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Osmotic adjustment increases water uptake, remobilization of assimilates and maintains photosynthesis in chickpea under drought

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Eight chickpea advanced breeding lines (ABLs) and their parents were evaluated for osmotic adjustment (OA), leaf carbohydrates and gas exchange under dryland field . These (ABLs) were derived from crosses between CTS 60543 × Kaniva and Tyson × Kaniva. Mean leaf water potential (LWP) fell down from –1.00 MPa at pre-stress level to about –2.25 MPa during terminal stress. Relative water content (RWC) showed periodic changes with alternate decrease or increase at certain interval, which also influenced the values of OA (low or high) in number of genotypes e.g. Kaniva, CTS 60543, Tyson and M 75. Significant variation in OA ranging 0.45 to 0.88 MPa was observed at high level of stress at –2.5 MPa. However, none of the genotypes showed stability of OA over the period of stress. Leaf starch declined even at mild stress (LWP,-1.6 MPa) resulting in an increase in hexose sugars and activation state of sucrose-phosphate synthase (SPS) that led to accumulation of sucrose. Both photosynthesis (P_{max}) and transpiration decreased concurrently in two chickpea lines M 129 and Tyson with increasing water stress. However, rate of decline in the photosynthesis slowed down even drought was further intensified. The observed periodic changes in OA, RWC and photosynthesis appeared to be associated with drought-induced changes in SPS and carbohydrates which modify water uptake of the leaves.

Keywords : *Cicer arietinum*, Water stress, Leaf water potential, Relative water content, Sucrose, Hexose, Sucrose-phosphate synthase, Photosynthesis, Transpiration, ABLs, Starch

Chickpea (*Cicer arietinum* L.) , an important winter food legume , is cultivated predominantly over 75-80% in water-limiting environment. The crop usually faces terminal drought during pod filling stage leading to significant reduction in the grain yield[1]. Among various traits, osmotic adjustment (OA) is considered as an important physiological trait for adaptation to drought[2]. The osmotic adjustment in chickpea has been reported to be ranged from 0 to 1.3 MPa[3]. Osmotic adjustment increases water absorption, maintains cell turgor, photosynthesis and leaf area duration, helps stomatal opening, delays senescence and death[4], reduce flower abortion[5], and improves root growth as water deficits develop[6]. The greater osmotic adjustment leads to higher growth rate and dry matter production in pigeonpea under drought[7] .

Genotypes of wheat and sorghum with a high capacity for OA had up to 60% higher yields than those with a low capacity[8,9]. The degree of OA has also been shown to be correlated with yield under dryland conditions in chickpea[8]. However, other workers[10] opined that osmotic adjustment may not have any direct effect on crop yields. Contradictory results suggested that chickpea probably cannot derive distinct advantage from osmotic adjustment in comparison to other crops. Therefore investigation was carried out to look for physiological significance of osmotic adjustment in chickpea under drought.

Materials and Methods

Experimental material—2-way crosses were made using chickpea genotypes with high osmotic adjustment (CTS 60543) and low OA (Kaniva) while another set having medium OA (Tyson) crossed with low OA (Kaniva). The populations were advanced to F_6 stage and eight advance breeding chickpea lines at F_6 stage were evaluated under rainfed for osmotic adjustment and other physiological traits. These breeding lines were M 39, M 51, M 55, M 75, M 86, M 93, M 110, M 129.

No pre-sowing irrigation was applied before planting the material on 31st October, 2004-05 at experimental site of Indian Institute of Pulses Research, Kanpur, India (20°27′N, 80°14′E). Irrigation was completely withdrawn throughout the crop season. The crop was subjected to complete dry weather till late podding stage with steady decrease in the soil moisture at given depth. The observations on

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the various physiological parameters were started at flowering, 70 DAS (days after sowing) and thereafter continued at 85, 92, 98, 101, 106, 113, 117 and 120 DAS.

Soil moisture—The soil moisture (composite samples) from profile 0-15 , 15-30 and 30-60 cm from top was measured between 1100-1200 hr by gravimetric method at 0, 60, 90, 97, 104, 111, 118 and 121 days after sowing.

Water relation characteristics—The measurement on water relation characteristics such as RWC, WP and OP was started upon the initiation of flower at 70 days and continued thereafter at 85, 92, 98, 101, 106, 113, 117 and 120 DAS till post-anthesis and pod filling. The leaf water potential and sampling for RWC and OP were simultaneously taken from the same plant in between 1100-1300 hr in sunny day covering all 11 accessions and 3 plants per replication for each line.

Relative water content (RWC)—The observations on RWC of 4th fully expanded leaf from top were taken. RWC of the leaf was finally determined by using formula [RWC= (Fresh weight-dry weight)/ (Turgid weight-dry weight)].

Leaf water potential (LWP)—The leaf water potential was measured by using Pressure Chamber (pms Instrument co., Corvallis, Oregon, USA).

Osmotic adjustment (OA)—Fifth leaf from the same plant was detached and put into the aluminium foil . The leaves were frozen in the liquid nitrogen for several days, thawed, and put into the 2ml hypodermic syringe and filtered. The filtered cell sap was used for measurements of osmotic potential (OP) using Wescor vapour pressure osmometer (USA) calibrated with standard solution of 100, 290 and 1000 Osmol solution. Osmalarity (in millimoles) of standard NaCl solutions (0.1, 0.2. 0.7 and 1 mol) was determined using osmometer and WP of corresponding NaCl solutions was calculated from the table[11]. The osmotic potentials of the leaves were determined from the linear equation derived from plot of osmolarity of the NaCl solutions and corresponding WP values of same NaCl solutions[11].

Osmotic potential at full turgor (OP_{100}) was calculated using following formula:

$$OP_{100} = (OP \times RWC)/100$$

OA was calculated from the difference in OP_{100} (at full turgidity) between leaves sampled at any given date and the least stressed OP, recorded at 92 DAS. Therefore, the ability of OA of any chickpea lines at any given time was estimated relative to the OP_{100} values at 92 days.

Leaf carbohydrates (starch, hexoses and sucrose)—The leaf carbohydrates were determined for the same plant with already known LWP and OP on 98, 101, 106, 113, 117, 120 DAS. Three randomly selected chickpea breeding lines e.g. M51, M 93, M129 and Tyson were used for starch and sugar (as osmolytes) estimation in leaves. The leaves closer to 4th/5th leaf of the same branch already sampled for RWC and OP, were detached, wrapped in aluminium foil and were immediately frozen in liquid nitrogen for several days. The frozen samples were taken out and oven-dried for further use. The dried leaf samples with known weight were dipped into 80% boiling alcohol. Standard extraction procedures[12] were followed to obtain a protein-free aqueous fraction containing soluble sugars (total soluble sugars including sucrose and hexoses). Sucrose[12] and total reducing sugars[13] (hexoses) were determined as per methods described. The residue obtained after alcohol extraction was washed and digested with 52% perchloric acid and starch was estimated[14].

Sucrose-phosphate synthase (SPS) activity—Breeding line M 129 and Tyson were selected for determining the leaf gas exchange (photosynthesis and transpiration) and sucrose phosphate (SPS) synthase activity. The activity per unit area of leaf was measured on 99, 102, 107, 114, 118 and 121 DAS. The samples 3rd to 5th leaf from top was taken immediately after photosynthesis measurement. SPS extraction and assay were done according to standard methods described in the literature[15]. The crude enzyme extract (supernatant) was loaded onto Sephadex G-25 columns (1×2.5 cm) and desalted by spinning at 800×*g* for 1 min in a refrigerated centrifuge. SPS activity was assayed with limiting substrates plus P*i* (V_{lim} limiting assay) or with saturating substrates (assay). The ratio of two activities (V_{lim}/V_{max}) is referred to as the activation state (per cent).

Gas exchange measurements—The steady state light-saturated (>1500 μmol photons $m^{-2}s^{-1}$) rates of photosynthesis (P_{max}) and transpiration in breeding line M 129 and Tyson were measured on a clear sunny day between 1200-1300 hr at 99, 102, 107, 114, 118 and 121 DAS along with sampling for leaf SPS. Fourth fully expanded leaf from top was used for the measurement under field conditions using fixed

exposed area of leaf at constant temperature 20°C and 60% relative humidity using open infra red gas analyzer (LI-COR 6400, USA).

Experimental design and statistical analysis—The experiment was laid out in a randomized block design (RBD) with three replications (three blocks). Each block consisted of the 11 genotypes in plots 3 m long by 1.5 m wide with 5 rows hand sown 0.3 m apart and with 0.1 m between plants. The number of plants per plot was about 150. The chickpea accessions and days after sowing (DAS) were treated as two treatments. Thus, total number of replications was 3 for each chickpea line. Three plants per replication were taken for various experiments. Data were analyzed using Statistical Software SPSS version 11. Analysis of variance (ANOVA) was conducted and significance of mean differences among treatments was tested using the least significant difference (LSD) method. Differences were considered to be significant if $P \leq 0.05$.

Results and Discussion

No rain was received between first observation on 70 DAS till 120 DAS (3rd March, 2005). Soil moisture at 0-15 cm depth declined to about 4% during pod filling at the stage of 100-120 DAS (Fig. 1). Leaf water potentials (LWP) after 92 DAS onwards declined steadily from –1.0 to –2.5 MPa till maturity along with decrease in soil moisture (Fig. 2). The slope calculated from the line of mean leaf water potentials (LWP) plotted against relative water content (RWC) indicated that decline in the LWP was very slow and gradual (Fig. 3).

RWC decreased with declining LWP when crop age increased from date of sowing, however mean RWC did not fall below 0.7 even at high stress (LWP –2.5 MPa; Fig. 4). As drought progressed, RWC repeatedly declined to lowest mean value of about 0.7 (Fig. 4). But RWC of the leaf subjected to stress restored partly or completely to pre-stress condition without any irrigation or rainfall (Fig 4). Thus, periodic change in RWC with alternate decreasing and increasing during a period of water stress suggested an internal mechanism, which helps to maintain RWC closer to normal pre-stress value and thereby preventing RWC to fall below a critical level. This pattern of RWC also influenced the leaf water potential that declined very slowly with faster

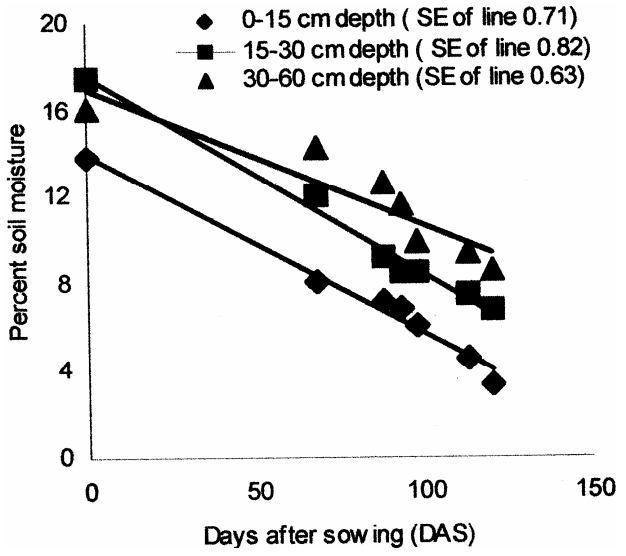

Fig. 1—Soil moisture profile at different depths and stages of crop growth.

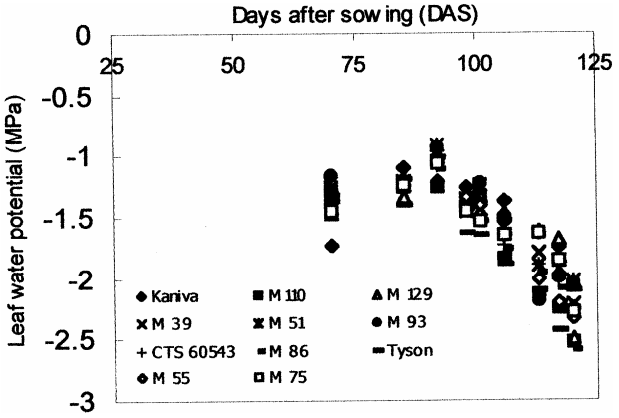

Fig. 2—Mean leaf water potentials of different genotypes with progressive increase in the water stress along with crop age (DAS). Each point represents mean of three replications.

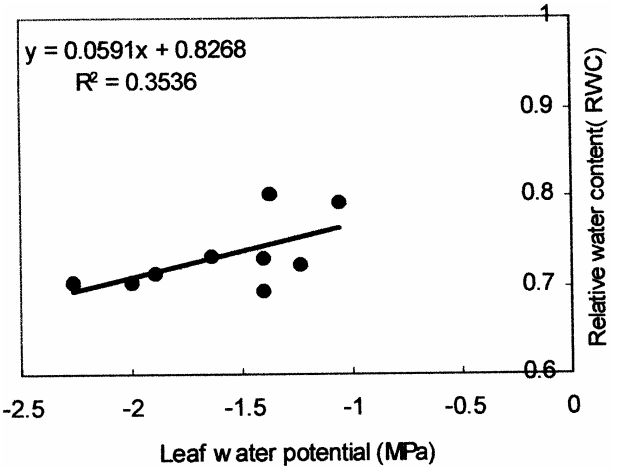

Fig. 3—Relationship of relative water content (RWC) with declining leaf water potentials (LWP). Each point represents mean RWC values of 11 chickpea lines at particular LWP.

decrease in the soil moisture (Fig. 3). Earlier studies[16] with pigeonpea have indicated that OA maintained leaf longevity by preventing RWC from falling below a critical level of about 32%.

The osmotic potential of all genotypes at full saturation (OP_{100}) was regressed with leaf water potential (Fig. 5). OP_{100} decreased linearly from 92 DAS onwards (Fig. 5). The rates of change in OP_{100} were significantly greater in M55, M39 and M 86 than CTS 60543 ($P<0.058$), with values falling to

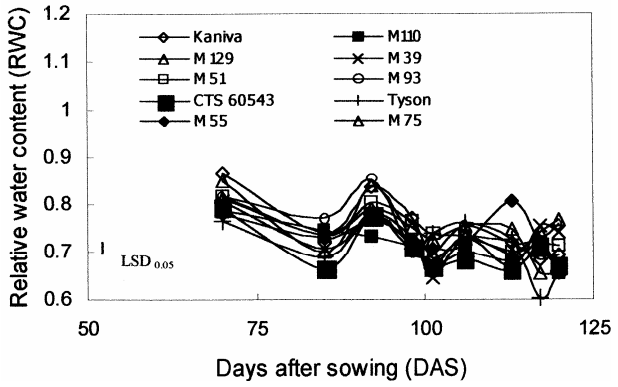

Fig. 4—Changes of mean Relative water content (RWC) with crop age or declining soil moisture. Each point represents mean of three replication.

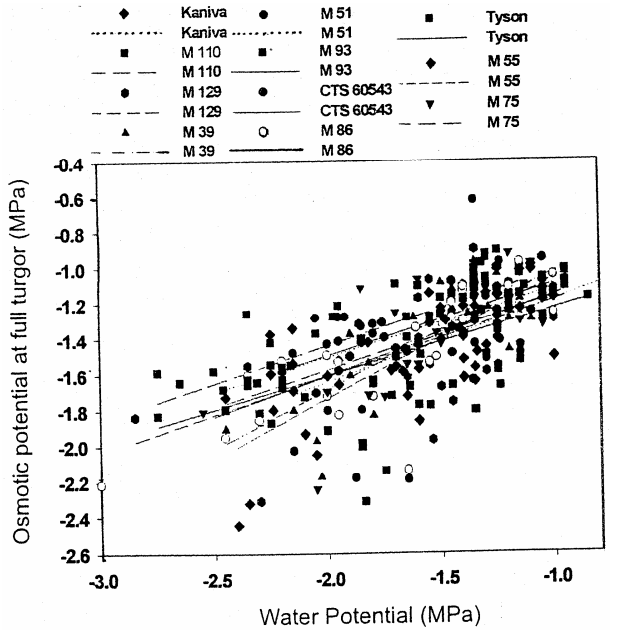

Fig. 5—Osmotic potentials at full turgor (OP_{100}) of chickpea lines with declining leaf water potentials. The slope of the line indicates the rate of osmotic adjustment (higher the slope more tendency of a genotype to adjust osmotically). Chickpea lines e.g. M 55, M 39, M 86 and Tyson have higher slope indicating higher tendency to adjust osmotically as compared to rest of the genotypes.

−1.9 to −2.3 MPa for the former, compared with −1.6 MPa for the latter, respectively.

The genotypic variation in OA ranging from 0.45 to 0.88 MPa was significant ($P\leq0.05$) at LWP below −2.0 MPa (Fig. 6). Our results showed lowest OA value (0.45 MPa) in CTS 60543 and maximum 0.88 MPa in breeding line M 86. Whereas, Kaniva had 0.62 MPa osmotic adjustment (Fig. 6). The significant genetic variation in OA from 0 to 1.3 MPa has been reported earlier in chickpea at very low LWP. However, earlier reports showed very high OA (1.3 MPa) in CTS 60543 at LWP, -3.0 MPa; while Kaniva did not show any OA under a trial conducted in Mediterranean climate of Australia[17]. The observed dissimilarities in OA for common genotypes tested at different locations suggested that OA is not a stable trait that varies depending upon the stress level, location or physiological stage of the plant.

Few genotypes such as CTS 60543, Tyson, M 75, Kaniva first showed increase in OA till 98 DAS, then declined at 113 DAS and then increased again at 120 DAS (Fig. 6). Thus, the osmotic adjustment in these genotypes expressed in a periodic manner similar to pattern as observed in case of RWC with ups and down at definite interval (Fig. 6a and b). Previous reports also indicated an increase in OA in chickpea followed by a complete disappearance or lowering of

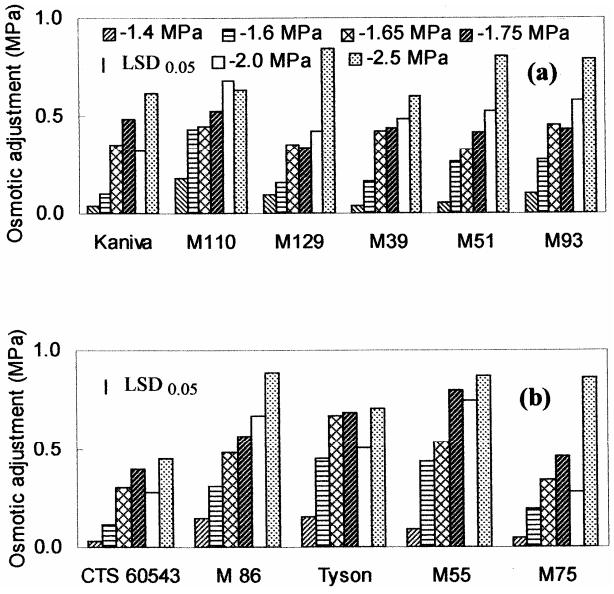

Fig. 6—Genotypic variation in the osmotic adjustment (a and b) in relation to declining leaf water potentials due to receding soil moisture level in respect to time (DAS). The bar represents the LSD for comparison between any two-histogram bar within or among the chickpea lines.

OA once it had reached to certain threshold level[17]. This suggested a possibility of repeated cycles of OA in chickpea under continued drought.

Leaf starch started degrading even in the mild stress at 98 DAS (about Ψ-1.6 MPa) and led to increase in hexoses (reducing sugars) in four chickpea lines M51, M93, M 129 and Tyson subjected to moderate or high water stress (Fig. 7). Like other C_3 species, starch occupies major pre-stored photosynthetic product in chickpea leaves. It has been reported that starch breaks down to release hexoses when photosynthesis declines below a threshold level[18] or drought could be the causal factor initiating starch breakdown and accumulation of soluble sugar[19]. Water stress further caused 2-3 fold increase in the activation state of sucrose-phosphate synthase (SPS; Table 1) that resulted in significant accumulation of sucrose (Fig. 8a). Results indicated that sucrose accumulation (Fig 8a) occurred with simultaneous lowering of hexoses (Fig. 7) as a result of starch breakdown. It appeared that both reducing sugars and sucrose might contribute solutes for osmotic adjustment or lowering of OP_{100} in chickpea

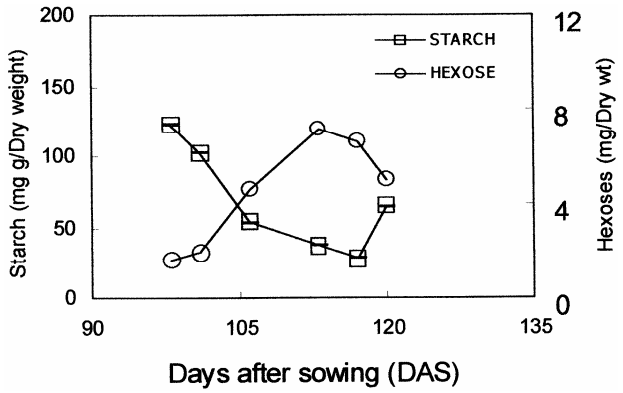

Fig. 7—Changes in the level of leaf starch and hexoses (means of 4 genotypes) with progressive increase in the water stress (DAS).

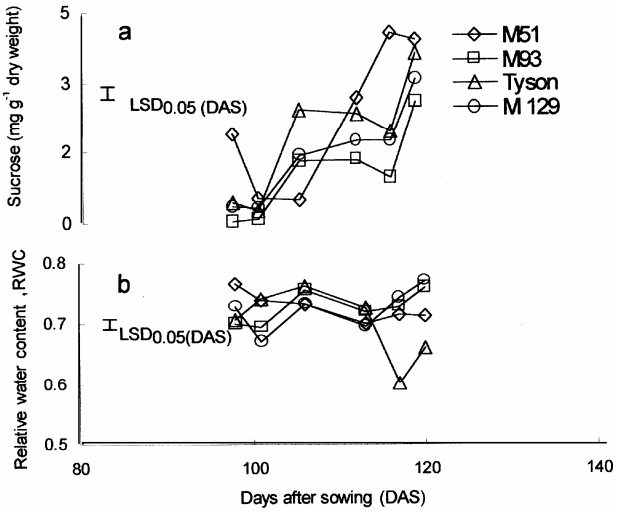

Fig. 8—Changes in the leaf sucrose (a) and relative water content (b) with increasing water stress as a result of receding soil moisture with time (DAS). [Each point represents mean of five replications (n=5)]

Table 1—Rate of gas exchange and activation state of sucrose-phosphate synthase in leaves of chickpea Tyson and advanced breeding line M 129 subjected to water deficits
[Values are mean ±SD of 5 replications]

			Tyson		
DAS	RWC	WP	P_{MAX}	Transpiration	SPS activation
99	0.71±0.02	-1.42±0.04	10.2±0.81	1.14±0.24	25±4.5
102	0.74±0.03	-1.65±0.04	8.6±1.26	0.88±0.083	28.53±5
107	0.76±0.06	-1.9±0.12	5.03±0.9	0.76±0.18	45.07±5
114	0.73±0.08	-2.1±0.18	2.6±0.78	0.41±0.13	57.3±3
118	0.6±0.03	-2.5±0.04	1.7±0.3	0.24±0.04	65.9±4.6
121	0.66±0.001	-1.7±0.06	5.7±0.58	0.81±0.05	23.6±3
M 129					
99	0.73±0.03	-1.32±0.07	12.8±1.6	1.87±0.11	11.8±1.56
102	0.67±0.02	-1.37±0.06	7.7±0.5	2±0.25	21.2±2.23
107	0.73±0.03	-1.47±0.09	6.7±1.3	1.24±0.36	30.5±2.48
114	0.7±0.032	-1.62±0.12	3.6±1.6	0.48±0.21	34.47±7
118	0.74±0.07	-1.7±0.09	2.1±1.5	0.37±0.075	48.43±3.5
121	0.77±0.05	-1.42±0.09	7.1±0.9	1.18±0.13	28.23±4.3
$LSD_{0.05}$	0.03	0.08	2.13	0.17	6.6

DAS = Days after sowing; RWC = Relative water content, WP= Water potential;
Pmax= Light-saturated rates of photosynthesis = µmol CO_2 $m^{-2}s^{-1}$ at >1500 PAR (µmol photons $m^{-2}s^{-1}$);
Transpiration rate = mmole H_2O $m^{-2}s^{-1}$; SPS = Per cent activation state of sucrose phosphate synthase

(Fig. 5). Similar to our findings, an increase in the sucrose content through transient increase in the activation state of SPS has been reported in spinach leaves subjected to drought[20]. Accumulation of sucrose appeared to be associated with increase in RWC (Fig. 8b), but decrease in OP_{100} (Fig. 5) i.e. Sucrose accumulation promoted osmotic adjustment. Subsequently, sucrose as osmolyte increased the gradient for water flux into the cell and maintained turgor by adjusting LWP and RWC (Figs 3, 4). Increased non-structural carbohydrates (starch and sugars) with enhanced remobilization of sucrose from stem to leaves in pigeonpea have been reported to be associated with leaf osmotic adjustment in pigeonpea under drought[7] . Accumulation of pinnitol derived from sucrose and proline as osmolytes has been reported in pigeonpea under water deficits[21]. Osmotic solutes in leaves increase gradient for water flux into the cell and maintains turgor[10].

The water stress resulted in a significant decline in the light-saturated rates of photosynthesis (P_{max}) and reduction in the transpiration rate in Tyson and M 129 (Table 1). However, when water stress was further intensified, at about 121 DAS, instead of declining, both P*max* and transpiration rates in test lines restored partially to normal value with concomitant increase in the RWC and WP (Table 1). The results showed reduction in the photosynthesis only at the beginning of stress development, however partial restoration of photosynthesis has been observed at high stress level which could be due to the high expression of osmotic adjustment transiently at lower leaf water potential (Table 1). Thus our results are consistent with earlier reports which indicated close association of osmotic adjustment with maintenance of high photosynthetic rates in other cool-season pulses[22] .The observed variation in OA and RWC with respect to increasing water stress, appeared to have close association with observed variation in the leaf carbohydrates in the chickpea lines subjected to drought (Figs 7, 8).

The transient decrease in the osmotic adjustment at WP -2.0 MPa (Fig. 6 a and b) observed in limited genotypes (Tyson, M 75, Kaniva, CTS etc) was likely to be associated with depletion in the osmolytes (reducing sugars or sucrose) (Figs 7, 8a). The results indicated that mild water stress helps in conversion of starch into reducing sugars followed by increase in SPS led to transient accumulation of sucrose contributing osmotic solutes (Table 1). The non-transportable carbohydrate, starch is converted to transportable form of sugar such as sucrose in the process of osmotic adjustment, thereby facilitates remobilization of pre-stored photosynthates from leaf to various sinks. When sucrose accumulated a threshold level, it increases the water flux into the leaf cells to regain the turgidity and improve LWP (Table 1) or RWC (Fig. 8 b). The leaf carbohydrates or sucrose and others as osmotic solutes started remobilizing as a consequence of regaining the leaf turgidity. Subsequently partial recovery of RWC (stress relief) led to decrease in the SPS activation state (Fig. 4 and Table 1), partial restoration of photosynthesis and reactivation of starch synthesis (Fig. 7). Thus, it is evident that osmotic adjustment prevents lowering of RWC below a critical level by efficient water uptake, restores photosynthesis and maintains positive carbon balance for longer stress period.

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