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Role of osmolytes in maintaining water balance and photosynthesis under drought and heat in Chickpea (*Cicer arietinum* L.)

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Abstract

The present investigation entitled "Role of osmolytes in maintaining water balance and photosynthesis under drought and heat in chickpea (Cicer arietinum L.)" was conducted at Research Farm & labolatory of Department of Plant Physiology (Division of Basic Science), Indian Institute of Pulses Research (IIPR), Kalyanpur, Kanpur, Uttar Pradesh. During the rabi season 2015-16. The chickpea (Cicer arietinum L.) various physiological traits which are essential for its adaptation to drought or water limiting environments. Among those, osmotic adjustment is considered to be one of the important traits conferring tolerance to terminal drought. The present study is aimed to investigate the role of osmotic adjustment in improving the water relation characteristics and maintaining photosynthesis under severe water stress. The 10 advanced breeding lines (ABLs) [C-7, C-8, C-9(M-51), C-11(M-86), C-16(M-55), C-19(M-93), C-20(M-39), C-21, C-214, C-235] derived from high and low osmotic adjustment (OA) were used for the experiments. The results showed varied degree of OA in the test genotypes in response to drought. The high osmotic adjustment variety Tyson and low OA Kaniva (ABLs) and other physiological traits along with high yielding varieties DCP 92-3 (heat sensitive) and JG 14 (heat tolerant) both of which are lacking osmotic adjustment used as check showed improved photosynthesis under drought when analysis was done using chlorophyll fluorescence imaging. In addition higher OA retained greater water and chlorophyll retention when subjected to water stress either in field condition or polyethylene glycol (PEG) 6000 induced drought. The solute accumulation in the leaves under water stress in this investigation proved to play vital role in mining water from deep soil. The experiment also showed positive results when heat and drought combindly imposed.

Keywords: Osmotic adjustment, chlorophyll fluorescence, osmolytes

Introduction

Chickpea (*Cicer arietinum* L.) is an important protein-rich cool-season food legume crop. Because it is cultivated predominantly in rainfed environments, it usually faces terminal drought during pod and seed filling, leading to significant reductions in grain yield (Siddique *et al.*, 2000) ^[26, 27]. However, not all observations of OA have shown positive physiological benefits. For example, while Jones & Rawson (1979) ^[13] reported that OA maintained higher rates of photosynthesis at low turgor in sorghum (Sorghum bicolor), Leport *et al.* (1999) ^[14] observed that photosynthesis was not maintained at high rates as leaf water potential (WP) decreased in chickpea. Moreover, in chickpea, OA was reversed during seed filling (Leport *et al.*, 1999) ^[14], presumably as assimilates were preferentially mobilized to the seed, and recycling of respired carbon within the pod has been suggested as a means of maintaining seed filling when leaf photosynthesis is low (Turner, 2003; Furbank *et al.*, 2004; Turner *et al.*, 2005) ^[20, 11, 21]. Furthermore, chickpea often experiences terminal heat during reproductive face due to climate change. Drought and heat are the most important constraints to chickpea production globally. It is estimated that drought and heat stresses together account for about 50% of the yield losses caused by abiotic stresses

In comparison to drought and other abiotic stresses, heat stress has received relatively less attention in chickpea breeding programs in the past. However, it has received considerable attention during the recent years. It is now well recognized that heat stress at the reproductive stage is increasingly becoming a serious constraint to chickpea productivity. This is because of: (i) large shift in chickpea area from cooler long-season environments to warmer short-season environments, (ii) increasing chickpea area under late sown conditions due to increasing cropping intensity, and (iii) expected overall increase in temperatures due to climate change (Gaur *et al.* 2012)^[10].

In India, the chickpea area reduced by 4.3 million ha (from 5.1 million ha to 0.8 million ha) in northern and northwestern India (Punjab, Haryana, Uttar Pradesh, Punjab), which has cooler long-season environments, and increased by 4.3 million ha (from 2.0 million ha to 6.3 million ha) in central and southern India (Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka), which has relatively warmer and short-season environments.

Thus, there has been a considerable increase in chickpea cultivation in areas which are prone to heat stress during reproductive development. Osmolyte accumulation (OA) is frequently cited as a key putative mechanism for increasing vields of crops subjected to drought conditions. The hypothesis is that OA results in a number of benefits that sustain cell and tissue activity under water-deficit conditions. It has been proposed as an effective tolerance mechanism for water deficits, which could be enhanced in crops by traditional plant breeding, marker-assisted selection or genetic engineering, to generate droughttolerant crops (Serraj & Sinclair, 2002) ^[24-25]. When water deficit develop various solutes accumulate in cells and subsequently tissue osmotic potential is reduced. Osmotic adjustment (OA) is derived from the net increase in cellular osmolality caused by the accumulation of solutes such as various ions (mainly potassium), sugars, poly-sugars (e.g. fructan), amino acids (e.g. proline), glycinebetaine, etc. Recently constitutive accumulation of natural solutes (e.g. glycinebetaine) and exotic solutes (e.g. mannitol) were engineered and tested for functionality in model plants such as tobacco. OA occurs when cellular water deficit exceeds a certain threshold.

Among various traits, osmotic adjustment (OA) is considered as an important physiological trait for adaptation to drought. The osmotic adjustment in chickpea has been reported to be ranged from 0 to 1.3 MPa. Osmotic adjustment increases water absorption, maintains cell turgor, photosynthesis and leaf area duration, helps stomatal opening, delays senescence and death, reduce flower abortion, and improves root growth as water deficits develop. The greater osmotic adjustment leads to higher growth rate and dry matter production in pigeon pea under drought. The degree of OA has also been shown to be correlated with yield under dry land conditions in chickpea. The present investigation is aimed to investigate the role of osmolytes accumulation in leaves towards maintaining water balance, photosynthesis and membrane stability during exposure to both combined stresses drought and heat.

Materials and Methods

A Field and laboratory experiment was conduct in the ICAR-Indian Institute of Pulse research, Kanpur (U.P.). Through Chlorophyll fluorescence of the leaves was measured by using Pulse amplified modulated fluorometer (Image analyzer Walz Germany) according to Schreiber *et al.* (1986) ^[23].

Chlorophyll fluorescence

Interaction studies Heat x Drought

In this experiment; the experiment were conducted to investigation for combined tolerance of drought and heat stress in chickpea genotype. The experiment was conducted under following series respectively.

Material

PEG 6000

- Water bath
- Chlorophyll fluorescence image PAM

Experiment 1st - Drought treatment

Excised leaves of different genotypes were placed in a test tube with 20% PEG for inducing drought *(in-vitro)* while the same placed in a test tube with distilled water was treated as control and exposed to continuous light for 48 hours.

Experiment 2nd - Heat treatment

After 48 hour, for heat temperature treatment, the same leaf sample were placed in a test tube with 20 ml distilled water and test tube transferred in a water bath in different temperature at 38°C and 40°C for 1 hours respectively.

Experiment 3rd - chlorophyll fluorescence assessment

After drought and high temperature treatment, fluorescence imaging and ETR were assessed by using IMAGE PAM (Walz, Germany).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence of the leaves was measured by using Pulse amplified modulated fluorometer (Image analyzer Walz Germany) according to Schreiber *et al.* (1986) ^[23]. Relative electron transport rate (*ETR*) of the leaf sample was determined by using the Hansatech leaf clip holder with PAR sensor, and was calculated using the following formula:

ETR = Quantum yield x PAR x 0.5 x ETR FACTOR

Where,

PAR= Photosynthetic Active Radiation

ETR factor = 0.84. This factor corresponds to the fraction of incident radiation absorbed by various leaf species.

Results and Discussion

Chlorophyll fluorescence

The effect of water stress on photosynthesis was studied by induced water stress using PEG 6000 and was precisely assessed by reduction in the photosynthesis in terms of Electron transport rate derived from the fluorescence parameters and fluorescence imaging. The values of ETR are considered to be a proxy measurement of photosynthesis. The results suggested that water stress caused inhibition of photosynthesis at cellular level.

The quantum yield denoted by the ratio of variable to maximum fluorescence (Fv/Fm) in light and dark-adapted leaves and was used to assess genotype's ability that how efficiently it makes utilization and conversion of light energy into photochemical energy. The dark-adapted leaves treated with PEG showed linear decrease in the quantum with increasing light intensity and reduction was more in stressed leaves as compared to control plants. However, light adapted leaves showed sharp decline in the quantum yield (ePS 2). The results indicated that interaction of high light with drought situation had more detrimental effect in inhibition of photosynthesis. Therefore high light under drought situation is an additional stress. A significant variation in quantum yield was observed when drought (PEG) was imposed. The results suggested that stresses such drought can cause adverse effects on photosynthesis.

Interaction studies between $Drought \times Heat$

Based on the above experiments, chickpea genotypes along with ABLs were assessed for ETR under simulated multiple stress conditions such as high temperature (38 °C, 39 °C, 40 °C, 45 °C respectively), high light from 0 to 700 μ mole m⁻²s⁻¹and water stress induced by PEG solution (Fig 1).

The genotypes showed higher ETR under multiple stresses have been identified as tolerant to drought, heat and high light. The fluorescence images of stressed and non stressed leaves were shown in the series of Figures 2 to 14. It was observed that at 40 °C, the quantum yield images of none of the genotypes were able to be captured hence at this high temperature light-adapted leaves were completely collapsed. Therefore comparisons among varieties and ABLs were made between two temperatures 38 and 40 °C. All the darkadapted leaves at 38 °C, the quantum yield (Fv/Fm) images of all the test lines were little affected as the electron carriers were all open that allows flow of electrons faster and efficient link between PS 2 and PS 1 established. However same temperature treated leaves in all the test lines showed drastic reduction in the quantum yield which were reflected in the quantum yield images in light -adapted leaves. Since the electrons acceptors under light are all closed or reduced, the flow of electrons have the restricted movement unless there are faster re-oxidation of the electrons carriers. Hence under high light, a pressure is created and actual assessment of photosynthetic efficiency can be precisely judged. There are changes in the quantum yield images among the test lines. Some of the promising lines treated with PEG as inducer of drought showed less reduction in the quantum yield in light-adapted condition as evidenced from, their least deviation from normal control. These test lines were C 9, C 23, C 7, C8, DCP 92-3, C 21, C 214, C 235, Tyson and Kaniva. Except Kaniva, all other test lines have significant osmotic adjustment. Therefore these lines were identified as superior performance under drought at 38 C. On the other hand, test lines evaluated at 40 °C, the quantum yield images could not be captured in most of test lines of light-adapted leaves except C9 and C 23 both under stressed and non-stressed condition (Fig 10).

In order to find out the effects of multiple abiotic stresses such as drought, high temperature and high light, the photosynthetic electron transport rate (ETR) of PEG treated (drought stressed) and non-stressed (water) of leaves was worked out at 38 °C in relation to progressive increase in the irradiance levels. It was observed that under non-stressed condition the photosynthetic ETR increased with increasing irradiance in all the test lines suggesting that high light intensity is not a detrimental factor for photosynthesis. However, photosynthetic electron transport rate in some of the ABLs such as C7 and C 8 under drought stressed condition (20% PEG treated) showed high photosynthesis even at light saturation point > 500 μ mole m⁻²s⁻¹ suggesting that these lines C 7 and C 8 derived from Tyson x Kaniva have been attributed by their ability to tolerate multiple abiotic stresses such as high light, drought and high temperature (38 °C).

The quantum yield (Fv/Fm) of PSII in dark, photochemical quenching (Qp) and electron transport rate (ETR) are related with the photosynthesis efficiency of the genotypes. Any reduction in this parameters indicate the down regulation of the photosynthetic process. If under stress, light energy absorbed by leaves can not be utilized for carbon fixation, it is dissipitated as chlorophyll fluorescence or in the form of heat. With increase in the water stress induced by PEG, quantum yield both under dark and light and photochemical quenching drastically declined and photosynthesis almost got inactivated at higher stress level. The varietal differences in fluorescence imaging in light and dark were also evident in this experiment and complete inhibition of quantum yield (Fv/Fm) imaging and e'PS2 observed at PEG and 40 °C.

The reduction in the photosynthesis in chickpea as a result of water stress has been reported by Basu et al. (2007) [2-5]. The inhibition of quantum yield (Fv/Fm) and photochemical quenching due to water stress with an increase in the nonphotochemical quenching (qNP) has been described by Basu et al. (1998)^[3] Photosynthetic processes in plants are highly sensitive and responsive to environmental stresses. In several species, chlorophyll fluorescence has been shown to be sensitive indicator of stresses including those caused by low temperature (Smillie and Hetherington, 1983)^[29], heat Havaux., 1992), nutrient deficiency (Conroy et al., 1986)^[8], and drought (Di Macro et al., 1988) [9]. Therefore, measurement of chlorophyll fluorescence has been proposed as a method of selecting drought, heat and cold tolerant genotypes. The effect of genotypes, stress and light and their interactions on fluorescence characteristics were highly significant suggesting that these factors strongly modify the photosynthesis of the genotypes Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sink for PS2 activity during mild drought (Cornic and Fresneau, 2002)^[7]. The data of Flagella *et al.* (1998) show that quantum yield of PS2, as related to Calvin cycle metabolism, is reduced only under drastic water deficit. Genty et al. (1987) ^[12] suggest, as possible causes of the drought-induced slowdown of electron flow subsequent to PQ, a direct impairment of electron flow from PQ via PSI, and an indirect inhibition due to a lack of utilization of reducing power and of dissipation of the light-induced proton gradient. Primary events of photosynthesis such as the electron transport capacity are very resilient to drought (Cornic et al., 1989)^[30].



Fig 1: Photosynthetic electron transport rate (E T R) in chickpea genotypes under combined stresses drought and heat.



Fig 2: Images of quantum yield of ABLs C 9 and C 23 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 38 °C



C-7 : dark –adapted leaf image of quantum yield (Fv/ Fm) (Top left stressed leaf of C-7 -20% PEG and top right control non-stressed leaf of C 7

C-8 : dark –adapted leaf image of quantum yield (Fv/ Fm) (bottom left stressed leaf of C-8 -20% PEG and bottom right control nonstressed leaf of C 8



C-7 : light –adapted leaf image of quantum yield (Fv/ Fm) (Top left stressed leaf of C-7 -20% PEG and top right control non-stressed leaf of C 7

C-8 : light –adapted leaf image of quantum yield (Fv/ Fm) (bottom left stressed leaf of C-8 -20% PEG and bottom right control non-stressed leaf of C 8

Fig 3: Images of quantum yield of ABLs C 7 and C 8 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 38 °C



Fig 4: Images of quantum yield of ABLs C 11 and C 12 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 38 °C



Fig 5: Images of quantum yield of ABLs C 19 and C 20 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 38 °C



Fig 6: Images of quantum yield of ABLs C-214 and C-235 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves at 38 °C



Fig 7: Images of quantum yield of ABLs DCP 92-3 and C-21 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and non-stressed leaves at 38 °C.



Fig 8: Images of quantum yield of ABLs Tyson and Kaniva in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves at 38 °C.



Fig 9: Images of quantum yield of ABLs C 7 and C 8 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 40 °C.



Fig 10: Images of quantum yield of ABLs C 9 and C 23 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 40 °C.



Fig 11: Images of quantum yield of ABLs C-11 and C-16 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 40 °C.



Fig 12: Images of quantum yield of ABLs C-19 and C-20 in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 40 °C.



Fig 13: Images of quantum yield of ABLs DCP 92-3 and C-21in dark vs light adapted leaves subjected to water-stressed (20% PEG) and non-stressed leaves 40 °C.



Fig 14: Images of quantum yield of ABLs Tyson and C-21in dark vs light adapted leaves subjected to water-stressed (20% PEG) and nonstressed leaves 40 °C.

Conclusions

It may be suggested from the present investigation that the osmotic adjustment may be an important parameter in selecting genotypes of chickpea for combined heat and drought resistance. Osmotic adjustment was shown to be under the control of a single or a multiple gene family and is highly heritable and may leads to yield benefit by the extraction of water from deeper soil layers.

Association between OA and yield was observed positive in some chickpea germplasms, however, in some other germplasms yield was contrast to the osmotic adjustment. Association between OA and maintenance of photosynthesis suggests that OA may provide benefit to yield under drought in chickpea.

References

- 1. Basu PS, Singh DN. Physiology and abiotic stress in chickpea. In: M. Ali, S. Kumar, and N. B. Singh (eds), Chickpea Research in India. Indian Institute of Pulses Research, Kanpur, India; c2003. p. 137-167.
- 2. Basu PS, Berger JD, Turner NC, Chaturvedi SK, Ali M, Siddique KHM. Osmotic adjustment of chickpea (*Cicer arietinum*) is not associated with changes in carbohydrate composition or leaf gas exchange under drought. Ann. Appl. Biol. 2007;150:217-225.
- 3. Basu PS, Sharma A, Sukumaran NP. Changes in net photosynthetic rate and chlorophyll fluorescence in potato leaves induced by water stress. Photosynthetica. 1998 Mar;35:13-9.
- Basu PS, Berger JD, Turner NC, Chaturvedi SK, Ali M, Siddique KH. Osmotic adjustment of chickpea (Cicer arietinum) is not associated with changes in carbohydrate composition or leaf gas exchange under drought. Annals of Applied Biology. 2007 Apr;150(2):217-25.
- 5. Basu PS, Ali Masood, Chaturvedi SK. Osmotic adjustment increases water uptake, remobilization of

assimilates and maintains photosynthesis in chickpea under drought. Indian Journal of Experimental Biology (CSIR). 2007;45:261-267.

- Basu PS, Ali M, Chaturvedi SK. Terminal heat stress adversely affects chickpea productivity in Northern India-Strategies to improve thermo tolerance in the crop under climate change. In: ISPRS Archives XXXVIII-8/W3 Workshop Proceedings: Impact of Climate Change on Agriculture. 23–25 February, New Delhi, India; c2009. p. 23-25.
- 7. Cornic G, Fresneau C. Photosynthetic carbon reduction and carbon oxidation cycles are the main electron sinks for photosystem II activity during a mild drought. Annals of Botany. 2002 Jun 15;89(7):887-94.
- 8. Conroy JP, Smillie RM, Küppers M, Bevege DI, Barlow EW. Chlorophyll a fluorescence and photosynthetic and growth responses of Pinus radiata to phosphorus deficiency, drought stress, and high CO2. Plant physiology. 1986 Jun 1;81(2):423-9.
- Di Marco G, Massacci A, Gabrielli R. Drought effects on photosynthesis and fluorescence in hard wheat cultivars grown in the field. Physiologia plantarum. 1988 Oct;74(2):385-90.
- 10. Devasirvatham V, Tan DK, Gaur PM, Raju TN, Trethowan RM. High temperature tolerance in chickpea and its implications for plant improvement. Crop and Pasture Science. 2012 Jul 9;63(5):419-28.
- 11. Furbank RT, White R, Palta JA, Turner NC. Internal recycling of respiratory CO2 in pods of chickpea (*Cicer arietinum* L.): the role of pod wall, seed coat, and embryo. Journal of Experimental Botany. 2004 Aug 1;55(403):1687-96.
- 12. Genty B, Briantais JM, Baker NR. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta (BBA)-General Subjects. 1989 Jan 27;990(1):87-92.

- Jones MM, Rawson HM. Influence of rate of development of leaf water deficits upon photosynthesis, leaf conductance, water use efficiency, and osmotic potential in sorghum. Physiologia plantarum. 1979 Jan;45(1):103-11.
- 14. Leport L, Turner NC, French RJ, Barr MD, Duda R, Davies SL, *et al.* Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. European Journal of Agronomy. 1999 Nov 1;11(3-4):279-91.
- 15. Ludlow MM. Contribution of osmotic adjustment to the maintenance of photosynthesis during water stress. InProgress in Photosynthesis Research: Volume 4 Proceedings of the VIIth International Congress on Photosynthesis Providence, Rhode Island, USA. Dordrecht: Springer Netherlands; c1986- 1987 Aug 10-15. p. 161-168.
- Maxwell K, Johnson GN. Chlorophyll fluorescence-a practical guide. Journal of experimental botany. 2000 Apr 1;51(345):659-68.
- 17. Morgan JM. Increases in grain yield of wheat by breeding for an osmoregulation gene: relationship to water supply and evaporative demand. Australian Journal of Agricultural Research. 2000;51(8):971-8.
- Morgan JM. Osmoregulation and water stress in higher plants. Annual review of plant physiology. 1984 Jun;35(1):299-319.
- Moinuddin, Khanna-Chopra R. Osmotic adjustment in chickpea in relation to seed yield and yield parameters. Crop Science. 2004 Mar;44(2):449-55.
- 20. Turner N. Adaptation to drought: lessons from studies with chickpea. Indian Journal of Plant Physiology; c2003. p. 11-7.
- 21. Turner NC, Davies SL, Plummer JA, Siddique KHM. Seed filling in grain legumes under water deficits with emphasis on chickpea (*Cicer arietinum* L.). Advances in Agronomy. 2005;87:211-250.
- 22. Schreiber U. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. Chlorophyll a fluorescence: a signature of photosynthesis. 2004, 279-319.
- 23. Schreiber U, Schliwa U, Bilger W. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynthesis research. 1986 Jan;10:51-62.
- 24. Serraj RA, Sinclair TR. Osmolyte accumulation: can it really help increase crop yield under drought conditions?. Plant, cell & environment. 2002 Feb;25(2):333-41.
- 25. Serraj, Sinclair. concluded that no yield advantage from OA had been demonstrated in any crop except at very low and uneconomic yields; c2002.
- 26. Siddique KH, Brinsmead RB, Knight R, Knights EJ, Paull JG, Rose IA. Adaptation of chickpea (*Cicer* arietinum L.) and faba bean (*Vicia faba* L.) to Australia. InLinking Research and Marketing Opportunities for Pulses in the 21st Century: Proceedings of the Third International Food Legumes Research Conference. Springer Netherlands; c2000. p. 289-303.
- 27. Siddique MRB, Hamid A, Islam MS. Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica. 2000;41:35-39.

- Summerfield RJ, Hadley P, Roberts EH, Min Chin FR, Rawsthrone S. Sensitivity of chickpea (*Cicer arietinum* L.) to hot temperatures du ring the reproductive period. Exp. Agiic. 1984;20:77-93.
- 29. Smillie RM, Hetherington SE. Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence *in vivo*: chilling, freezing, ice cover, heat, and high light. Plant Physiology. 1983 Aug 1;72(4):1043-50.
- Cornic GL, Le Gouallec JL, Briantais JM, Hodges M. Effect of dehydration and high light on photosynthesis of two C 3 plants (*Phaseolus vulgaris* L. and Elatostema repens (Lour.) Hall f.). Planta. 1989 Jan;177:84-90.