions. Relevance to anhydrous biology. Plant Physiol., 86: 754-758.

Crowe, L.M., R. Mouradin, J.H. Crowe, S.A. Jackson and C. Womersley, 1984. Effects of carbohydrates on membrane stability at low water activities. Biochim. Biophys. Acta, 861: 131-140.

Delauney, A.J., C.A.A. Hu, P.B. Kavi-Kishor and D.P.S. Verma, 1993. cloning of ornithine aminotransferase cDNA from vigna acontifolia by trans-complementation in *Escherichia coli* and regulation of proline biosythesis. J. Biol. Chem., 268: 18673-18678.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem., 28: 350-356.

Gaff, D.F., 1977. Desiccation tolerant vascular plants of Southern Africa. Oecologia, 31: 95-108.

Gaff, D.F., 1980. Protoplasmic Tolerance of Extreme Water Stress. In: Adaptation of Plants to Water and High Temperature Stress. Turner, N.C. and P.J. Kramer (Eds.), Wiley-Inter science: New York, pp: 207-231.

Gaff, D.F. and B.R. Loveys, 1984. Abscisic acid content and effect during dehydration of detached leaves of desiccation tolerant plants. J. Exp. Bot., 35: 1350-1358.

Gaff, D.F. and B.R. Loveys, 1993. Abscisic acid levels in drying plants of a resurrection grass. Trans. Malaysian Soc. Plant Physiol., 3: 286-287.

Ghasempour, H.R., D.F. Gaff, R.P.W. Williams and R.D. Gianello, 1998a. Contents of Sugar In: Leaves of drying desiccation tolerant flowering plants, particularly grasses. Plant Growth Regul., 24: 185-191.

Ghasempour, H.R., E.M. Anderson, R.D. Ginello and D.F. Gaff, 1998b. Growth inhibitor effects on protoplasmic drought tolerance and protein synthesis in leaf cells of the resurrection grass, *S. stapfianus*. Plant Growth Regul., 24: 179-183.

Ghasempour, H.R., E.M. Anderson and F. Gaff Donald, 2001. Effects of growth substances on the protoplasmic drought tolerance of leaf cells of the resurrection grass, *Sporobolus stapfianus*. Aust. J. Plant Physiol., 28: 1115-1120.

Gianello, R.D., J. Kuang, D.F. Gaff, H.R. Ghasempour, C.K. Blomstedt and J.D. Hamill, 2000. Proteins correlated with desiccation tolerance in a resurrection grass Sporobolus stapfianus. Chapter 20. Plant responses to environmental stress. Scientific Publishers, Oxford, pp: 161-166.

Hoagland, D.R., 1937. Some aspects of the salt nutrition of higher plants. Botanical Rev., 3: 307-334.

Kaiser, K., D.F. Gaff and WH. Jr. Outlaw, 1985. Sugar conents of leaves of desiccation-sensitive and desiccation-tolerant plants. Naturwisscnschaft, 72: 608-609.

KaviKishor, P.B., Z. Hong, G.H. Milao, C.A.A. Hu and D.P.S. Verma, 1995. Overexpressin of Δ^1-pyrroline 5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol., 108: 1387-1394.

Kennedy, J.F., 1987. Carbohydrate Analysis: A Practical Approach. Chaplin, M.F. and J.F. Kennedy (Eds.), Oxford: IRL.

Kuang, J., D.F. Gaff, R. Gianello, C.K. Blomstedt, A.D. Neale and J.D. Hamill, 1995. Changes *in vivo* prote in complements in drying leaves of the desiccation-tolerant grass *Sporobolus stapfiamus* and the desiccation-sensitive grass *S. pyramidalis*. Aust. J. Plant Physiol., 22: 1027-1034.

Levitt, J., 1980. Responses of Plants to Environmental Stresses. Water, Radiation, Salt and Other Stresses II, Academic Press, New York.

Lowry, O., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem., 193: 239-249.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry which is more generally applicable. Anal. Biochem., 83: 346-356.

Shackel, K.A. and A.E. Hall, 1983. Comparison of water relations and osmotic adjustment in sorghum and cowpea under field conditions. Aust. J. Plant Physiol., 10: 423-435.

Shwab, K.B. and F.D. Gaff, 1986. Suger and ion contents of drought tolerant plants under water stress. J. Plant Physiol., 125: 257-265.

Smith-Espinoza, C.J., J.R. Phillips, F. Salamini and D. Bartels, 2005. Identification of further *Craterostigma plantagineum cdt* mutants affected in abscisic acid mediated desiccation tolerance. Mol. Gen. Genomics, 274: 364-372.

Stanley, L., M. Almansouri and J.M. Kinet, 2004. Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Sci., 167: 9-18.