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Rajat Chaudhary
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110 (U.P.), India

Mukesh Kumar
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, (U.P.), India

RS Sengar
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, (U.P.), India

Pushpendra kumar
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, (U.P.), India

SK Singh
Department of Genetics and Plant Breeding, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, (U.P.), India

Yogesh kumar
Department of Soil Science, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, (U.P.), India

Correspondence
Rajat Chaudhary
Department of Agricultural Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110 (U.P.), India

Effect of Salinity stress on Photosynthesis and expression of salt tolerant genes in Chickpea (*Cicer arietinum* L.)

Rajat Chaudhary, Mukesh Kumar, RS Sengar, Pushpendra Kumar, SK Singh and Yogesh Kumar

Abstract
Chickpea (*Cicer arietinum* L.) is a salt sensitive leguminous crop species, but some genetic variation for salinity tolerance exists. The experiment was conducted in pots, in which fifteen genotypes of chickpea were subjected to 0, 40 or 60 mM NaCl added to the soil to determine the variation in salt tolerance. Photosynthetic rate were measured in the reproductive of both saline and non- saline condition. The results showed photosynthesis rate decreased under both the salinity levels. The genotypes CSG 8962, L 550 and JG 16 are found the most tolerant among studied genotypes under both salinity levels (S1 and S2). The HKT1 gene was expressed higher in susceptible genotype (K 850) in leaves and roots under both the salinity levels. P5CS gene was expressed higher in susceptible genotype (K 850) in leaves tissue. In root tissue, no expression was observed. LEA2 gene was detectable under the water deficit condition in leaves and roots under both the salinity levels. The transcription factor WRKY and NAC were expressed higher in leaves and roots in tolerant genotype CSG 8962. The genotype CSG 8962 showed high levels of tolerance compared for the other cultivars. The results suggest that chickpea cultivars tolerant of salinity have better growth potential than do sensitive ones.

Keywords: salinity, photosynthesis, salt tolerant index, high-affinity potassium transporter, NACL, and salt tolerance indices.

Introduction
Chickpea (*Cicer arietinum* L.) is a legume crop and belongs to the family Fabaceae. It is self-pollinated, diploid (2n=2x=16) with a genome size of 740 Mbp. It is an annual crop that can complete its life cycle in 90 to 180 days depending on the prevailing meteorological conditions. It is the second most important legume crop after dry beans (Varshney *et al*., 2012) [31]. The genus Cicer originated in South-Eastern Turkey and spread to other parts of the world. Chickpea is grown in 54 countries with nearly 90% of its area covered in developing countries (Gaur *et al*., 2012) [10]. Almost 80% of global chickpea is produced in Southern and South-Eastern Asia. Chickpea production is 13.10 million tonnes from 13.50 million ha. the area with an average productivity of 971 kg/ha worldwide. India has first ranked in the world, contributing 68% of the global chickpea production. In India pulse crops are cultivated in 9.92 million ha, producing a total of 9.88 million tonnes with an average yield of 995.96 kg/ha (FAOSTAT, 2014) [8].

Chickpea seeds provide a source of dietary protein (21%) and carbohydrates (40%) for humans and animal feed in some developing countries (Flowers *et al*., 2010; Gaur *et al*., 2012) [9, 10]. Moreover, chickpea not only improves soil fertility by fixing atmospheric nitrogen (N), but it also requires low or less N inputs as it can fix up to 70% of its N requirements (Flowers *et al*., 2010) [9]. Chickpea productivity is constrained by several abiotic stresses (Singh *et al*., 1994; Gaur *et al*., 2007 and Chaudhary *et al*., 2016) [8, 11, 4] and salinity is one of the most important determinants of crop growth over a range of environments. In Australia and India, salinity has already become a major deterrent to crop production, including legumes. In India alone, about 13 million ha are currently affected by salinity. In Uttar Pradesh salt affected area was about 13.69 lakh ha (www.cssri.org). Salinity adversely affects the plant growth due to low osmotic potential and nutrient imbalance. These factors affect the physiological and biochemical activities and growth and development of plants (Munns and James, 2003) [25].

The Na^+ transport and the candidate genes involved in salt tolerance, the high-affinity potassium transporter (HKT) gene family, salt overly sensitive (SOS) pathway, and Na^+/H^+ antiporter (NHX) gene family mediate Na^+ transport in different parts of plants and are prospective genes involved in ion 'exclusion' and/or 'tissue tolerance' in plants. Arabidopsis class I transport gene localised on the plasma membrane, AtHKT1;1, has been involved in the removal of Na^+ from xylem sap into xylem parenchyma thereby reducing Na^+ accumulation in leaves (Sunarpi *et al*., 2005) [29]. At cell level, low levels of Na^+ in cell cytoplasm, to avoid ion toxicity to cell metabolism, is achieved by sequestration of Na^+ into vacuoles mediated by tonoplast-localized Na^+/H^+ exchanger 1 (NHX1) or by excluding Na^+ from cytoplasm to extracellular spaces controlled by plasma-membrane localized salt overly sensitive 1 (SOS1; a Na^+/H^+ antiporter) (Hasegawa, 2013; Bassil and Blumwald, 2014; Deinlein *et al*., 2014) [15, 26, 6]. Both the genes (NHX1 and SOS1) are powered by the H^+ gradient across the tonoplast and plasma membrane, respectively, which needs the activity of vacuolar and plasma membrane H^+-ATPases and vacuolar H^+-pyrophosphatase (Bassil and Blumwald, 2014; Roy *et al*., 2014) [2, 27]. Exploring the physiological mechanisms and the candidate genes involved would improve our understanding of mechanisms of salt tolerance in chickpea.

Materials and Methods

Experimental Site: The experiment was conducted at field laboratory research and experiment station of Department of Agricultural Biotechnology, SVPUA&T, Modipuram, Meerut, which is situated at North West plain Zone 26.47^0N (latitude), 82.12^0E (longitude) and at 113 m above mean sea level. The composition and structure of the soil are presented in table 1.

Table 1: Soil structure at experimental site.

Soil	Per-cent
Sand	35.2
Silt	48.6
Clay	16.2
pH	8.8
Organic carbon	0.3
Available N	128.4 kg/ha
Available P_2O_5	13.5 kg/ha
Available K_2O	355.7 kg/ha

Materials: The total fifteen chickpea germplasm which were collected from IIPR, Kanpur, Uttar Pradesh and NBPGR, Delhi. The germplasm belongs to different geographical areas and the details regarding pedigree, type, characteristic and year of release is given in table 2.

Table 2: Description of chickpea genotypes screened against salinity condition

S. N.	Name	Type	Pedigree	Year of Release/ Notification	characteristic
1	L550(ICC 4973)	Kabuli	PB 7 x Rabat	1977	Moderately resistant to pod borer
2	K850	Desi	Banda Local x Etah Bold	1978	Large seeded, performs well under irrigatedas well as rainfed conditions
3	Pusa372 (BG-372)	Desi	P 1231 x P1265	1993	Moderately resistant to wilt, blight & root rot., Small seed, light brown
4	Pusa 362 (BG362)	Desi	(BG 203 x P179) x (BG 303)	1995	Tolerant to wilt, Bold seeded
5	KWR108	Desi	Selection from germplasm line P 108	1996	Resistant to wilt, Seeds are dark brown and small.
6	PKV Kabuli-2 (KAK2)	Kabuli	(ICCV 2 x Surutato 77) x ICC 7344	1998	Bold seeded
7	CSG8962(Karnal Chana 1)	Desi	Selection from GPF 7035	1998	Performs well under saline and sodic soils; Wilt resistant
8	JG11 (ICCV 93954)	Desi	(Phule G-5 x Narsinghpur bold) x ICCC 37	1999	Resistant to wilt, moderately resistant to root rot. Bold seeded
9	JG16 (SAKI 9516)	Desi	(ICCC 42 x ICCV 88506) x (KPG 59 x JG 74)	2000	Perform well under drought situation, moderately resistant to wilt
10	ICCV05107	Desi	ICC 4958 x ICCV 92311	2002	An intermediate yielding variety
11	ICCV95334(JGK 3)	Desi	[(ICCV 2 x Surutato 77) x ICC 7344] x Blanco Lechozo	2006	Recommended for late sown irrigated condition and moderately resistant to wilt
12	JAKI 9218 (ICCV 93952)	Desi	(ICCC 37 x GW5/7) x ICCV 17	2007	Resistant to fusarium wilt, root rot and collar rot, resistant to lodging shattering, rainfed / irrigated conditions
13	JG14	Desi	[(GW 5/7 × P327) × ICCL 83149]	2009	Moderate resistant to wilt, dry root and pod borer, heat tolerant, Recommended under late sown conditions
14	ICCV07112	Desi	-	-	Resistant to wilt and heat tolerant
15	ICCV88105	Desi	-	-	-

Experiment

Fifteen genotypes of chickpea, for salt tolerance experiment, were grown in non-draining, plastic pots (25 cm diameter, 21 cm height). The genotypes included Desi (small seeded, thick testa) and Kabuli (large-seeded, thin testa) types. Seeds were sown for analysis of salt (NaCl) tolerance. Ten seeds of each fifteen genotypes were sown in a separate pot. After sowing, soil moisture was maintained by watering pots with distilled water. After germination of seeds, the plants were treated with different concentrations of NaCl equivalent of 0 mM, 40 mM and 60 mM at reproductive phase. To create the irrigation water of desired salinity level, required quantity of NaCl were throughly mixed with irrigation water to the pots. The pot experiment was performed in complete randomized design (CRD) with three replication. The meteorological data were recorded by an automatic weather station of Indian Institute of Farming System and Research (IIFSR) Modipuram, Meerut, India. The meteorological data (2013-14) indicated that weekly minimum and maximum temperature during the crop season ranged from 5.68 to 14.70 and 17.14 to 27.18 respectively. Total rainfall received was 1.81 mm during the entire crop season and relative humidity was found to be very from 62.62 to 97.35% (Fig.1a). However, meteorological data (2014-15) indicated that weekly minimum and maximum

temperature during the crop season ranged from 5.14 to 14.62 and 14.14 to 31.83 respectively. Total rainfall received was 1.23 mm during the entire crop season and relative humidity was found to be very from 67.59 to 95.32% calculated as standard meteorological weather during experiment conducted from November 2013 to March 2014 and November 2014 to March 2015 (Fig.1a & 1b).

Photosynthesis rate

Leaf net photosynthetic rates (Pn) were measured during salt (NaCl) stress at the flowering stages of both stressed and non-stressed plant. The Pn was measured on the penultimate leaves using an LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA). When measuring Pn, the photosynthetic photon flux density (PPFD), provided by a 6400- 02 LED light source, was set to 1500 mmol m^{-2} s^{-1}. The temperature and CO_2 concentration in the leaf cuvette were set to 25 ºC and 360 ppm (ambient CO_2 concentration in the greenhouse), respectively. Humidity in the cuvette was controlled by circulation of the air through the desiccant. A steady flow rate of 500 mmol s^{-1} was maintained in the leaf chamber. Three individual leaves (penultimate position) per plant were measured for 5 plants of each temperature.

A. Fundamental calculations for photosynthesis and related parameters

$$P_{net} = 1 + \frac{\Delta CO2}{L}$$

Where P_{net} is net photosynthesis rate, ΔCO_2 is a change in CO_2 concentration from some reference point, and L is leaf area or leaf mass. Transpiration is usually calculated in an analogous manner.

B. Leaf internal CO_2 concentration - Using the Ohm's law analogy,

$$P_{net} = g_s*(C_a - C_i)$$

Where P_{net} is net photosynthesis rate, g_s is stomatal conductance to CO_2 (which is equal to stomatal conductance to water vapour / 1.6) and C_a and C_i are the ambient and leaf internal concentrations of CO_2, respectively.

Molecular parameter

Sample collection: Five chickpea genotypes (ICCV 07112, CSG 89662, JG 14, K 850, and KWR 108) were selected for transcript analysis. The samples of germplasm after 24 hr of treatments (0, 40, and 60 mM) were collected for RNA isolation from shoot and root of the plant by both control and stressed plant at same time.

Isolation of total RNA: Total RNA was isolated from the control and treated salt stress chickpea plantlets of selected variety by using Genei Pure Kit. The total-RNA was subjected to spectrophotometer using RNase-free water as blank: absorbance was recorded at 260/280 and 260/230.

Gel electrophoresis: 1.2g agarose was added to 100 ml MOPS buffer and melted in a microwave oven. After cooling, 720 µl of 37 per cent formaldehyde and 2.5 µl of ethidium bromide from 10X stock were added and poured into electrophoresis tray for solidification.

Sample preparation for RNA loading: One volume of 5X loading dye was mixed with 4 volumes of isolated RNA sample, incubated at 65°C for 5 min. and chilled on ice for 5 minutes before loading. The horizontal electrophoresis unit (Merck, Pvt. Limited, India) with tank buffer (MOPS buffer) was run at 45V for 30 min. prior to loading. Electrophoresis was continued after loading samples at 50V for 45 min. and gels were observed under UV-Transilluminator and documented by Alpha Digi Doc (Alpha Innotech Corporation, U.S.A.).

c-DNA synthesis

First Strand cDNA Synthesis: Thaw Total RNA from chickpea (shoot and root) supplied on the ice. Thaw oligodT and or Random hexamer, dNTP mix and 5X Assay Buffer at room temperature (20-25°C). Store all components on ice, soon after thawing. Add 1-5 µl mRNA (10-100 ng) or total RNA (100 ng-5 µg) and Nuclease-Free Water make up the volume to 9 µl. Add 2 µl of Random Hexamer or 1 µl Oligo(dT)18 primer or gene specific primer. Place the Test and Control tubes at 65°C for 10 minutes in a dry bath and then at room temperature for 2 minutes to remove any secondary structures. Spin the tubes briefly.

Component Volume

Rnasin	1 µl
100mM DTT	1 µl
5X Assay Buffer for M-MuLV RT	4 µl
30mM dNTP mix	2 µl
MMuLV Reverse Transcriptase	1 µl
Nuclease-Free Water.	1 µl
Total Volume	20 µl

Mix the contents well and incubate at 37 °C for 1 hour in dry bath. Incubate at 94 °C for 2 minutes. Quickly place tubes on ice and spin briefly. This step denatures RNA-cDNA hybrids.

PCR amplification condition: Following condition was standardised for the amplification of different genes with different primers which were performed by using Thermal Cycler (PTC-100, Bio-Rad, USA).

Stage	Step	Temp. (^{0}C)	Duration	No. of cycle
1	Initial denaturation	95	5 min.	1
2	Denaturation	94	1 min.	35
3	Annealing (vary with primers)	55	1/2 min.	
4	Extension	72	1 min.	
5	Final extension	95	7 min.	1
6	Storage	4^{0}C		

Electrophoresis of the amplified PCR products: About 20 µl of the amplified reaction mixture from each PCR tube with 3 µl of loading dye were loaded onto 1.2% agarose gel along side 100 base pair DNA ladder as molecular weight marker (Merck, Pvt. Limited, India). Electrophoresis was done at 50 volts for initial 30 min. and then 70 volts for 1 hr in a horizontal electrophoresis unit system (Atto, Japan). The buffer used was 1x TAE at pH=8.0. The DNA bands in the

gel were visualised on a UV transilluminator and documented using a gel documentation system (Alpha DigiDoc, Alpha Innotech Corporation, USA).

Table 3: List of gene specific primers used for gene expression analysis

S. No	Primer	Forward	Reverse
1	LEA2	CGACAACGGAAGGGCAAATG	CAAGACTAAACTTTGTGCAGTCC
2	WRKY	CTCATTCACACAAGTTCCTGC	GTCCTTTGTGAAAGGGCTGG
3	P5CS	TGGTCCCTAGAGGCAGTAACA	TGTCAACAACCCCTCAACTCC
4	Actin	GCAGCATCAGGTATGGCAGT	TTCCATCAAGTTCCCCTCTT
5	NAC	GCTCGGGTACCTCCTACTC	TTCCTCAGCTTGGACCACTT
6	HKT1	TTCCTCGGTGGCGAAATCTT	TCTCAACGTCACAGCGATCC

Result

The studied genotypes showed a wide range of salt tolerant index (RSTI) for photosynthesis rate at S1 ranged from 0.041 (JAK 19218) to 0.121 (CSG 8962) with an average mean of 0.077. While at S2, photosynthesis rate ranged from 0.032 (JAK 19218) to 0.074 (L 550) with an average mean of 0.046. The salt susceptibility index (SSI) for photosynthesis rate at S1 ranged from 0.79 (JG 11) to 1.58 (ICCV 95334) with an average mean of 1.21. While at S2, photosynthesis rate ranged from 1.36 (ICCV 88105) to 2.94 (K 850) with an average mean of 1.90 (Table 4). The Geometric mean of productivity (GMP) for photosynthesis rate at S1 ranged from 7.35 (JAK 19218) to 12.55 (CSG 8962) with an average mean of 9.86. While at S2, photosynthesis rate ranged from 6.45 (JAK 19218) to 9.79 (L 550) with an average mean of 7.12(Fig. 2).

Expression profile of selected gene related to salt stress

The total RNA of five chickpea genotypes namely ICCV 07112, CSG 8962, JG 14, K 850 and KWR 108 from leaves and root at non- saline and saline condition (S1 and S2) were isolated. To study the transcript abundance of the following genes P5CS, HKT1, LEA, NAC and WRKY with semiquantitative PCR.

Proline (P5CS) gene expression analysis: The P5CS (Delta(1)-pyrroline-5-carboxylate synthetase) gene expression analysis of selected genotypes studied under control (non-stress) and salt stress (S1 and S2) condition from leaves and root at reproductive stage has been presented in fig. 3.1. The transcript of P5CS gene was highly accumulated in leaves of all the genotypes in control (non-saline) as well as saline condition (S1 and S2). The maximum accumulation being recorded at salinity level 2 (S2) in leaves tissue. In the case of roots, P5CS gene showed no expression in all the genotypes at both the salinity level and control.

High-affinity Potassium Transporter (HKT1) gene expression: High-affinity Potassium Transporter (HKT1) gene expression analysis of selected genotypes studied under control (non-stress) and salt stress (S1 and S2) condition from leaves and root at reproductive stage has been presented in fig. 3.2. The transcript of HKT1 gene was not detected in control (non-saline) condition. This gene was accumulated in leaves of all the genotypes at both the salinity levels. The maximum accumulation being recorded at salinity level 2 (S2) in leaves tissue. In the case of roots, HKT1 gene showed expression in all the genotypes at both the salinity levels except control.

Late Embryogenesis Abundant (LEA2) gene expression: Late Embryogenesis Abundant (LEA2) gene expression of selected genotypes studied under control (non-stress) and salt stress (S1 and S2) condition from leaves and root at reproductive stage has been presented in fig 3.3. The transcript of LEA2 gene was accumulated in leaves of all the genotypes in the control as well as saline condition (S1 and S2). The maximum accumulation being recorded at salinity level 2 (S2) in leaves tissue. In the case of roots, LEA2 gene showed expression in all the genotypes at both the salinity levels except control.

WRKY transcription factor expression analysis: The transcription factor WRKY expression of selected genotypes studied under control (non-stress) and salt stress (S1 and S2) condition from leaves and root at reproductive stage has been presented in fig 3.4. The transcript of WRKY gene was accumulated in leaves of all the genotypes in control (non-saline) as well as saline condition (S1 and S2). The maximum accumulation being recorded at salinity level 2 (S2) in leaves tissue. In the case of roots, LEA gene showed expression in all the genotypes at both the salinity levels except control.

NAC transcription factor expression analysis: The transcription factor NAC expression of selected genotypes studied under control (non-stress) and salt stress (S1 and S2) condition from leaves and root at reproductive stage has been presented in fig.3.5. The transcript of NAC gene was not detected in control (non-saline) condition. The transcript of NAC gene was accumulated in leaves of all the genotypes at both the salinity levels (S1 and S2). This gene is highly expressed in leaves of all the genotypes at salinity level 2 (S2). In case of roots, NAC gene showed expression in all the genotypes at both the salinity levels except control.

Discussion

Salt stress impairs reproductive development in plants due to the possible accumulation of toxic ions (Na^+) in reproductive tissues, reduced supply of assimilates to reproductive tissues due to decreased leaf area and reduced photosynthesis, water restriction and/or hormonal imbalances (Munns 2002) [24]. Salinity damaged leaf tissues and decreased photosynthesis in chickpea (Khan *et al.*, 2016) [19], the salt stress deleterious effect is reduced by change the structural organization of thylakoids. Salt stress on photosynthetic membrane results in the loss of grana stacking. In the present study, photosynthesis rate decreased under saline condition. The STI value is maximum for L 550 and CSG 8962 and minimum for K850 under saline condition. Under stress conditions (salinity and drought), photosynthetic pigments decreased in concentration, suppression in the mesophyll conductance and the stomata closure at moderate to severe stress (Flowers *et al.*, 2010) [9].

Expression analysis of salinity related genes

The high-affinity potassium transporter (HKT) gene family, mediate Na^+ transport in different parts of plants and are prospective genes involved in ion exclusion and/or tissue

tolerance in plants. HKT is responsible for reducing shoot Na^+ by removing Na^+ from xylem in roots as it flows towards the shoot (Davenport *et al.*, 2007; Hamamoto *et al.*, 2015) [5, 4]. However, in *Arabidopsis* leaves, HKT1 was expressed in the plasma membrane of xylem parenchyma cells to retrieve Na^+ from xylem sap (Sunarpi *et al.*, 2005) [29] and time- and tissue-dependent expression of AtHKT1 determines Na^+ distribution in plant organs/tissues (Hamamoto *et al.*, 2015) [4]. The expression of HKT1 in leaves tissues was lower in genotypes CSG 8962 and KWR 108, compared with genotype K 850. In roots, the similer result was observed. Similarly, expression of HKT1 in leaves and roots was also down-regulated in tolerant genotypes (CSG 8962) compared with sensitive genotypes (K 850) (Kader *et al.*, 2006; Zhou *et al.*, 2016) [18, 33]. A possible physiological explanation is that increased expression of HKT1 may have resulted in the higher influx of Na^+ into the cytosol of plant tissues in K850, compared with CSG 8962, making it more salt-sensitive (Kader and Lindberg, 2005; Kader *et al.*, 2006) [17, 18] So, expression of HKT1 may also differ between petiole and leaflets of chickpea leaf thereby unloading and storage of more Na^+ into petiole thereby avoiding high concentrations of Na^+ in leaf blades.

In plants, proline is synthesized from two precursors, L-Glutamate (Glu) and Arginine/Ornithine (Orn) (Hu *et al.*, 1992) [16]. Although Glutamate pathway is believed to be dominant in many stressful and normal conditions, except for the case of excessive nitrogen (Delauney *et al.*, 1993) [7]. Proline biosynthesis of Glu is mostly occurring in the cytosol of plant cells and in the chloroplast when faced with water deficit (Lehmann *et al.*, 2010) [20]. Under stressful conditions, it is accumulated in the cytosol to induce water diffusion into cells (Matoh *et al.*, 1987) [23], while in the absence of stress, proline is transported to organelles, particularly vacuole and plastid. The P5CS (delta-1-pyrroline-5-carboxylate synthase) enzymes are believed to be rate limiting and play a significant role in the regulation of the proline level in plants (Yoshiba *et al.*, 1997) [32]. The expression of P5CS gene in leaves tissues was higher in genotype K 850 while no expression was observed in root tissues. It showed that when the expression of P5CS gene is increased, proline accumulation reaches higher levels. The overexpression of P5CS gene has resulted in higher survival rate, improved tolerance and higher yield under osmotic stresses in Plants. This tolerance is the result of accumulating proline in higher levels than control plants. The similer finding is also observed by Ueda *et al.*, (2001) [30]; Amini *et al.*, (2015) [1]. The expression pattern of P5CS gene, as an indicator of the way it affects proline accumulation in plants.

The presence of LEA (Late embryogenesis abundant) proteins has been associated with cellular tolerance to dehydration, which may be induced by freezing, saline conditions, or drying. LEA2 gene encodes a protein of LEA group and may be involved in various plant developmental processes and abiotic stress responses. Mostly legume LEA transcripts and proteins have similar accumulation patterns as those found in LEA proteins. The *M. truncatula* microarrays showed that water deficit conditions imposed with NaCl treatments (200 mM) induce the accumulation of transcripts from LEA genes (Manfre *et al.*, 2009) [21]. In the present study, LEA2 gene is expressed in developing parts of leaves and roots under saline condition. The genotypes CSG 8962 and KWR 108 showed higher expression in leaves in comparison to roots under saline condition. LEA2 gene is expressed under water deficit caused by salinity or environmental changes. The similar result is also found by Gu *et al.*, (2012) [13]; Battaglia and Covarrubias (2013) [3]; Marjani *et al.*, (2014) [22].

Under salt stress, transcriptional factors greatly influence gene expression and stress signalling in plants (Golldack *et al.*, 2014) [12]. The transcription factors NAC and WRKY (more DEGs with lower expression in CSG 8962) were differentially expressed between genotypes (tolerant and susceptible). Transcription factors can induce or repress the function of genes involved in salt tolerance (Roy *et al.*, 2014) [27] and their interaction with downstream genes and regulatory/signalling networks, together with their expression, could have a central role in the regulation of salt tolerance in plants. This work suggests that a number of transcription factors are influencing the genotypic difference for salt tolerance in chickpea. The transcription factors NAC and WRKY are expressed under the saline condition in leaves and roots. WRKY and NAC both are expressed higher in genotype CSG 8962 (tolerant). The WRKY plays a central role in ABA signalling pathway. Transcription factors have very diverse functions and numerous transcription factors are characterized under salt stress, however, transcriptional reprogramming under salt stress is poorly known (Peleg *et al.*, 2011) [26]. Therefore lower or higher transcript abundance of different transcription factors can have different impacts on plant salt tolerance.

Table 4: Salt tolerance indices of fifteen chickpea genotypes with S1 and S2 for Photosynthesis rate.

Genotypes	S1				S2			
	GM	SSI	STI	Rank	GM	SSI	STI	Rank
L550	11.26	1.09	0.097	4	9.79	1.55	0.074	1
K850	9.58	1.27	0.07	6	0	2.94	0	13
Pusa372	8.69	1.06	0.058	8	6.77	1.8	0.035	9
BG362	12.47	1.56	0.119	2	9.29	2.18	0.066	4
KWR108	9.38	1.1	0.068	7	7.52	1.75	0.043	6
KAK2	8.72	1.34	0.058	8	6.98	1.92	0.037	8
CSG8962	12.55	1	0.121	1	9.72	1.78	0.073	2
JG11	8.06	0.79	0.05	10	6.61	1.49	0.034	10
JG16	12.43	1.58	0.119	2	9.78	2.1	0.073	2
ICCV05107	8.04	1.12	0.05	10	7.16	1.5	0.039	7
ICCV95334	11.12	1.58	0.095	5	8.51	2.14	0.056	5
JAK19218	7.35	1.19	0.041	12	6.45	1.59	0.032	12
JG14	12.36	1.49	0.117	3	9.35	2.11	0.067	3
ICCV07112	8.23	1.14	0.052	9	6.55	1.8	0.033	11
ICCV88105	7.52	0.95	0.043	11	6.72	1.36	0.035	9

Gm- Geometric mean, SSI- Salt Susceptibility Index and STI- Salt Tolerance Index

S1- Salinity level 1(40 mM) and S2- Salinity level 2 (60 mM)

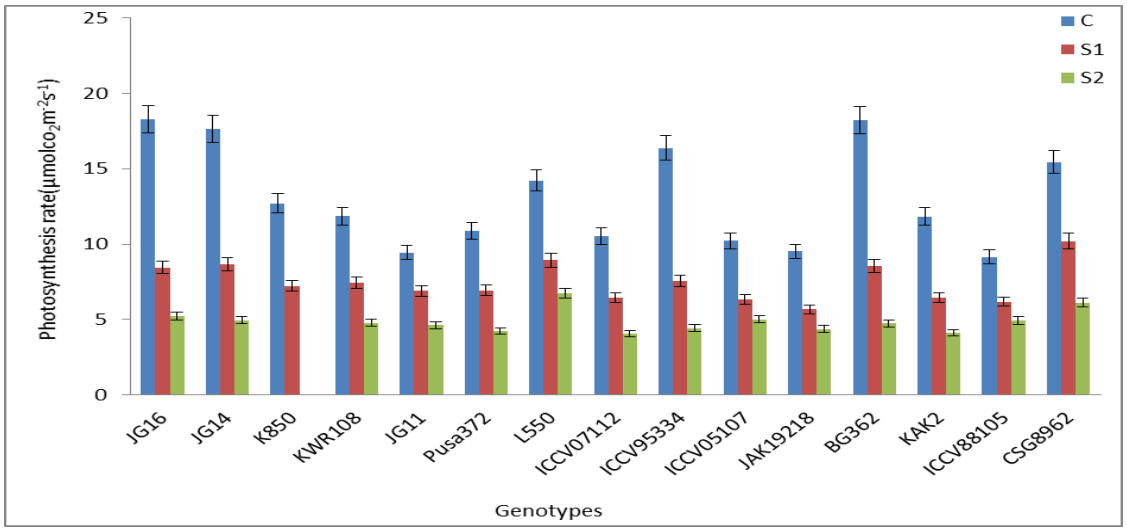
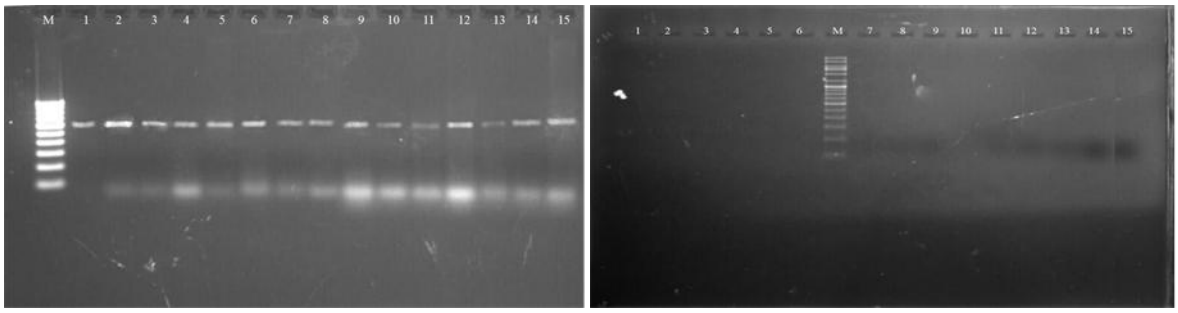
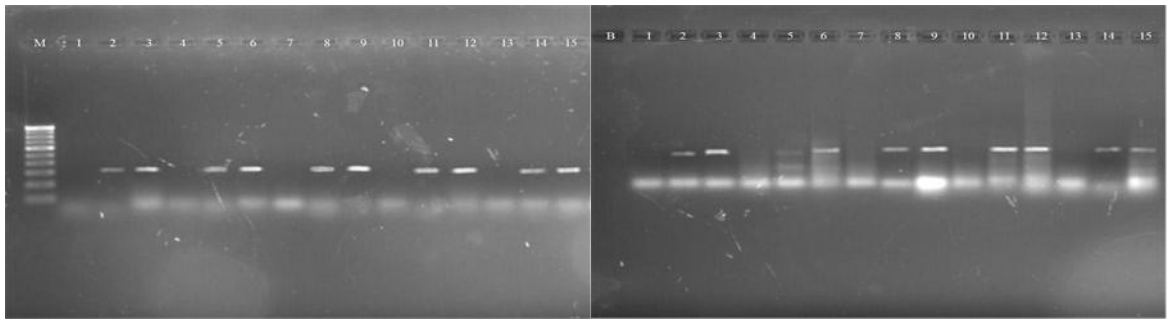


Fig 2: Photosynthesis rate of fifteen genotypes of chickpea under control (C), salinity level 1(S1) and salinity level 2 (S2).



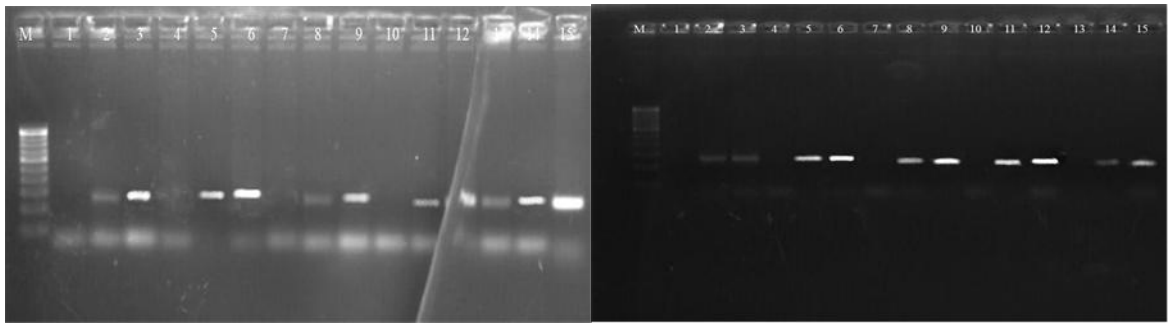
(a) **(b)**

Fig 3.1: Expression analysis of P5CS gene (a) leaves tissue (b) root tissue M- denotes 100 bp ladder, Lane 1- ICCV 07112(Control), Lane 2- salinity level 1 (S1), Lane 3- salinity level 2 (S2); Lane 4- CSG 8921(Control), Lane 5- S1 Lane 6- S2; Lane 7- JG 14(Control), Lane 8- S1, Lane 9- S2 Lane 10- K 850(Control), Lane 11- S1, Lane 12- S2 Lane 13- KWR 108(Control), Lane 14- S1, Lane 15- S2.



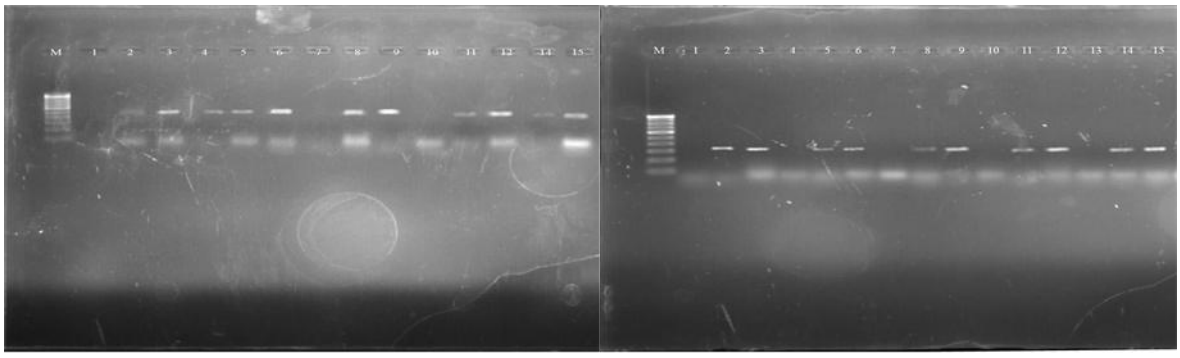
(a) **(b)**

Fig 3.2: Expression analysis of HKT1 gene (c) leaves tissue (d) root tissue M- denotes 100 bp ladder, Lane 1- ICCV 07112(Control), Lane 2- salinity level 1 (S1), Lane 3- salinity level 2 (S2); Lane 4- CSG 8921(Control), Lane 5- S1 Lane 6- S2; Lane 7- JG 14(Control), Lane 8- S1, Lane 9- S2 Lane 10- K 850(Control), Lane 11- S1, Lane 12- S2 Lane 13- KWR 108(Control), Lane 14- S1, Lane 15- S2.



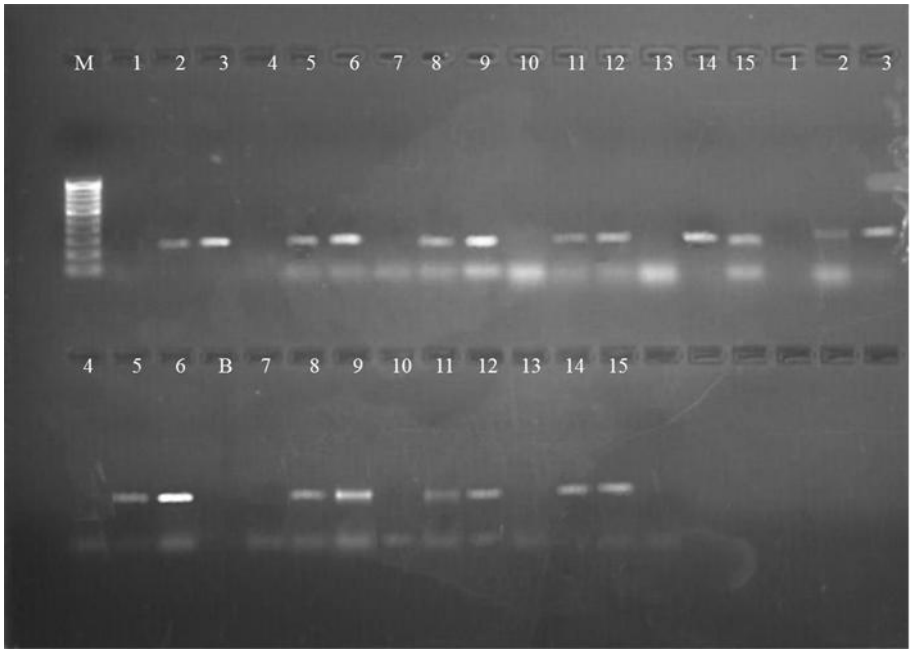
(a) **(b)**

Fig 3.3: Expression analysis of LEA2 gene (e) leaves tissue (f) root tissue M- denotes 100 bp ladder, Lane 1- ICCV 07112(Control), Lane 2- salinity level 1 (S1), Lane 3- salinity level 2 (S2); Lane 4- CSG 8921(Control), Lane 5- S1 Lane 6- S2; Lane 7- JG 14(Control), Lane 8- S1, Lane 9- S2 Lane 10- K 850(Control), Lane 11- S1, Lane 12- S2 Lane 13- KWR 108(Control), Lane 14- S1, Lane 15- S2.



(a) **(b)**

Fig 3.4: Expression analysis of WRKY gene (g) leaves tissue (h) root tissue M- denotes 100 bp ladder, Lane 1- ICCV 07112(Control), Lane 2- salinity level 1 (S1), Lane 3- salinity level 2 (S2); Lane 4- CSG 8921(Control), Lane 5- S1 Lane 6- S2; Lane 7- JG 14(Control), Lane 8- S1, Lane 9- S2 Lane 10- K 850(Control), Lane 11- S1, Lane 12- S2 Lane 13- KWR 108(Control), Lane 14- S1, Lane 15- S2.



(a) **(b)**

Fig 3.5: Expression analysis of NAC gene (i) leaves and root tissue (j) root tissue M- denotes 100 bp ladder, B- blank, Lane 1- ICCV 07112(Control), Lane 2- salinity level 1 (S1), Lane 3- salinity level 2 (S2); Lane 4- CSG 8921(Control), Lane 5- S1 Lane 6- S2; Lane 7- JG 14(Control), Lane 8- S1, Lane 9- S2 Lane 10- K 850(Control), Lane 11- S1, Lane 12- S2 Lane 13- KWR 108(Control), Lane 14- S1, Lane 15- S2.

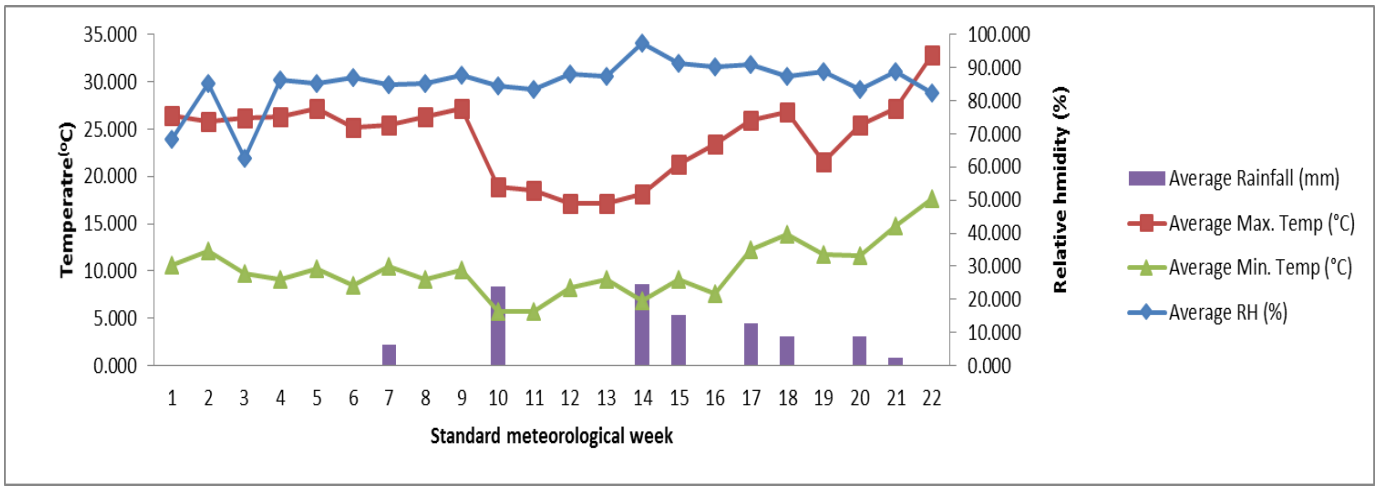


Fig 1a: Standard meteorological weather during November 2013 to March 2014.

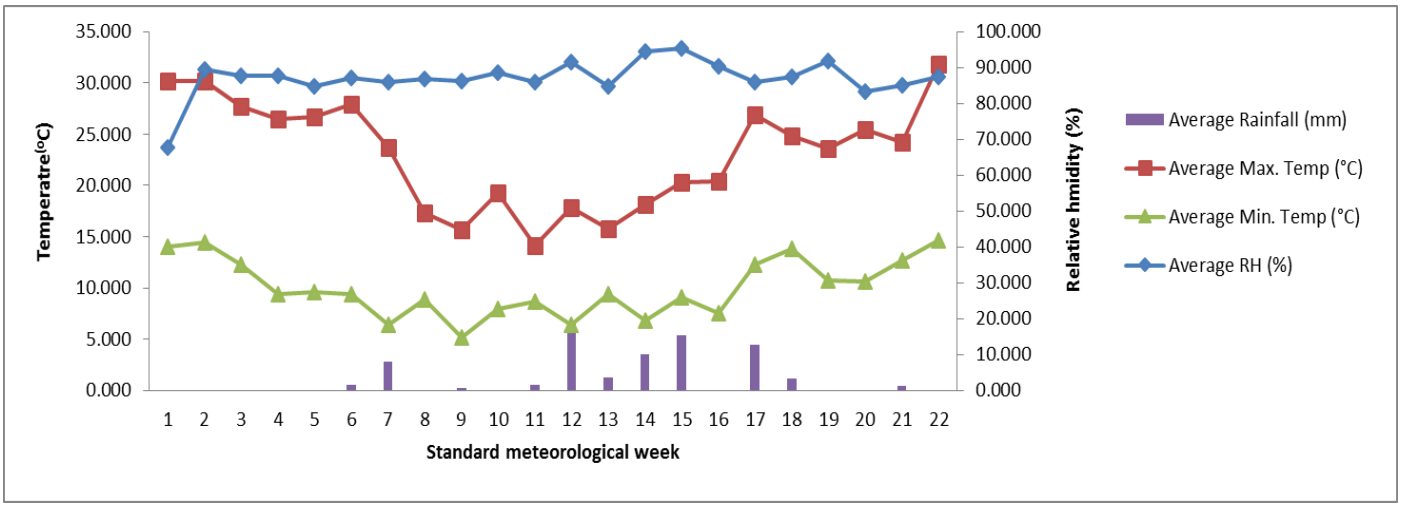


Fig 1b: Standard meteorological weather during November 2014 to March 2015.

Conclusions

On the basis of above finding, following useful conclusions, both having fundamental and applied values, may be drawn. Salinity stress is a main problem in the world which is responsible for the crop loss annually. Responses of cultivated plants, which differ in salt tolerance, are an important phenomenon in distinguishing plant salinity relations. Photosynthesis rate were decreased. Gene expression analysis of HKT1, P5CS and LEA2 genes in leaves and roots at both salinity level were detected. The transcription factor WRKY and NAC were detected under saline condition. The genotype CSG 8962, L550 and JG 16 were least effected under the saline condition in comparison to other genotypes under study. On the basis of above finding may expedite and help to develop more salt resilient chickpea genotype without affecting growth and yield.

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