CAMTA1 Transcription Factor Regulates Salinity And Drought Tolerance In Chickpea (*Cicer arietinum* L.)

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Research Article

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Abstract

Various abiotic stresses like drought, salinity, high temperature, and chilling adversely affect plant growth and productivity. Terminal drought stress is one of the major concerns which limits the growth and yield of chickpea. CAMTA (Calmodulin binding transcription activator) plays a vital role in stress tolerance in plants. In this study, we have selected a *CAMTA1* gene to explore its role against salinity and drought stress in an economically important crop, chickpea (*Cicer arietinum* L.). *CAMTA1* gene was then over-expressed in chickpea and was exposed to drought and salinity. The over-expression of *CAMTA1* enhanced the activities of various antioxidant enzymes (ascorbate peroxidase; APX, catalase: CAT, glutathione S-transferase; GST, superoxide dismutase; SOD, monodehydroascorbate reductase; MDHAR). The reduced stress markers TBARS and H_2O_2 enhanced the survival of plants against both stresses. The physiological parameters (net photosynthesis; P_N, transpiration; E, stomatal conductance; g_s, photochemical quenching; qP, non-photochemical quenching; qN, and electron transport rate; ETR) were improved in the transgenics under both the stresses, that protected the plants from damage. This investigation verified that the *CAMTA1* gene provides tolerance against drought and salinity by maintaining biochemical, physiological, and morphological performances, and could be exploited for genetic engineering strategies to overcome the stresses in other economically important crops.

Introduction

Chickpea (*Cicer arietinum* L.) ranks second in area and third in the production among food legumes and is a good source of protein for humans. It is the most common grain legume in 40 countries worldwide, including arid and semi-arid regions (Rao et al. 2002). Water deficit is one of the significant constraints for the reduction in crop yield. Plants respond to drought stress and acclimatize through various physiological and biological changes.

Various environmental factors affect plants, such as biotic and abiotic stress. Abiotic stress includes salinity, chilling, drought, heat stress, and temperature. Drought stress is one of the major constrains for the reduction in crop yield and productivity. It occurs when the available water in the soil is reduced, and atmospheric conditions cause a considerable loss of water by evapo-transpiration. Drought tolerance is seen in almost all plants, but it varies from species to species (Jaleel et al. 2007). It reduces plant growth by affecting various physiological and biochemical processes such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, and nutrient metabolism (Farooq et al. 2009). Terminal drought (soil moisture stress that occurs at the crop's pod filling and seed development stage, with increasing severity at the end of the season) is a major constrain to chickpea production during the closing stages of its reproductive phase (Gaur et al. 2008). It affects both elongation and expansion growth (Anjum et al. 2003a). Plants have different mechanisms to adapt under water stress conditions, including drought escape, drought avoidance. Due to terminal drought, plants lose their ability to survive and decrease crop yield and productivity. Several transcription factors have been revealed in plants, having crucial roles during plant growth, development, and under various stresses.

Transcription factors regulated by calcium (Ca^{2+}), which act as a secondary messenger, play a vital role in several signaling pathways of growth and development as well as in stress signaling and adaptation. Calmodulin (CaM) is one of the prominent Ca^{2+} sensors in eukaryotic cells, including plants. During calcium signaling, stimulus-response involves a set of Ca^{2+} sensor proteins and Ca^{2+} binding proteins (Galon et al. 2010). Three major types of calcium sensor proteins are calmodulin (CaM) like proteins (CMLs), calcium-dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBLs) (Poovaiah et al. 2013). When there is an increase in the intracellular Ca^{2+}, calmodulin (CaM) interacting with calcium undergoes a conformational change, thus activates the target gene and other stress-responsive genes. Hence, to explore the role of the *CAMTA1* gene under drought stress, a TF named CAMTA (Calmodulin binding transcription activator) was expressed in chickpea. CAMTA is composed of multiple functional domains, including nuclear localization signal (NLS), a CG-1 DNA binding domain which includes CGCG and CGTG motif, TIG domain which is an immunoglobulin-like fold involved in non-specific DNA binding, ANK repeat, which helps in protein-protein interaction, and a varying number of IQ motifs in a conserved manner. The previous report identified the family of six *Arabidopsis* genes encoding calmodulin-binding transcription activator (CAMTAs), also referred to as signal responsive protein (SRs) or ethylene-induced CaM binding proteins (EICBP).

In this study, we have chosen the *CAMTA1* gene based on their protective role against drought, as revealed in *Arabidopsis thaliana* (Pandey et al. 2013), and we have further explored its protective role against salinity and drought stress in an economically important crop chickpea. We have successfully transformed the *CAMTA1* gene in chickpea and validated the *CAMTA1* gene in chickpea and determined its possible role against drought stress. The expression of *CAMTA1* was found to be elevated in all transgenic lines during qRT-PCR analysis. Several stress-responsive biochemical and physiological performances were estimated to explore the role of *CAMTA1* in drought stress, and enhancement was found in most of the parameters evaluated that validated that over-expression of *CAMTA1* significantly enhanced drought tolerance in chickpea.

Materials And Methods

2.1 Plant material and experimental design

Chickpea cultivars of P-362 were obtained from Gobind Ballabh Pant University, Pantnagar, Uttarakhand. The seeds were surface sterilized with mercuric chloride (0.1%, w/v) for 5 min followed by washing with sterile distilled water, and then with 70% ethanol for 3 min followed by washing with sterile distilled water. Seeds were kept overnight for imbibition, and the water was removed the next day. The seeds were then allowed to germinate in the dark for 2–3 days. The germinated seedling was placed in Murashige and Skoog's (MS) salts containing B5 vitamins, sucrose (3% w/v), and agar (0.8% w/v) adjusted to pH 5.8. The medium was autoclaved at 121 psi for 20 min. The culture was incubated in the culture room under cool white fluorescent light at 24 ℃ for 16 h of photoperiod. For the preparation of cotyledonary node explants (CNs), the cotyledons and axillary shoots were removed from 17 days old seedlings.

2.2 Construct preparation and plant transformation

CAMTA1 gene was cloned in pCAMBIA 1301 plant expression vector. This vector consists of hygromycin (*hpt*II) selection marker (plant) and kanamycin selection marker (bacterial). The *CAMTA1* gene was cloned in the pCAMBIA 1301 vector and was followed by plasmid isolation. Restriction digestion was performed with two restriction enzymes *Bgl*II and *Bst*EII. The presence of insertion of the *CAMTA1* gene was confirmed by gel electrophoresis (Supplementary Fig. S1).

2.3 *Agrobacterium* strain and plasmid

Agrobacterium strain LBA4404 was used for transforming the binary expression vector pCAMBIA1301. YEB medium was prepared by adding appropriate antibiotics (Kanamycin 50 mg/l, Streptomycin 50 mg/l, and Rifampicin 20 mg/l) and grown overnight at 28 °C and was harvested by centrifugation at 4,000 rpm and the pellet was re-suspended in 25 ml of MS medium containing 100 µM acetosyringone. O.D. of the culture was between 0.8 to1.0 at 600 nm. Excised nodes were sonicated for 30 sec and then incubated for 20 min at 24 °C in dark. The explants were blotted dry in sterile filter paper and co-cultivated in MS medium plates containing BAP and 100 µM acetosyringone for 48 h under normal growth conditions. The explants were then transferred to MS medium containing cefotaxime (500 mg/l) and incubated for 7 days under 16/8 h L/D cycle.

2.4 Selection and regeneration

The regenerated shoots were transferred to MS medium containing hygromycin for the selection of transformed cells. Different concentrations of hygromycin (5, 10, and 15 mg l^{-1}) were used. After three subsequent selections, the surviving shoots were transferred to a regeneration medium containing (Zeatin 0.02 mg/l, Silver nitrate 17 mg/l, and Chlorocholine chloride 0.2 mg/l). Seeds were grown in a cup filled with soilrite for 14 days and used for grafting. After 14 days, the shoots were grafted onto the germinated seedlings and transferred to the plant growth chamber (Adaptis 1000 PG, Conviron, Canada). The plants were transferred to the glasshouse for hardening and acclimatization and subsequent flower development and pod setting (Supplementary Fig. S2).

2.5 Gene expression analysis of chickpea by qRT-PCR

To analyze gene expression, total RNA was isolated from the leaves of chickpea plants and control plants, using Spectrum plant total RNA kit (Sigma-Aldrich, USA). cDNA was prepared using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). This cDNA was used as a template for the quantification of total transcript using 2x Mastermix SYBR green dye (Thermo Scientific, USA) in a 7500 real-time PCR machine (Applied Biosystems, USA). The chickpea actin gene was used as a reference control. The relative expression of the gene was analyzed and calculated using the $2^{\wedge\wedge}C_T$ method (Livak and Schmittgen. 2001).

2.6 Biochemical analysis of transgenic chickpea under drought and salinity stress

Total protein was extracted from the chickpea leaves by using the Total Protein Extraction Kit (Merck, Germany) and was quantified with the Bradford method (1976) at 595 nm (Spectramax 340 PC, Molecular Devices, USA). For the analysis of enzyme activities, fresh leaves of chickpea (200 mg) were crushed in liquid N_2, and the extract was prepared with 2 ml of 100 mM phosphate buffer (pH 7.5) having 1 mM EDTA and 1% polyvinylpyrrolidone (PVP) and centrifuged for 15 min at 14,000rpm at 4 °C. The resultant supernatant was used for the assay of different enzymes.

To estimate SOD (EC 1.15.1.1) activity in chickpea, 1 ml of enzyme extract was prerpared according to Beauchamp and Fridovich (1971). The absorbance of the reaction mixture was measured at 560 nm, where 1 unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the nitro blue tetrazolium (NBT) reduction in the light.

The estimation of H_2O_2 was also performed and measured spectrophotometrically after a reaction with potassium iodide (KI). The reaction mixture consisted of 0.5 ml 0.1% trichloroacetic acid (TCA) leaf extract supernatant, 0.5 ml of 100 mM potassium phosphate buffer, and 2 ml reagent (1M KI w/v in Milli-Q water). The reaction was developed for 1 h in darkness, and absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with a known concentration of H_2O_2.

The method of Nakano and Asada (1981) was used to estimate APX (EC 1.11.1.11) activity in chickpea. The reaction mixture consists of 1.0 ml of reaction buffer potassium phosphate (pH 7.0) with 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.1 ml of enzyme extract. The absorbance of the activity was measured at 290 nm. For the calculation, the extinction coefficient of 2.8 mM^{-1}cm was used.

Catalase (EC 1.11.1.6) enzyme activity was performed, followed by Chandlee and Scandalios (1984) using a reaction mixture containing 0.1 M phosphate buffer, 100mM H_2O_2. The reaction mixture was centrifuged at 10,000 rpm for 20 min at 4 °C. The absorbance was measured at 240 nm.

Glutathione S-transferase (GST) (EC 2.5.1.13) activity was estimated, according to Habig et al. (1974), using a reaction mixture containing 100 mM potassium phosphate buffer (pH 7.5) with 1 mM CDNB (1-chloro, 2,4 dinitrobenzene), 10 mM GSH (reduced), and 0.8 ml of enzyme extract. The reaction mixture was incubated for 5 min. The absorbance was measured at 340 nm for 2 min at 15 sec time interval.

The estimation of monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4) was followed according to Vanacker et al. (1988). The reaction mixture consists of 100 mM potassium phosphate buffer (pH 7.5), L-ascorbic acid (25 mM) and NADH (0.2 mM). The absorbance was measured at 340 nm for 2 min at 15 sec time interval.

2.7 Measurement of physiological parameters

Various physiological parameters were measured of transgenic chickpea plants expressing *CAMTA1* gene along with treated and untreated control plants. The water use efficiency (WUE), net photosynthetic

rate (P_N), stomatal conductance (gs), transpiration (E), Fv/Fm (variable to maximum fluorescence) proportion, qP (photochemical quenching), NPQ (non-photochemical quenching), and ETR (electron transport rate) were recorded in entirely open leaves with a photosynthetic system, Li-6400 (LI-COR, USA). The level of CO_2 in the leaf chamber was retained at 400 µmol (CO_2) mol^{-1} air. The photosynthetic photon flux density (PPFD) was retained at 300 µmol (photons) m^{-2} s^{-1}. The level of VPD (vapor pressure deficit) was lower than 2 kPa. The leaf temperature was at 25 °C, and RH (relative humidity) was 55–60%. All the physiological parameters were recorded between 08:00 to 11:00 h.

2.8 Statistical analysis

All the values reported in this work are the means of three replicates. Standard errors were calculated using the means of three replicates. The data were analyzed using DMRT (Duncan's Multiple Range Test) for the analysis of the significant difference between the means ($p < 0.05$).

Results

3.1 Screening of transgenic events with PCR

PCR analysis of the putative transformants expressing the *CAMTA1* gene was performed along with the treated and untreated control plants. A specific set of primers were used for the analysis of transgenic chickpea plants. The desired band obtained from the internal set of primers was a 800 bp amplicon, while control plants showed no amplification.

3.2 Relative expression analysis of *CAMTA1* gene by qRT-PCR

The relative expression of the *CAMTA1* gene was analyzed by qRT-PCR with gene-specific primers and, Chickpea actin primer was taken as an endogenous control. The result showed a higher transcript level ranging from 11 to 15 fold change under drought stress (Fig. 1).

The putative transformed chickpea plants were subjected to drought and salinity stress. The plants showed enhanced growth as compared to treated control and wild-type plants (Fig. 2). After 7 days of drought treatment in chickpea, the transgenic plants expressing *CAMTA1* gene recovered under well-watered conditions.

3.3 Physiological performance of *CAMTA1* gene under drought stress and salinity

Various physiological parameters have been investigated, which showed enhanced expression of *CAMTA1* gene against drought as well as in salinity. Net photosynthesis (P_N: µmol CO_2 m^{-2} s^{-1}) of *CAMTA1* under drought and salinity were enhanced compared with WT plants. The highest activity was observed to be 5 µmol CO_2 m^{-2} s^{-1} in (T-5) event under drought and salinity (Fig. 3a and b).

The transpiration rate (E) was enhanced in transgenic events harbouring the *CAMTA1* gene compared to WT plants. The highest activity observed was to be 1.2 mmol H_2O m^{-2} s^{-1} in (T-2) event in drought and 0.9 mmol H_2O m^{-2} s^{-1} in (T-5) event under salinity stress (Fig. 3c and d).

The stomatal conductance (g_s) was highest in T-2 and T-4 events upto 0.048 mol $m^{-2}s^{-1}$ as compared to wild-type plants and was found to be 0.035 mol $m^{-2}s^{-1}$ under drought stress, while under salinity stress, the highest stomatal conductance observed was 0.045 mol $m^{-2}s^{-1}$ in T-5 event, as compared to 0.021 mol m^{-2} s^{-1} in wild-type plants (Fig. 3e and f).

The coefficient of photochemical quenching (qP) was found to be enhanced in all the transgenic events under drought and salinity stress compared to wild-type plants. The non-photochemical quenching (NPQ) was slightly higher in transgenic plants (0.5) than in treated control (0.49) but was higher in wild-type plants (0.65) both in drought as well as in salinity stress conditions (Fig. 3g and h).

The expression of electron transport rate (ETR) was higher in transgenic (T-5) event and was found to be 70 µmol e- $m^{-2}s^{-1}$ as compared to treated control plants and was observed to be 56 µmol e- $m^{-2}s^{-1}$, and a slight increase of 95 µmol e- $m^{-2}s^{-1}$ in wild-type plants under drought stress. Under salinity stress conditions, the expression of transgenic events was slightly higher as compared to treated control plants than in wild-type plants (Fig. 3i and j).

3.4 Biochemical analysis of *CAMTA1* gene under drought and salinity

However, various biochemical parameters were also investigated for the expression of the *CAMTA1* gene in chickpea both in drought and salinity stress. In our study, the expression of ascorbate peroxidase (APX) was higher in the transgenic lines 11.05 µmol $min^{-1}mg^{-1}$ P^{-1} (T-2 line) as compared to treated control plants which were observed to be 7.50 µmol min^{-1} mg^{-1} P^{-1} under drought stress, while in salinity, the expression of APX was found to be higher 10 µmol min^{-1} mg^{-1} P^{-1} in T-1 line and 7.5 µmol min^{-1} mg^{-1} P^{-1} in treated control plants (Fig. 4a and b).

The activity of catalase (CAT) enzyme was shown to be higher at 7 µmol min^{-1} mg^{-1} P^{-1} in T-4 line as compared to 4.50 µmol min^{-1} mg^{-1} P^{-1} in the treated control plants under drought stress. Under salinity stress, the expression of CAT was higher 7.5 µmol min^{-1} mg^{-1} P^{-1} in T-4 line while it was 3.50 µmol min^{-1} mg^{-1} P^{-1} in treated control plants (Fig. 4c and d).

The activity of glutathione-S-transferase (GST) was found to be enhanced by 9 µmol min^{-1} mg^{-1} P^{-1} in the T-1 line while it was found to be 3 µmol min^{-1} mg^{-1} P^{-1} in treated control plants under drought and salinity stress (Fig. 4e and f).

The total protein content was also found to be enhanced by 4.5 mg g^{-1} FW in the T-3 line, as compared to 4.25 mg g^{-1} FW in treated control and 3 mg g^{-1} FW in wild-type (WT) plants under drought and salinity

stress (Fig. 4g and h).

The activity of superoxide dismutase (SOD) was found to be enhanced as 6.5 U mg^{-1} P^{-1} in T-4 line, as compared to treated control which was found to be 4.5 U mg^{-1} P^{-1} under drought stress, while in salinity, the expression was found to be increased as 6 U mg^{-1} P^{-1} in T-2 line as compared to treated control which was found to be 4 U mg^{-1} P^{-1} (Fig. 4i and j).

The activity of monodehydroascorbate reductase (MDHAR) was also found to be higher as 2 µmol min^{-1} mg^{-1} P^{-1} in T-1 line as compared to treated control and was found to be 0.9 µmol $min^{-1}mg^{-1}$ P^{-1} under drought stress; while under salinity stress, the expression was higher upto 1.6 µmol min^{-1} mg^{-1} P^{-1} in T-4 line as compared to 0.7 µmol $min^{-1}mg^{-1}$ P^{-1} in the treated control (Fig. 4k and l).

The activity of thiobarbituric acid (TBARS) was also investigated under drought and salinity stress. TBARS was found to be 4.5 µmol g^{-1} FW in T-4 line and 5.5 µmol g^{-1} FW as compared to treated control plants under drought stress, while in salinity, the expression of TBARS was found to be 2.5 µmol g^{-1}FW in T-3 line and 4 µmol g^{-1}FW in treated control plants (Fig. 5a and b).

The expression of hydrogen peroxide (H_2O_2) was also studied under drought and salinity stress. The expression was higher in all the transgenic lines and was found to be 2.5 µmol g^{-1} FW in T-5 line and 3.1 µmol g^{-1} FW in control plants under drought stress, while in salinity, the expression was higher as 2.5 µmol g^{-1} FW in T-3 line as compared to 4 µmol g^{-1} FW in control plants (Fig. 5c and d).

The antioxidant enzymes such as APX, CAT, GST, SOD, MDHAR increases under drought and salinity stress (Kumar et al. 2021). All the above parameters suggested that the *CAMTA1* gene enhances antioxidant enzymes activities and protects plants under drought and salinity stress.

Discussion

Many studies have shown that the CAMTA1 transcription factor plays a vital role in drought tolerance in plants. In *Arabidopsis*, the mechanism of CAMTA1 TF has been well established. CAMTA1, upon binding with the promoter of the *cis*-acting element, undergoes a conformational change and activates other stress-responsive genes under various abiotic stress (Pandey et al. 2013), but various roles of CAMTA1 are still to be explored. To explore the role of *CAMTA1* gene in chickpea, a tolerant variety of P-362 has been used to analyze various stress-responsive parameters subjected to drought and salinity stress.

Under stress conditions, plants produce reactive oxygen species, which is very harmful to plant growth and induces oxidative stress by generating ROS such as superoxide radicals, hydroxyl radicals, hydrogen peroxide, and alkoxy radicals (Munne-Bosch and Penuelas 2003; Esfandiari et al. 2008). However, various biochemical parameters were also investigated for the expression of the *CAMTA1* gene in chickpea under drought and salinity stress. The expression of SOD was found to be higher in all the transgenic plants as compared to treated control plants. The activity of APX is also enhanced in all the transgenic lines

compared to treated control plants, as APX scavenges peroxides by converting ascorbic acid and helps in the elimination of toxic H_2O_2 from plants.

Catalase is also one of the crucial antioxidant enzymes involved in regulating the intracellular level of H_2O_2 (Prasad et al. 1995). It converts H_2O_2 into H_2O with the regeneration of $NADP^+$, hence plays an important role in stress conditions (Jimenez et al. 1998). In this study, catalase activity was found to be increased in all the transgenic lines compared to treated control plants.

The increased level of ROS causes oxidative stress to biomolecules such as nucleic acid, lipids, and proteins (Mittler 2002). Among ROS, hydrogen peroxide is a toxic compound and is highly injurious to plants resulting in lipid peroxidation and membrane injury (Sairam et al. 1998; Baisak et al. 1994; Menconi et al. 1995). Hence, the activity of H_2O_2 was found to be lowered in all the transgenic lines compared to treated control plants that provide tolerance against stress conditions.

Glutathione S-transferase (GST) is known to express at different stages of plant development. It conjugates GSH to an array of electrophilic compounds of exogenous and endogenous origins (Cummins et al. 2011). GST activity was enhanced in all the transgenic lines compared to treated control plants that provide tolerance against stress.

The activity of monodehydroascorbate reductase (MDHAR) was also enhanced in all the transgenic lines compared to treated control plants. One of the main cellular components that is damaged by ROS are lipids (peroxidation of unsaturated fatty acid). TBARS is widely used as an oxidative marker, that is formed when there is an increase in lipid peroxidation and causes cellular damage and toxicity. The expression of TBARS was found to be lower in all the transgenic lines as compared to treated control plants. The results showed that the lower value of TBARS causes less toxicity against stress conditions.

The physiological parameters were also investigated of the *CAMTA1* gene both in the drought and salinity stress. Drought stress is one of the most important limiting factors which limits plant growth and productivity. It affects both elongation and expansion (Anjum et al. 2003a; Bhatt and Srinivasa Rao 2005; Kusaka et al. 2005; Shao et al. 2008). It also affects plant water relations, due to which there is a decrease in carbon assimilation and results in an imbalance between electron excitation, hence results in the production of ROS (Abid et al. 2018). Due to the closing of stomata under drought stress, there is a decrease in the CO_2 fixation and reduces transpiration rate. In our study, the net photosynthesis (P_N) showed enhanced expression of the *CAMTA1* gene in all the transgenic events (T-1 to T-6) as compared to a slight decrease in wild-type plants during drought and salinity stress. Transpiration rate and stomatal conductance are also enhanced in all the transgenic events as compared to wild-type plants in drought and salinity stress. Photochemical quenching (qP) was higher in all the transgenic events as compared to wild-type plants and a slight decrease in treated control plants in drought and salinity stress. The non-photochemical quenching (qN) has a protective role in oxidative stress. The expression of the *CAMTA1* gene in non-photochemical quenching was slightly higher in transgenic events as compared to treated control in drought and salinity stress. Due to the over-excitation of photosystem II, the electron transport

rate increases, which leads to heat consumption, and hence there is a decrease in photosynthetic rate. The expression of ETR in the transgenic plants was higher as compared to treated control plants, while a slight decrease in untreated control plants.

We hypothesize that *CAMTA1* acts as a positive regulator under drought and salinity stress based on our investigations. The parameters mentioned above showed enhanced expression in the transgenic events as compared to wild-type plants. Under drought and salt stress, the *CAMTA1* gene plays a protective role which provides plants to withstand tolerance under stress conditions.

Conclusions

The present study explored the role of the CAMTA1 transcription factor, which has not been investigated in detail concerning its role in both biochemical and physiological parameters. The enhanced expression in all transgenic lines showed that the *CAMTA1* gene provides tolerance against drought stress. The reduced stress marker, TBARS, and H_2O_2 enhanced the overall performance of the transgenic plants and overcame oxidative stress. The increased level of photosynthesis (P_N), stomatal conductance, and non-photochemical quenching (qN) enhanced the physiological performance of chickpea under drought and salinity stress. The above result showed that *CAMTA1* regulates drought and salinity tolerance in chickpea. The overall study of the *CAMTA1* gene can be used to develop tolerant chickpea in arid and semi-arid regions and may prove effective under drought and salinity abiotic stresses.

Declarations

The authors declare that they have no known competing financial interests.

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Author's contribution

Meenakshi: Conceptualization, Investigation, Methodology, Validation, Roles/Writing - original draft, Writing - review & editing, **Anil Kumar**: Formal analysis, Investigation, **Arvind Kumar Dubey**: Formal analysis, **Varun Kumar**: Investigation, **Shiv Narayan**: Investigation, **Samir V. Savant**: Supervision, **Veena Pande**: Supervision, **Pramod Arvind Shirke**: Formal analysis, **Indraneel Sanyal**: Funding acquisition, Project administration, Supervision, Writing - review & editing.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Figures

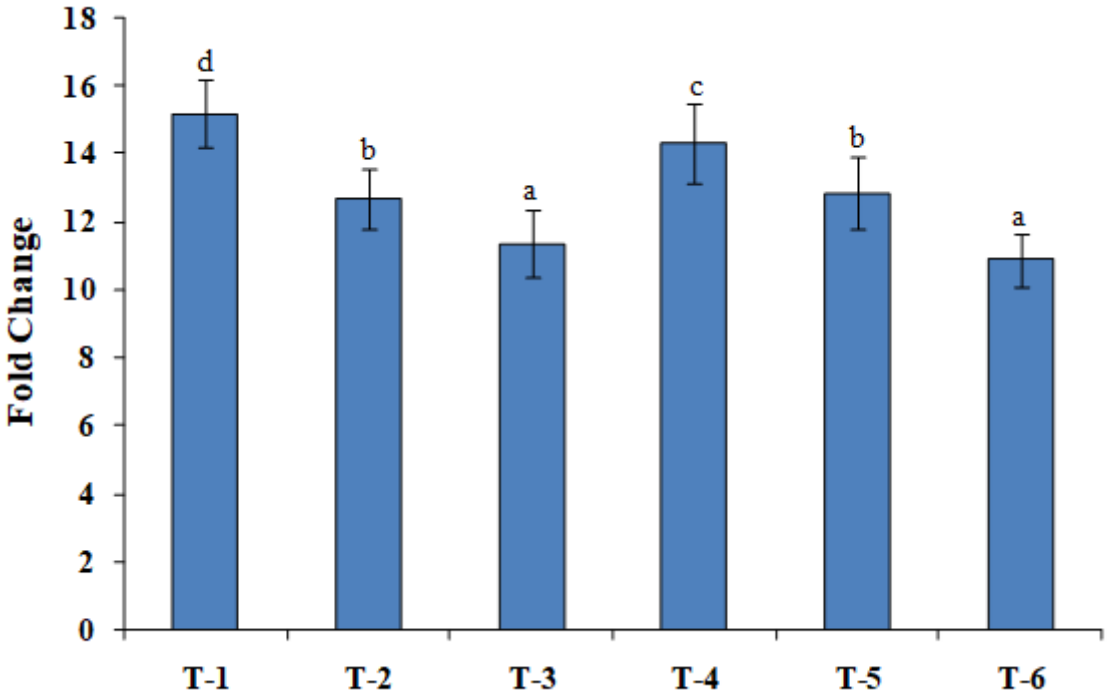

Figure 1

Relative expression of CAMTA1 gene in transgenic chickpea plants by qRT-PCR analysis.

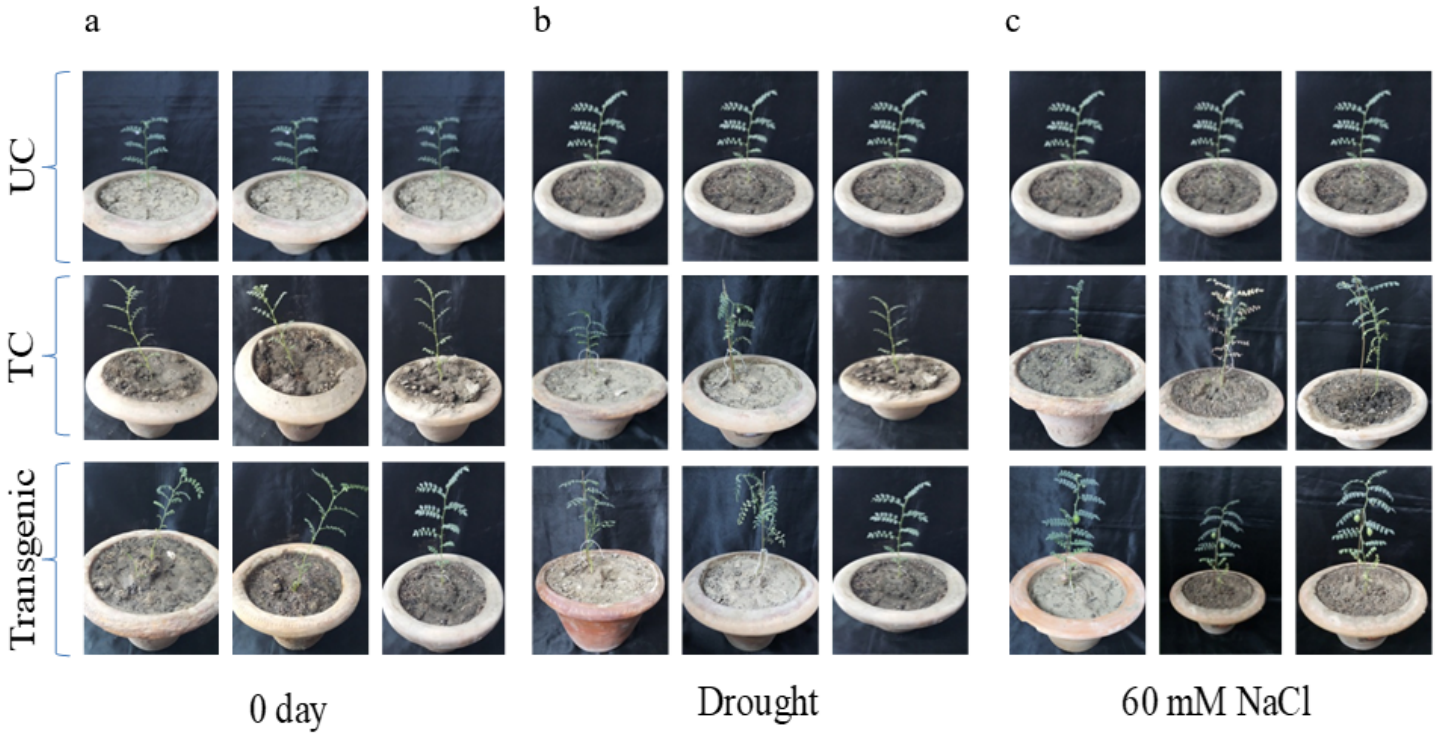

Figure 2

Morphological appearance of chickpea plants under drought and salinity stress. a After 7 days of drought treatment. b salinity stress by 60 mM NaCl. c transgenic plants expressing CAMTA1 gene recovered well as compared to wild-type plants under well watered conditions.

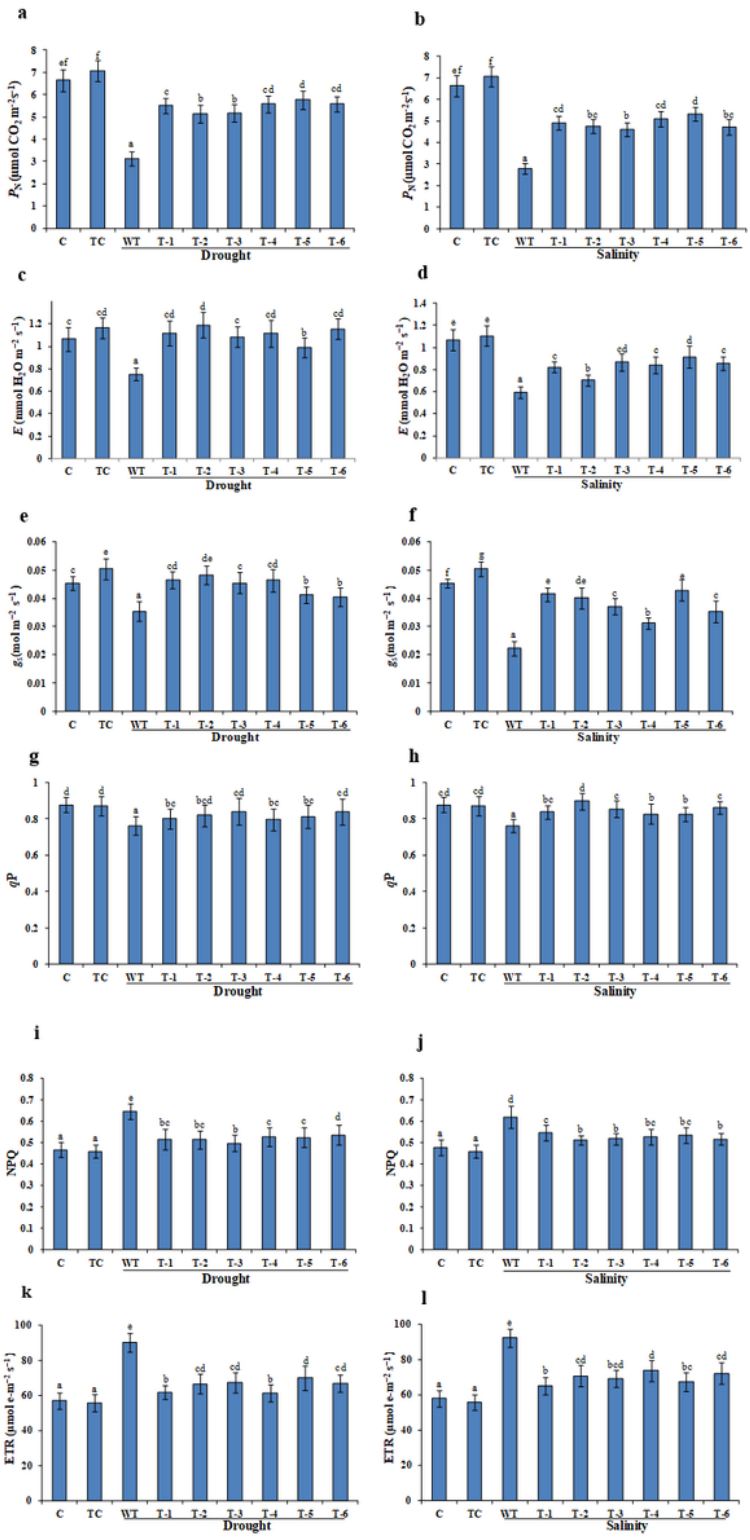

Figure 3

Physiological performances of CAMTA1 gene in chickpea against drought and salinity stress. a and b represents photosynthesis (PN). c and d represents transpiration rate (E). e and f represents stomatal conductance (gs). g and h represents photochemical quenching (qP). i and j represents non-photochemical quenching (NPQ). k and l electron transport rate (ETR). Duncan's Multiple Range Test

(DMRT) was used for the analysis of significant difference between the mean ($p < 0.05$) has been compared. All the values are the means of three replicates ± SD.

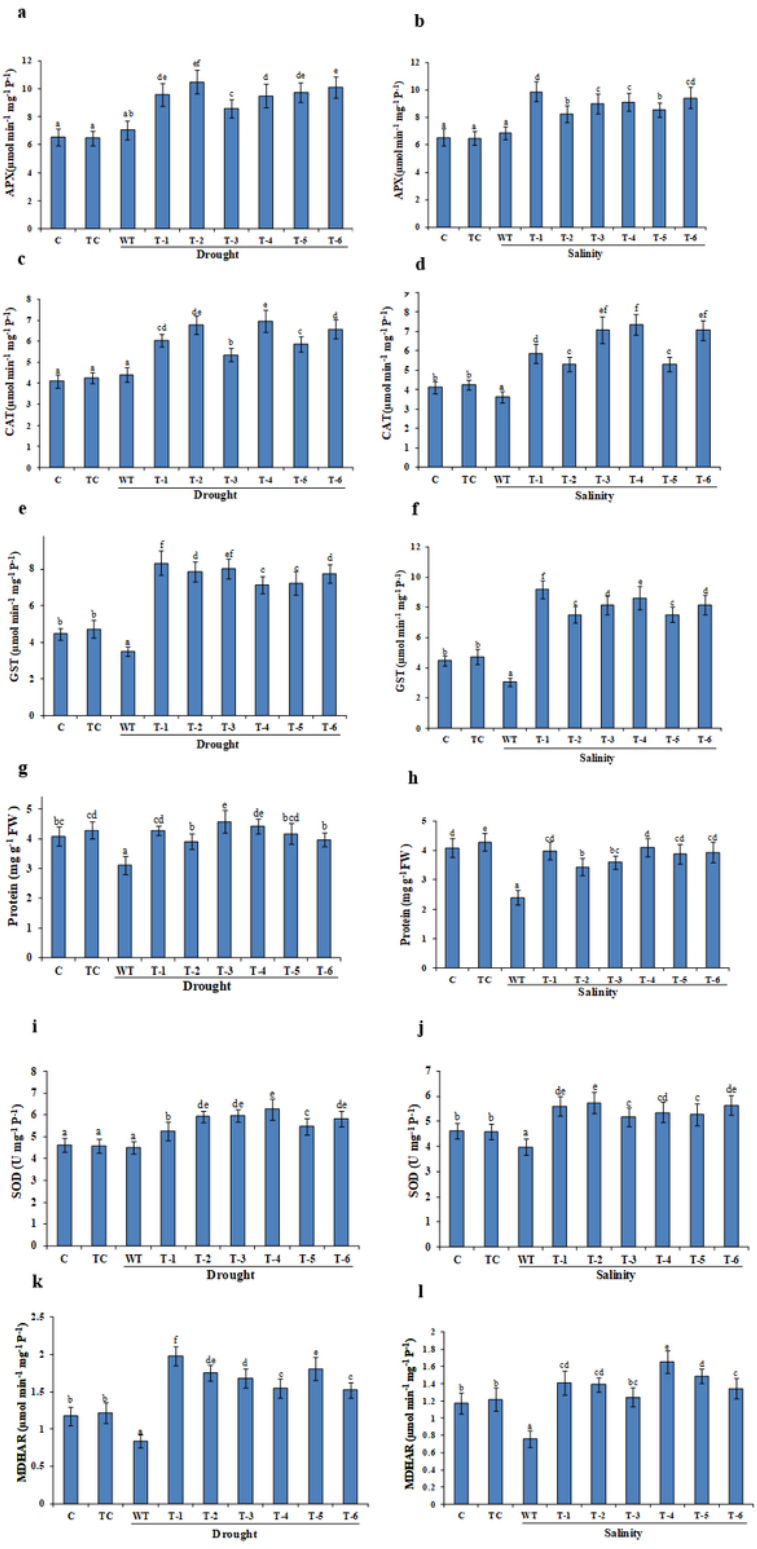

Figure 4

The level of antioxidant enzymes under drought and salinity stress. a and b represents ascorbate peroxidase (APX). c and d represents catalase (CAT). e and f represents glutathione S-transferase (GST). g and h represents protein estimation of CAMTA1 gene. i and j represents superoxide dismutase (SOD). k

and l represents monodehydoascorbate reductase (MDHAR) showed enhanced expression of transgenic lines as compared to Treated Control (TC) and Wild-Type (WT) plants. Duncan's Multiple Range Test (DMRT) was used for the analysis of significant difference between the mean ($p < 0.05$) has been compared. All the values are the means of three replicates ± SD.

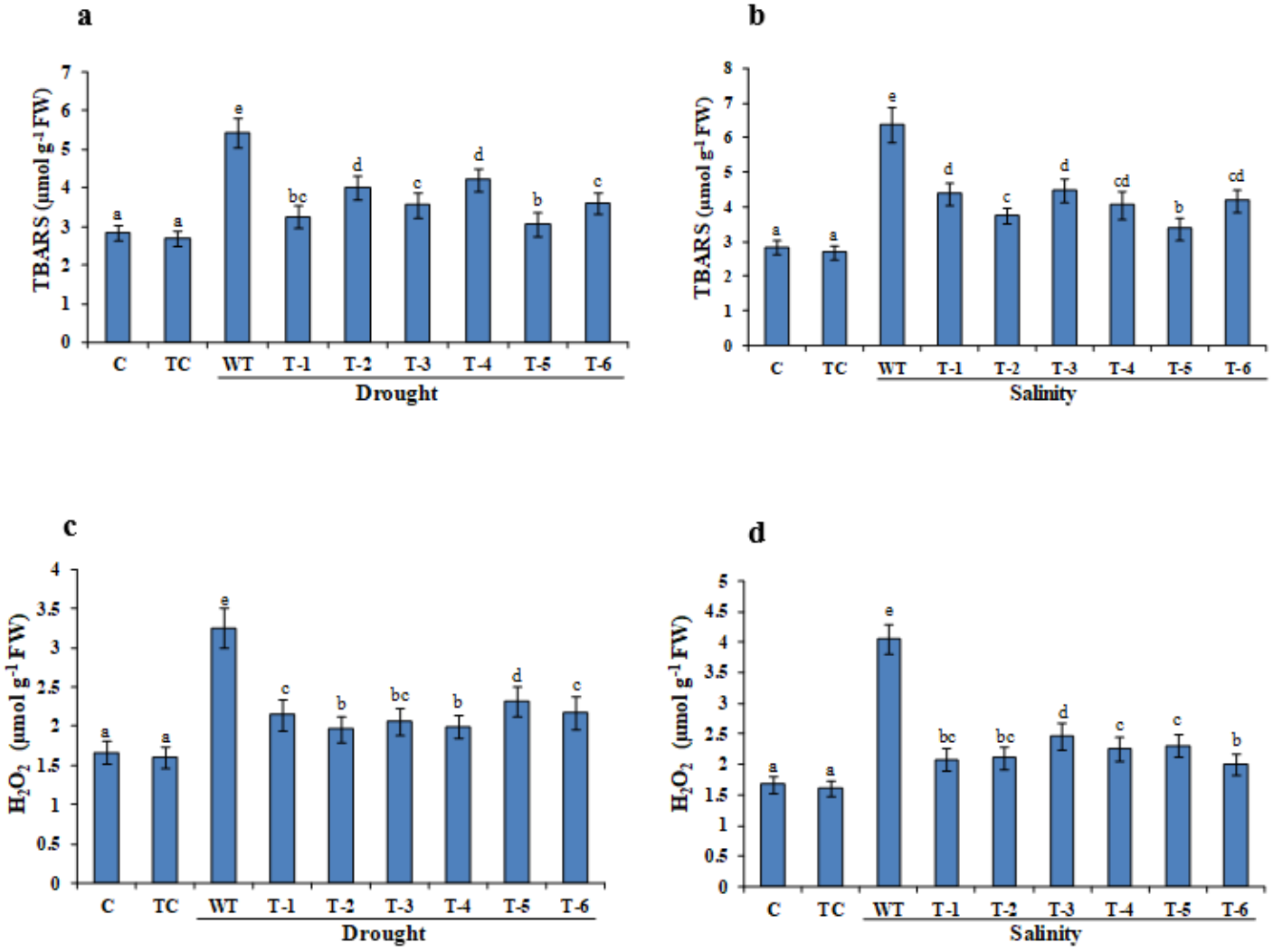

Figure 5

The level of stress markers under drought and salinity stress. a and b TBARS was found to be low as compared to treated control and wild type plants. c and d the level of H2O2 was also found to be less as compared to Treated Control (TC) and Wild-Type (WT) plants. Duncan's Multiple Range Test (DMRT) was used for the analysis of significant difference between the mean ($p < 0.05$) has been compared. All the values are the means of three replicates ± SD.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Fig.S1schematicrepresentationofCAMTAconstruct.docx

- Fig.S2Transformationinchickpea.docx