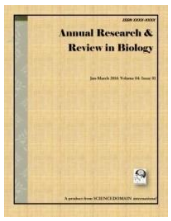

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Analysis of Stress Responsive Genes in Capsicum for Salinity Responses

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VKM and RS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EN, NR and KMG involved in literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study is an endeavor to gain proper understanding about salt tolerance mechanism in plants; an attempt was made to characterize the differential expression of stress responsive genes, sodium potassium content proline content in three capsicum cultivars having different salt sensitivity level.
Place and Duration of Study: School of Bio Sciences and Technology, VIT University, Vellore of India between June 2013 to May 2014.
Methodology: Capsicum cultivars (salt tolerant, salt moderate sensitive and salt susceptible) were treated with different concentration of NaCl such as 25mM, 50mM, 100mM, 150mM and 200mM. Gene expression studies under different salt treatment were done for the following genes: osmotic adjustment (CaPROX1), osmotin like protein (CaOSM1), aquaporin (CaPIP2), dehydrin responsive gene (CaDREBLP1), ring domain zinc finger protein gene (CaKR1), membrane protein (CaChi2), endoplasmic reticulum ubiquitine ligase (CaRMa1H1) and cell death repressor (CaBI1). Proline content and sodium and potassium ion content also measured.

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Results: The result indicated that genes CaDREBLP1, CaRMa1H1, CaKR1, CaOSM1 were up regulated while CaPROX1, CaPIP2 genes were down regulated under salt stress. But no significant difference was noticed in gene expression level of CaBI1 and CaChi 2 gene.
Conclusion: The higher gene expression level of stress responsive genes viz. CaDREBLP1, CaRMa1H1, CaKR1, CaOSM1 may involved in different level of salt tolerance among selected cultivars. Thus differential transcript modulation of these genes in capsicum cultivars indicates their role lending the salt tolerance in salt tolerant cultivar than sensitive.

Keywords: Stress responsive gene; biotic; abiotic; osmolytes; Salt tolerance; transcript expression.

ABBREVIATIONS

CADREBLP1	-	*Capsicum annuum DREB-LIKE PROTEIN 1*
CAOSM1	-	*Capsicum annuum OSMOTIN- LIKE PROTEIN*
CAPROX1	-	*Capsicum annuum PROLINE OXIDASE/DEHYDROGENASE*
CACHI2	-	*Capsicum annuum CHITINASE CLASSII*
CABI-1	-	*Capsicum annuum BAX INHIBITOR1*
CAPIP2	-	*Capsicum annuum AQUAPORIN*
CARMA1H1	-	*Capsicum annuum RING DOMAIN CONTAINING PROTEIN*
CAKR1	-	*Capsicum annuum CYS-3-HIS ZINC FINGER PROTEIN*

1. INTRODUCTION

Abiotic stress has been always limiting factor for plant growth and distribution [1,2]. Plant have been evolved a numbers of defenses at molecular and cellular levels apart from the physical and chemical barriers, plants have a number of defenses at molecular and cellular levels [3-6]. In addition to that plant also modulates its low molecular osmolytes such as glycine betaine [4,8-9], proline, sugars alcohol and amines to maintain their homeostasis. Few searchers have also reported abundance of ABA in salt tolerant verities than salt stress sensitive [10-12]. Apart from low molecular weight osmolytes, and phytohormones, stress related genes might play crucial role in tolerance but direct evidence is generally lacking. Previous works have elucidated the role of SOS pathway's genes in salt tolerance [1,13-15] which has been expressed differentially in sensitive and tolerant varieties under salt stress conditions. Mutant plant studies have produced some crucial breakthrough to give better insight in salt tolerance mechanism [16]. Apart from this several reports have also attempted to develop abiotic stress-tolerant crop plants, by over-expressing the abiotic stress responsive genes [17,18]. Studies with transgenic plants confirm the conception that monitored gene expression can lead to enhanced stress tolerance [19,20].

Since scanty of reports are available on transcript regulation of stress responsive genes from various pathways in capsicum under salt stress. Therefore, crafting a connection between the genes expression modulation and higher degree of tolerance within a genotype may provide a better new insight to analyze the tolerance mechanism. Present study is designed to understand the molecular mechanisms by comparing transcript expression of stress responsive genes, involved in diverse functions such as transcript regulation (ring domain zinc finger protein gene (CaKR), osmotic adjustment (CaPROX1), water, solutes movement (CaPIP2), osmolytes production (osmotin like protein (CaOSM1), membrane structural protein (CaChi2), endoplasmic reticulum ubiquitine ligase (CaRma1H1) and programmed cell death (cell death repressor gene (CaBI-I)) in three capsicum varieties having different salt tolerance level under various level of salt stress. Objective of this study is to detect and analysis the gene expression profile and to fetch a connection between salt tolerance and stress responsive genes.

2. MATERIALS AND METHODS

2.1 Plant Material and Salt Treatment

Seeds of three capsicum cv. susceptible cultivar (CO1), tolerant (G4) and moderately sensitive (K2) [21] were collected from Coimbatore Agriculture University and allowed to grow in soil pots under controlled green house conditions at VIT University, Vellore. 10 plants were placed in

six sets of plants (three replicates) for each cultivar and allowed to growth for 60 days. Then plants were treated with 500 ml solution of six different concentration of NaCl such as 25 mM (2.5 dS/m), 50 mM (4.95 dS/m), 100 mM (9.8 dS/m), 150 mM (14.6 dS/m) and 200 mM (19.6d S/m) respectively for 30 days. Control plants were treated with distilled water. This study is laboratory based and each experiment performed in this study was conducted in triplicates.

2.2 Proline Estimation

The proline content was estimated from 1g leaf sample of capsicum plant treated with different salt treated according to the protocol mentioned by Bates et al. [22].

2.3 Sodium and Potassium ions Estimation

Both Na^+ and K^+ content measurement from 0.5g leaf sample were conducted in capsicum plant treated with different salt treated using flame photometry [23].

2.4 RNA Isolation

All glassware plastic wares, mortar and pestle used in RNA isolation were treated with 0.1% (V/V) DEPC for 24 h followed two times autoclaving and oven drying at 80ºC for 48 h. 50µg of leaves sample was homogenized with liquid nitrogen, resulting powder was mixed in 1 ml of trizol reagent (invitogen) and incubated at room temperature for 15min at room temperature followed by addition of chloroform. Mixture was centrifuged at 12000xg for 15min at 4ºC and supernatant was precipitated at -20ºC for 30 min after adding isopropanol. The air dried pellets were dissolved in RNAse free water and equal volume of saturated phenol was mixed. The mixture was centrifuged at 12000xg for 10min. Resulting supernatant was taken in new tube and equal volume of chloroform was added followed by centrifugation for 10minutes at 12000xg. Supernatant was taken out in new tube followed by addition of 2.5 volume of 70% ethanol 1/10 volume of 3M sodium acetate and it was incubated at -20ºC for overnight for precipitation. Then mixture was centrifuged at 13000xg for 25 min and pellet were dried and dissolved in RNAse free molecular grade water. The quality of total RNA was checked by spectrophotometric absorbance at 260 and 280 nm.

2.5 First Strand c-DNA Synthesis

cDNA was synthesized using RNA three replicate isolated from three set of salt treated as well as control plant using oligo (dT) primer (900ng). To the RT-PCR reaction, 2µl dNTPS (10 mM), reverse transcriptase 1µl, 5X assay buffer and 2µl DTT, 1µl RNAse inhibitor and 6µl RNA (200ng) and make up to 20µl with DEPC treated molecular grade water. The reaction was carried out at 37ºC for 60min and 72ºC for 15 min in eppendorf thermo cycler. The cDNA was tested for amplification using β-actin specific primer for 30 cycles.

2.6 Primer Designing

Gene specific primers (Table1) were designed manually based on the sequence information available at NCBI GenBank database (http://www.ncbi.nlm.nih.gov) and thermo-dynamic properties were analyzed with help of Vector NTI software.

2.7 Primer Optimization and PCR Normalization

The gene specific primers were used to carry out transcript expression analysis using PCR. The reaction was made up using 2X buffer, 200 µM dNTPS, reverse primer 1µl, forward primer 1µl DNA polymerase 2 µg, make up the volume up to 20µl with RNAse free molecular water. The thermal cycling included initial denaturation (95ºC, 5 min), followed by 30 cycles of denaturation (95ºC, 30s), primer annealing (65ºC, 30s) and primer extension (72ºC, 1 min) followed by final extension 72ºC for 15 min. For ascertaining equal RNA loading in PCR reaction, actin was used as an internal control. For each gene transcript analysis three replicates of c DNA from three set of treated plant were used.

2.8 Statistical Analysis

All the experiment was performed in triplicate. The analysis of variance (Two-way ANOVA) between the control and sodium bicarbonate supplemented groups was carried out using Tukey's multiple comparisons test at $p < 0.05$ in Graph pad Prism (Version 6.0). Figures represent in mean and their standard deviation.

3. RESULTS

3.1 Proline Content

Fig. 1 shows the proline content in leaves of three capsicum cultivars. More accumulation was observed in salt tolerant cultivar as compared to susceptible and moderately sensitive cultivar when plants were exposed to salt stress.

3.2 Sodium and Potassium Ion Content

When plant were exposed to salt there was significant difference in accumulation of Na^+ and K^+ in leaves of salt treated plant and untreated control plant. The highest level of Na^+ was observed in susceptible cultivar (CO1) followed by followed by resistant cultivar (G4) while it was least detected in moderately susceptible cultivar (K2) (Fig. 3). But highest level of K^+ level was noticed in G4 cultivar followed by CO1 and K2 (Fig. 2).

Comparative study of Ion content and proline

Fig. 4 clearly demonstrates relative increase of proline (Fig. 4A) and sodium ion content (Fig. 4C) among capsicum cultivars CO1, K2 and G4. The increase in sodium ion and proline is found be more prominent in G4 than other two selected cultivars. Fig. 4B shows that decrease was more profound in moderately sensitive cultivar (K2) followed by salt susceptible (CO1) and salt resistant cultivar G4.

3.3 Expression Study

DREBLP1 expression enhanced in all plants exposed to salt differed significantly among the genotypes, and significantly increased in plants exposed to salt. DREBLP1 gene transcripts were found to gradually increasing as the salt concentration increased. However the highest expression level was observed in salt tolerant cultivar (G4) followed by moderate sensitive cultivar (K2) while it was least expressed in salt susceptible cultivar (CO1) (Fig. 5).

CaKR1 expression level induced in all plants exposed to salt varied significantly among the genotypes, and significantly increased in plants exposed to salt. The transcripts expression level of CaKR1 was not traceable in untreated control of selected cultivars. However higher expression level was found to be in tolerant cultivar (G4) than moderate sensitive (K2) susceptible cultivar (CO1) (Fig. 5).

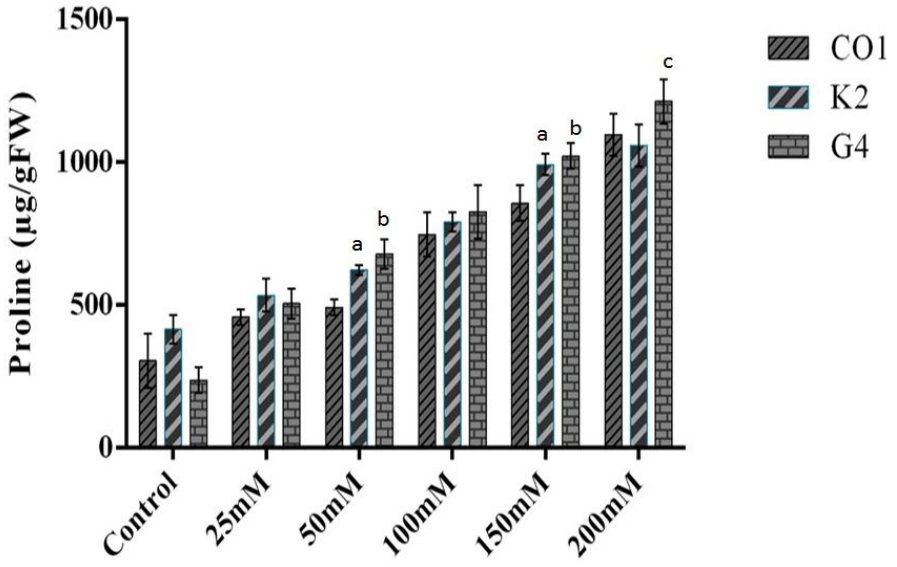

Fig. 1. Proline content of Capsicum cultivars CO1, K2 and G4 were estimated in the plants treated with sodium chloride and control. 0 mM (1), 50 mM (2), 100 mM (3), 150 mM (4), 200 mM (5) of sodium carbonate supplemented cultures Values are expressed in mean ± SD (n=3). Letters a, b, c represents to significant value between capsicum Cultivars CO1 x K2, CO1 x G4 and K2 x G4 ($p < 0.05$)

Table 1. Primers of capsicum used in analysis of stress responsive genes

Gene	Accession no.	Forward primer	Reverse primer
CAPIP2	GI 10430410	GCT TCC ATA ACTT GCG GTG CCC	CAGTATGTGAACGGCAGTAATTGTGACC
CaDREB	GI40647094	GAAGCCCTCCGACCATTGAAGTTG	CCCAACCACTTACTTATGCTGCTACACG
CaPROX1	Fj9115491	GGACCGTTATGAATCTCTCTGATTCTGGG	GCGGGTTGAATCGTTGAGTCTTCTGC
CaRMa1H1	AY513612.2	CGGTCATCTTTACTGCTGGCCTTG	CCTTCTCATTCTTGGGCTACTGCTG
Ca KR1	DQ862464.1	GTGTTCTTCCTCCTTGCCTTTGTGAC	CTAAGTGAACCAGGGCATTCACCAT
CaBI1	FJ719768.1	CGCAATCGGCTTCTCGCAGTCG	GCAACCACATGAGGAGGGAGACACC
CaOSM1	AY262059.1	GTGTTCTTCCTCCTTGCCTTTGTGAC	CTAAGTGAACCAGGGCATTCACCAT
CAChi2	AF091235.1	CAGGGACTTGTTTGAACGGATGC	GACACCGTAGCCTGGCACTCG
CaAct	AY572427.1	CGCAGGAATCCAGTCACG	ACCCTCATGACATCACTTCTCTATT

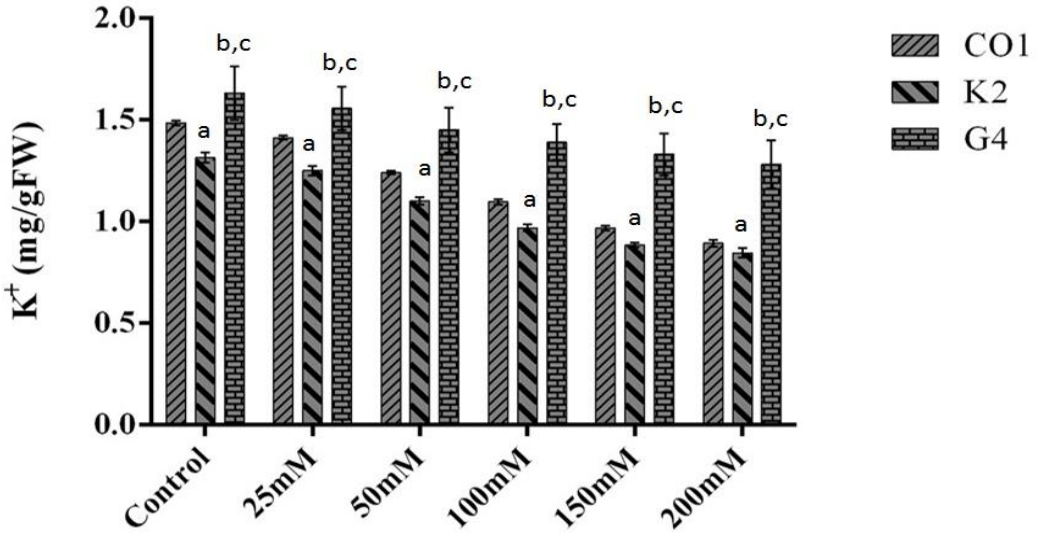

Fig. 2. Potassium ion content of Capsicum cultivars CO1, K2 and G4 were estimated in the plants treated with sodium chloride and control. 0 mM (1), 50 mM (2), 100 mM (3), 150 mM (4), 200 mM (5) of sodium carbonate supplemented cultures Values are expressed in mean ± SD (n=3). Letters a, b and c represents to significant value between capsicum Cultivars CO1 x K2, CO1 x G4 and K2 x G4 ($p < 0.05$)

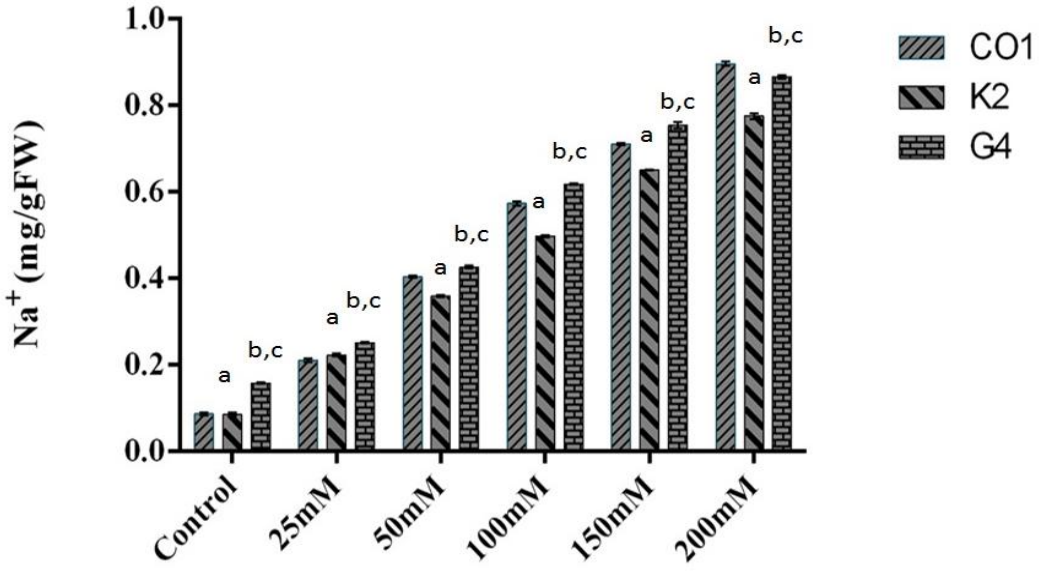

Fig. 3. Sodium ion content of Capsicum cultivars CO1, K2 and G4 were estimated in the plants treated with sodium chloride and control. 0 mM (1), 50 mM (2), 100 mM (3), 150 mM (4), 200 mM (5) of sodium carbonate supplemented cultures Values are expressed in mean ± SD (n=3). Letters a, b and c represents to significant value between capsicum Cultivars CO1 x K2, CO1 x G4 and K2 x G4 ($p < 0.05$)

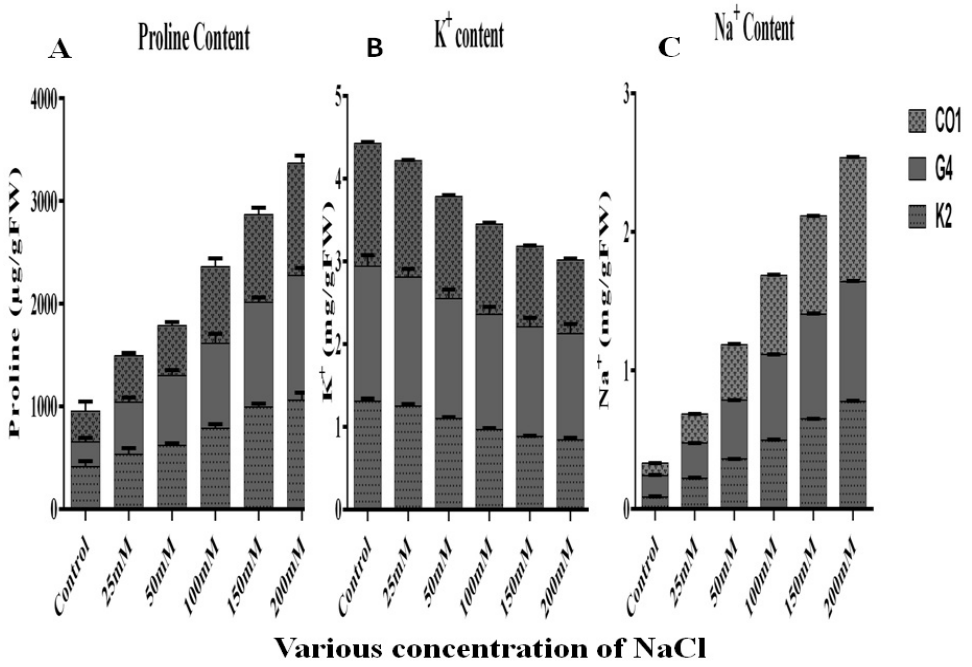

Fig. 4. Comparative increase and decrease Proline (A) and potassium (B), Sodium (C) ion content of Capsicum cultivars CO1, K2 and G4 were estimated in the plants treated with sodium chloride and control. Control, 50 mM, 100 mM, 150 mM, 200 mM of sodium Chloride. Values are expressed in mean ± SD (n=3)

The gene transcripts expression levels of CaPROX1 decreased significantly in tolerant cultivar (G4) and moderate sensitive cultivar (K2) as the plants were exposed to salt. But not such significant reduction was noticed in susceptible cultivar (CO1). However more reduction in transcript expression was observed in tolerant cultivar than moderate sensitive cultivar (Fig. 5).

CaOSMO1 expression enhanced in all plants exposed to salt differed significantly among the genotypes, and significantly increased in plants exposed to salt. The extent of increase was significantly higher in salt tolerant cultivar (G4) as compared to moderate sensitive (K2) cultivar while CaOsmo1 was least expressed in leaves of salt susceptible cultivar (CO1). But transcripts were not very low in untreated control plants of all selected cultivars (Fig. 5).

CaPIP2 expression level reduced in all plants exposed to salt differed significantly among the genotypes, and significantly decreased in plants exposed to salt. However the decrease in expression level was most in G4 cultivar followed by moderately sensitive cultivar (K2). But in case of salt susceptible cultivar (CO1) expression level was nearly same in differently salt treated plant (Fig. 5).

CaRaM1H1 expression induced in all plants exposed to salt differed significantly among the genotypes, and significantly increased in plants exposed to salt. Gene transcripts were not detectable in control plant of all selected cultivar. The highest expression was noticed in salt tolerant cultivar (G4) followed by moderately sensitive cultivar (K2) while its expression level was least in salt susceptible cultivar (CO1) (Fig. 5).

CaBI1 expression significantly increased in plants exposed to salt but plant exposed to salt not differed significantly among the genotypes.However there was profound difference in expression level between untreated control and salt treated plants (Fig. 5).

CaChi2 expression significantly enhanced in plants exposed to salt but plant exposed to salt not differed significantly among the genotypes tolerant (G4), moderately sensitive (K2) and susceptible cultivar (CO1) (Fig. 5).

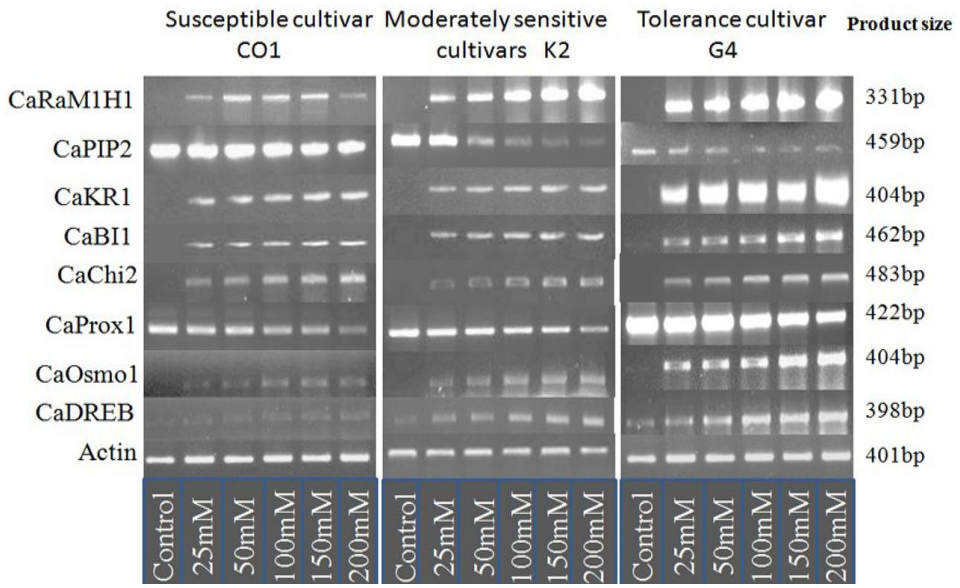

Fig. 5. Transcript expression profiling of stress responsive genes of three capsicum cultivars

4. DISCUSSION

A plenty of works debated on role of osmolytes for improving tolerance against abiotic stress [24]. Proline is one of widely distributed osmolyte in plant life. Proline is synthesized in plants through two alternated pathways: L-orthinine and L-glutamate pathways. In present study proline accumulation induced in all plants exposed to salt differed significantly among the genotypes. This significant variation is well supported by the gene expression level of CaPROx1, one of proline degrading enzyme. Higher expression may lead into low accumulation of proline plant. As CaPROX1 expression in highest in case of G4 cultivar control plant so the proline content in lowest in G4 cultivar plant. But as the salt concentration increases the expression level of this gene is decreased which results in increase in proline content in G4 cultivar. The expression of CaPROX1 is varying among the cultivars which could be the reason for no significant difference among the selected cultivars.

This may be one of the possible reasons for difference in salt sensitivity level among the selected capsicum cultivars. Due to similar chemical nature Na^+ replaces K+ from its target site. Results indicates that selected capsicum cultivars may follow different strategies to exclude the Na^+ and maintain the K^+.

Previous expression studies concerned with salt tolerance have mainly focused on the plant physiological and molecular responses of individual variety under different level of salinity. Our previous study has also demonstrated effect of salt on growth attributes and hormones in these selected cultivars [25].

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Differential transcript expression of stress responsive genes in capsicum is not well spoken to address the salt tolerance mechanism. Dehydrin responsive element binding factor (DREBLP1) is a member transcript factor family which regulates expression of stress responsive genes on exposure to different biotic and abiotic factors [26]. Some of earlier reports also highlight the potential of DRE gene in providing tolerance against different abiotic stresses [27-29]. In present study transcripts analysis of CaDREPLP1 also match with previous results. Our previous on phytohormones on salt exposure results indicates significant increase in ABA [25]. Loss function study in Arabidopsis and tobacco not only illuminated the role of DRE under abiotic stress but also its independency to ABA. In addition to that over-expression of DRE/CBF1 gene in transgenic Arabidopsis has also elucidated enhanced level salt and freezing tolerance [30]. In addition to that over-expression of DRE/CBF1 gene in transgenic Arabidopsis has also elucidated enhanced level salt and freezing tolerance. In current study, level of increase in the gene expression in salt tolerant

cultivar (G4) over the moderate sensitive (K2) and salt susceptible cultivar (CO1) infers the involvement of DRE for difference in level salt tolerance and acclimation under salt stress. The result thus indicates that the differential gene expression pattern among selected cultivars may be one of the reasons for difference in sensitivity level against salt.

CaKR1 is member of ankyrin repeat zinc finger protein transcription factor family which is mainly involved in signaling [31] and development mechanisms [32]. Earlier studies reported that induction of zinc-finger transcription factor under salt and other abiotic stresses in rice plays crucial role in stress resistance [11]. Enhanced gene expression of ankyrin repeat zinc finger gene was noticed not only under biotic stress but also under abiotic stress and different chemical treatment [33]. In addition to this ankyrin repeat transcription factor genes were found to be organ- specific mainly expressed in root and flower but not in healthy leaves and stem [34]. Expression of CaKR varied in individual cultivar depending on their tolerance levels. Study on two rice cultivars sensitive (IR29), tolerant (Pokkali) and current study on capsicum three cultivars tolerant (G4), moderate sensitive (K2) and susceptible difference (CO1) in the transcription expression level were witnessed [35]. This result advocates the role of CaKR1 for difference in sensitivity level among selected cultivars.

However, over-expression and silencing studies of PROX gene demonstrated modulated transcript expression which results to marginal difference in free proline accumulation with limited impact on plant development and growth [36]. Few recent studies have highlighted the necessity of proline catabolism in meristematic and expanding cell for sustained development [37]. Δ1-Pyrroline 5-carboxylate synthase (P5CS) and suppression of Proline oxidase (PROX1) increased the proline content conferred the tolerance to salinity as well as cold [38,39]. Variation in the extent of reduction in gene expression level among salt tolerant cultivar (G4), moderate sensitive and susceptible cultivar suggests the direct involvement of PROX1 in imparting tolerance to salinity in Capsicum. Increase in proline content during environmental stress is caused by activation of its biosynthesis and deactivation of its degradation. Capability to accumulate proline in response to environmental stress is highly variable between or within species [40]. However in present study significant variation in gene expression between control plant and plant exposed to salt indicates that that CO1, K2 and G4 cultivar may also imply same strategies to maintain the osmotic homeostasis for proline.

In earlier study in pepper and tomato enhanced transcript expression of osmotin gene has been found potential candidate for enhanced resistance against biotic and abiotic stresses [41,42]. Furthermore, constitutive over-expression of osmotin gene has raised the salt tolerance level in soybean [43]. In current study, difference in the extent of transcript accumulation of CaOSMO1 gene advocates the possibility for contribution of CaOSMO1 gene in tolerance against salinity.

Plasma membrane intrinsic protein (PIPs) constitutes the largest and evolutionary most conserved subfamily of aquaporins that facilitates the transport of water and small neutral solutes or gases [44]. Earlier studies on compressive gene expression in Arabidopsis and tobacco have witnessed the down regulation of aquaporins under salt and drought, which would be one of the molecular tricks to maintain the cell turgor pressure [45,46]. In present study the difference in CaPIP gene expression was not found to be significantly related to salt concentration as well among the cultivars. This result suggests that decrease in CaPIP2 gene expression may not be playing direct role in difference tolerance among the selected cultivars.

CaRm1H1 genes is part of diverse ubiquitine ligase family which regulated many crucial cellular processes such as cell cycle progression, cell signaling, DNA repair, protein trafficking under biotic and abiotic stress [47-49]. Transgenic tomato study on CaRma1H1 has also provided evidence for its involvement in tolerance mechanism [50]. Some recent studies also elucidated the RINGE3's role in the cellular responses to stress hormone abscisic acid as well as environmental stresses in higher plants [51,52]. As our earlier study on Capsicum has already reported about significant ABA increase in response to salinity, this may be one possible way to increase in CaMa1H1 transcript expression. In addition to this transgenic study in Arabidopsis also crystallizes role of CaRma1H1 gene in aquaporins regulation [11]. Present results also support involvement of CaRma1H1 gene for difference in sensitivity level against among sensitive (CO1), Moderate sensitive (K2) and tolerant cultivar (G4).

Many studies have already provided convincing evidence about intimacy of conserved cell death suppressor, BAX inhibitor-1 (BI-1) in attenuating endoplasmic reticulum stress-mediated programmed cell death [53,54]. Recently transgenic studies on Arabidopsis, barley and tobacco have also pointed out the importance of BAX inhibitor-1 in biotic and abiotic stress tolerance [53-56]. However no profound difference was observed among the susceptible (CO1), moderate sensitive (K2) and tolerant cultivar (G4). This result indicates that CaBI1 may not assert tolerance to capsicum cultivars.

Chitinase is one of the most important enzymes which mainly indulged in pathogenic tolerance in plant. Apart from biotic stress it plays crucial role in abiotic stresses such as oxidative, osmotic [11,57]. Further over-expression study in Arabidopsis gives compelling evidence for its key role in osmotic tolerance and bacterial resistant [58]. In this study induction of CaChi2 gene was observed in all three capsicum cultivars as the salt concentration increased. Transgenic tobacco study has already reported that over-expression of endochitinases CHIT33 and CHIT42 provides the tolerance to salt, heavy metals and bacterial pathogenic. This difference in transcript expression level between tolerant and sensitive cultivar may be crucial factor for imparting salt tolerance in tolerant variety.

Chitinase in one of the most important membrane bound enzyme which is mainly indulged with resistance against biotic stress. In addition to biotic stress, it also imparts resistance in plant under oxidative and osmotic stress. Further over-expression study in Arabidopsis presented convincing evidence for its involvement in osmotic tolerance as well as bacterial resistance. Results of present study suggest that Cachi2 gene may not be directly involved in salt tolerance for capsicum cultivars.

5. CONCLUSION

Crop improvement of salt tolerance is obligatory to meet the growing demand of food. Detailed knowledge of genes, protein and mechanism can help in developing stress tolerant varieties. Differential study of physiological and transcriptional parameters in cultivars with diverse sensitivity level against stresses could provide new insight to update the knowledge in this aspect. For Capsicum cultivar G4, K2 and CO1, such a study was conducted for leaves and we observed many of selected gene were induced under salt exposure. Although there was increase in proline as the salt concentration increased. But no significant difference was observed among cultivars. But capsicum cultivars followed different mechanisms for retaining sodium content in leaf. Our data shows that CaDREBLP1, CaRMa1H1, CaKR1, CaOSM1 genes were observed to be up regulated while CaPROX1, CaPIP2 genes were observed to be down regulated under salt stress (Fig. 5). But no significant difference was noticed in gene expression level of CaBI1 and CaChi2 gene. From present study we conclude that higher gene expression level of stress responsive genes viz. CaDREBLP1, CaRMa1H1, CaKR1, CaOSM1 will be the possible candidates for different level of salt tolerance among selected cultivars. Further investigation of these four genes by over-expression can give better understanding for salt tolerance mechanism adopted by plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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