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EFFECTIVENESS OF GRAFTING TO IMPROVE SALT TOLERANCE IN CHICKPEA (*CICER ARIETINUM* L.)

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Abstract: The effect of sodium chloride salinity on plant growth was studied in seven cultivars in chickpea. With increasing salinity level resulted in a progressive absorption of Na^+ in determinant of K^+ , thereby increasing Na^+:K^+ ration and causing an ionic disequilibrium which possibly suppressed plant growth. Genotype, K 850 showed maximum germination under 1.71 mMNaCl. Genotype CSG 8890 showed maximum reduction in radical as well as plumule length and minimum with Bio 201. Grafting high yielding but salt- susceptible chickpea cultivars onto salt resistant/tolerant root stocks is a sustainable strategy to overcome saline stress. The positive response of grafting exerted by tolerant rootstocks or scion-rootstock interactions on yield and pod characteristics of chickpea under saline conditions is attributed to several physiological and biochemical changes. In this paper, the importance of chickpea grafting, strategies to select appropriate rootstocks, scion-rootstock interaction for growth, yield and quality characteristics, as well as the tolerance mechanisms that (grafted) plants deploy to circumvent or minimize the effects of salt stress in root zones are discussed.
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Key Words: Salt, NaCl, Na/K ratio, Plant growth, salt resistant, salt susceptible, *Cicer arietinum* L. genotypes , Grafting

Introduction

Salinity, particularly in arid and semi-arid land is a problem of great concern to agriculture not only in India but also many other parts in the world. In many places areas of the world, characterized by a short rainy winter and a long dry summer, non-saline soils may gradually become salinized to varying degree with intensive irrigation. The problem of secondary salinization, in particular are serious in developing countries, since they can be responsible for loss of once productive agricultural land (Chavan and Karadge, 1986). The extent and rate of salt accumulation in the soils is a function of irrigation water quality, irrigation methods and quantity, soil properties and annual precipitation.

Salinity generally inhibits the growth of plants which may either due to osmotic reduction in water availability, specific ion toxicity usually associated with either excessive chloride and sodium intake and ion imbalances (Bernstein 1963, Gorham et al. 1985, Cheeseman, 1988 and Judith et al. 2017). Plants grown in saline conditions have higher contents of Na^+ and Cl^- ions imbalances. Plants grown in saline conditions have higher contents of Na^+ and Cl^- ions and low K^+ contents in the developing tissues, both affecting the establishment of equilibrium necessary for the normal metabolic reactions (Bange 1959, Greenway and Manns, 1980 and Cusido et al , 1987) . Differences in salt tolerance occur not only in different species of the same genus but also in different varieties of the same species. Grafting is a horticultural technique whereby tissues of plants are joined so as to continue their growth together (Colla et al. 2010 & 2013). The upper part of the combined plant is called the scion while the lower part is called the rootstock. The success of this joining requires that the vascular tissues grow together and such joining is called inosculation. The technique is most commonly used in asexual propagation of commercially grown plants for the horticultural and agricultural trades (Singh et al, 2017 & Singh et al., 2020) . An experiment was conducted to determine the effect of grafting on salt and salt mixtures on the plant growth of chickpea (*Cicer arietinum* L.).

Materials and Methods

Seeds of seven chickpea cultivators viz., C235, K 850, BG 256, CSG 8962 Karnal Chana-1, CSG 8890, Bio104 and Bio 201 sown into petri dishes layered with whatman filter paper. Four levels of salinity were created to give 0, 0.43, 0.86 and 1.71 mM (20 ml of salt solution) NaCl into the petri dishes. In control 20 ml distilled water was used the electrical conductivity value of the salt was 4.23, 7.36 and 8.0 ds/m, respectively. Each petri dish was used after saturation with 20 ml of salt solution daily after draining out previous solved solution. The whatman filter papers were also replaced after every 2 days. Observations were recorded after every alternate day on germination percentage under different treatments after 15 days of culture.

Fresh plants were dried at 80^0C for 24 hours. The samples were then ground in a blender for sodium and potassium estimation. For the estimation of sodium and potassium 500 mg dry weight of the tissue was digested in 5 ml of tri acid mixture (Concentrated 10, HNO_3 : 4 $HClO_4$:1 H_2SO_4) in a corning necked flasks for overnight. Refluxing in digestion block at 180^0 C for 30 minutes resulted in decolouration of the digested solution which was followed by addition of 20 - 30 ml of deionized water. It was filtered by whatman filter paper and sodium and potassium were estimated by flame photometer from the stock solution using NaCl and KCl as standard.

The shoot of each genotype was cut from the middle of the second internode starting from soil surface for grafting. Three different procedures were used for preparation of scion and root stock.

1, Saddle Grafting
2. Whip Tongue grafting (Mid Grafting)
3. Side grafting

Saddle grafting:

Diagonal cuts of equal length were made on the scion and stocks of equal girth in the form of saddle to match each other. These two components were brought together facing cambium layers, tide securely and coated with grafting compounds (aluminium foil, straw pipe and wax)

Whip tongue Grafting:

The girth of the plants selected were approximately 0.8 cm in diameter. Diagonal cuts on both scion and root stock were made. Approximately more than 1 cm long plantlets were chosen for grafting purpose. Long smooth sloping cut was made in scion at one stroke root stock was prepared by making cut in reverse downward direction starting about ¼ of the distance from the top. The scion and the root stock were interlocked in such a way that the cambium layer matched on both the sides of completed grafts and secured in position with grafting material.

Side Grafting: The scion was placed latterly on the side of a root stock. The scion was prepared by removing all the lateral branches and vegetative growths were removed near the point of graft. The long , slopping and wedge shaped cut was made for scion. Soon after bringing together the root stock and scion, the point of the union was sealed with grafting material.

Three kinds of materials such as straw pipe, aluminium foil and wax were used for assembly of root stock and scion. A direct cut of 0.5cm size was made in the root stock. The first leaf on the root stock was removed by blade to facilitate easy slip over a straw pipe/ aluminium foil/wax assembly. The length of straw pipe was selected depending on the length of the root stock. This assembly was slipped down to union point of scion and root stock to provide proper strength. The scion was sliced from both sides, removing the skin of the stalk with the help of blade and the inserted in the cut of the root stock. The straw pipe/aluminium foil was pulled down to the joint while wax was pasted to the joint. The pots were filled with gravel. The planting material for root stock was grown into pot containing sand. The grafted plants in pot containing sand were kept in bigger pot and watered twice a day. The water was also sprayed over the gravel to keep condition humid and cool around the pots. The pots were protected from the direct light by covering from all sides with the black opaque sheets. The sprouts coming up from the nodes of the root stock were removed daily to retain true scion only . After 5 days, the grafted plants were shifted to light conditions. The plants were watered with 1/4 Hoagland solution as per their requirement. After days of grafting, assembly of the straw pipe/aluminium foil/wax was removed by cutting them gently from one side down and the pulling apart the two folds.

Results and Discussion

In general, increasing the dose of NaCl decrease the germination percentage (table 1). The maximum reduction in germination (25%) was noticed at 1.71 mM NaCl. However, least reduction in germination percentage (96.36%) was observed at 0.43 mM NaCl the salt mixtures having NaCl and $CaCl_2$ and $MgCl_2$ were used in different ratios as chickpea is reported to be more sensitive to chlorides than sulphates (Manchanda et al 1981 and Manchanda and Sharma, 1989). Among various genotypes used K 850 showed maximum germination (62%) under 1.7 1 mM NaCl is followed by BG256 (60%). However high reduction (25 - 46.15%) in germination percentage was noticed for sensitive genotypes CSG 8890, Bio 104 and Bio 201 (Table 1)

The effect of salinity level on seedling vigour is reflected as radicle and plumule length (table 2). There was considerable difference among genotypes in respect of reduction in radicle and plumule length under saline condition. Maximum reduction in radical length (59.12%) was noticed with genotype CSG 8890 and minimum with Bio 201 (14.78%). Besides other genotypes showed moderate reduction in radical length (table 2). Genotype Bio 102 also showed minimum reduction (3.89%) in plumule length. However, there was a large reduction in plumule length (24.72%-28,0%) in CSG 8890, CSG 8962 karnal chana 1 and K850 (table 2). The response of genotype to salinity varied significantly irrespective of different concentration of NaCl. All the genotypes showed differential performance to germination percentage, radicle and plumule length. These parameters for effective index for discriminating effect under various treatments and genotypes. However, Dua and Sharma in 1996 advocated that germination is not a good index of salinity tolerance rather survival percentage could be considered as one of the component of salinity tolerance of pigeonpea. Several other reports (Akbar and Yabuno,1977) also indicated that no correlation existing between tolerance at germination and later growth stages in different crops. Further, it was postulated that survival percentage should be taken as selectively better indicator of genotypes having high yield under salinity (Dua, 1991 and Munns et. al., 2008).

Varying degree of salinity symptoms were observed under different levels of salinity in salt sensitive and resistant genotypes. These symptoms were intensify on increasing doses of salt. Their symptoms ranged from yellowing, browning, leaf senescence to partial and complete death of the plants. Among various genotypes used, more intensive symptoms were observed in salt sensitive genotypes as compared to salt tolerant genotypes. The results similar to finding of present investigation on initiation and development of symptoms under different levels of salinity has also been reported in various crops viz, wheat (Bhardwaj and Rao, 1960 & Balasubramanian and Sarin, 1974), cowpea (Balasubramanian and Sarin, 1976), mungbean (Balasubramanian and Sarin, 1976), gram (Bhardwaj and Rao, 1960, Lauter and Munns, 1982 louder and 1986, & Mamo et al., 1996) Broad bean (Dua et al. 1989) and pea (Dua et al. 1989).

The radicle and plumule length was also found very effective index in discriminating chickpea genotypes under different levels of salinity. The relative reduction in plumule and radicle length may be taken as a more reliable index than germination percentage as these are not easily affected by environmental condition. The results obtained earlier in chickpea (Dua and Sharma, 1996 ,1997 and Dua 1998) are also in agreement with the finding of present investigation.

There were no significant differences among genotypes with regards to Na^+/K^+ uptake ratio indicating no significant difference in tolerant levels to NaCl.

Since the Survival rate of *in vitro* derived plantlet in pot under saline condition was very low, grafting procedure were standardized to increase the survival if in vitro derived plantlets, the details of these experiments were depicted in table 4. Out of three grafting methods used, mid grafting method showed maximum (40.50%) success followed y side grafting (18.07%). However, minimum success (3.08%) was achieved while saddle grafting method was used. Out of three method of joining scion and root stock, straw pipe method showed best response (35.85%) interms of survival of successful grafted plants. Similar results were also found in Solanaceace family (Keatinge et al, 2014).

Table 1: Relative genotypic sensitivity under different salinity level in chickpea

Genotype	Germination (%)	Radicle length (%) Reduction as against control	Plumule length (%)
C 235	55.56±0.56	48.61±0.40	19.44±0.11
K 850	60.56±0.52	52.55±0.44	24.72±0.16
BG 256	54.53±0.51	41.26±0.47	15.83±0.13
CSG 8962 Karnal Channa-1	69.00±0.65	55.32±0.52	27.44±0.14
CSG 8890	27.75±0.32	59.12±0.56	28.00±0.1121
Bio 104	30.78±0.23	46.96±0.36	13.89±0.11
Bio 201	44.44±0.33	14.78±0.12	03.89±0.07

Table 2: Survival (%) of explant under different NaCl concentration

Genotype	NaCl Concentration			
	Control (%)	0.25%	0.5%	1.0%
C 235	100	90.90	80.00	54.39
K 850	100	96.36	86.36	62.00
BG 256	100	96.36	74.55	60.00
CSG 8962 Karnal Chana-1	100	100.0	61.82	54.54
CSG 8890	100	88.33	61.67	25.00
Bio 104	100	88.33	35.00	25.00
Bio 201	100	88.33	63.63	46.15

Table 3: Na^+/K^+ uptake of different genotypes

Genotype	Ratio	Uptake of Na^+ (ppm)	Uptake of K^+ (ppm)	K^+/Na^+ ratio ± SE
CSG 8962 Karnal chana-1				
1.0.5% NaCl	-	0.97±0.01	0.12±0.01	0.12±0.01
2.0.5% NaCl:$CaCl_2$	1:1	0.69±0.01	0.11±0.02	0.16±0.02
3.0.5%NaCl:$CaCl_2$:$MgCl_2$	5:3:1	0.70±0.03	0.12±0.03	0.17±0.04
CSG 8890				
1.0.5% NaCl	-	1.45±0.02	0.13±0.02	0.09±0.02
2.0.5% NaCl:$CaCl_2$	1:1	1.18±0.04	0.14±0.01	0.12±0.04
3.0.5%NaCl:$CaCl_2$:$MgCl_2$	5:3:1	0.43±0.05	0.12±0.02	0.19±0.03
Bio 104				
1.0.5% NaCl	-	1.17±0.06	0.12±0.05	0.10±0.07
2.0.5% NaCl:$CaCl_2$	1:1	1.60±0.05	0.17±0.05	0.11±0.05
3.0.5%NaCl:$CaCl_2$:$MgCl_2$	5:3:1	0.53±0.05	0.10±0.02	0.19±0.04

Table 4: Comparison of different grafting method in chickpea

S. No.	Method of grafting	Method of joining			Total (%)
		Aluminium foil	Wax	Straw Pipe	
1.	Side Grafting	00	00	15	18.07
2.	Mid Grafting	00	04	78	40.59
3.	Saddle Grafting	00	00	02	03.08
	Total	00	10.00	35.85	

Reference:

[1]. Akbar M and Yaburo T (1977). Breeding for saline resistance varities of rice. IV Inheritance of delayed type panicle sterility induced by salinity. Japan J. Breeding: 27 : 237-240.

[2]. Balsubramanian V. and Sarin MN (1974), An analysis of growth of salt stresses wheat seedlings. Indian Journa of Plant Physiology. 16 :22-27.

[3]. Balsubramanian V. and Sinha SK (1976). Effects on salt stress on growth, nodulation and nitrogen fixation in cowpea and mungbean. Physiology Plantarum 36:197-200.

[4]. Bange GGJ (1959). Interactions in the potassium and sodium absorption by intact maize seedlings. Plant and Soil 11: 17-29.

[5]. Bernstein L (1963). Osmotic adjustment of plants to saline media. II dynamic phase . American Journal of Botany 48: 909-918.

[6]. Bhardwaj SN and Rao IM (1960). Physiological studies on salt tolerance in crop plants. IX Effect of NaCl and Na2CO3 on seedling respiration and growth of wheat and gram. Indian Journal of Plant Physiology 3:56-71.

[7]. Colla G., Rouphael Y., Leonardi C. and Bie, Z (2010). Role of grafting in vegetable crops

grown under saline conditions. Sci. Hortic. , 127, 147–155. [CrossRef]

[8]. Colla G., Rouphael Y., Jawad R., Kumar P., Rea E. and Cardarelli M. (2013). The effectiveness of grafting to improve NaCl and $CaCl_2$ tolerance in cucumber. Sci. Hortic. 164, 380–391. [CrossRef]

[9]. Chavan PD and Karadge BA (1986). Growth, mineral nutrition, organic constituents and rate of photosynthesis in *Sesbania grandiflora* L. grown under saline conditions Plant and Soil 93: 395-404.

[10]. Cheeseman JM (1988). Mechanism of salinity tolerance in plants. Plant Physiology 87: 547-550.

[11]. Cusido RM, Plazon J, Altabella T and Morales C (1987). Effect of salinity on soluble protein, free amino acids and nicotine contents of *Nicotiana rustica* L. Plant and Soil 102: 56-60.

[12]. Dua RP, Sharma SK and Mishra B (1989). Response of broad bean (Vicia faba) and pea (Pisum sativum) varieties to salinity. Indian Journal of Agricultural Sciences 59:729-731.

[13]. Dua RP (1991). Differential response of chickpea (*Cicer arietinum* L.) genotypes of salinity. Indian Journal of Agricultural Sciences (Cambridge) 119:367-71.

[14]. Dua RP and Sharma SK (1997). Suitable genotypes of gram (Cicer arietinum L.) and mechanism of their tolerance to salinity . Indian Journal of Agricultural Sciences. 67: 102-106.

[15]. Dua RP (1998). Salinity tolerance in chickpea. Indian Journal of Plant Physiology 3:102-106.

[16]. Gorham J, Wyn Jones RG and McDonnell E (1985). Some mechanism of salt tolerance in crop plants. Plant and Soil 89: 15-40.

[17]. Greenway H and Munns R (1980). Mechanism of salt tolerance in non-halophytes. Annual Review of Plant Physiology 31:149-190.

[18]. Judith Atieno, Yongle Li, Peter Langridge, Kate Dowling, Chris Brien, Bettina Berger, Rajeev K. Varshney and Tim Sutton (2017). Exploring genetic variation for salinity tolerance in chickpea using image-based phenotyping Published online 2017 May 2. doi: 10.1038/s41598-017-01211-7

[19]. Keatinge J.D.H., Lin L.J., Ebert A.W., Chen W.Y., Hughes J.A., Luther G.C., Wang J.F. and Ravishankar M. (2014). Overcoming biotic and abiotic stresses in the Solanaceae through grafting: Current status and future perspectives. Biol. Agric. Hortic. 30, 272–287. [CrossRef]

[20]. Lauter DJ and Munns DW (1982). Salt response of chickpea and cowpea genotypes to different ionic composition. Agron. Abs. 213.

[21]. Lauter DJ and Munns DW (1996). Salt tolerance of chickpea during vegetative growth and at different humidity. Australian Journal of Plant Physiology 14: 171-180.

[22]. Mamo T, Richtes C and Heigtag B (1996). Salinity effects on growth and ion content of chickpea (*C. arietinum L*) and Lentil (*Lens culnaris medic.*) varieties. Journal of Agronomyb and Crop Sciences 176: 235-247.

[23]. Manchanda HR, Sharma SK, Dixit ML, and Singh S (1981). Possibility of growing chickpea in chloride and sulphate dominant saline conditions. In all India Seminar on Water Resources- Its Developments and Management, Chandigarh, India . PP: 19-30.

[24]. Manchanda HR and Sharma SK (1989). Tolerance of chloride and sulphate salinity in chickpea. Journal of Agricultural Sciences 113: 407-410.

[25]. Munns R and Tester M. (2008). Mechanisms of salinity tolerance. Vol. 59:651–681. [PubMed] [Google Scholar]

[26]. Singh H., Kumar P., Chaudhari S. and Edelstein M. (2017). Tomato grafting: A Global perspective. HortScience , 52, 1328–1336. [CrossRef]

[27]. Singh H., Kumar P., Kumar A., Kyriacou M.C., Colla G. and Rouphael Y. (2020). Grafting Tomato as a Tool to Improve Salt Tolerance. Agronomy 10: 1-22 ; (www.mdpi.com/journal/agronomy).

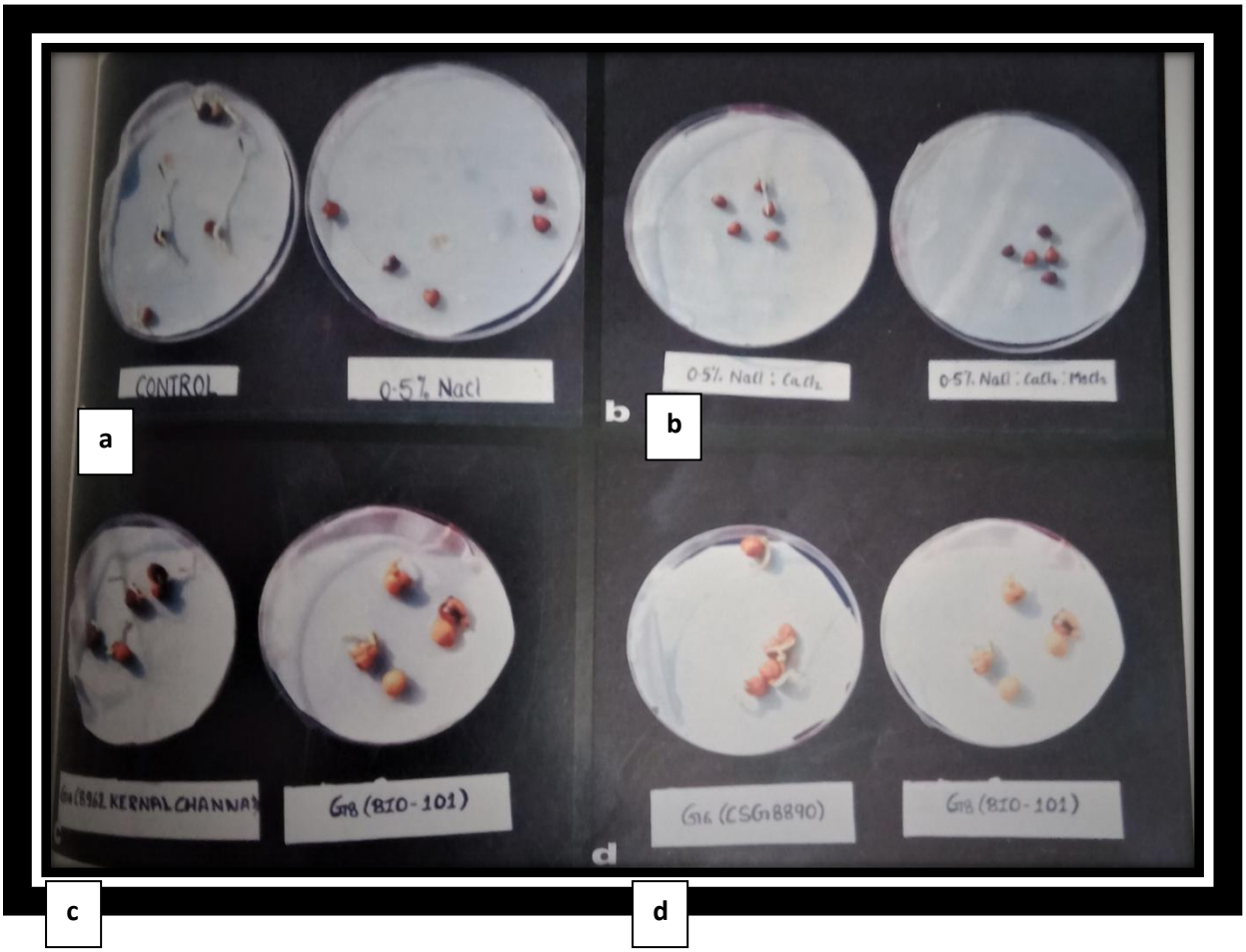

Fig.1: Effect of salt and salt mixtures on seedling emergence of chickpea genotypes :

a. Difference between control and 0.5% NaCl
b. Difference between 0.5% NaCl: $CaCl_2$: and 0.5% NaCl:$CaCl_2$:$MgCl_2$
c. Difference between tolerant and test genotypes
d. Difference between tolerant and test genotypes

Fig 2: Grafting of root stock and scion of differenet genotypes of chickpea :

a) Preparation of the scion
b) Preparation of the root stock
c) Union of scion and root stock using straw pipe
d) & (e) Conditioning of grafted plants
f) Grafted plant showing successful union of root stock and scion
g) Failure of grafting showing wilting and drying on grafted portion
h) Successfully growing of grafted plants showing stems with simple leaf of mother plant and normal leaf of grafted plant

Not show:
g) Failure of grafting showing wilting and drying on grafted portion
h) Successfully growing of grafted plants showing stems with simple leaf of mother plant and normal leaf of grafted plant

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