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Research Article

Genetic Variability and Root System Architecture Adaptation in Chickpea (*Cicer arietinum* L.) under Salt and Drought stress

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Abstract

Root architectural traits have potential role under abiotic stresses; however, genetic variability need to be explored in chickpea to determine the contribution of root system architecture (RSA) under salt and drought stresses. A hydroponic experiment was conducted to explore the RSA of twenty chickpea genotypes grown under salt (SL) and drought (DR) stress with three replicates. Salt stress was established as 60 mM NaCl, whereas Polyethylene Glycol-8000 (PEG-8000) was used to develop the -0.9 bars drought stress. Three weeks old seedlings were harvested, images were taken, and root system architecture was evaluated using image J Smartroot software. Root samples were preserved and analyzed for antioxidant stress contents. Results statistically analyzed by R-studio of R software 4.1.0 explored that in both SL and DR the tolerant genotypes were Wanhar (40%,74%), 14005 (50%, 67%) and PB091(33.3%, 31.9%) having vigorous RSA, ascorbate peroxidase (APX) and catalase (CAT) activity in root as compared to control. Whereas the genotypes Bhakkar (12%,0.9%), Bulksar (24%) and 15033 (29%,18%) have sorted as sensitive or non-adaptive showing decrease in RSA and antioxidant enzyme activity in both salt and drought condition relative to control. In general, a clear genetic variability can be observed in chickpea genotypes owing to differential behavior with respect to RSA (87%) and antioxidant defense system (51.8%) as indicated by principal component analysis. Although systematic breeding approaches have led to the development of varieties with maximum yield potential, but environmental abiotic constraints limit the target production. This study can be supportive to breeders for the screening of chickpea varieties at seedling stage on the base of root system architecture and antioxidant activity that can thrive well in salt and drought stress without compromising yield performance.

Keywords: Hydroponics; Salt stress; Water deficit; Legumes; Antioxidant enzyme; Root growth

1. Introduction

Globally salinity and drought are the major constraints limiting chickpea growth and production as major enzymes involved in metabolic processes like photosynthesis, lipid and carbohydrate metabolism, cell wall component biosynthesis, primary metabolite synthesis, and protein alteration are affected by these stresses (Garg et al., 2016).

Chickpea (*Cicer arietinum* L.) is the 3rd legume crop that grows in semi-tropical and temperate areas, cultivated on 13.7 million hectare land, with 14.2 million tons per annum production (FAOSTAT., 2019). Chickpea ranking 7th among legume crops in which reduction of yield occur due to drought (Daryanto et al., 2015; Chen et al., 2017) Similarly salinity stress affect the chickpea at early stage of germination and growth (Khan et al., 2014).

Root architecture is a potential trait for the appropriate establishment of plant in soil. Optimum root system architecture is required for the efficient acquisition of soil resources (Ahmad et al., 2019). Salt and drought stress induced root apical meristem activity is critical in root architectural modification of chickpea (Khandal et al., 2017). Its root architectural morphology determines the seedling radicle elongation into a profound taproot system alike other dicotyledons. Taproot starts branching into laterals within six to seven days of sowing even before emergence. Taproot shows potential geotropic movement whereas lateral roots initially grow at angle of 45° for some distance then moves downward (Purushothaman et al., 2013). The tolerant genotypes have larger root length and higher root to shoot biomass ratio, contribute in foraging capacity of roots under stress conditions (Bhaskarla et al., 2020).

Disorganized conformational change in functional proteins along with reduced photosynthetic rate and deactivated enzyme activity is considered as major consequence of salt stress (Silva et al., 2011). In chickpea salt and drought stress up-regulates the enzymatic antioxidant activities to fight with reactive oxygen species (ROS) (Mushtaq et al., 2021). ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins and nucleic acids. Drought stress increases the formation of ROS like superoxide (O_2), hydrogen peroxide (H_2O_2), singlet oxygen, and hydroxyl radical (OH) (Curz, 2008). ROS is a double-edged sword under abiotic stress as under low levels acts as signaling molecule by regulating cytosolic ion homeostasis via modulating the activity of different ROS-sensitive ion channels whereas at higher

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concentrations causes damage to macromolecules (Demidchik, 2017). A sensitivity of chickpea crop to abiotic stress like salinity and drought have been reported previously at different growth stages (Ramamoorthy et al., 2016). However, in terms of response to abiotic stresses a significant genetic variability has been reported in chickpea germplasm despite of being having small genetic base (Turner et al., 2013).

Provision of resources and amendment of soil is not always a practical solution, despite strategies to develop morphological desired traits as well as physiological and metabolic adaptive responses in crops to combat impacts of salt and drought stress can be crucial. One of the rationale approaches is to consider root system architecture as roots are the initial part of plant that encounter the surrounding environment and counteract the stresses in rhizosphere. Therefore, present study has been designed to evaluate twenty chickpea genotypes at seedling stage under both salt and drought stress with respect to root system architectural variation. It has been hypothesized that tolerant genotypes produce vigorous root system architecture and have well responsive antioxidant defense system. The objective is to identify the possible novel constitutive as well as induced root traits contributing under salt and drought stress conditions. This study will be a way forward in breeding programs to develop drought and salt resistant varieties by considering root system architectural traits, antioxidant enzyme activity and genetic variability.

2. Materials and Methods

2.1. Plant material and experimental setup

Seeds of twenty chickpea genotypes named as Bhakkar-11,15033, Wanhar-2000, Paidar-91, Pb-2008, 15030, C-44, PB-91,10008,13036,14005, PB-2000, CM-98, Thal-2006, Bittal-98, 15024, 13012, Bittal-2016, 11030, Bulkasar were collected from Ayub Agriculture Research Institute (AARI), Faisalabad (Table 1). Controlled experimental conditions were used by hydroponic system to apply treatments. Experimental setup was arranged with respect to completely randomized statistical design with two factor factorial arrangements, replicated thrice. Following treatments were applied as C: Control without Salt and Drought stress, SL: Salt stress using 60 mM sodium chloride (NaCl), DR: Drought stress (-0.9 bars) using polyethylene glycol (PEG-8000). Seeds were sown in sand using trays and irrigated with distilled water on daily basis until 7 days to maintain optimal growth conditions before transplantation. At 8th day seedlings were transplanted to hydroponic boxes containing standard nutrient solution and aerated using aeration pumps. On 13th day half doses of stress treatments were applied whereas rest half was applied on 14th day. At 21st day plants were harvested and data for root and shoot fresh biomass was recorded and root samples were preserved at -20 ºC for enzymatic antioxidants and reactive oxygen species. For total plant biomass (TPB) samples were placed in oven for drying at 65 ºC for 72 hours.

2.2. Root imaging and smart root analysis

Root imaging of plants was performed at 21st day after harvest. High resolution digital camera was used to take images using flatbed covered with black sheet (Fig. 1). The images were processed using image J based Smart Root software for root system related attributes (Lobet et al., 2011). Primary root length (PRL), lateral root length (LRL) and lateral roots number (LRN) and total root length (TRL) was measured from smart root software whereas lateral root density was measured according to Kiran et al. (2019) as LRN/PRL. Indexing was performed on mean data of root related traits to get clear evaluation of root architectural behavior of chickpea.

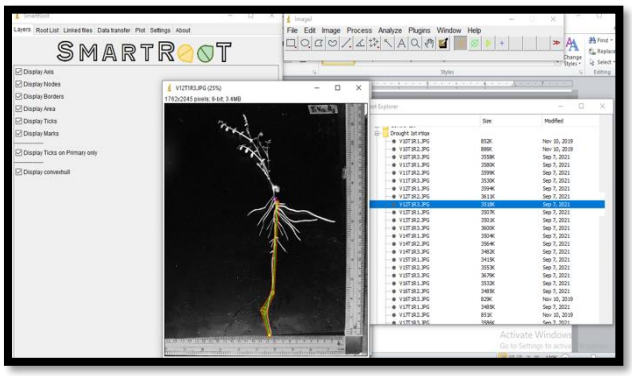

Fig. 1. Processing of 21-days old chickpea root images on smart root for root system related analysis.

2.3. Determination of antioxidant enzyme activity and content in response to stress in root

2.3.1. Ascorbate peroxidase (APX)

Reaction mixture for the ascorbate peroxidase was prepared by using 50 mM potassium buffer (pH 7.0), 0.5mM ascorbic acid and 0.1 mM hydrogen peroxide in 40 µL of enzyme extract. The absorbance was measured at 290 nm on spectrophotometer (Cecil CE 7400). APX was calculated in µg mL^{-1} (Nakano and Asada, 1981).

2.3.2. Catalase Activity (CAT)

For catalase activity determination method of Aeb, 1984 was adopted with a few modifications. In 0.1mL of enzyme extract, 3mL of reaction mixture containing 50mM phosphate buffer (pH 7.8), 5.9 mM hydrogen peroxide was added. The reaction mixture was kept aside for 3 minutes and the after 30 seconds absorbance was measured at 240nm using spectrophotometer (Cecil CE 7400). Catalase content was measured in µg mg^{-1}.

2.3.3. Malondialdehyde (MDA)

Malondialdehyde activity was determined by method explained by (Ohkawa et al., 1979). Extract was

Table 1: Genotypic variation in Chickpea for Total root length and Total Biomass under Salinity and Drought stress. C represents control treatment; SL represents salt stress and DR represents Drought stress

	Total Root Length (cm)				Total Plant Biomass			
Chickpea Genotypes	**C**	**SL**	**DR**	**Mean**	**C**	**SL**	**DR**	**Mean**
Bhakkar-11	86.76±43.83	65.38±8.35	29.91±6.41	60.68^{a}	241.20 ±2.95	230.33±11.48	229.30±17.55	233.81^{a}
15033	97.83±57.58	43.57±12.58	20.05±2.82	53.82^{abc}	153.06 ±15.26	224.30 ±12.64	252.63±70.53	210^{abcd}
Wanhar	46.74±8.12	62.02±14.80	83.97±2.85	40.91^{bcdefg}	283.50 ±17.72	183.76±15.14	198.90±37.12	222.05^{abc}
Paidar-91	67.45±4.84	47.00±10.19	27.66±5.12	47.97^{abcdef}	161.76 ±15.43	86.16±9.89	159.56±57.98	135.83^{e}
Pb-2008	66.04±10.75	46.92±11.38	25.53±1.80	46.16^{abcdef}	180.03±11.18	102.33±9.45	322.33±166.54	201.56^{abcd}
15030	77.93±14.98	60.76±9.46	27.25±1.76	55.31^{abc}	267.26±18.67	184.83 ±10.43	236.26±75.53	229.45^{ab}
C-44	69.31±24.66	64.07±3.25	26.08±7.63	53.16^{abcd}	150.03±14.30	259.13±23.01	213.50±76.15	20755^{abcd}
PB-91	57.42±19.25	59.16±5.12	51.04±10.69	55.87^{abc}	159.76±12.83	184.36 ±15.37	216.20±64.77	186.77^{abcd}
10008	50.22±14.41	62.40±7.65	31.11±4.37	47.91^{abcdef}	170.10 ±11.14	133.36±13.19	239.00±98.90	180.82^{abcd}
13036	51.25±9.35	61.15±7.50	37.63±5.34	51.01^{abcdef}	202.23±11.18	174.23 ±25.67	283.86±121.20	220.11^{abc}
14005	58.42±8.81	65.02±8.58	49.39±16.75	57.61^{ab}	173.93 ±14.30	176.66±21.55	185.06±68.96	178.55^{abcd}
PB-2000	76.50±3.93	68.45±8.09	24.87±9.12	55.97^{abc}	160.03±11.55	145.26±16.30	142.80±22.40	149.36^{de}
CM-98	57.56±5.24	77.47±3.73	33.33±10.28	53.79^{abc}	158.20±16.51	168.73±27.91	214.90±19.62	180.61^{abcd}e
Thall-2006	47.65±6.04	36.29±6.67	34.73±2.15	39.56^{cdefg}	148.56 ±11.33	155.20 ±19.00	315.83±119.03	206.53^{abcd}
Bittal-98	42.68±5.41	32.86±10.44	26.90±3.23	34.15^{fg}	146.53±8.52	152.36 ±22.53	152.36 ±61.56	161.92^{de}
15024	31.17±2.72	32.95±1.72	18.49±5.74	27.53^{g}	153.30±13.33	198.96 ±18.20	188.43±5.13	180.23a^{bcde}
13012	35.15±7.78	46.90±8.85	23.97±1031	35.34^{efg}	150.80±39.29	215.73 ±67.3	278.06±18.66	214.86^{abcd}
Bittal-201	46.73±1.50	46.06±9.69	27.46±2.57	40.07^{bcdefg}	163.83 ±37.41	224.68±5.23	184.00±15.34	190.84^{abcde}
11030	57.81±1.04	26.79±8.34	22.62±2.72	35.75^{defg}	188.30 ±9.56	183.86±23.86	187.733±3.59	186.63^{abcd}e
Bulksarv	80.32±4.76	36.07±10.00	40.11±1.95	52.17^{abcde}	158.83 ±14.20	160.13 ±27.31	179.10±25.18	166.02^{bcde}
Mean	60.30^{a}	50.71^{b}	29.60^{c}		178.56^{b}	177.22^{b}	220.74^{c}	
	LSD for treatment at 5%		17.78		**LSD for treatment at 5%**		**65.51497**	
	LSD for genotype at 5%		**6.88**		**LSD for genotype at 5%**		**25.373**	
	LSD for genotype*treatment at 5% 30.80				**LSD for genotype*treatment at 5%**		**113.4753**	

prepared by adding 0.6% thiobarbuturic acid in 2 mL of trichloroacetic acid. Heated the mixture at 100°C for 20 minutes using water bath and cooled immediately for 20 minutes and centrifuged at 10,000 rpm for supernatant separation. The absorbance was noted at 532 nm using spectrophotometer (Cecil CE 7400).

2.3.4. Hydrogen peroxide (H_2O_2)

In 1mL of 0.1 % (w/v) trichloroacetic acid, 0.5g fresh sample was homogenized. At 12000 rpm the homogenate was centrifuged for 15 minutes to separate the supernatant. In reaction mixture containing 0.1 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1M potassium iodide, 0.1 mL of the supernatant was added. The absorbance was noted at 390nm using spectrophotometer (Cecil CE 7400) (Terzi et al.,2014).

2.4. Data analysis and visualization

Principle component analysis (PCA) was performed to explain the genetic variability. Agricolae package of R software was used to perform two way analysis of variance (ANOVA). Least significant difference test (LSD) at 5% level of probability was applied to check the mean effect of treatments on genotypes and vice versa.

3. Results

For validating statistically, R-studio of R software 4.1.0 (Team, 2020) was used to generate biplots by taking treatments variables as principal components and vectors denote the response variable. PCA biplots were generated using R studio of R software under three variable treatments C: Control, SL: Salt stress and DR: Drought stress. The cos2 value in the biplot depicts the distribution of genotypes with respect to genetic variability using color intensities (Das et al., 2017).

3.1. Total plant biomass (TPB)

The weight of living plant material contained above and below a unit of ground surface area at a specific point in time is known as plant biomass (W). The biomass or weight of organic matter assimilated by a community or species per unit land area per unit time is referred to as production. The cumulative percentage of 71.1% indicates moderate level of variation between the genotypes with respect to TPB production.

The angle between SL and DR is more than 90° which depicts the opposite trends of genotypes in both treatments (Fig. 2a).

In SL greater percent increase was observed in genotypes C-44 (72%), 15033 (46%), 13012 (43%) and Bittal-2016 (37%) with respect to control whereas highest decline in biomass was observed in Paidar-91 (46%), Pb-2008 (43%), Wanhar (35%), 15030 (30%) as compared with control. In DR conditions highest percent increase of 112, 84, 79, 65 and 42% was observed in Thall-2006, 13012, Pb-2008, 15033 and C-44, respectively, in comparison with control. With respect to stress induced alleviation in DR conditions the highest decrease was observed in Wanhar (29%), 15033 (11%), Pb-2000 (10%), Bhakkar-11 (4%) and Paidar-91 (1.3%) than control. The genotype Pb-2008 showed higher

biomass under DR however, it performed negatively in SL whereas the response of Pb-2000 was vice versa (Table 1).

3.2. Root system architecture (RSA)

Root system architecture is not just important for nutrient and water absorption and translocation it also works potentially as primary line of defense against biotic and abiotic stress. A significant genetic variability exists between the chickpea genotypes for RSA as indicated by the cumulative percentage of 87% between two principal components and have more authenticated by the cos2 value (Fig 2b; 3b). The degree of angle between vectors

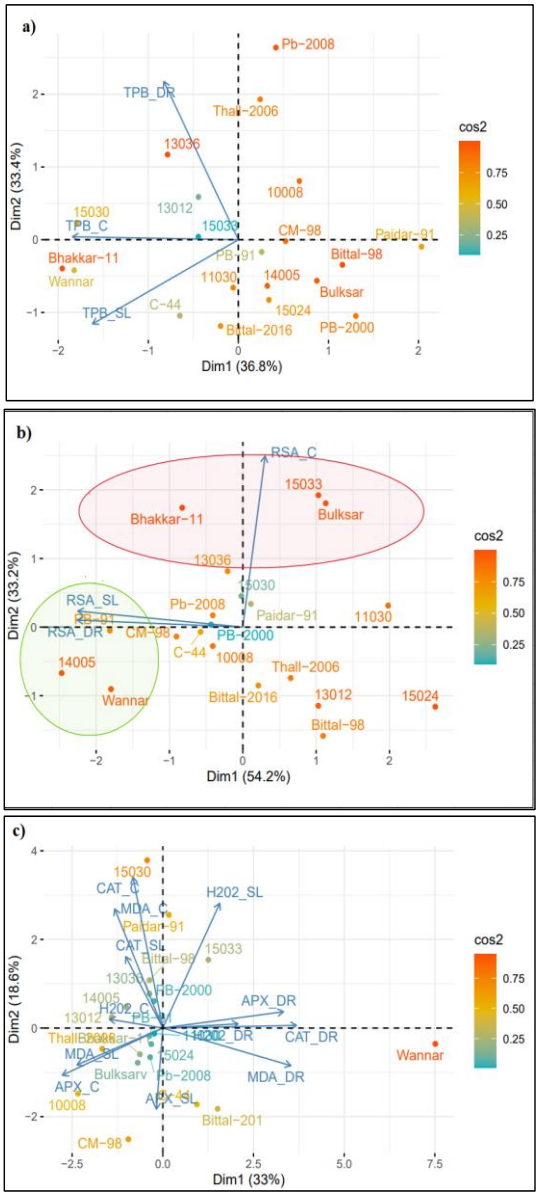

Fig. 2: a) Biplot generated to determine the root system architectural behavior of twenty chickpea genotypes under variable treatment regimens (b) Total Plant biomass. (c) Enzyme activity in root. C: Control without Salt and Drought stress, SL: Salt stress using 6 0mM sodium chloride (NaCl), DR: Drought stress using -0.9 bars polyethylene glycol (PEG-8000).

on biplot depicts the correlation in treatments as in this case the angle between SL and DR is less than 90° which indicates their same effect on genotypes in comparison to control.

Under both salt and drought all genotypes have shown an increase in RSA as compared to control except genotypes Bhakkar-11(12%,0.9%), Bulksar (24%, 24%),15033(29%,18%) and 11030(12%,21%) which showed a percent decrease. The highest percent increase as compared to control was observed in genotype Wanhar (40%, 74%), 14005 (50%, 67%). PB-91(33.3%, 31.9%) respectively (Table 1).

3.3. Antioxidant enzyme activity and ROS content in roots

The principal component analysis performed for antioxidant enzyme activity showed a cumulative percentage of 51.8%. The highest percentage of 33% was shown by PC1 and it was highly influenced by the APX, CAT activity under DR. Under Salt stress only genotype Wanhar showed percent increase of 31% for APX activity while all genotypes showed percent decrease with range of 2 to 46% as compared to control. The genotypes having lowest percent decrease were Thall-2006 (30.7%), Bhakkar-11 (37.6%), 15024 (38.1%), 13036 (40.8%), Paidar-91 (41%), 15030 (48%) whereas in DR all genotypes showed percent increase for APX activity than control except 15024 which showed a percent decrease of 0.6% considered as non-significant. Significantly higher increase was observed in Wanhar (1475%), Bittal-98 (248%), 15030 (217%), 15033 (169%) and C-44(137%) while all other genotypes showed non-significant activity. So, the genotype Wanhar is resistant under both salt and drought stress whereas genotypes Bittal-98, 15030, 15033 and C-44 are considered resistant in drought (Fig 2c; 3a).

Under salt stress highest percent increase of CAT activity was estimated in Bulksar (10%), PB-91 (3%) and 11030 (3%), whereas percent decrease for CAT activity was estimated in Bittal-2016(36%), Bhakkar-11(37%), 13036 (38%), Paidar-91 (44%), and15030 (48%) in comparison with control. Under drought stress significant percent increase in CAT activity as compared to control was estimated in Wanhar (1243%), 15024 (301%), C-44 (260%), Bittal-2016, CM-98, PB-91(188,183 and 182%) respectively. Overall all genotypes showed percent increase in CAT activity under DR but most of the genotypes showed non-significant effect (Fig 2c; 3a).

Under salt stress significant percent increase in H_2O_2 content have been observed in genotypes 15030 (187.5%), 15033 (133%), Wanhar (128%), Paidar-91 (47%), PB-91 (27%), Bittal-98 (20%) whereas genotypes C-44, 13036 and CM-98 showed non-significant change in comparison with control. With respect to percent reduction highest values were observed in Pb-2008 (68%), 10008 (64%), PB-2000 (58%), 13012 (48%),11030 (23%). Under drought stress all genotypes have shown percent increase in H_2O_2

content except PB-2000 which showed percent decrease of 5%. The significant increase in H_2O_2 content was observed in Wanhar (526%), Bittal-2016 (516%), C-44 (410%), PB-2008 (318%), Bittal-98 (300%), 13036 (276%), CM-98 (209%) and 11030 (194%) while all other genotypes showed non-significant behavior (Fig 2c; 3a).

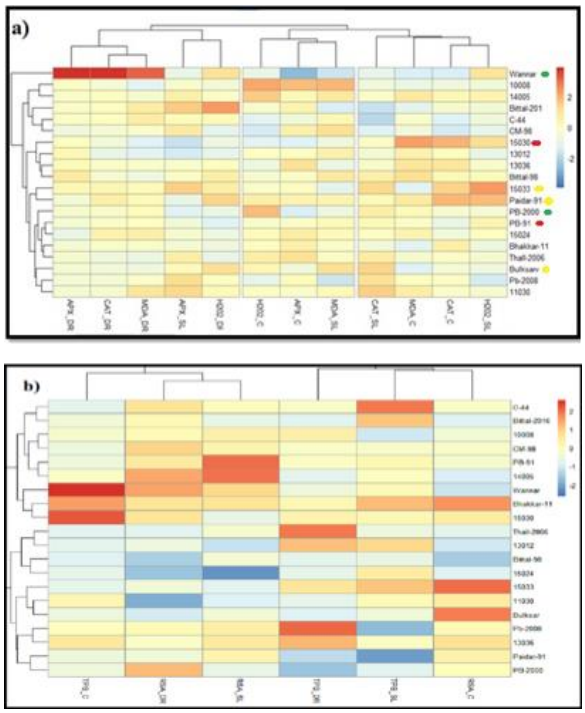

Fig. 3: a) Heatmap depicting relative enzyme activity in root of twenty chickpea genotypes under variable treatment regimes (b) Heatmap depicting differential RSA behavior associated with biomass production in under variable treatment regimes. C: Control without Salt and Drought stress, SL: Salt stress using 60mM sodium chloride (NaCl), DR: Drought stress using -0.9 bars polyethylene glycol (PEG-8000).

Lipid peroxidation is a major consequence of salt stress and higher MDA content is indicative of excessive lipid peroxidation in chickpea genotypes. Under salt stress only three genotypes Bulksar (50%), 10008 (40%) and CM-98 (8%) showed significant percent increase for MDA as compared to control whereas all other genotypes showed decrease in MDA as compared to control. So, the genotypes showing percent decrease in MDA can be considered as tolerant to NaCl stress. The lowest values were observed in Pb-2008 (77%), 15030 (75%), 13036 (69%), Pb-2000 (60%) and Wanhar (58%). Under drought regimes highest increase in MDA content was observed in genotypes Wanhar (736%), Bittal-2016 (316%), 15033 (270%), Bhakkar-11 (222%), C-44 (191%) whereas the lowest increase was observed in 15030 (31%), 13012 (12%) and Bittal-98 (20%) in comparison with control (Fig 2c; 3a).

4. Discussion

The present study has been designed to evaluate the genetic variability in twenty chickpea genotypes under both SL and DR stresses taking in consideration the potential role of RSA and antioxidant defense system to maintain vigor under oxidative damage. Chickpea is characterized by taproot system which emerges from the seeds comprising first order lateral root and the presence of sparsely and densely second order branches (Gaur et al., 2015). One of the important plant responses associated with root length modifications is imperative in estimating plant stress tolerance. Proliferative and deep root system is favorable in various crops, for drought tolerance including chickpea (Varshney et al., 2011). The genotype Wanhar, 14005 and PB-91 have vigorous root system architecture (Fig 1a) in both salt and drought stress which is in accordance with the study conducted in wheat and chickpea by Kashiwagi et al., (2015). In the present study most of the genotypes maintained the root growth except Bhakkar-11, Bulksar, 15033 and 11030 which significantly decreased the root growth under stress. Genotypes which have shown vigorous RSA in DR have also maintained the RSA in SL which is in accordance with study executed by Mann et al. (2019). Osmotic stress application resulted in differences in the RSA between the eight genotypes (Drysdale, Giles, Sakha94, Irena, Veery, Klassik, Gemmiza7, Gemmiza12), where genotypes were divided into adapted genotypes that have non-significant decreased values in LRN and TRL, while non-adapted genotypes have a significant decrease in LRN, TRL and root surface area (SA) (Azab et al.,2021). In current study Fig. 1b depicts the green highlighted (Wanhar, 14005, PB-91) as resistant/adapted and red highlighted (Bhakkar, Bulksar, 15033, 11030) as susceptible/non-adapted genotypes in accordance with the study performed by Azab et al., (2021). According to Yadav et al. (2022) GRASTF gene has been identified as differential responsive gene to drought stress by improving root morphology in tolerant chickpea genotypes as compared to sensitive varieties. Similarly in another study salinity tolerance specific loci CaLG04, CaG05 and CaG06 has been identified as key determinant of salt tolerance in chickpea salt tolerant genotypes by Atieno et al. (2021).

Enzymatic antioxidant activity was also estimated in chickpea under both DR and SL stress. The extent of DR and SL severity also depends on the genotypic capability to cope with oxidative stress. In SL reactive oxygen species contents increased as compared to control. The altered levels of MDA and H2O2 compared with control potentially indicates the stressed conditions of plant. Enzymatic antioxidants work to scavenge the ROS in the plants. SOD is the first line of defense against the ROS scavenging, it converts superoxide anion O2- into H2O2 whereas H2O2 is converted by CAT and APX into H2O (Hasanuzzaman et al., 2020). Genotype Wanhar have showed 31% increased APX in SL whereas in DR a significant increase of 75% have encountered which depicts its strong antioxidant defense mechanism against stress whereas reduced the MDA content in SL and increased MDA content in DR correlates with its ability

of enhanced enzyme activity to resist against the stressed regimes. This sort of behavior has also been observed by Alsahli et al. (2019) in wheat, maize and rice.

With respect to categorization of drought tolerant and susceptible genotypes in wheat and chickpea genotypes, Rasool et al. (2013) have described higher APX and CAT activity as indication of tolerance so in our studied genotype Wanhar, Bittal-98, 15030, 15033 and C-44 have higher APX activity whereas Wanhar, 15024, C-44, Bittal-2016, CM-98 and PB-91 have highest CAT activity. Under salt stress, genotype Wanhar have showed higher APX but for CAT genotypes Bulksar, PB-91 and 11030 have showed increasing trend and this differential behavior can be attributed as genetic variability of chickpea under SL and DR stresses (Fig 1c, 2a). Varieties with higher genetic potential of producing APX render them to detoxify effect of H2O2 in cell organelles and regulate biological process including cell cycle. This provokes growth and development under salt and drought stress described by Sofo et al. (2015). Similarly, in current study in varieties Wanhar, Bulksar, PB-91 production of higher concentration of APX and CAT reduced the oxidative stress of H2O2 subsequently enhanced cell cycle and developed extensive root system. The differential behavior of chickpea genotypes under both SL and DR stress depicts their genetic variability with respect to SL and DR induced oxidative stress tolerance. Results are in agreement with the data produced by Zhang et al.(2021) in olive and plantago respectively. According to Shriti et al. (2023) MYB transcription factors are involved in abiotic stress responses. Heterologous expression of drought and salt stress responsive gene MYB78 in chickpea lower water loss in drought stress and Na+ in salt stress, whereas enhance APX and CAT activity and hydrogen oxide production.

5. Conclusions

Overall, a clear genetic variability can be seen in chickpea genotypes owing to differential behavior with respect to RSA and antioxidant defense system. In both SL and DR the tolerant evaluated genotypes were Wanhar, C-44, 14005 and PB091 whereas the genotypes Bhakkar, Bulksar and 15033 have been sorted as sensitive or non-adaptive under SL and DR stress. Significant variation was observed in case of biochemical attributes APX, CAT, MDA and H2O2 among stress tolerant and sensitive genotypes. Similarly Improvement in RSA related attributes PRL, LRL, LRN, TRL was also observed in tolerant genotypes as compared to sensitive genotype. Since, these genotypes were not studied for RSA and Antioxidant potential under drought and salt stress before current study. Therefore, genotypes evaluated at seedling stage based on RSA and stress responding biochemical can potentially pave ways for breeders to study at genetic level, identify candidate gene of stress tolerance and produce genotypes which have higher yield as well as better adaptability potential under stressed regimes.

Author Contributions

A. Kiran and R. Sultana: Conceptualization, Methodology, Writing- Original draft preparation. **M. Noor and R. Iqbal**: Data curation, Writing- Original draft preparation. **A. Sharif and I. Javed**: Writing-Reviewing and Editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest

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