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Yield Correlation of Chickpea (*Cicer Arietinum* L.) Genotypes Based on Physiological and Morphological Traits for Salt Tolerance

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Abstract: *Thirty genotypes of chickpea (Cicer arietinum L.) were evaluated to estimate correlation of physiological and morphological traits with yield under salt stress conditions. Correlation of different characters on plant seed yieldunder salt stress conditions was studied with relative water content, membrane stability index, sodium pottasium ratio, days to maturity, days to flowering, pods per plant, seeds per pod, 100 seed weight and plant height.Under saline stress condition seed yield per plant exhibited highly significant positive correlation with days to maturity (0.562), pods per plant (0.690), RWC (0.906) and MSI (0.864), and negative correlation of seed yield with days to flowering (-0.495) and Na: K ratio (–0.894) was observed, which shows that the plants having high yield under salt stress have lower amount of Na:K ratio, traits like 100 seed weight showed (0.864) relative less significant correlation value and other traits do not show significant correlation with plant seed yield. The lower the ratio, higher was yield per plant indicating that the plants that were able to exclude the sodium and prevent it from going to stem showed better tolerance to salinity. Also the other physiological parameters kike RWC and MSI showed positive correlation with seed per plant under salt stress condition. The traits like higher mean seed yield per plant under saline stress, higher pods per plant, higher RWC, higher MSI and a low stem Na:K ratio are associated with tolerance to salinity in chickpea*

Keywords: *Correlations, salt stress and chickpea.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) (2*n* = 16) king of pulses ranking second in world food legume production (FAOSTAT, 2013). It is well known as one of potential crop to meet the protein hunger. The yield loss in chickpea due to salinity has been estimated to be approximately 8 to 10 per cent of total global production (Flowers *et al*., 2010). In India, *desi* type of chickpea, accounts for nearly 90 per cent of total area under cultivation and remaining (10%) cultivated area being occupied by *kabuli* type. Chickpea is a deep-rooted, hardy, dry land crop and can grow to full maturity despite conditions that would prove fatal for most of the crop plants. It is grown on marginal land and rarely receives fertilizers or protection from diseases and insect pests (Singh and Reddy, 1991).

India is the world's biggest producer, with an annual production of around 8.88 Mt, grown in an area of about 9.21mha. representing 68 per cent of total world chickpea production and average yield of 995 kg ha^{-1} (http://agricoop.nic.in/).

World is losing around two thousand hectares of farm soil daily to salt induced degradation, salt spoiled soils worldwide is 20 per cent of all irrigated lands which is an area equal to France. Extensive economic losses due to salinity include costs of $27 billion plus loss of crop value per year. Every day for more than 20 years, an average of two thousand

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hectares of irrigated land in arid and semi-arid areas across 75 countries have been degraded by salt, according to a study by UN University's Canadian based Institute for Water, Environment and Health (UNU-INWEH, 2014).Vadez *et al*. (2007) reported a strong relationship ($r^2 = 0.50$) was found between the seed yield under salinity and the seed yield under a non-saline control treatment, indicating that the seed yield under salinity was explained in part by a yield potential component and in part by salinity tolerance per se.

Turner *et al*. (2013) concluded the role of pods per plant, seed numbers, seed yield and yield components, pollen viability, in vitro pollen germination and in vivo pollen tube growth to evaluate reproductive success. The increased salt tolerance, as measured by yield under salinity or relative yield under saline conditions, was positively associated with higher pod and seed numbers and higher shoot biomass but not with time to 50 per cent flowering nor with the number of filled pods in the non-saline treatment. Pod abortion was higher in the salt sensitive genotypes, but pollen viability, in vitro pollen germination and in vivo pollen tube growth were not affected by salinity in either the salt tolerant or salt sensitive genotypes. The concentrations of sodium and potassium ions were significantly higher in the sensitive than in the tolerant genotypes. Sodium and potassium ions accumulated in leaves and in pod wall, whereas accumulation in the seed was much lower.

MATERIALS AND METHODS

Selection of Experimental Material

Sets of thirty genotypes were shortlisted for carrying out detail analysis. The susceptible lines were included in the study in order to have a comparison and validation of the results obtained in the experiment. These pots were saturated with normal irrigation water and stress was induced in pots by making saline solution selected genotypes were evaluated with two replications in single environment under single date of sowing (22 Nov. 2014), under controlled greenhouse condition and the observations were recorded in each replication in pots.

Induction of Salt Stress in Pots

Chickpea plants were grown under saline and normal soil conditions in 13cm diameter pots which contain about 6.5 kg of soil taken from the IARI farm with initial electric conductivity = 0.4ds/m, pH 8.1 and it was fertilized with di-ammonium phosphate (DAP) about 2g per pot. The salt treatment was applied in pots to induce saline stress by treating the normal soil by 80 mM solution of NaCl with normal tap water in a sufficient volume to wet the soil to field capacity to saturate the entire pot soil. There is the requirement of about approximately 1.50 L/pot of solution to completely saturate the each pot to bring it to field capacity.

80 moL/m3 (8ds/m) = 4.68 mg NaC1 in 1 L water

This corresponds to an application of 7.60 g NaCl per pot, equivalent to 1.17 g NaCl per kg of soil. The saline treatment was applied 4 to 5 days before carrying out sowing. Thereafter, pots were watered with tap water containing no significant amount of NaCl, and maintained close to field capacity to avoid an increase in salt concentration in the soil solution the EC of individual pots was monitored weekly with conductivity meter and the considerable EC of about 7.5 to 8.5ds/m was maintained in each pot by further adding the saline solution. Non-saline treated controls were brought initially to field capacity with non-saline water with equal volume required for reaching the field capacity.

Physiological Parameters

1. ***Membrane stability index (MSI) (as per Blum and Ebercon, 1981):*** Membrane Stability Index (MSI) was calculated by taking 100 mg fresh leaf sample in test tube and immersing it in 10 ml of distilled water. This test tube was kept in water bath at 45°C for 30 min. It was allowed to cool at room temperature and then water conductivity of sample (C_1) was measured using Electrical Conductivity Meter. Again, the test tube was kept in water bath at 100°C for 10 min. and subsequently cooled to room temperature and the final conductivity meter reading of the sample (C_2) was measured. The Membrane Stability Index (MSI) was calculated using following formula.

$$MSI = [1 - C_1/C_2] \times 100$$

2. ***Relative water content (RWC) (as per Barrs and Weatherley, 1962):*** RWC = [(Fresh Weight – Dry Weight)/(Turgid Weight – Dry Weight)] × 100
3. ***Measurement of sodium and potassium ratio:*** Plant parts are detached like shoot, subsequently dried and grinded to prepare samples. Digestion was done in Diacidic reagent (HNO_3:$HCLO_4$ at 2:1 ratio) and Potassium and sodium was measured by flame photometry, by simultaneously running the blank that did not contain plant samples.

Morphological traits

Data was recorded on various traits like Shoot length (SL),Root length (RL), Fresh shoot weight (FSW), Shoot dry weight (SDW), Fresh root weight (FRW),Root dry weight (RDW), Days to maturity (DTM), Days to 50 per cent flowering (DTF), Plant height (PH),Filled pods per plant (FPP), Seeds per pod (SPP), 100 seed weight (TW), Plant seed yield (PSY).

Statistical Aanalysis

The data of individual characters was analysed statistically and all statistical observation were carried out on the mean value of the two replications. Variance, correlation were performed for all the observations using SPSS *vs.* 20 software. Means were separated using LSD (least significant difference) at $P < 0.05$. Analysis of variance was used to compare the effects of stress and non-stress conditions on genotypes.

Estimation of Correlation

Phenotypic correlation coefficients were estimated by using the statistical software package SPSS *vs.* 20 (IBM Corp.). The formulae suggested by Al-Jibouri *et al.* (1958) for calculation of correlation by using the variance and covariance estimates from analysis of variance and covariance tables.

Phenotypic correlation coefficient,

$$r_{p12} = \sigma^2_{p12} / (\sigma_{p1} \cdot \sigma_{p2})$$

Where σ^2_{p12} and σ^2_{g12} are the covariance between character X_1 and X_2, respectively; σ_{p1} and σ_{p2} are the phenotypic standard deviation for character X_1 and X_2, respectively.

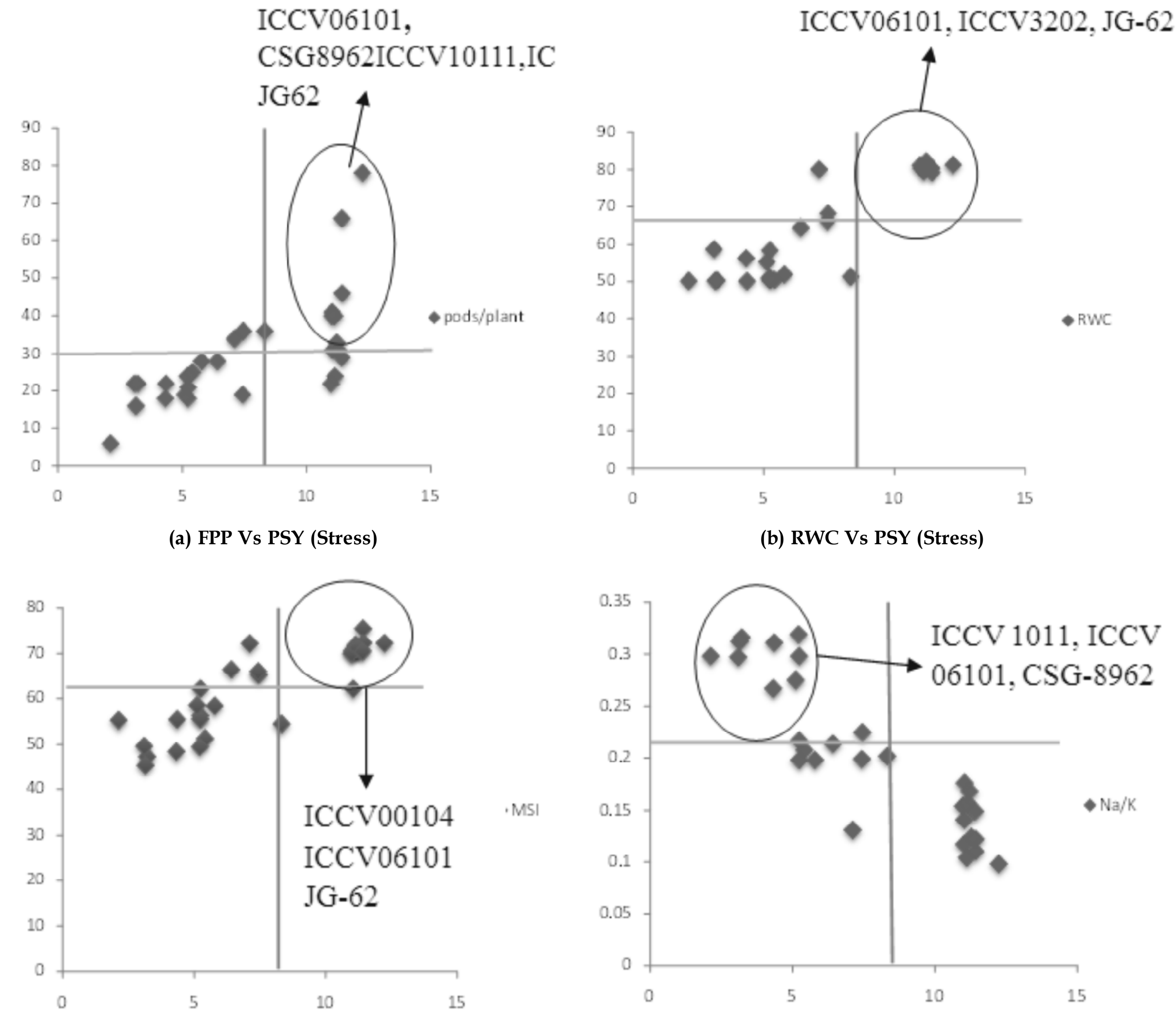
RESULTS

Correlation indicates the magnitude of association between pairs of character and forms the basis of selection index, thereby aiding the breeder in crop improvement. The estimates of phenotypic correlation coefficients between ten characters of different chickpea genotypes are given in (Table 1).

Table 1
Estimates of correlation coefficient between different characters with PSY under stress in 30 chickpea genotypes

	Correlations									
	Days to flowering	*Days to maturity*	*Plant height*	*Pods per plant*	*Seed per pod*	*100 seed weight*	*Relative water content*	*Membrane stability index*	*Sodium: Potassium*	*Seed yield per plant*
Days to flowering	1	–.330	–.218	–.253	.072	–.556**	–.454*	–.412*	.349	–.495**
Days to maturity	–.330	1	.666**	–.026	.528**	.461*	.609**	.509**	–.453*	.562**
Plant height	–.218	.666**	1	–.039	.400*	.170	.429*	.315	–.241	.333
Pods per plant	–.253	–.026	–.039	1	–.207	–.249	.604**	.613**	–.706**	.690**
Seed per pod	.072	.528**	.400*	–.207	1	.208	.311	.297	–.183	.285
100 seed weight	–.556**	.461*	.170	–.249	.208	1	.337	.320	–.265	.388*
Relative water content	–.454*	.609**	.429*	.604**	.311	.337	1	.890**	–.855**	.906**
Membrane stability index	–.412*	.509**	.315	.613**	.297	.320	.890**	1	–.891**	.864**
Sodium: Potassium	.349	–.453*	–.241	–.706**	–.183	–.265	–.855**	–.891**	1	–.894**

**. Correlation is significant at the 0.01 level (2–tailed). *. Correlation is significant at the 0.05 level (2–tailed).



(a) FPP Vs PSY (Stress) **(b) RWC Vs PSY (Stress)**

(c) MSI Vs PSY (Stress) **(d) Na/K Vs PSY (Stress)**

Figure 1: Maximax–minimax graphical plot showing genotype distribution on yield per plant under stress Vspods per plant and other physiological parameters

Note: Y–axis (a) FPP, (b) RWC, (c) MSI, (d) Na/K; *X* axis–PSY (stress)

PSY (Plant Seed Yield)

Under saline stress condition seed yield per plant exhibited highly significant positive correlation with days to maturity (0.562), pods per plant (0.690), RWC (0.906) and MSI (0.864), while traits like 100 seed weight showed (0.864) relative less significant correlation value and negative correlation of seed yield with days to flowering (–0.495) and Na: K ratio (–0.894) was observed, which shows that the plants having high yield under salt stress have lower amount of Na:K ratio.

RWC (Relative Water Content)

RWC content in plant tissue showed highly significant correlation with days to maturity (0.609), FPP (0.604) and MSI (0.890) while it displayed the significant negative correlation with days to flowering (–0.454) and Na:K ratio (–0.855). This shows that RWC content positively affects the pods per plant and MSI while negatively correlated with Na:K ratio which can give us rough pictures for salt tolerant genotypes on the basis of these parameters.

MSI (Membrane Stability Index)

If we see the correlation of MSI with other morphological traits and physiological traits it displayed highly significant positive correlation with days to maturity (0.509), FPP (0.613) and RWC (0.890). While association was negative with Na:K ratio (–0.891) and days to flowering (–0.412).

Na:K (Sodium: Potassium ratio)

The association of Na:K ratio (stem) among various morphological and physiological traits showed the significant correlation between each other while it displayed significant negative correlation with the characters like FPP (–0.706), RWC (–0.855), MSI (–0.891) and days to maturity(–0.453).

DTM (Days to maturity)

The correlation between several morphological yields related traits and traits of agronomic importance, days to maturity was significant positively correlated with PH (0.666), SPP (0.528) and TW (0.461).

PH (Plant height)

The plant height shows significant positive correlation with days to maturity (0.666) and SPP (0.400). The other traits and their correlation with each other are mentioned in (Table 4.7). Determination of correlation coefficients is criteria important for the selection of favourable plant genotypes for effective chickpea breeding programmes. Correlation coefficients in general show associations among independent characteristics and the degree of linear relation between these characteristics. The knowledge of correlation among above discussed traits with the seed yield and yield contributing characters will help in selection of superior chickpea genotypes in breeding programme for salt tolerance

DISCUSSION

Chickpea (*Cicer arietinum* L.) is an outstanding crop amongst the most pulse crop of India. Chickpea per acre production in the nation is insufficient to fulfill the customer's request. It is chiefly because of the poor genetic composition of the cultivars and non-accessibility of good quality seed of varieties with high yield potential and resistance against biotic and abiotic stress. Hence, the improvement of enhanced cultivars of chickpea is the need of the day. Determination of relationship between yield and yield related qualities criteria is important for the selection of favourable plant types for effective chickpea breeding programmes. Correlation coefficients in general show associations among independent characteristics and the degree of linear relation between these characteristics. Therefore the significant goal of this study was to assess the relative significance of different salt tolerance related characteristics that may add to yield dependability under saline condition for further breeding endeavours in chickpea for saline environment.

Correlation studies of characters are very useful and important to know the suitability of various traits for selection because selection of particular trait may affect relative traits in bringing desirable or undesirable changes. By and large an immediate determination of yield may not be powerful as it is complex element and is quantitatively acquired with low heritability. Accordingly high genotypic and environment interaction are prone to limit the change. Concurrent expression of characters may be either because of pleiotropic and hereditary linkage.

In the event that the relationship is because of complex interaction of genes, it is hard to partition these impacts by selecting a specific character so related. It is in this manner essential to build up relationships between attributes, it is difficult to separate these effects by selecting a particular character so related. In case of genetic linkage an association can be reversed provided the linkage is not very closed. It is therefore important to establish correlations between traits, before launching any breeding programme. Further the component character of yield exhibit different association among themselves and also with yield. The genetic advance may also be limited by unfavorable association with desired attributes. Hence the knowledge of association between plant yield and other morpho-physiological characters with yield under terminal salt conditions are essential for planning a sound breeding programme. In the present investigation, correlation estimates were obtained from 30 genotypes for seed yield under salt stress with other morpho-physiological characters. The correlation between seed yield per plant under saline stress conditions exhibited highly significant positive correlation with days to maturity (0.562), filled pods per plant (0.690, RWC (0.906) and MSI (0.864). Negative correlation between seed yield under saline stress was seen with days to flowering (–0.495) and Na: K ratio (–0.894) which signifies that the plants having high yield under salt stress have lower amount of Na:K ratio in their stem. Thus for selection of salt tolerant genotype can be assisted by studying these correlated traits. The study of correlation of physiological parameters like RWC content in plant tissue shows highly significant correlation with days to maturity (0.609) , filled pods per plant (0.604) and MSI (0.890) while it displayed the significant negative correlation with days to flowering (–0.454) and Na:K ratio (–0.855). This shows that RWC content positively affects the pods per plant and MSI while negatively correlated with Na:K ratio which can give us rough picture for salt tolerant genotypes on the basis of these parameters. While, If we see the correlation of MSI with other morphological traits and physiological traits it displayed highly significant positive correlation with days to maturity (0.509), filled pods per plant (0.613) and RWC (0.890) while association was negative with Na:K ratio (–0.891) and days to flowering (–0.412).

The association of Na:K ratio in stem among various morphological and physiological traits showed significant correlation between each other. It displayed significant negative correlation with the characters like pods per plant (–0.706), RWC (–0.855), MSI (–0.891) and days to maturity (–0.453). When comparison for potassium content was done among chickpea genotypes, it was found that the values of potassium content in shoot of tolerant genotypes were significantly higher than that of susceptible genotypes, whereas the values of potassium content in root of susceptible genotypes were significantly higher than that of tolerant genotypes Sivasankaramoorthy (2013). So the negative correlation for Na:K ratio content in stem with the plant yield under salt stress indicates that those genotypes are salt tolerant. The correlation between several morphological yield related traits and traits of agronomic importance, days to maturity was significant positively correlated with plant height (0.666), seed per pod (0.528) and 100 seed weight (0.461).

The plant height shows significant positive correlation with days to maturity (0.666) and seed per pod (0.400). Genotypes possessing high RWC in saline conditions can be considered as salt tolerant and helps the plant to thrive well under stress conditions. Chickpea genotypes with good salt tolerance would give higher yield under stress conditions. This would help to increase the chickpea production in salt affected nontraditional chickpea growing area as well as sustain crop production in degrading land.

There was significant reduction in pods per plant under salt stress condition. It showed positive correlation with all physiological parameters like RWC and MSI but negative correlation with Na:K ratio under salt stress condition. According to Vadez *et al*. (2007) the major trait related to salinity tolerance was the ability to maintain a large number of filled pods in stress conditions too. There was not significant variation between saline and normal conditions of growth in a genotype indicating that this is least affected character by the environment. Number of pods per plant, seeds per plant and seed yield played a major determining factor for yield under saline stress. Pod abortion was generally reported to be higher in the salt sensitive genotypes.

The concentrations of sodium and potassium ions, but not chloride, in the seed were significantly higher in the sensitive than in the tolerant genotypes. Sodium and potassium ions accumulated in leaves and in pod wall, whereas accumulation in the seed was much lower. The differences for this trait existed from genotype to genotype and plant group to plant group. The influence of salt stress was immense on the Na:K ratio and formation of pods thereby the seed yield per plant. It is pertinent to mention that the seed weight was not influenced by salt stress.

Many workers have reported that the salinity caused yield reductions due to the effect of sodium ion rather than the toxicity caused by chloride (Tester and Davenport 2003; Kader and Lindberg

2005). These workers further reported that the sodium toxicity actually affects potassium availability and its distribution in the cytoplasm and membrane and thus salinity is more of a function of potassium availability. Those plants which are able to maintain a low sodium in the stem either by inhibiting its intake in the roots or vacoulising it and decreasing its concentration in shoots there by having a low Na:K ratio in the stem were found to be tolerant to salinity (Hajibagheri *et al*., 1989; Carden *et al*., 2003). The concentration of potassium is generally static and is around 100 mM, while the resistant ones limit the sodium concentration to lower levels so as to not increase its toxicity effects (Kader and Lindberg 2005). Potassium acts as an activating substance for most of cystolic enzymes. Sodium though cannot do this function, but being identical in its structure to potassium binds to the sites of potassium in the enzyme in a competing fashion rendering the enzyme defunct, thus hindering the actual function of the enzyme (Maathuis and Amtmann 1999; Carden *et al*., 2003).

CONCLUSION

The correlation studies indicate the strong relation between the stem Na/K ratio and yield per plant. The lower the ratio, higher was yield per plant indicating that the plants that were able to exclude the sodium and prevent it from going to stem showed better tolerance to salinity. Also the other physiological parameters kike RWC and MSI showed positive correlation with seed per plant under salt stress condition. The traits like higher mean seed yield per plant under saline stress, higher pods per plant, higher RWC, higher MSI and a low stem Na:K ratio are associated with tolerance to salinity in chickpea. Greater genetic gains can be obtained by using these parameters in selection for salinity tolerance

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