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Bioregulators protected photosynthetic machinery by inducing expression of photorespiratory genes under water stress in chickpea

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Abstract

Globally, water deficit is one of the major constraints in chickpea (*Cicer arietinum* L.) production due to substantial reduction in photosynthesis. Photorespiration often enhances under stress thereby protecting the photosynthetic apparatus from photoinhibition. Application of bioregulators is an alternative to counter adverse effects of water stress. Thus, in order to analyze the role of bioregulators in protecting the photosynthetic machinery under water stress, we performed an experiment with two contrasting chickpea varieties, *i.e*., Pusa 362 (Desi type) and Pusa 1108 (Kabuli type). Water deficit stress was imposed at the vegetative stage by withholding water. Just prior to exposure to water stress, plants were pretreated with thiourea (1,000 mg L^{-1}), benzyladenine (40 mg L^{-1}), and thidiazuron (10 mg L^{-1}). Imposed water deficit decreased relative water content (RWC), photosynthetic rate (P_N), quantum efficiency of PSII (F_v/F_m), and enhanced lipid peroxidation (LPO). However, bioregulator application maintained higher RWC, P_N, F_v/F_m, and lowered LPO under water stress. Expression of Rubisco large subunit gene (*RbcL*) was low under water stress both in the Kabuli and Desi type. However, bioregulators strongly induced its expression. Although poor expression of two important photorespiratory genes, *i.e.,* glycolate oxidase and glycine decarboxylase H subunit, was observed in Desi chickpea under imposed stress, bioregulators in general and cytokinins in particular strongly induced their expression. This depicts that the application of bioregulators protected the photosynthetic machinery by inducing the expression of *RbcL* and photorespiratory genes during water deficit stress.

Additional key words: chlorophyll fluorescence; drought; photochemical efficiency.

Introduction

Chickpea (*Cicer arietinum* L.) is the fourth most important legume crop cultivated mostly on marginal lands in arid and semiarid regions of the world. Globally, it is cultivated on over 13.5 million hectares with an annual production of 13.1 million tons (FAOSTAT 2013). Among the chickpea growing countries, India is the largest producer and consumer of chickpea contributing to 70% of the world's total production (FAOSTAT 2013). It is a rich source of proteins, essential amino acids, and vitamins, such as riboflavin, niacin, thiamin, and folate (Jukanti *et al*. 2012). Based on a seed size and colour, chickpea is grouped into two market classes, namely Desi and Kabuli. Globally about 80% of total production is contributed by Desi cultivars and these are relatively more tolerant to abiotic stress than the Kabuli type. Chickpea is traditionally cultivated in marginal and rainfed areas (Rao *et al*. 2002). Drought is the major constraint in chickpea production as 90% of world's chickpea is grown under rainfed conditions (Kumar and Abbo 2001). It causes 40–50% reduction in the yield globally; the productivity of chickpea remains constant for the past six decades (Ahmad *et al*. 2005, Varshney *et al*. 2010). Plants grown under drought conditions show a lower relative water content, stomatal conductance, and transpiration in order to conserve water. Consequently, CO_2 fixation is reduced and P_N decreases, resulting in lesser assimilate production for growth and yield of plants (Xu *et al*. 2010). Water deficit stress thus results in the decline of photosynthesis, disturbance of metabolism and finally the death of plant (Farooq

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Abbreviations: BA – benzyladenine; Chl – chlorophyll; CKs – cytokinins; DM – dry mass; F_v/F_m – quantum efficiency of PSII; FC – field capacity; FM – fresh mass; GDCH – glycine decarboxylase H subunit; GOX – glycolate oxidase; IPT – isopentenyl transferase; LPO – lipid peroxidation; P_N – net photosynthetic rate; RbcL – Rubisco large subunit; RuBP – ribulose-1,5-bisphosphate; RWC – relative water content; TDZ – thidiazuron; TU – thiourea.
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et al. 2008).

Understanding the physiological and molecular responses to water deficit stress is essential for a holistic perception of plant resistance mechanisms to water-limited conditions. RWC of leaves is an important characteristic which is directly related to a soil water content (Sarker *et al.* 1999). Stress-exposed plants immediately lower down RWC of their leaves (Grover *et al*. 2004). LPO is another system most commonly ascertained to oxidative damage and hence is regarded as an indicator of water deficit stress. Drought stress has been reported to substantially increase the malondialdehyde content in green bean genotypes (Yasar *et al*. 2008). Moreover, photosynthesis is aptly regarded as the most important physiological process in plants which is particularly sensitive to effects of water deficiency (Ashraf and Harris 2013). Even a small decrease in the water potential of a plant causes its stomata closure and decreases the intensity of photosynthetic assimilation of CO_2 (Rivero *et al*. 2009). As the degree of hydration of tissues decreases, the photosynthetic apparatus of plants undergoes functional changes, which further develop into disruption of its structure (Chernyad'ev 2005). However, many other processes, such as photochemical efficiency of photosystems, can be also affected by severe water stress (Dubey 2005), while chlorophyll (Chl) fluorescence parameters usually do not change during mild water stress (Rulcová and Pospíšilová 2001, Vomáčka and Pospíšilová 2003).

Presently, there is a greater research interest in identifying and employing bioregulatory molecules, which can be used to improve the stress tolerance in crops under field conditions. Exogenous applications of bioregulators provide an alternative approach to counter stress conditions (Kaur *et al*. 1998) as they revert inhibitory effects of water stress (Mehta *et al*. 2006). Bioregulators are likely candidates for playing a role in the transformation of stress-related signals into changes in gene expression needed to affect appropriate adaptation to suboptimal environmental conditions.

Among bioregulators, cytokinins (CKs) are known to regulate several aspects of plant growth and development, including the response of plants to abiotic stress (Rivero *et al*. 2007). Increasing evidence suggests that CKs are involved in stress responses (Havlová *et al*. 2008). CKs are well reported for reducing the perilous effect of drought stress (Ali *et al*. 2011) by protecting the photosynthetic machinery and selectively regulating the expression of certain genes involved in protection under water stress (Chernyad'ev 2005). Increased endogenous CK contents through isopentenyl transferase (*IPT*) transformation has been found to be associated with improved drought tolerance in various plant species (Zhang *et al*. 2010) including creeping bentgrass (Merewitz *et al*. 2010, 2011a). CKs, which regulate multiple functions in plant cells, were shown to control the induction and stimulation of critical plant protein synthesis and increase the stability of the photosynthetic apparatus during water deficiency. Merewitz *et al*. (2010, 2011b) reported that *IPT* transgenic plants, having a high zeatin content, exhibited higher P_N and F_v/F_m under drought stress in creeping bentgrass. The quantum efficiency of PSII, determined as variable to maximum Chl fluorescence ratio (F_v/F_m), decreased in plants pretreated with water but mostly not in those pretreated with CKs (Haisel *et al*. 2006). CKs have been also reported to induce the synthesis of photorespiratory enzymes (Rivero *et al*. 2009) and Rubisco under water stress conditions.

Benzyladenine (BA), a synthetic CK, shows a protective effect against water deficit and appears that it has a long lasting effect (Bano *et al*. 2010). Thidiazuron (TDZ) is another urea-based CK, nondegradable by cytokinin oxidase enzyme, which stimulates endogenous CK biosynthesis (Zhou *et al*. 1994). In addition, a certain thiol molecule, such as thiourea (TU), is known to maintain the redox state (–SH/-S-S- ratio) of the cell and its proper functioning under stress conditions (Srivastava *et al*. 2011). Also among the various thiols tested at the field, TU is the most cost-effective and ecofriendly one in nature.

Rubisco is the main enzyme in the CO_2 assimilation pathway. The rate of photosynthesis in higher plants depends primarily on the activity of Rubisco (Parry *et al*. 2002). Various evidence on water stress-induced alterations indicate that the content and activity of Rubisco actually control photosynthetic carbon assimilation (Reddy *et al*. 2004). The amount of Rubisco in leaves is controlled by the rate of synthesis and degradation of the enzyme, even in stressful environments. Drought stress induces reduction in the contents and activities of photosynthetic carbon-reduction cycle enzymes, including the key enzyme Rubisco. Decreased synthesis of Rubisco under drought was evidenced by a rapid decrease in the abundance of Rubisco large subunit (RbcL) in cucumber (Zhang *et al*. 2013). Loss of Rubisco activity has been also reported in several plant species under drought (Parry *et al*. 2002, Chaitanya *et al*. 2003).

As photorespiration is directly linked to photosynthetic metabolism and depends on recycling of ribulose-1,5-bisphosphate (RuBP) in the Benson–Calvin cycle. Severe drought stress should also result in reduced rates of photorespiration (Biehler and Fock 1996). The photorespiration pathway can protect the photosynthetic apparatus against photoinhibition by sustaining photon utilization in nonassimilatory electron flow (Osmond *et al*. 1997). This function of photorespiration could be particularly important when electron consumption by CO_2 assimilation is reduced in drought-stressed leaves (Osmond and Grace 1995). We studied the significance of photorespiration in water-stressed leaves by checking the expression levels of two major photorespiratory enzymes, *i.e.,* glycolate oxidase (GOX) and glycine decarboxylase H subunit (GDCH), in order to determine whether tolerance to water stress resulted in an increased

abundance of the enzymes of the photorespiratory pathway. Our results showed that during water stress, all the applied bioregulators, especially CKs, resulted in the induction of photorespiration and enhanced the expression level of Rubisco, which ultimately contributed to the protection of photosynthetic machinery.

Materials and methods

Plant material and growth conditions: The present study was carried out at the Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi (28°N, 77°E, about 250 m a. s. l.). Two popular varieties of chickpea were selected for the study, *i.e.*, Pusa 362 (Desi type) and Pusa 1108 (Kabuli type); the seeds were obtained from Division of Genetics, Indian Agricultural Research Institute, New Delhi. Sowing was done in 0.3 m diameter earthen pots filled with clay loam soil and farmyard manure in a 4:1 ratio during the winter season inside a net house of Division of Plant Physiology, IARI, New Delhi. All recommended agronomic practices were followed to raise healthy crop plants. A recommended basal dose of fertilizers in the form of urea, single super phosphate, and murate of potash were applied. Experiments were conducted in a factorial completely randomized design (CRD) with four replications using aforementioned chickpea varieties.

Water-stress treatment: The pot experiment was divided into two sets. In the first set of potted plants (half of the total number of pots), water stress was imposed by withholding water until symptoms of wilting were visible. This stage was reached by withholding water for eight days when soil moisture diminished to around 5% and leaf RWC to around 60% (Fig. 2*A*). The second set of the potted plants (half of the total number of pots) was used for well-watered treatment, which means optimal soil moisture, *i.e.,* soil moisture between 25–30% (~100% FC) (Fig. 1). It was maintained by regular watering.

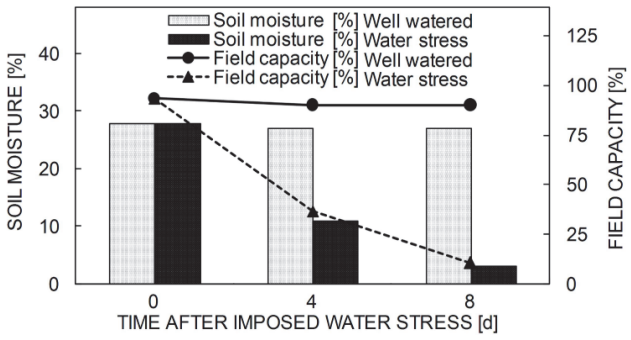

Fig. 1. Soil moisture content and field capacity recorded during the course of water-stress treatments.

Bioregulator treatments: Before imposition of the water stress, the plants of both sets were treated with foliar application of optimum doses of TU (1,000 mg L^{-1}), BA (40 mg L^{-1}), and TDZ (10 mg L^{-1}), while water spray was used as control. The optimum doses for each bioregulator were selected according to our previous work (Vineeth *et al*. 2015), where the foliar application of varying doses of TU (0, 500, 1,000, 1,500 ppm), BA (0, 20, 40, 60 ppm), and TDZ (0, 10, 15, 20 ppm) were evaluated for the dose optimization in terms of the photosynthetic rate and grain yield.

Soil moisture: The soil moisture content was estimated periodically during the imposed water-stress treatment. For the estimation of soil moisture, soil samples of 25 g of fresh mass (FM) were taken from the root zone of the potted plants. Then the samples were kept in the oven at 105°C, and dried till constant dry mass (DM) was achieved. The soil moisture content was calculated according to Faulkner *et al*. (1989) and expressed in percentage [%]. Fully saturated soil at 100% of field capacity (FC) contained 30% moisture. By using this relation, FC [%] was estimated at different water stress periods (Fig. 1). The soil moisture content was calculated as [(FM – DM)/DM] × 100.

Relative water content (RWC): Leaf RWC was determined from FM, DM, and turgid mass (TM) using the formula RWC [%] = [(FM − DM)/(TM − DM)] × 100. Leaf tissue (1 g) was immersed in deionized water and kept in the dark for 12 h at 4°C. After this, leaves were removed from deionized water, gently blotted dry, and weighed for TM. Samples were then dried in an oven set to 80°C for at least 72 h and weighed for DM (Barrs and Weatherley 1962).

Photosynthesis and chlorophyll (Chl) fluorescence: Rate of photosynthesis was measured using portable infrared gas analyzer (IRGA), *LI-6400XT* model (*Li-COR*, USA). The rate of photosynthesis (P_N) and quantum efficiency of PSII (F_v/F_m) was measured by operating the IRGA in the closed mode. The top most fully expanded leaf was enclosed in the assimilation chamber. Photosynthesis was measured while the CO_2 concentration changed over a definite time interval. The system automatically calculated photosynthesis on the basis of preloaded flow and leaf area. All the parameters were determined during the course of the experiment between 10:00 and 11:30 h by providing artificial light source of saturated intensity 1,000 μmol(photon) m^{-2} s^{-1}. F_v/F_m was evaluated as the ratio of the variable fluorescence (F_v) to the maximal fluorescence (F_m). Leaf clips were used to adapt individual leaves to darkness for 30 min prior to reading the F_v/F_m ratio. Three readings were taken per pot on each sampling day.

Lipid peroxidation (LPO): LPO was estimated as the thiobarbituric acid reactive substances according to the method of Heath and Packer (1968). Leaf samples (0.5 g) were homogenized in 10 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 × *g* for 15 min. To 1.0 ml aliquot of the supernatant, 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95°C for 30 min in the water bath and then cooled under room temperature. After centrifugation at 10,000 × *g* for 10 min, the absorbance of the supernatant was recorded at 532 nm in the UV-visible spectrophotometer (*Specord Bio-200*, *Analytik Jena*, Germany). The thiobarbituric acid reactive substances (TBARS) content was calculated according to its extinction coefficient, *i.e*., 155 mM^{-1} cm^{-1}. The values for nonspecific absorbance at 600 nm were subtracted.

RNA isolation and semiquantitative RT-PCR: Expression analysis of *RbcL*, *GOX,* and *GDCH* genes were carried out by semiquantitative RT-PCR. Total RNA from leaf tissue was extracted using Trizol reagent according to the recommendations of the manufacturer (*Invitrogen*, USA). One microgram of total RNA was reverse transcribed using gene specific primers and *Qiagen* one step RT-PCR kit. Linear amplification for semiquantitative RT-PCR was obtained with 28 cycles. The gene specific primers were designed manually as *RbcL* (F 5'-TGG TCT TAC CAG TCT TGA TCG-3'; R 5'-ACG ATA GGA ACA CCC AAT TCT C-3'), *GOX* (F 5'- ATC GAC GTG AGC AAG ATA GAC-3'; R 5'-TCA CAT CCT TCC AGC TTA GAG-3'), and *GDCH* (F 5'- TGG GCT TCT TCN ACT GCC AAT GC-3'; R 5'-TCC AYC CAT CTT CRT ANG GGC TTG-3'). Eukaryotic initiation factor (*IF4Ac*) was used as an internal standard (F 5'- CAT TGG CAA TCA CCC AGA GTG-3'; R 5'- ACT AAT GAC ACT TGT GCA CGT C-3'). Reactions were performed using *QB* 96 thermal cycler (*QB*, England) under the following conditions: initial PCR activation step: 15 min at 95°C, reverse transcription: 30 min at 50°C, denaturation: 1 min at 94°C, annealing: 1 min at 56°C, extension: 1 min at 72°C, and final extension: 10 min at 72°C. The amplification products were electrophoresed on 1% agarose gel at 70 V in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0) using 100 bp plus DNA ladder (*Thermo Fisher Scientific*, USA). Gels were stained with ethidium bromide and visualized on *Uvi Pro Gel* documentation system (*Uvitec*, England). The band intensities of the acquired images were determined using densitometer (*AlphaImager 2000*, USA). Each densitometric value was expressed as the mean ± SD.

Statistical analysis: Data were subjected to statistical analysis using three way factorial analysis of variance (*ANOVA*) (Panse and Sukhatme 1967). Means were tested by the least significant difference at $P \leq 0.05$ according to the *Duncan*'s multiple range test using the *OPSTAT* software (*CCS HAU*, Hisar, India). All the measurements were performed four times for each treatment and the means and standard error (SE) were reported.

Results and discussion

Bioregulators act as modulators of plant responses to water stress. In the present study, no effect of the bioregulator dose on RWC was recorded under well watered conditions in both chickpea varieties. However, RWC was reduced under water stress and maximum reduction was recorded after foliar application of water spray (control) (Fig. 2*A*). Similar results have been reported earlier (Grover *et al*. 2004). Foliar application of bioregulators during water stress maintained higher RWC values than that of the control. Maximum RWC in the Desi variety was estimated after the application of TU. RWC response to bioregulators was relatively higher in the Kabuli variety as compared to the Desi type. Reduction in RWC under water stress was higher in the Kabuli type than that of Desi one. Among all the three bioregulators, plants treated with TU maintained the highest RWC values under water stress in both the varieties (Fig. 2*A*). Similar results on RWC were also reported after the application of TU in wheat (Anjum *et al*. 2011). However, application of BA has been reported to decrease RWC by 86 to 55% in senna (*Cassis anustifolia* Vahl) under water stress (Singh *et al*. 2001).

In both chickpea varieties, LPO increased under water stress (Fig. 2*B*). Similarly, substantially enhanced malondialdehyde content was also reported earlier under drought in green bean genotypes (Yasar *et al*. 2010). The application of the bioregulators lowered LPO under water stress (Fig. 2*B*). The treatment with CKs (BA and TDZ) caused maximum prevention of LPO in the Desi type. In the Kabuli type, TU and TDZ were the most effective. Criado *et al*. (2009) reported that exogenous CK application increased cell membrane stability in wheat cultivars under drought stress. The positive effect of CKs on stress tolerance of plants is related to their protective effects from the oxidative stress by preventing the formation of free radicals (Zhang and Schimidt 2000). CK reduced LPO under water stress in many species (Lai *et al*. 2007). TU has also been reported to restrict LPO in cluster bean (Garg *et al*. 2006) and mustard (Srivastava *et al*. 2011).

P_N in both chickpea varieties decreased under water stress (Fig. 2*C*). Drought stress has been reported to decrease the rate of photosynthesis (Lawlor 2002). The reduction in photosynthetic activity under stress occurs due to decline in CO_2 availability caused by the restriction of CO_2 diffusion (Flexas *et al*. 2007) and inhibition of ribulose-1,5-bisphosphate (RuBP) synthesis (Lawlor 2002, Rivero *et al*. 2009). Mild water stress

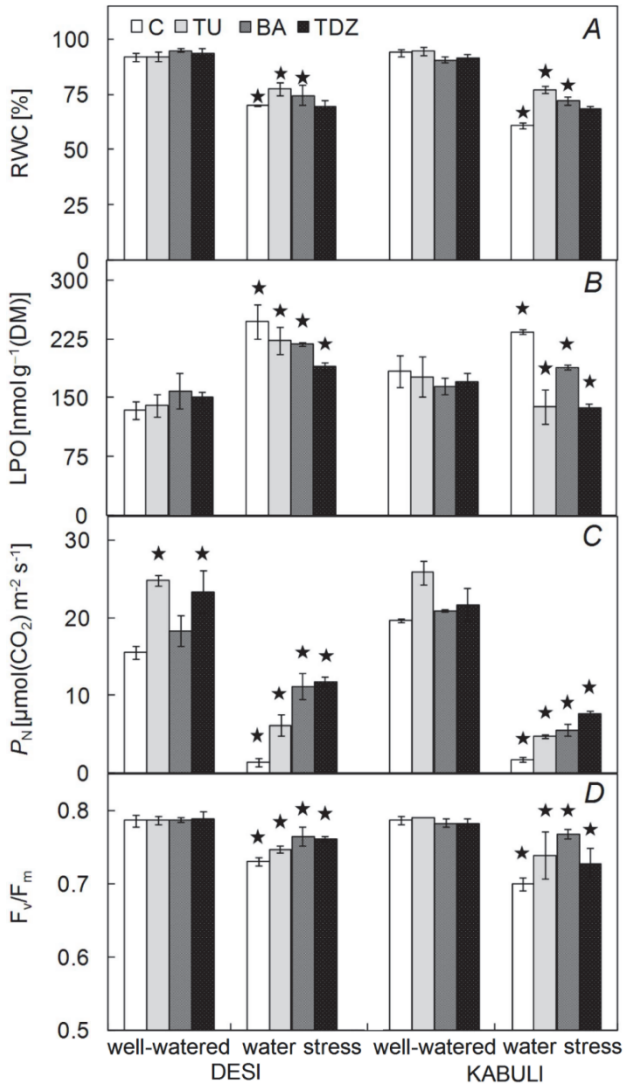

Fig. 2. Effect of bioregulators under well watered and water-stress conditions on relative water content (RWC) (*A*), lipid peroxidation (LPO) (*B*), net photosynthetic rate (P_N) (*C*) and quantum efficiency of photosystem II (F_v/F_m) (*D*) in chickpea varieties. Data represent the means of four replicates ± SD. * – significantly different at $p \leq 0.05$. C – control; TU – thiourea; BA – benzyladenine; TDZ –thidiazuron.

reduces photosynthesis due to stomatal limitations while under severe drought stress reduction in CO_2 fixation is caused by the breakdown of photosynthetic machinery (Tambussi *et al*. 2000).

Under well watered condition, TU, BA, and TDZ enhanced P_N and maintained it under water stress (Fig. 2*C*). TU has been reported to increase photosynthetic efficiency (Sahu *et al*. 1993, Mathur *et al*. 2006). TU appears to have more diverse biological activities because of its –SH group (Sahu *et al*. 1993) probably by maintaining redox homeostasis through its ROS scavenging activity and hence large accumulation of photosynthates (Shanu *et al*. 2013). CKs increase the stability of photosynthetic apparatus during water stress (Chernyad'ev 2005). Phenylurea CKs have been reported to improve photosynthetic activity in maize (Lazova and Yonova 2010). TDZ treatment also induced photosynthetic CO_2 assimilation in soybean plants (Vakilionová *et al*. 1991).

Under well watered condition, quantum efficiency of PSII (F_v/F_m) in the bioregulators-treated plants did not significantly vary. However, under imposed water stress conditions, the F_v/F_m ratio was reduced in both varieties. The plants treated with bioregulators (TU, BA, and TDZ) maintained higher F_v/F_m compared with the control (water spray) under water stress in both chickpea varieties. Foliar spray of BA maintained the higher F_v/F_m ratio as compared to other treatments in both chickpea varieties (Fig. 2*D*). Merewitz *et al*. (2010, 2011b) reported that *IPT*- transgenic plants, having a high zeatin content, exhibited higher F_v/F_m under drought stress in creeping bentgrass. Nonstomatal effects of CK have been reported including alleviation of the negative effects of water stress on photochemical activities of PSI and PSII, and a content and activity of Rubisco by applied CKs (Chernyad'ev and Monakhova 2003, Nyitrai 2005).

Semiquantitative RT-PCR analysis of *RbcL* gene resulted in amplicons of about 579 bp. Under the water stress conditions, the strong reduction in expression was observed in the water-stressed Desi plants, while bioregulators-treated plants exhibited strong expression under water stress. Higher expression of *RbcL* gene was observed after the foliar application of TU under water stress condition (Fig. 3*A*). In the Kabuli type, a lesser reduction in *RbcL* expression was observed under water stress in the control plants. Further, comparatively higher *RbcL* gene expression in the Kabuli plants was found after the foliar application of BA under well watered and water stress conditions (Fig. 3*A*). Loss of Rubisco activity has been reported in several plant species under drought (Parry *et al*. 2002, Chaitanya *et al*. 2003). Rubisco large and small subunits often degrade due to any kind of stress (Wilson *et al*. 2002). Wingler *et al*. (1998) reported that the stress-related decline in Rubisco was delayed in transgenic tobacco plants that produced more CKs. BA treatment has been reported to increase Rubisco activity in wheat (Xie *et al*. 2004). CKs application has been reported to alleviate negative effects of water stress by modulating content and activity of Rubisco (Chernyad'ev and Monakhova 2003, Nyitrai 2005).

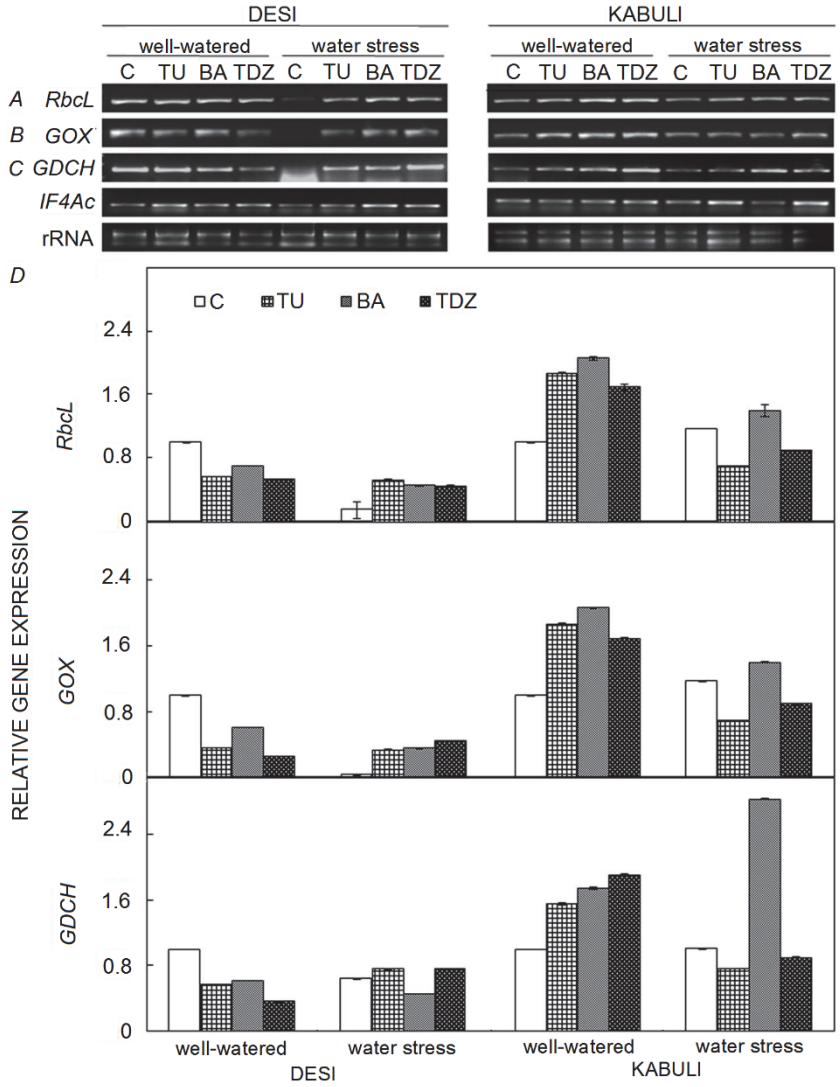

Fig. 3. Semiquantitative RT-PCR analysis of (*A*) Rubisco large subunit (*RbcL*), (*B*) glycolate oxidase (*GOX*), and (*C*) glycine decarboxylase H subunit (*GDCH*) genes under well-watered and water-stress conditions upon bioregulators treatment in Desi and Kabuli chickpea genotypes. (*D*) Quantification of gene expression relative to initiation factor *IF4Ac* gene. C – control; TU – thiourea; BA – benzyladenine; TDZ – thidiazuron.

Photorespiration was determined by analyzing the expression of two major photorespiratory genes, *i.e., GOX* and *GDCH*. Transcript expression analysis of the *GOX* gene resulted in amplicons of about 496 bp. Very low expression of *GOX* was observed in control plants under water stress in Desi chickpea. However, the plants treated with the bioregulators (TU, BA, and TDZ) exhibited strong expression under water stress. Further, higher expression of *GOX* was obtained with the foliar application of BA and TDZ under water stress (Fig. 3*B*). Gene expression of *GOX* in the Kabuli type was found to be higher as compared to Desi under water stress. The bioregulators (TU, BA, and TDZ)-treated Kabuli plants showed strong expression under well watered condition. Furthermore, higher expression of *GOX* in the Kabuli type was obtained with the foliar application of BA under water stress (Fig. 3*B*).

Photorespiration is essential in protecting the photosynthetic apparatus under water stress (Kozaki and Takeba 1996). Ludt and Kindl (1990) found that *GOX* mRNA decreased during drought in lentil (*Lens culinaris*). Transgenic tobacco with an elevated content of endogenous CKs showed increased GOX activity compared with nontransformed control plants (Synková *et al*. 2004). Under water deficit conditions, *IPT*-transgenic tobacco has been reported to maintain a physical associations among chloroplast, peroxisomes, and mitochondria, indicating CK-mediated occurrence of photorespiration, enabling protection of photosynthetic process and its beneficial role during water stress (Rivero *et al*. 2009). Under water stress, production of CKs has been reported to protect photosynthetic process by inducing photorespiration (Rivero *et al*. 2009).

Transcript expression analysis of *GDCH* gene resulted in amplicons of about 388 bp. In Desi chickpea, under water stress, strong reduction in expression of *GDCH* gene was observed in the control plants. However, the plants treated with the bioregulators (TU and TDZ) exhibited higher expression levels under water stress condition (Fig. 3*C*). In Kabuli chickpea, under water stress, expression of *GDCH* gene was comparable to that under well watered condition in the control plants.

However, bioregulators (TU, BA, and TDZ)-treated plants exhibited higher levels of expression under well watered condition. Interestingly, the plants treated with BA exhibited very strong expression of *GDCH* gene under water stress (Fig. 3*C*). The improved drought tolerance in *IPT*-transgenic tobacco has been reported due to the upregulation of photorespiration under water stress (Rivero *et al*. 2009, Rivero *et al*. 2010, Zhang *et al*. 2010). In contrast, reports on reduction of photorespiration under water stress were also made earlier (Biehler and Fock 1996, Rizhsky *et al*. 2002). Several reports have indicated that changes in hormone homeostasis, brought about by the expression of *IPT* gene resulted in enhanced drought tolerance (Rivero *et al*. 2009, Rivero *et al*. 2010).

Conclusion: Our present study might conclude that in both Desi (Pusa 362) and Kabuli (Pusa 1108) chickpea, all three bioregulators, especially CKs, induced the water-stress tolerance by restricting LPO and enhancing the gene expression of the photorespiratory enzymes (GOX and GDCH) and Rubisco. We suggest that this protected the photosynthetic machinery as supported by higher photosynthetic rate and quantum efficiency of PSII under water stress. This may be considered as a very important trait of water-stress tolerance that may increase the yield of chickpea under these conditions.

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