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Physiological and biochemical adaptation of chickpea (*Cicer arietinum* L.) genotypes under moisture stress

VAISHALI SHARMA, JAGMEET KAUR, SARVJEET SINGH, INDERJIT SINGH, SATVIR KAUR and NORAH JOHAL

Department of Genetics and Plant Breeding, Punjab Agricultural University, Ludhiana-141004, India; E-mail: jagskaur@pau.edu

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ABSTRACT

Six chickpea genotypes *i.e.* tolerant (BGD1094, ILC 3279 and L555) and sensitive (GL29095, GL12003 and GNG2171) categorized on the basis of lysimetric screening for moisture stress conditions were evaluated for physiological and biochemical studies. Tolerant genotypes exhibited higher photosynthetic rate, Relative Water Content (RWC) and leg haemoglobin content in comparison to sensitive genotypes. Proline, total soluble sugars, superoxide dismutase, peroxidase and catalase activities increased among all the genotypes but tolerant ones showed higher upheaval and under moisture stress conditions (rainfed) in contrast to sensitive genotypes. Starch content reduced correspondingly under moisture stress with maximum decline (32.36%) observed in GL12003. The accumulation of osmolytes and higher antioxidative enzymatic activity in tolerant genotypes imparted tolerance to moisture stress in comparison to the sensitive ones.

Key words: Antioxidant enzymes, Chickpea, Drought tolerance

Chickpea (*Cicer arietinum* L.) is one of the most important grain legumes grown over 13.5 m ha with an average productivity of about 967.6 kg/ha, of which India solely contribute about 68% (FAOSTAT 2015). In arid and semi-arid regions, chickpea is generally grown under rainfed conditions. Other than moisture, cold, heat and salinity are major abiotic stresses which hamper the chickpea production. Chickpea faces two types of drought situations such as, terminal drought where soil moisture continuously decreases towards the end of the growing season and intermittent drought where soil moisture may be depleted if winter rain is irregular and insufficient. The crop faces drought stress either when the water supply to roots is interrupted or when transpiration rate is very high. Legume plants have at least two ways to resist drought *i.e.* drought avoidance via efficient stomata regulation and drought tolerance via osmotic adjustment (Vadez *et al.* 2008).

Plants respond to drought stress and become accustomed through various physiological and biochemical changes including changes of water use efficiency, pigment content, osmotic adjustment and photosynthetic activity (Farooq *et al.* 2009). High relative water content (RWC) and low excised-leaf water loss are linked with drought tolerance. The drought stress often leads to oxidative stress in plants due to higher leakage of electrons towards O_2 during photosynthetic and respiratory processes causing enhanced production of reactive oxygen species (ROS). The ROS such as superoxide radical (O_2), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and alkoxy radical (RO) are highly reactive and can change normal cellular metabolism through oxidative damage to membranes, nucleic acids and proteins (Mittler 2002). When the crop experiences stress conditions, there is modulation of the activities of antioxidant enzymes which leads to enhanced cellular protection (Kaur *et al.* 2012). Plant cells respond defensively to oxidative stress by reducing the concentration of ROS and maintaining antioxidant defense compounds and osmolytes. Proline is one of the common osmolytes which increase in plants under moisture stress and help the plants to maintain cell turgidity (Moayedi *et al.* 2011). The damage caused during stress cold ultimately stress yield. Therefore, in the present study attempt has been made to elucidate various physiological and biochemical adaptations in the selected chickpea genotypes through lysimetric screening for moisture stress.

MATERIALS AND METHODS

Six genotypes of chickpea (*Cicer arietinum* L.) were raised in the experimental field of Department of Genetics and Plant Breeding, Punjab Agricultural University, Ludhiana under irrigated (control) and rainfed conditions. The crop was sown in the month of November 2015 and six genotypes (categorized as tolerant *viz.*, BGD 1094, ILC 3279, L 555 and sensitive *viz.*,GL 29095, GL 12003, GNG 2171 on the basis of lysimetric screening) were evodited in a randomized block design with three replications. Each genotype was accommodated in paired row of 3m length at row spacing of 40 cm.The physiological (relative water content, photosynthetic rate) and biochemical (total soluble sugars, superoxide dismutase, peroxidase and catalase activity) parameters were estimated from leaves during reproductive stage. Starch content was estimated from the seed sample at maturity. Leg ahaemoglobin content was estimated from the nodules during reproductive phase.

Physiological parameters: Relative leaf water content was estimated according to the method of Weatherley (1950) from second and third leaves and was calculated as: RWC

= (Fresh weight-Dry weight /Turgid weight-Dry weight) X100. Photosynthetic rate was recorded as µmole CO_2 m^{-2} s^{-1} by using Portable Photosynthesis System (LI-6400XT, LICOR). Leg haemoglobin content from the fresh nodules was determined by using drabkin's solution as per the method described by Wilson and Reisenauer (1963).

Biochemical parameters: Proline content both in leaves and seeds was extracted using 3% sulfosalicylic acid and estimated by reacting it with acidic ninhydrin reagent (Bates *et al.* 1973). Total soluble sugars and starch content were extracted with 80% ethanol and estimated (Dubois *et al.* 1956).

Enzymatic estimation: The enzyme extract for superoxide dismutase (SOD) and peroxidise (POX) was prepared from 0.1g fresh leaf sample with 0.1M potassium phosphate buffer (pH 7.5) containing 1% PVP, 1mM EDTA and 10mM β-mercapto ethanol. The enzymes were estimated as per the protocols given by Marklund and Marklund (1974) for superoxide dismutase and Shannon *et al.* (1966) for peroxidise. The enzyme catalase (CAT) was extracted from 0.1g fresh leaf sample with 50mM sodium phosphate buffer containing 1% PVP and estimated by the methods of Chance and Maehley (1955).

Protein profiling: Protein of the seed of irrigated and rainfed crop was analyzed using SDS PAGE by Laemmli (1970).

Yield parameters: Three plants were taken randomly from each plot and average seed yield per plant was recorded and expressed as in grams/plant. Harvest index **(**HI) defined as the ratio of seed yield to the total biomass at maturity and was expressed in per cent.

RESULTS AND DISCUSSION

Physiological parameters

Photosynthetic rate: Photosynthetic rate decreased under rainfed condition with maximum decline observed in sensitive genotypes (54.76%), where as it was 11.65% in tolerant genotypes. Among sensitive genotypes, GL 29095 showed a maximum reduction (59%) in the photosynthetic rate under rainfed condition. This can be attributed to decline in stomatal conductance under moisture stress (Krouma 2009). Photosynthetic rate of L 555 (tolerant genotype) showed 8% decline under rainfed condition as compared with irrigated condition.

Relative water content: For efficient physiological functioning and growth processes of crop, optimum relative water content is essential and is known as potential physiological marker in many crops. In the present study, RWC significantly decreased in all genotypes under moisture stress condition, but these reductions in tolerant genotypes were less (9.64%) as compared to sensitive ones (26.14%) (Table1). Among tolerant genotypes BGD 1094 and L 555 showed least reduction (9%) while among sensitive genotypes GL 12003 showed maximum reduction (29%) in RWC under moisture stress. This decline may be attributed to higher water loss through stomatal regulation during photosynthesis and inefficient water utilization assimilation under moisture stress (Lobato *et al.* 2008). The lesser decline in RWC of tolerant genotypes in comparison to sensitive ones could be due to efficient control mechanisms to maintain cell and tissue hydration under water stress by regulating stomatal opening. The alterations in RWC in response to water stress have also been reported by Kaur *et al.* 2016.

Leg haemoglobin content: The leg haemoglobin content in nodules of the genotypes correlated with RWC having the highest decline of 49% in GL 12003 (sensitive) and the lowest of 14% in L 555 (tolerant). Decline in leg haemoglobin content has also been reported in common bean subjected to severe moisture stress condition which may be due to restriction of carbohydrate transport from leaves to nodule (Figueriedo *et al.* 2008). There can be production of O, radicals in stressed genotypes which are reported in *Medicago truncatula* showing reduced leg haemoglobin content (Mhadhabi *et al.* 2009).

Biochemical parameters

Total soluble sugars, proline content and starch content: Proline is the most important organic solute accumulate in higher plants under drought conditions (Sumera and Asghari 2010). There was low accumulation of total soluble sugars and proline content in leaves and seeds under irrigated conditions, while it enhanced noticeably under rainfed condition (Table 2). The mean value of proline content in leaves increased from 1.23µmoles/g to 2.52µ moles/g and in seeds from 1.97µ moles/g to 4.64µ moles/g under rainfed conditions. Higher increase was observed in

Table 1. Photosynthetic rate, RWC and leghaemoglobin content of chickpea genotypes under different conditions

Genotype	Photosynthetic rate (µmole CO_2 m^{-2} s^{-1})		RWC (%)		Leghaemoglobin content (mg/g fresh nodule weight)	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
GL 29095	10.32±1.23	4.21±1.12	71.25±0.75	54.64±0.84	7.54±0.24	4.24±0.25
GL 12003	11.32±0.89	5.13±0.84	76.34±0.84	54.36±0.57	6.22±0.21	3.15±0.24
GNG 2171	11.12±0.89	5.48±1.51	77.64±0.75	57.35±1.21	5.34±0.31	3.48±0.54
BGD 1094	12.34±1.12	10.89±0.89	76.38±1.21	69.54±0.67	6.18±0.34	5.12±0.31
ILC 3279	10.26±1.35	8.67±1.12	76.34±0.84	68.25±0.57	5.63±0.41	4.78±0.25
L 555	10.36±1.48	9.56±1.78	75.89±0.87	68.78±0.68	7.54±0.52	6.45±0.52

Data represent mean± standard error of triplicates

Table 2. Proline content (leaves and seeds), total soluble sugars and starch content in chickpea genotypes under irrigated and rainfed conditions

	Proline content (leaves) (µmoles/g dry weight)		Proline content (Seeds) (µmoles/g dry weight)		Total soluble sugars (mg/g dry weight)		Starch content (mg/g dry weight)	
Genotype	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**
GL 29095	1.02±0.20	1.86±0.46	1.52±0.21	3.12±1.31	32.31±1.35	46.31±1.52	34.18±2.03	24.26±2.14
GL 12003	0.95±0.16	1.63±0.51	1.63±0.16	3.06±1.14	29.65±1.67	42.14±1.53	37.48±1.58	25.35±1.64
GNG 2171	1.16±0.21	1.93±0.34	1.56±0.12	3.16±1.06	32.58±1.61	40.24±0.61	36.43±2.12	26.47±2.06
BGD 1094	1.34±0.34	3.26±0.25	2.36±0.31	6.14±1.14	36.57±2.02	65.24±1.25	54.23±2.07	41.48±2.11
ILC 3279	1.53±0.41	3.19±0.14	2.31±0.22	6.25±1.12	36.81±1.39	67.26±1.36	52.34±1.87	42.34±1.92
L 555	1.38±0.42	3.25±0.51	2.43±0.32	6.10±0.76	38.12±1.46	68.31±2.15	49.28±2.16	40.58±2.31
Mean	1.23	2.52	1.97	4.64	34.34	54.92	43.99	33.41

Data represent mean ± standard error of triplicates

tolerant genotypes BGD1094 (58.90%) and ILC 3279 (63.04%) in leaves and seeds, respectively. Variation in total soluble sugars estimated in dry chickpea leaves is represented in Table 2. Maximum sugar content was observed in L 555 (38.12 mg/g dry weight) and minimum in GL 12003 (29.65 mg/g dry weight) under irrigated condition. In order to maintain the cell turgor, total soluble content was increased under moisture stress conditions. Genotype ILC 3279 showed maximum increase of 42.57% in TSS content under moisture stress condition relative to control conditions. There was inter-relationship between starch and soluble sugars concentration. Starch content under moisture stress decreased by 32.36%, 29.02% and 27.34% in the seeds of sensitive genotypes GL 12003, GL 29095 and GNG 2171 respectively. Moisture stress however did not show a pronounced decrease in the starch content of tolerant genotypes. In the seeds of tolerant genotype L 555, the moisture stress reduced starch content only to 17.65%.

The Present study indicated an increase in soluble sugars and proline, while storage compound starch declined as the stress increased. Changes in quantity of soluble sugars in association with moisture stress may be due to increased sugar biosynthesis, conversion of storage forms of carbohydrates to soluble sugars, breakdown of cell wall polysaccharides and changes in rate of sugar transport. Under moisture stress condition, lowered water potential is accompanied by breakdown of starch by hydrolytic enzymes amylases into glucose and maltose that increases the osmotic concentration of cell. As a result, cellular turgor, expansion growth, uptake of water and minerals through root are maintained. Proline acts as protective osmolyte which accumulates faster than other amino acids, shows diverse role in drought tolerance reactive oxygen species scavenger, and protection from oxidative damage and stabilizing enzymatic proteins against desiccation. Enzymes involved in proline biosynthesis elevates under drought stress, whereas those of degradation are inhibited (Sumithra and Reddy 2004).

Antioxidant enzymes: Water deficit stress influences the anti oxidative defense mechanisms to a great extent, by increasing the activity of some specific enzymes which plays a vital role in plant's tolerance to stress. Among the antioxidant enzymes, superoxide dismutase (SOD) constitutes the first line of defense via detoxification of superoxide radicals to H_2O_2 (Sairam and Saxena 2000). In the present study, activity of SOD was found to be higher in leaves of tolerant genotypes as compared to sensitive genotypes under control and rainfed conditions (Fig.1). Highest activity was observed in tolerant genotype ILC 3279 (351.42 unit enzyme/g FW), whereas lowest activity was noticed in GNG 2171 (275.60 unit enzyme/g FW) under rainfed conditions. Higher superoxide dismutase activity during drought stress protects plants from oxidative injury (Arora *et al.* 2002).

The specific activity of peroxidase in the leaves of tolerant genotype ILC 3279 was increased by 34.07% under moisture stress condition, whereas the activity of peroxidase in the leaves of sensitive genotypes did not increase considerably (*i.e.* 22.21 and 23.05 % increase were reported in GL 29095 and GNG 2171, respectively). Higher level of peroxidase activity resulted into higher capacity to decompose H_2O_2 more rapidly (Patel and Hemantaranjan 2012).

Catalase eliminates H_2O_2 by breaking it down directly to water and oxygen. Under control condition the highest activity of catalase was found in tolerant genotypes *i.e.* L 555 (943.13 ΔA/min/gFW) and BGD 1094 (934.56 ΔA/min/gFW), whereas lowest activity was observed in sensitive genotype *i.e.* GNG 2171 (597.40 ΔA /min/gFW). There was sharp increase in catalase activity observed in ILC 3279 (27.70%) when exposed to moisture stress condition (Fig. 3).

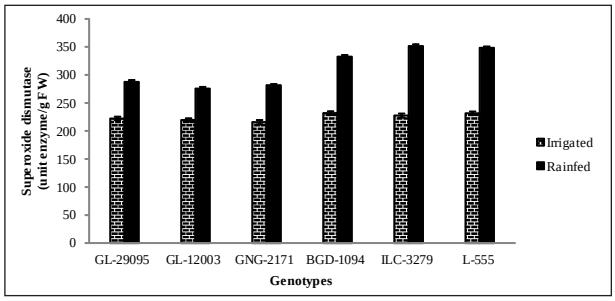

Figure 1. Superoxide dismutase activity of chickpea genotypes under irrigated and rainfed conditions

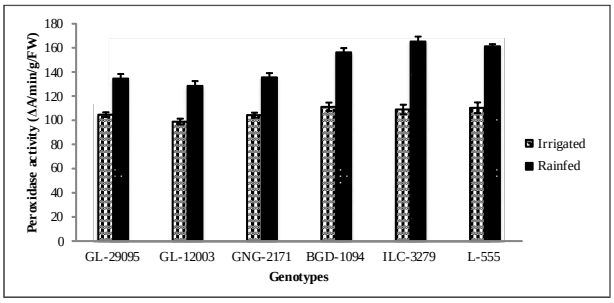

Figure 2. Peroxidase activity of chickpea genotypes under irrigated and rainfed conditions

Increased activity of catalase enzyme develops a potential for defense against damage as observed in maize genotypes (Helal and Samir 2008).The least increase was observed in sensitive genotype GL 29095 (16.41%) under moisture stress. The decrease in catalase activity observed under water deficit stress could be either caused by inhibition of new enzymes or photo inactivation (Basu *et al.* 2010).

Electrophoretic analysis of total proteins in seeds from irrigated condition revealed that bands near to 96 KDa molecular weights were more intense in BGD 1094 and L 555; and less intense in GNG 2171. In seeds derived from rainfed condition, differences were more evident. Bands of

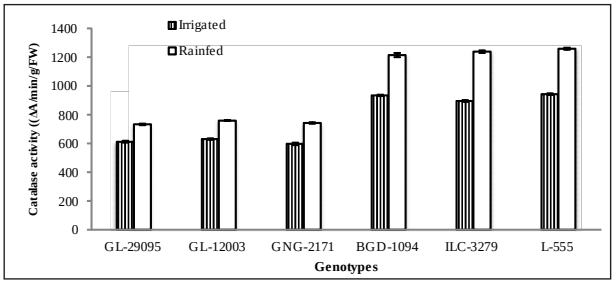

Figure 3. Catalase activity of chickpea genotypes under irrigated and rainfed conditions

lesser intensity near 96 KDa molecular weights were visible in BGD 1094, ILC 3279 and L 555. However, 96 KDa molecular weight proteins were less intense in GNG 2171. Bands of 66 KDa molecular weights were more intense in control than stress treatments. 29 KDa bands were more intense in GNG 2171 both in irrigated and rainfed conditions. Bands with molecular weight 20.1 KDa and 14.3 KDa were observed with high intensity under irrigated than rainfed conditions (Fig. 5). Molecular markers have been used to study the extent of genetic variation. The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops (Nisar *et al.* 2007). The genotypic variations in banding pattern were higher in present research in analogy to water stress treatments. These results were in accordance with finding of Iqbal and Bano (2009) in wheat. Severe drought stress had effect on protein banding patterns. However, other water stress treatments showed no significant effect as observed in

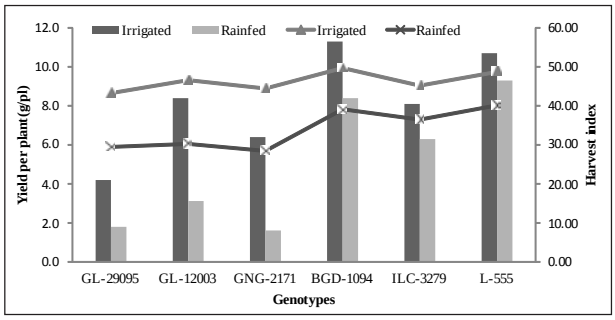

Figure 4. Yield per plant and harvest index of chickpea genotypes under irrigated and rainfed conditions

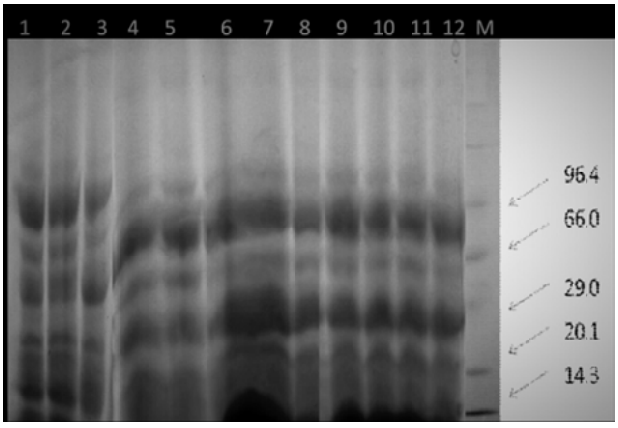

Figure 5. Banding pattern in chickpea genotypes (GL 29095, GL 12003, GNG 2171, BGD 1094, ILC 3279 & L 555) under irrigated and rainfed conditions by SDS-PAGE
Lanes: M - Protein molecular weight marker (14.3-96.4 KDa), Lane : 1- GL 29095 (irrigated), Lane : 2- GL 12003 (irrigated), Lane : 3- GNG 2171 (irrigated), Lane: 4- BGD 1094 (irrigated), Lane: 5- ILC 3279 (irrigated); Lane: 6- L 555 (irrigated); Lane 7- GL 29095 (rainfed), Lane : 8- GL 12003 (rainfed), Lane : 9- GNG 2171 (rainfed), Lane: 10- BGD 1094 (rainfed), Lane: 11- ILC 3279 (rainfed); Lane: 12- L 555 (rainfed)

chickpea (Mansourifar *et al.* 2011).

Yield attributes: Under control condition, genotypes BGD 1094 (11.3g) and GL 29095 (4.2g) recorded maximum and minimum yield per plant, respectively. Sensitive genotypes, GNG 2171 (74.84%) and GL 12003 (62.86%) showed maximum yield reduction under moisture stress condition. The tolerant genotype L 555 recorded minimum yield per cent reduction (13.08) due to moisture stress.

Harvest index: Harvest index is one of the important yield contributing attributes and the data pertaining to it is presented in Fig. 4. Under rainfed condition, harvest index was reduced in all the genotypes and maximum decline was observed in sensitive genotypes GNG 2171 (35.98%), GL 12003 (35.06%) and GL 29095 (31.84%). Higher photosynthetic rate due to high RWC of leaves resulted into minimum decline in harvest index of tolerant genotypes (19.4%). Environmental stresses such as water shortages especially during grain filling cause reductions in photosynthesis and remobilization of stored materials, rate

and duration of grain filling and grain weight (Sadeghipour 2008). Oberoi *et al.* (2015) has reported that poor capacity of the anti oxidative defense system in sensitive cultivars seems to be partly responsible for reduced yield potential under drought stress.

An assessment of the results shows that moisture stress tolerant genotypes having higher RWC, photosynthetic rate and regulated enzymatic defense mechanism resulted into higher yield in comparison to sensitive genotypes under drought.

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