The Bioscan 8(2): 569-574, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

DIFFERENTIAL SENSITIVITY OF CHICKPEA GENOTYPES TO SALICYLIC ACID AND DROUGHT STRESS DURING PRE-ANTHESIS: EFFECTS ON TOTAL CHLOROPHYLL, PHENOLICS, SEED PROTEIN AND PROTEIN PROFILING

PRADEEP KUMAR PATEL* AND A. HEMANTARANJAN
Department of Plant Physiology, Institute of Agricultural Sciences,
Banaras Hindu University Varanasi - 221 005, INDIA
e-mail: pradeepk.bhu@gmail.com

KEYWORDS
Chlorophyll
Chickpea
Drought
Pre- anthesis
Salicylic acid

Received on :
21.11.2012

Accepted on :
12.04.2013

***Corresponding author**

ABSTRACT
The work was conducted with the purpose to evaluate the effect of salicylic acid (SA) under drought stress on chlorophyll pigment, phenolics, seed protein and protein profile in four chickpea (*Cicer arietinum* L.) genotypes (*i.e.,* Tyson, ICC 4958, JG 315 and DCP 92-3). The experiment was carried out in a complete randomized design with three replications. Drought stress was imposed during the pre-anthesis phase. Reduction in relative injury was observed in plants treated with SA at the threshold level of 1.5mM. Drought stress reduced the total chlorophyll and percentage of seed storage protein, where increases the level of total phenolics content were observed under drought stress and this was further induced by SA. The genotype ICC 4958 perform better than Tyson, JG 315 and DCP 92-3 under drought stress with SA treatment. Moreover, it is also noteworthy that drought did not change significantly the 1-D protein profile of chickpea genotypes. This suggests that chickpea could be induced to tolerate drought using 1.5mM of SA.

INTRODUCTION

Drought is one of the most important environmental stresses limiting the productivity of crop plants around the world (Bohnert *et al*., 1995). Grain legumes, in general, and chickpea, in particular, appear to have more sensitivity towards drought as compared to cereals. Chickpea is a cool-season legume in the northern regions of India and is also being cultivated in warm season environments in the central and southern parts of the country. In this crop, yield losses might be the result of intermittent drought during the vegetative phase, drought during reproductive development or terminal drought at the end of the crop cycle (Serraj *et al*., 2004). Drought stress decreases the rate of photosynthesis (Kawamitsu *et al*. 2000). Plants grown under drought conditions have a lower stomatal conductance in order to conserve water. Consequently, CO_2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants. Diffusive resistance of the stomata to CO_2 entry probably is the main factor limiting photosynthesis under drought (Boyer, 1970). Mild or moderate drought stress, stomatal closure (causing reduction of leaf internal CO_2 concentration (Ci)) is the major reason for reduced rates of leaf photosynthesis (Chaves, 1991; Cornic, 2000; Flexas *et al*., 2004). Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (Iturbe-Ormaetxe *et al*., 1998). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species such as superoxide radical (O2·), hydroxy radical (·OH), hydrogen peroxide (H_2O_2) and alkoxy radical (RO·) in chloroplasts, mitochondria and peroxisomes (Smirnoff, 1993). This suggests that measures in mitigating negative effects of drought on chickpea can be taken, including the application of exogenous salicylic acid.

Salicylic acid (SA) is a naturally existing phenolic compound and is considered to be a potent plant growth regulator because of its diverse regulatory role in plant metabolism. Phenolic compounds have strong free radicals scavenging capacity (Hall and Cuppett, 1997). Evidences exist that externally applied SA can increase the plant tolerance to several abiotic stresses, including osmotic stress (Wang *et al*., 2010), heavy metal stress (Moussa and El-Gamel, 2010) and also influence a range of diverse processes in plants, including seed germination, stomatal closure, ion uptake and transport, membrane permeability, photosynthesis and plant growth rate (Aftab *et al*., 2010). Patel *et al*. (2011) recently reported that SA sustained antioxidant system under drought stress particularly in chickpea.

The alteration of total chlorophyll and phenolics contents and protein synthesis or degradation is among the fundamental metabolic processes that may influence drought stress

tolerance (Ouvrard *et al*.,1996; Jiang and Huang, 2002). Both quantitative and qualitative changes of proteins have been detected during the stress (Riccardi *et al*. 1998; Ahire *et al*., 2005; Kottapalli *et al*., 2009). Alterations of proteins under drought stress conditions have been studied widely in many plant species, but not predominantly in chickpea. Therefore, the present investigation was designed to further explore and elaborate the ameliorative role of SA, in chickpea subjected to pre-anthesis drought stress, and to investigate the changes in total chlorophyll, phenolics, storage seed protein content and protein profiles in chickpea genotypes differing in drought tolerance.

MATERIALS AND METHODS

Site description

The experiment was carried out during *rabi* (cool) season 2009-10 and 2010-11 in rain- protected wire- house at the Horticulture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The experimental area lies between latitudes 25.18ºN, longitude 83.0ºE and has an altitude of 123.93m. The average of climatic conditions calculated during the entire growth period is as follows: maximum /minimum temperatures, relative humidity (RH) were28.0ºC / 13.6ºC, 71.3/36.5 % respectively, and the average hrs sunlight was 6.9.

Plant materials and treatments

Seeds of chickpea (*Cicer arietinum* L.) genotypes (Tyson, ICC 4958, JG 315 and DCP 92-3) were obtained from Indian Institute of Pulse Research (IIPR-ICAR), Kanpur, India. Seeds of uniform size were selected and surface sterilized with 0.2% $HgCl_2$ solutions followed by repeated washing with double distilled water (DDW). For treatments with SA, Salicylic acid (Molecular Weight: 138.12 Sigma Aldrich, Chemie GmBH, Munich, Germany) was dissolved in absolute ethanol and then added drop wise to water (ethanol/water: 1/1000 v/v) (Williams *et al*., 2003). Thereafter, 10 seeds of each genotype for each treatment were soaked for 6h in distilled water without SA (0mM SA) taken as control (T0) and1.0 and 1.5mM SA taken as T1 and T2 respectively. Seeds were subsequently sown (10 per pot), size (30cm X 30cm) filled with farm soil having 12-14% moisture at the time of sowing, plants and were thinned to six uniform plants per pot at the first true leaf stage. The experimental soil was sandy loam containing organic carbon 0.31%, available nitrogen 228.00kg ha^{-1}, available phosphorus 17.00 kg ha^{-1}, available potassium 180.00 kg ha^{-1} and pH 7.3 in water. There were 36 pots per treatment, including three replications of each experimental treatment. All the pots were applied with the standard dose of fertilizer for chickpea, 20, 40, 20 kg ha^{-1} of N, P_2O_5 and K_2O respectively.

Drought stress applications

Each genotype was grouped in two sets *e.g.*, irrigated and drought imposed at pre-anthesis, thereafter called early drought stress (EDS). Drought stress treatment was imposed at the early and late stage by controlling irrigation schedule and it was instigated at 50 days after sowing (DAS). Control plants (irrigated) were given three irrigations (at 25, 50 and 65 DAS) from the date of sowing to maturity. Early drought stressed plants received only two irrigations (25 and 65 DAS) (fig.1). Observations were taken on normal and stressed plants at 58 days after sowing.

Methodology

Total chlorophyll

Total chlorophyll content was determined in first fully expanded leaves from top at pre- anthesis drought (*i.e.* 58 DAS) in normal and stressed plants by the method of Yoshida *et al*. (1972).

Total phenolics

The total phenolicswere measuredat 765nm in first fully expanded leaves from top at pre- anthesis drought (*i.e.* 58 DAS) by using Folin Ciocalteu reagent (McDonald *et al*., 2001).

Protein

The protein content was determined in first fully expanded leaves from top at pre- anthesis drought (i.e. 58 DAS) in normal and stressed plants by the method of Lowry *et al*. (1951).

Protein profiling

Sodium dodecyl sulphate polyacrylamide 1- D gel electrophoresis (SDS-PAGE) was carried out in seed storage protein developed under pre- anthesis drought stress condition according to the method of Laemmli (1970). Gel was stained with Coomasie Blue R250 and distained with 5% MeOH/ acetic acid mixture. Protein Molecular Weight Markers GeNei™, visible on SDS-PAGE after staining with Coomassie Brilliant Blue R-250 (Broad Range 0.5mL, No.105975 PMWB) were used gel electrophoresis unit (SCI PLAS TV400Y standard twin- plate maxi gel unit, SCI PLAS LTD, 22 Cambridge BC4 OFJ, U.K. was used.

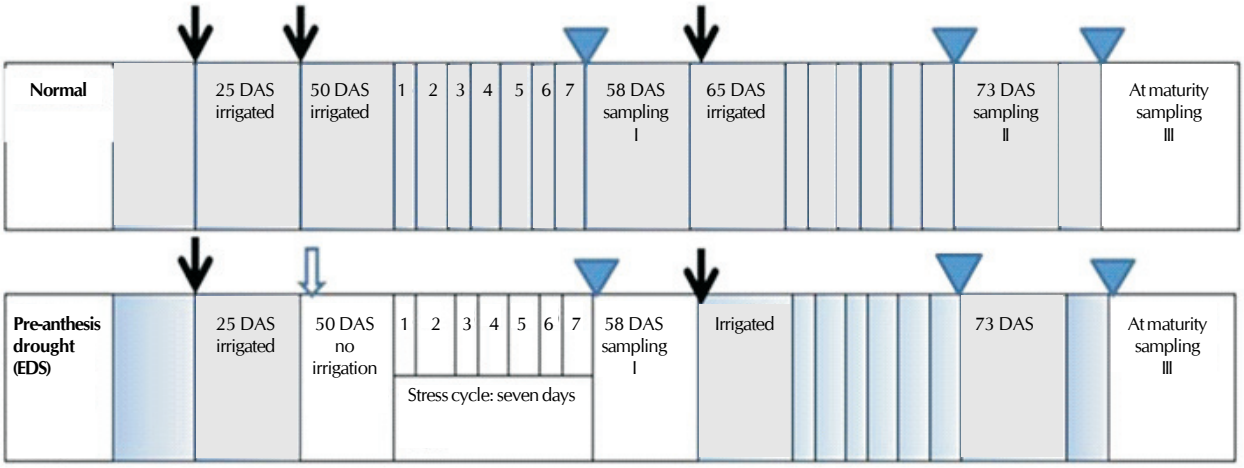
Statistical analysis

Samples were collected in three replicates and each replicate/ sample was assayed twice. The design of the experiment was completely randomized design (CRD) and data was analyzed for analysis of variance (*ANOVA*) and means were compared by the least significant difference (LSD) test and those at $P < 0.05$. Standard error of the mean was also calculated (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Total Chlorophyll

The present study revealed that the chlorophyll content decreased under drought stress in all four chickpea genotypes. Significant differences were observed in genotype, treatment as well as in interaction (genotype × treatment). Genotype JG 315 and DCP 92-3 registered the maximum reduction in chlorophyll concentration under drought stress. When considering percentage of reduction, as compared to control, the maximum was for genotype JG 315 (58%) and the minimum for Tyson (11.1%) (Fig. 2). Drought decreased the total chlorophyll concentration to a large extent in the four chickpea genotypes. The results are in agreement with Nyachiro *et al*. (2001) that described a significant decrease of chlorophyll caused by water deficit in six *Triticum aestivum* cultivars. Decreased or unchanged chlorophyll levels during



(Solid arrow) indicate the irrigation time with respect to DAS, (empty arrow) indicate the water with holding time with respect to DAS, (solid triangle) indicated the sampling time (I and II for physiological and biochemical parameters and III for yield attributing parameters), DAS indicate days after sowing.

Figure1: Schematic representation of experimental layout

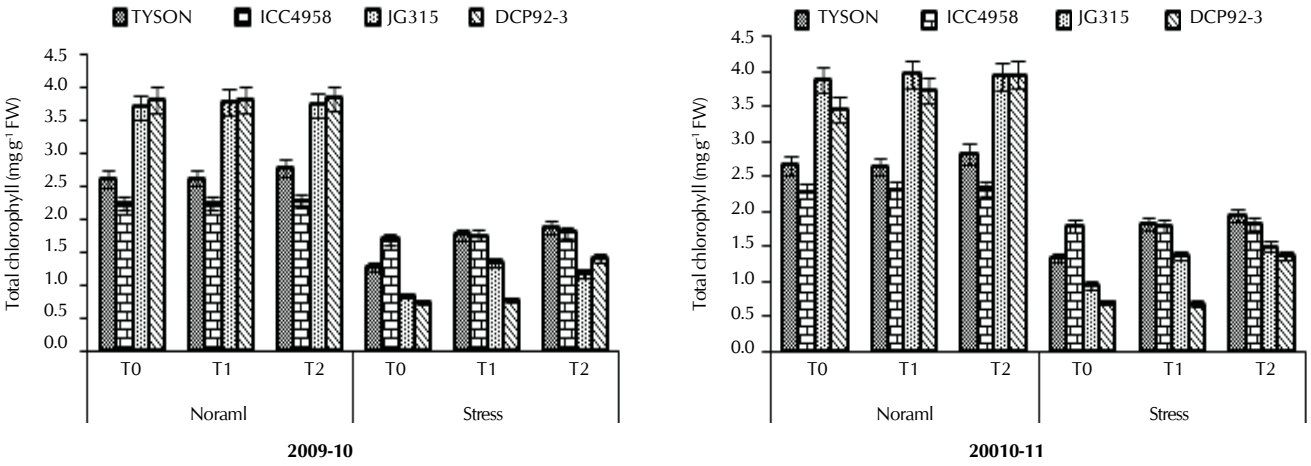


Figure 2: Effect of salicylic acid (SA) on leaf total chlorophyll content (mg g^{-1} FW) in four chickpea (*Cicer arietinum* L.) genotypes (Sampling time – I *i.e.,* at 58 DAS). (T0 = 0 mM SA, T1 = 1.0 mM SA, T2 = 1.5 mM SA). Data shown are mean +SE.

drought stress have been reported in other species, depending on the duration and severity of drought (Kpyoarissis *et al*., 1995). A decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Herbinger *et al*., 2002).

Salicylic acid maintained the level of chlorophyll could be attributed to its stimulatory effects on antioxidant enzymatic activity (Patel *et al*., 2011) that protect the chlorophyll breakdown by scavenging the reactive oxygen species (ROS). Our results are in agreement with those of Rajasekaran and Blum (1999) who reported that salicylic acid protects chlorophyll, maintained photosynthesis and enhanced the growth of jack pine seedlings under drought.

Total phenolics

Phenolic compounds have antioxidant properties because of their ability to scavenge free radicals and active oxygen species such as singlet oxygen, free radicals and hydroxyl radicals. The antioxidant properties of phenolic are mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Results revealed that total phenolics increased under drought and on an average, the maximum level was noticed at pre- anthesis drought. At this stage the genotype differences were significant. Tyson and ICC 4958 showed higher phenolics content as compared to JG 315 and DCP 92-3 in response to SA over the control. Maximum total phenolics content in plants treated with 1.5 mM SA under drought stress was (6.19mg g^{-1}fresh weight) in ICC 4958 and minimum in DCP 92-3 (5.06mg g^{-1}fresh weight). In this work, an increase in the level of phenol with either drought or SA treatments was observed (Fig. 3). Our results are in agreement with those that reported the ability of phenolic

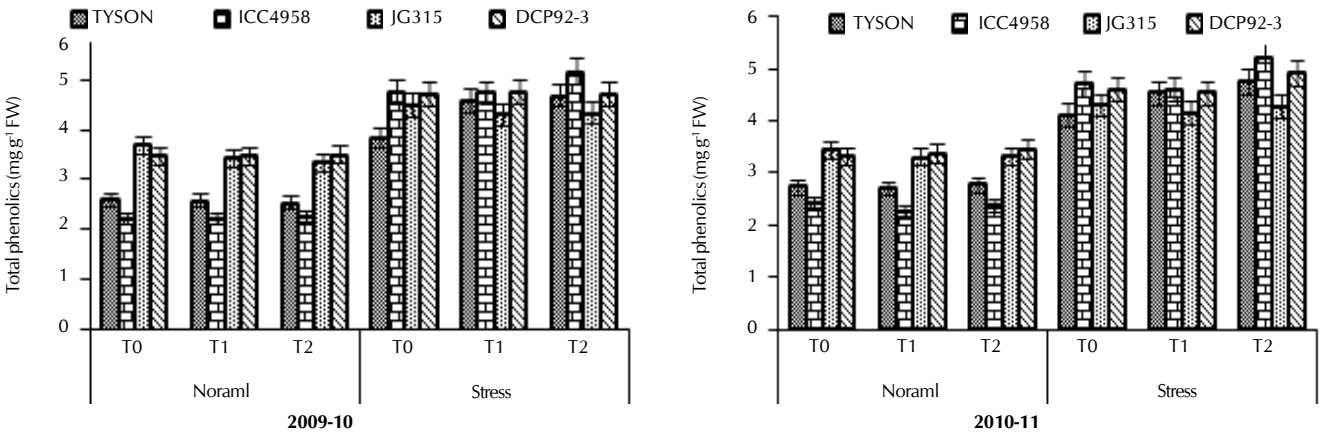


Figure 3: Effect of salicylic acid (SA) on total phenolics content (mg g^{-1} FW) activity in four chickpea (*Cicer arietinum* L.) genotypes (Sampling time – I *i.e.,* at 58 DAS). (T0 = 0 mM SA, T1 = 1.0 mM SA, T2 = 1.5 mM SA). Data shown are mean +SE

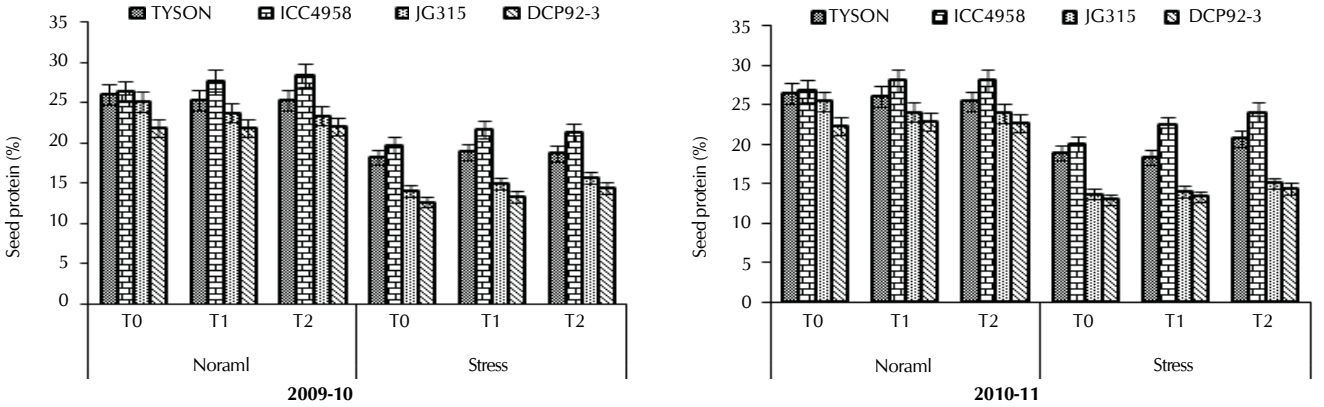


Figure 4: Effect of salicylic acid (SA) on seed protein (%) in four chickpea (*Cicer arietinum* L.) genotypes (Sampling time – I *i.e.,* at 58 DAS). (T0 = 0 mM SA, T1 = 1.0 mM SA, T2 = 1.5 mM SA). Data shown are mean +SE.

compounds to scavenge free radicals and active oxygen species (Duh *et al*., 1999; Odabasoglu *et al*., 2004).

Seed protein

The percentage of seed storage protein in chickpea genotypes under drought stress was significantly reduced. The maximum percentage of seed storage protein was recorded under control condition, and the minimum in seed which was developed under drought stress condition at pre- anthesis stage. Under normal and stress condition the maximum protein percentage was observed in the genotype ICC 4958 (28.3 and 21.3%) followed by genotype Tyson (25.3 and 18.6%) whereas, the minimum was noticed in JG 315 (23.3 and 15.6%) and DCP 92-3 (21.9%, 14.3%) in the treatment of SA@1.5 Mm (Fig. 4). The seed protein content in our studies might have decreased because of a reduction in the allocation of nitrogen by the stress to the developing seeds. Carvalho *et al*. (2005) noticed a 50 % reduction in protein and oil content of lupin seeds developed under water stress conditions. The present studies indicated that variations existed in the protein content of the seeds produced by the plants stressed at pre- anthesis stages. The larger decrease in the seeds storage protein at pre - anthesis stage might have occurred because of the greater effect of drought stress on seed-filling processes. These observations suggested that allocation of nitrogen its utilization in the seeds might be a key determinant in deciding the sensitivity of the seed development phase in drought-stressed plants.

Protein profiling

SDS – PAGE was done with seed proteins of drought stressed samples. Results revealed that all samples were amplified in 9 major bands out of 12. The all bands were monomorphic. The smallest protein was ~ 20 k Da and highest ~ 66k Da. Results revealed that drought stress at pre-anthesis stage did not significantly change the 1-D protein profile of chickpea genotypes, with the exception that the band intensity of a polypeptide with molecular mass in closer to (~) 24.5, 26 and 36.6 KDa under treatment of SA @ 1.0mM (T1) and SA @ 1.5mM (T2) were increased partly in all chickpea genotypes viz. Tyson, ICC 4958 JG 315 and DCP 92-3 under drought stress. Genotypes Tyson and DCP 92-3 noticed high band intensity at treatment T2 and T1 respectively whereas, the genotypes ICC 4958 and JG 315 showed high band intensity on both the treatment *i. e.* T1 and T2. The level of this polypeptide was higher in T1 and T2 rather than T0 (Fig. 5). Moreover, profile expressing SA treatments with chickpea showed a higher density of some protein bands. This indicates a role of exogenous SA in the induction of more defensive proteins. Formation of new proteins and protein accumulation is considered a way and an indicator of resistance towards

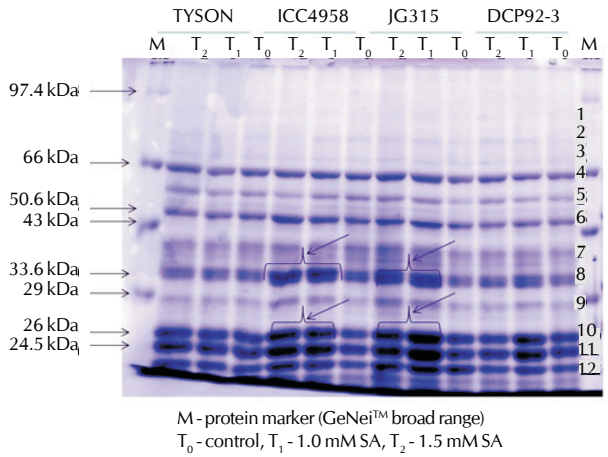


Figure 5: Effect of salicylic acid (SA) on SDS-PAGE profiles of seed storage protein in four chickpea (*Cicer arietinum* L.) genotypes grown under pre- anthesis drought (Sampling time – III *i.e.,* at maturity). The arrow indicates the increased band intensity in response to the drought stress treatment

drought. In the present experiment, SA treatments induced the formation of new dense protein bands of (~) 24.5, 26 and 36.6 KDa in chickpea seed. This indicates that SA plays an important role in the induction of drought resistance. This role may occur through accumulation of certain proteins and/ or formation of new polypeptides which are so called dehydrin responsive proteins (DRPs).

CONCLUSIONS

The present study reveals that genotypes ICC 4958 showed less degradation of chlorophyll pigment and have higher accumulation of phenols in comparison to Tyson, JG 315 and DCP92-3 at the threshold level of SA @ 1.5mM. On the basis of the performance of chickpea genotypes at different levels of SA especially at pre- anthesis stages of development, it is concluded that pre- anthesis stage was sensitive under drought stress, which could be in part mitigated by pre-soaking SA treatment for improving drought tolerance in chickpea. Besides these the study also reveal that SDS-PAGE analysis of the proteins did not detect significant qualitative changes in protein synthesis in stressed plants along with SA treatment and control. It strongly suggests that chickpea can be considerably tolerant to drought at the level of 1.5mM SA.

ACKNOWLEDGEMENTS

We extend our sincere thanks to the University Grant Commission (UGC) for a Ph.D. research fellowship and the Indian Institute of Pulse Research (IIPR- ICAR) Kanpur, India for kindly providing the chickpea genotypes. We also thankful to Dr. S. A. Ansari and his team members for support in SDS-PAGE analysis in the Physiology and Molecular Biology laboratory, Tropical Forest Research Institute (TFRI) Jabalpur, M.P. India.

REFERENCES

Aftab, T., Khan, M. M. A., Idrees, M. and NaeemMoinuddin, M. 2010. Salicylic acid acts as potent enhancer of growth, photosynthesis and artemisinin production in *Artemisia annua* L. *J. Crop. Sci. Biotech.* **3:** 183-188.

Ahire, R. K., Kale, A. A., Munjal, S. V. andJamdagni, B. M. 2005. Induced water stress influencing proline accumulation, protein profiles and DNA polymorphism in chickpea. *Indian. J. Plant Physiol.* **10:** 218-224.

Bohnert, H. J., Nelson, D. E. and Jensen, R. G. 1995. Adaptations to environmental stress. *Plant Cell.* **7:** 1099-1111.

Boyer, J. S. 1970. Leaf enlargement and metabolic rates in corn, soybean and sunflower at various leaf water potentials. *Plant Physiol.* **46:** 233-235.

Carvalho, I. S., Chaves, M. and Pinto Ricardo, C. 2005. Influence of water stress on the chemical composition of seeds of two lupins (*Lupinusalbus* and *Lupinusmutabilis*). *J. Agron. Crop Sci.* **191:** 95–98.

Chaves, M. M. 1991. Effects of water deficits on carbon assimilation. *J. Exp. Bot.* **42:**1-16.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture – not by affecting ATP synthesis. *Trend Plant Sci.* **5:** 187–188.

Duh, P. D., Tu, Y. Y. and Yen, G. C. 1999. Antioxidant activity of aqueous extract of harnjyur (*Chyrsanthemummorifolium* Ramat). *Lebensm Wissensch Technology.* **32:** 269-277.

Flexas, J., Bota, J., Loreto, F., Cornic, G. and Sharkey, T. D. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. *Plant Biol.* **6:** 1–11.

Gomez, K. A. and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research, A. *Willy- Interscience Publication, New York.*

Hall, C. A. and Cuppett, S. L. 1997. Structure-activities of natural antioxidants.141–170 In: Auroma O. I. and Cuppett S.L. (eds), Antioxidant methodology in vivo and in vitro concepts. *AOCS Press, Champaign, IL.*

Herbinger, K., Tausz, M., Wonisch, A., Soja, G., Sorger, A. and Grill, D. 2002. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiol. Biochem.* **40:** 691–696.

IturbeOrmaetxe, I., Escuredo, P. R., Arrese-Igor, C. and Becana, M. 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant Physiol.* **116:** 173–181.

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

Kawamitsu, Y., Driscoll, T. and Boyer, J. S. 2000. Photosynthesis during desiccation in an Intertidal Alga and a Land Plant. *Plant Cell Physiol.* **41(3):** 344-353.

Kottapalli, K. R., Rakwal, R., Shibato, J., Burow, G., Tissue, D., Burke, J., Puppala, N., Burow, M. and Payton, A. 2009. Physiology and proteomics of the water-deficit stress response in three contrasting peanut genotypes. *Plant Cell Environ.* **32:** 380-407.

Kpyoarissis, A., Petropoulou, Y. and Manetas, Y. 1995. Summer survival of leaves in a soft-leaved shrub (*Phlomisfruticosa*L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. *J. Exp. Bot.* **46:** 1825–1831.

Laemmli, U. K. 1970. Cleavage of structure proteins assembly of the head of bacteriophage T4. *Nature.* **22:** 680-685.

Lowry, O. H., Rosebrough, N. J., Farr, A. J. and Randall, R. J. 1951. Protein measurement with the folin-phenol reagents. *J. Biol. Chem.***193:** 265–275.

Manivannan, P., Abdul Jaleel, C., Sankar, B., Kishorekumar, A., Somasundaram, R., Lakshmanan, G. M. A. and Panneerselvam, R. 2007. Growth, biochemical modifications and proline metabolism in *Helianthus annuus*L. as induced by drought stress. *Colloids and*

Surfaces B: Biointerfaces. **59:** 141–149.

McDonald, S., Prenzler, P. D., Autolovich, M. and Robards, K. 2001. Phenolic content and antioxidant activity of olive extracts. *Food Chem*. **73:** 73-84.

Moussa, H. R. and El-Gamel, S. M. 2010. Effect of salicylic acid pre-treatment on cadmium toxicity in wheat. *Biologia Plantarum*. **54:** 315–320.

Nyachiro, J. M., Briggs, K. G., Hoddinott, J. and Johnson-Flanagan, A. M. 2001. Chlorophyll content, chlorophyll fluorescence and water deficit in spring wheat, *Cereal Res. Comm*. **29:** 135– 142.

Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Halici, M.andBayir, Y. 2004. Comparision of antioxidant activity and phenols content of three lichen species. *Phytothera Research*. **18:** 938–941.

Ommen, O. E., Donnelly, A., Vanhoutvin, S., Van Oijen, M. andManderscheid, R. 1999. Chlorophyll content of spring wheat flag leaves grown under elevated CO2 concentrations and other environmental stresses within the ESPACE-wheat project. *Europ. J. Agron*. **10:** 197-203.

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

Patel, P. K., Hemantaranjan, A., Sarma, B. K. and Singh, R. 2011. Growth and antioxidant system under drought stress in Chickpea (*Cicer arietinum* L.) as sustained by salicylic acid. *J. Stress Physiol. Biochem*. **7:** 130-144.

Rajasekaran, L. R. and Blum, T. J. 1999. New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. *J. Plant Growth Regul*. **18:** 175-181.

Riccardi, F., Gazeau, P., De Vienne, D. and Zivy, M. 1998. Protein changes in response to progressive water deficit in maize. *Plant Physiol*. **117:** 1253-1263.

Serraj, R., Krishnamurthy, L., Kashiwagi, J., Kumar, J., Chandra, S. and Crouch, J. H. 2004. Variation in root traits of chickpea (*Cicer arietinum* L..) grown under terminal drought. *Field Crops. Res*. **88:** 115–127.

Smirnoff, N. 1993. The role of active oxygen in response of plants to water deficit and desiccation. *New Phytologist* **125:** 27–58.

Wang, L. J., Fan, L., Loescher, W., Duan, W., Liu, G. J. and Cheng, J. S. 2010. Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. *BMC Plant Biol*.**10:** 34.

Williams, M., Senaratna, T., Dixon, K. and sivasithamparam, K. 2003. Benzoic acid induces tolerance to biotic stress caused by *Phytophthoracinnamomi* in Banksai attenuate. *J. Plant Growth Regul*. **41:** 89–91.

Yoshida, S., Forno, D. A. J., Cock, H. and Gomez, K. A. 1972. Determination of chlorophyll in plant tissue. In: Laboratory manual for physiological studies of rice. *2eds. International Rice Research Institute (IRRI)*.