Salt stress tolerance index of cowpea (Vigna unguiculata L. Walp.) genotypes for cowpea genetic improvement in saline soil condition

Trustinah Trustinah
National Research and Innovation Agency
Nuryati Nuryati
National Research and Innovation Agency
Rudy Soehendi
National Research and Innovation Agency
Purwantoro Purwantoro
National Research and Innovation Agency
Yuliantoro Baliadi
National Research and Innovation Agency
Gatut Wahyu Anggoro Susanto
National Research and Innovation Agency
Apri Sulistyo
National Research and Innovation Agency
Heru Kuswantoro
National Research and Innovation Agency
Febria Cahya Indriani
National Research and Innovation Agency
Runik Dyah Purwaningrahayu
National Research and Innovation Agency
Kartika Noerwijati
National Research and Innovation Agency
Made Jana Mejaya
mmejaya@yahoo.com
National Research and Innovation Agency
Sholihin Sholihin
National Research and Innovation Agency
Arief Harsono
National Research and Innovation Agency
Yusmani Prayogo

National Research and Innovation Agency

Research Article

Keywords: cowpea, tolerance, genotype, salinity, yield

Posted Date: May 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9331256/v1

Additional Declarations: No competing interests reported.

Research Article

Salt stress tolerance index of cowpea (*Vigna unguiculata* L. Walp.) genotypes for cowpea genetic improvement in saline soil condition

Trustinah Trustinah[1], Nuryati Nuryati[1], Rudy Soehendi[1], Purwantoro Purwantoro[1], Yuliantoro Baliadi[1], Gatut Wahyu Anggoro Susanto[1], Apri Sulistyo[1], Heru Kuswantoro[1], Febria Cahya Indriani [1], Runik Dyah Purwaningrahayu[1], Kartika Noerwijati[1], Made Jana Mejaya[1*], Sholihin Sholihin[1], Arief Harsono[1], Yusmani Prayogo[1]

[1] Research Center for Food Crops, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor KM. 46, Cibinong, Bogor Regency 16911, West Java, Indonesia.

*Corresponding author email: mmejaya@yahoo.com; trustinah02@yahoo.com

ABSTRACT

Salinity is a major constraint to cowpea (*Vigna unguiculata* L. Walp) production in coastal and saline-prone areas. The objective of this study was to characterize cowpea genotypes grown under saline soil conditions based on morphological traits, yield, and stress tolerance indices, and to identify promising genotypes for genetic improvement in saline-prone areas. This study consisted of two trials namely screen house trial and field trial. Twenty-five cowpea genotypes were planted on screen house in Malang using two replications of factorial completely randomized design. Two environments, saline soil (salt-stress) and non-saline (normal) conditions, were used as the first factor. The field trial was conducted in saline soil (with the ESC 7-12 dS m^{-1}) during the dry season from July to September 2018 in Lamongan Regency, East Java Province, Indonesia. A randomized block design with 22 replications was used for the field study. The study showed that the salinity level in this test was classified as moderate to severe. Stress Tolerance Index (STI) and yield under salt stress (Ys) (0.89**) and yield under non-salt stress (Yp) (0.54**) were found to be positively correlated. In both stress and non-stress situations, genotypes with high STI also generated high yields. Pod quantity and seed output exhibited the greatest stress under salt stress conditions, with stress intensity greater than 0.70. Cowpea genotypes varied in terms of yield and yield components. All of the characters observed varied, by which 72.80% could be explained by four factors. Four clusters of cowpea genotypes were identified, each cluster consisting of 11, 3, 7, and 4 genotypes. Based on seed yields, four genotypes MLG 17013, MLG 17042, MLG 17055, and KT 6 had yields of more than 2.0 t.ha^{-1}. In order to lessen the effects of climate change, cowpea varieties could be genetically improved for salinity tolerance in saline soil using the stress-tolerant accessions.

Key words : cowpea, tolerance, genotype, salinity, yield

INTRODUCTION

Cowpeas (*Vigna unguiculata* (L.) Walp.) contain anti-nutritive and nutritious substances that are vital to human health. Cowpeas range in moisture content from 11 to 17%, ash from 3.13 to 3.97%, fat from 0.58 to 1.42%, protein from 15.5 to 20.76%, carbohydrates from 58.46 to 63.48%, and folic acid from 100.13 to 131.57 µg/ml. They are crucial for nutritious food in the human diet because they include amino acids that complement cereals (Jayathilake et al., 2018) (Karuwal et al., 2021). Cowpea's other traits include early maturation (60–70 days), resistance to drought and acidity in the soil, and the ability to be planted in less fertile regions (Trustinah et al., 2007) (Özdemir *et al*., 2009). Cowpea, with these features, has the potential to be grown in suboptimal environments, including saline soil.

In the development of food crops, salinity has grown to be a major issue, particularly in coastal regions. In Indonesia, salinity is typically found on coastal agricultural land due to sea water intrusion, tide influence, or sea level rise brought on by climate change. An estimated 12,020 million hectares, or 6.20% of Indonesia's total geographical area, are near the shore and susceptible to salt, spread along the west and east coasts of Aceh Province, north coast of Java, Mbay area in Flores Island (East Nusa Tenggara Province), the coast and tidal plains spread almost throughout Indonesia, while deltas (estuarine) spread throughout the provinces in Kalimantan and some in Sumatra (Karolinoerita & Annisa, 2020). The high concentration of dissolved salts in the soil reduces the roots' ability to absorb nutrients, which is the cause of the salinity issue. Nonsaline (EC <1.0 dS m^{-1}), very slightly saline (EC 1.1-2.0 dSm^{-1}), moderately saline (EC 2.1-4.0 dSm^{-1}), saline (EC 4.0-8.0 dSm^{-1}), very saline (EC 8.1-16.0 dSm^{-1}), and very strongly saline (EC >16.0 dSm^{-1}) are the different categories of soil salinity (Jones, Jr., 2003).

Previous research on the cowpea genotypes in saline soil had been reported that under salt stress conditions, seed germination significantly delayed and reduced the performance of yield, fresh weight, pod length, pod number, and plant height. of cowpea(Özdemir et al., 2009) (Bashandy & El-Shaieny, 2016) (Islam *et al*., 2019). Yield loss depends on the stress level. Cowpea genotypes varied in yield and yield components at soil electrical conductivity of 9.5 dS m-1; seed yield and pod number per plant indicated the maximum stress level with stress intensity over 0.80 (Trustinah & Nugrahaeni, 2021), even on 10.5 dS. m^{-1} no yield was recorded (Himaya *et al*., 2019). Increasing irrigation water salinity had an impact on vegetative development. Plant height and leaf count decreased by 38% and 66%, respectively, when irrigation water salinity rose above 3500 parts per million (Salih & Kia, 2013). This is also shown in mungbean. Stunted plant development, necrosis and chlorosis on both young and old leaves, smaller leaves, a delay in pod ripening, and, in extreme cases, yellowing of the leaves are all signs of the effects of salt on mungbean (Trustinah *et al*., 2019) (Sehrawat *et al*., 2018).

Numerous techniques have been employed to determine a genotype's stress tolerance under normal and stressed conditions, such as Stress Tolerance Index by (Fernandez *et al*., 1992) on mungbeans, (Ravelombola *et al*., 2017) on cowpea, (Kaur *et al*., 2023) on chickpea, and Salt Response Index on asparagus bean by (Chen *et al*., 2007).

The availability of cowpea varieties that are adaptive on saline soils will increase cowpea productivity while at the same time filling protein deficiencies in general, improving nutrition and increasing farmers' income (Harnowo *et al*., 2016). The objective of this study was to characterize cowpea genotypes grown under saline soil conditions based on morphological traits, yield, and stress tolerance indices, and to identify promising genotypes for genetic improvement in saline-prone areas.

MATERIALS AND METHODS

This study consisted of two trials namely screen house trial and field trial.

1. Screen house trial

The first experiment was carried out at the screen house of the Indonesian Legumes and Tuber Crops Research Institute (ILETRI) at Kendalpayak Village, Pakisaji Sub-district, Malang District, East Java Province, Indonesia, during the 2018 dry season. The cowpea genotypes used in this study consisted of breeding lines, released varieties, local accessions, and introduced materials, all obtained from the ILETRI, Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Republic of Indonesia.

Experimental design

A factorial completely randomized design with two replications was used to evaluate twenty-five cowpea genotypes. The two environments (salt stress and non-salt stress) were the initial factor. A pot with 6.5 kg of soil was used to plant cowpea. Phonska fertilizer (15% N, 15% P_2O_5, 15% K_2O, 10% SO_4) was applied to the soil at a rate of 0.95 g per pot prior to planting. Salt stress using 150 mM NaCl solution. The plants are watered with saline solution until the soil electrical conductivity reach above 8 dS m^{-1} and then non-saline water was used to irrigate it. Controlling weeds, pests, and diseases is a major part of plant upkeep.

Data measurements

Seed weight, number of seeds per pod, pod length, pod number, number of nodes, number of branches, and plant height were among the plant data recorded in this experiment. The Hanna portable EC meter model H1993310 was used to measure the electrical conductivity of the soil on a regular basis.

Data aanalysis

Cowpea genotype tolerance was evaluated using stress intensity (SI), stress tolerance index (STI) (Fernandez et al., 1992), and plant growth score on salinity utilizing injury criteria on a 4-6 scale and the Salt Response Index (SRI)(Chen et al., 2007). Salt injury was scored using a grading scale as follows: 0 = no injury, with 100% plant survival and no visible damage; 1 = mild injury, characterized by yellowing of a small portion (approximately one-fifth) of the leaf apex and margins; 2 = moderate injury, where about half of the leaf apex and margins became chlorotic; 3 = severe injury, marked by extensive yellowing of most leaf apexes and margins; and 4 = extreme injury, indicated by leaf abscission, stem shrinkage, and eventual plant death

The indices were calculated according to the formulas proposed by Fernandez (1992) and (Chen *et al*. 2007) as follows:

Stress intensity (SI) = $1- (\bar{Y}_s / \bar{Y}_p)$

Stress tolerance index (STI) = $(Y_p)\ (Y_s) / (\bar{Y}_p)^2$

Salt response index (SRI) = $(Y_s/Y_p) \times 100\%$

In these equations, Yp represents the yield of a given genotype under non-stress conditions, Ys denotes the yield under stress conditions, $\hat{Y}$p is the mean yield in the non-stress environment, and $\hat{Y}$s is the mean yield in the stress environment.

2. Field trial

The field trial was conducted in the dry season of 2018 at Lohgung Village, Brondong sub-district, Lamongan district, East Java province, Indonesia. The test was carried out in the dry land area near the coast, located about 500 m from the sea. The genotypes used in the field trial were the same plant materials described in the screen house experiment, obtained from ILETRI, IAARD, Ministry of Agriculture, Republic of Indonesia.

Experimental design

Three replications of a randomized block design were employed in the field trial. Each genotype was planted on a 1.6 m x 4 m plot (4 rows, 4 m long), with two plants per hole spaced 40 cm by 10 cm apart. Fertilizers were given with phonska fertilizer 250 kg ha^{-1}, respectively.

Data measurements

Observations included seed yield per plot, seed yield per plant, 100-seed weight, number of pods per plant, number of seeds per pod, pod length, days to harvesting, days to flowering, leaf width, leaf length, number of branches, and plant height. Soil electrical conductivity was periodically measured using a Hanna portable EC meter (model HI993310).

Data analysis

ANOVA was used to analyse the data, followed by the Duncan Multiple Range Test (DMRT) test at a significant level of 5%. Pearson Correlation was utilized for correlation analysis, grouping genotype used Principal Component Analysis (PCA) and clustering K-Mean in R Studio 4.1.3 software (Posit, 2022).

RESULTS AND DISCUSSION

The qualitative characters

The cowpea genotypes have varied for qualitative characters such as leaf shape, pod color, and seed color. Cowpea leaves consist of three leaves (trifoliate) with alternating locations and vary in shape from oval to lanceolate. There were variations in size and color of seeds. Most accessions had ovate leaf shape, purple flower color, cream dark pod color, round pod shape, brown to yellowish seed color (Figure 1).

Figure 1. Characteristics of cowpea leaves and seeds.

1. Screen house trial

Soil EC exceeding 9 dSm^{-1} indicated a high level of saline stress during the trial. Plant growth is significantly impacted by this disease. Three weeks after planting (WAP) is when salt toxication symptoms start to show, and the damage gets worse until the sixth week after planting. A score of 1-2 on the salt injury grading criteria indicated that the cowpea genotype responded to salinity stress in different ways, ranging from moderate tolerance to sensitive (**Table 1**).

Table 1. Salt injury grading criteria of 25 cowpea genotypes under stress of salinity

Grading	Cowpea performance	Number of cowpea genotypes		
		3 WAP	5 WAP	7 WAP
0	no salt injury, 100% survival without damage	0	0	0
1	mild salt injury, indicated by small area (approximately 1/5) of leaf apex and leaf margin turning yellow	25	2	0

2	moderate salt injury when 1/2 leaf apex and leaf margin became chlorotic	0	23	24
3	severe salt injury when majority of apexes and leaf margin turned sallow	0	0	1
4	extreme salt injury when leaves fell off, stem became shrunken, and the plant eventually died	0	0	0

WAP=weeks after planting

The range of stress intensity values was 0.25 to 0.88, indicating moderate to high levels of stress. Pod number and seed output were the most stressed of those characteristics, with stress intensity exceeding 0.70. There was a 74% fall in seed weight and a 76% decrease in pod number. (**Figure 2**). **Figure 3** shows the mean of agronomic traits under normal (non salt) and salt stress conditions, while **Figure 4** shows the plant height under normal (blue line) and salt stress (red line) conditions at 3 weeks after planting (WAP) to 11 WAP.

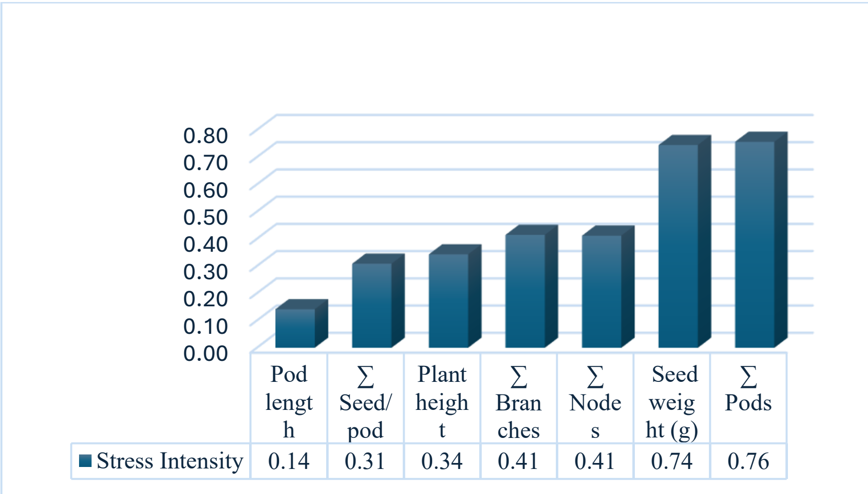


Figure 2. Stress Intensity (SI) of agronomic traits

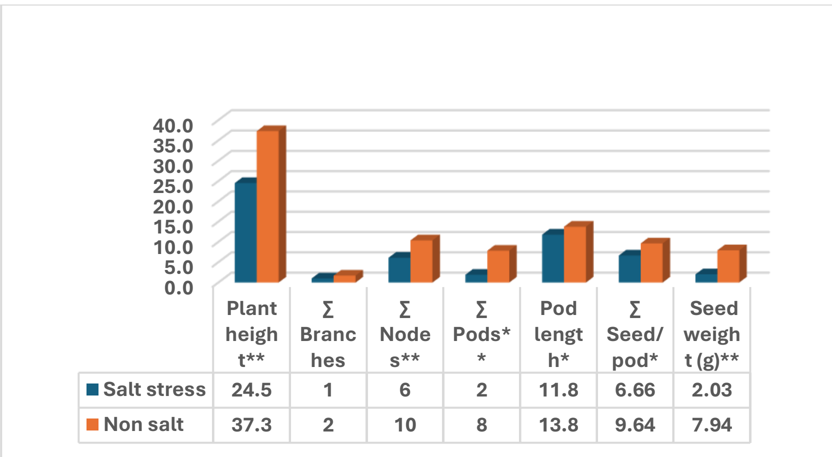


Figure 3. Mean of agronomic traits under normal (non salt) and salt stress conditions

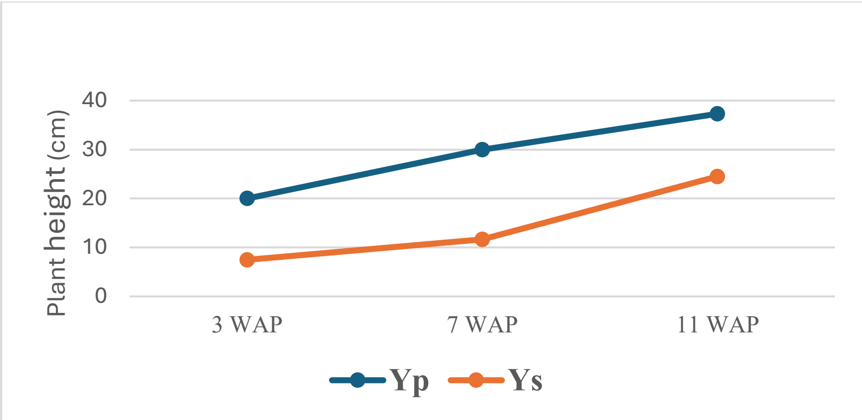
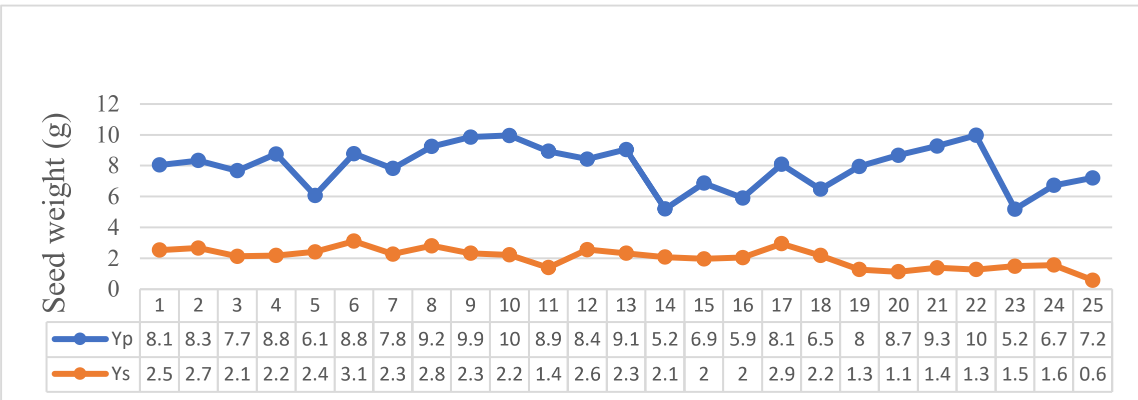


Figure 4. Plant height under normal (blue line) and salt stress (red line) conditions at 3 weeks after planting (WAP) to 11 WAP

Yield (seed weight) losses are very high, ranging from 60-92% with an average 73.3%. Genotype, environment, and the relationship between genotype and environment were all significant for seed weight, according to an analysis of variance. This suggested that, in contrast to non-salt stress, various cowpea genotypes reacted differently to salt stress. There was no correlation between seed weight (yield) in non-stress and salt stress environments. The findings indicated that a genotype was not always associated with a high yield in a salt environment or a non-stress environment. The positive correlation was observed between Stress Tolerance Index (STI) and yield on salt stress (0.89**) and yield under non-salt stress (0.54**). It was shown that genotypes with high STI also produced high yields under both stress and non-stress conditions. There are 10 genotypes with STI above the average (0.26), namely genotype number 1, 2, 4, 6, 8, 9, 10, 12, 13, and 17 (**Figure 5a and 5b**). Additionally, (Ravelombola *et al.*, 2017) on cowpea, (Allel *et al.*, 2019) on barley, and (Kaur *et al.*, 2023) on chickpea revealed positive correlations between factors on stress environment (Ys) and STI.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Yp	8.1	8.3	7.7	8.8	6.1	8.8	7.8	9.2	9.9	10	8.9	8.4	9.1	5.2	6.9	5.9	8.1	6.5	8	8.7	9.3	10	5.2	6.7	7.2
Ys	2.5	2.7	2.1	2.2	2.4	3.1	2.3	2.8	2.3	2.2	1.4	2.6	2.3	2.1	2	2	2.9	2.2	1.3	1.1	1.4	1.3	1.5	1.6	0.6

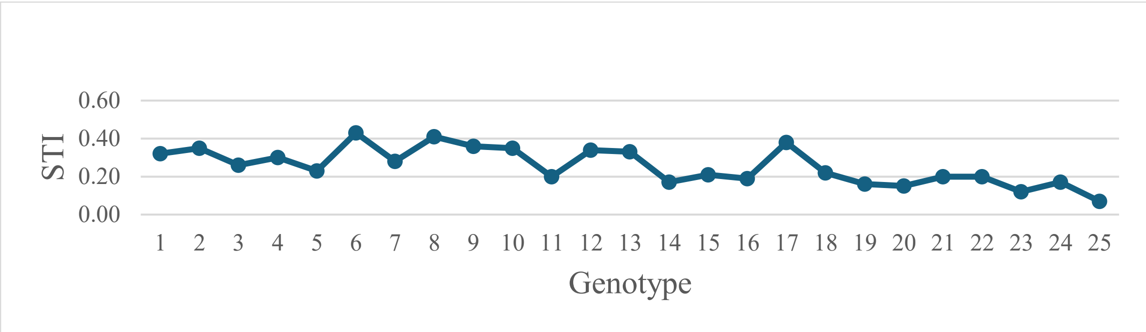


Figure 5 a and 5 b. Seed weight and Stress tolerance index (STI) of 25 cowpea genotypes traits under normal (non salt) and salt stress conditions

2. Field Trial

Electrical conductivity of soil

Irrigation used irrigation water with water electrical conductivity 5.6 dS.m$^{-1.}$ The salinity level at planting was moderate to severe with soil electric conductivity 7 dS.m^{-1}, and at the sixth week after planting, the salinity increased to 10-12 dS.m^{-1} (**Figure 6**).

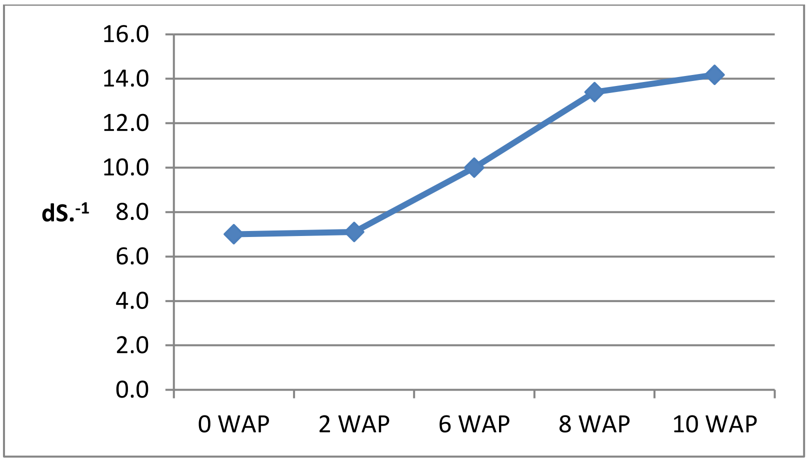


Figure 6. Electrical conductivity of soil during plant growth.

According (Taufiq & Purwaningrahayu, 2013) (Alharbi, 2017) (Karolinoerita & Annisa, 2020), The buildup of salt from the bottom layer, which is transported by water during the evaporation process, causes the electric conductivity of topsoil to increase. It was also reported by (Lacerda *et al*., 2011) that Salinity levels above 2.2 dS m^{-1} reduced dry-season maize performance; however, these salts were effectively leached by high rainfall during the rainy season. Consequently, the negative impact on cowpeas was eliminated, resulting in significant vegetative biomass despite a decrease in pod production.

The salt symptoms poisoning cowpea appeared two week after planting which indicated by young leaves with necrosis and chlorosis; however recovered in the vegetative and reproductive phases. According to certain research, dwarf plant growth, necrosis and chlorosis in both young and old leaves, smaller, greener-colored leaves, rapid leaf loss, and, in extreme cases, yellowing and drying out of the leaf margins are signs of salt poisoning in salinity (Wahid *et* al., 2004) (Trustinah *et al*., 2016).

The quantitative characters

The results showed that the genotypes have significant variation for all observed traits. Plant height ranged from 40-66 cm, number of branches 1-3, leaf length 10.7-14.7 cm, leaf width 3.0-9.6 cm, days to flowering 36-45 days after planting (DAP), days to harvesting 61-70 (DAP), number of pods 5-11, pod length 11.8-18.5 cm, number of seeds per pod 9-16 seeds, 100 seeds weight 10.27-17.12 g, weight of seed per plant 20.8-75.2 g, seed yield 1.03-2.31 t ha^{-1}. The average value for the observed traits for plant height 55.47 cm, number of branches 1.89, leaf length 12.58 cm, leaf width 7.29 cm, flowering at 40 DAP, harvesting at 65 DAP, pods number 8, pod length 16.0 cm, number of seeds per pod 13 seeds, weight of 100 seeds 13.08 g, seeds weight per plant 49.16 g, and seed yield 1.63 t ha^{-1} (Table 2).

Table 2. Descriptive statistics of 25 cowpea genotypes

	Average	Standard Deviation	Minimum	Maximum	Median
Plant height (PH), cm	55.47	6.36	40.0	66.0	55.0
Number of braches (NB)	1.89	0.35	1.4	2.6	1.80
Leaf Length (LL), cm	12.58	1.01	10.7	14.7	12.20
Leaf width (LW), cm	7.29	1.84	3.0	9.6	7.70
Rasio LL/LW (RLW)	1.95	0.96	1.27	4.64	1.59
Flowering day (FD) DAP	39.56	2.35	36	45	39
Harvesting Day (HD) DAP	65.16	2.54	61	70.0	66
Number of pod (NP)	7.56	1.53	4.8	11	7.90
Pod length (PL), cm	16.00	1.52	11.8	18.5	16.30
Number of seed per pod (SP)	13.11	1.92	9	16.2	13.40
Seed weight per plant (SW)	49.16	12.65	20.8	75.2	48.50
Wieght of 100 seeds (W100S), g	13.08	1.53	10.27	17.12	13.09
Yield (Y), t ha^{-1}	1.63	0.35	1.03	2.31	1.67

Response of cowpea to salinity was also reported by previous researchers. (Bashandy & El-Shaieny, 2016) observed that yield of cowpea irrigated with 4800 ppm saline water varied from 27-52 g, which was similarly reported in the current study. Other research showed that at the soil EC of 9.52 $dS.m^{-1}$, the pod yield was 3.83 g, the pod length was 8.0 cm, the seed yield was 2.80 g, and the hundreds of seed weight was 12.96 g, while in non-saline condition (soil electrical conductivity 0.74 $dS.m^{-1}$) seed yield 6.75 g, pod yield 9.17 g, pod length 9.9 g, and weight of 100 seeds 9.9 cm (Ďzdemir *et al.*, 2009). (Himaya *et al.*, 2019) also reported that Cowpea production was greatly reduced by rising salinity levels; no yield was seen in treatment water EC=10.5 dS.m^{-1}. In an earlier evaluation of 125 cowpea genotypes under moderate to high saline conditions (soil electrical conductivity 9.50 dS.m^{-1}), (Trustinah & Nugrahaeni, 2021) reported that the highest stress level was demonstrated by seed yield and pod count, with stress intensity exceeding 0.80.

The correlation among traits

According to Table 3's correlation analysis results, yield (Y) was positively correlated with number of branches (NB) (r=0.30), number of pods (NP) (r=0.33), and negatively correlated with days to flowering (FD) (r=-0.38*) and days to harvesting (HD) (r=-0.22). The weight of 100 seeds (W100S) showed a negative correlation with the number of pods per plant (NP) (r=-0.46*), a negative correlation with plant height (PH) and seed yield (Y) (-0.26), and a positive correlation with the number of branches (NB) (r=0.18), days to flowering (FD) (r=0.47*), and days to harvesting (HD) (r=0.29) (**Figure 7**). (Mofokeng *et al.*, 2020) also reported a positive correlation between seed yield and branch number (0.30**), yield with pod number (0.71**), and yield with 100-seed weight (0.33**). A positive correlation between plant height and seed yield (0.18** and 0,86**) was reported by (Gerrano *et al.*, 2019) (Ceritoğlu & Erman, 2020).

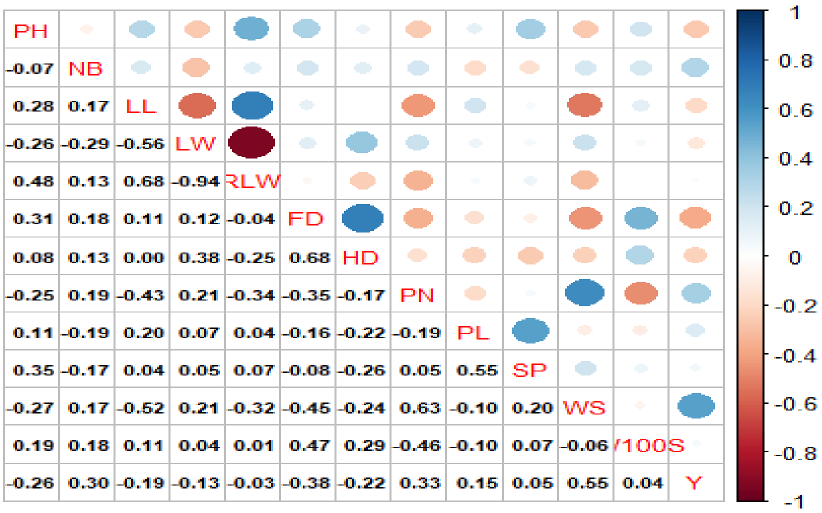


Figure 7. Correlation coefficient matrix showing associations of agronomic traits among cowpea genotypes. plant height (PH), number of branches (NB), leaf Length (LL), leaf width (LW), ratio LL/LW (RLW), flowering day (FD), harvesting Day (HD), number of pod (NP), pod length (PL), number of seed per pod (SP), seed weight per plant (SW), weight of 100 seeds (W100S), yield (Y).

The principal components

The principal components analysis of twenty-five cowpea genotypes for morphological traits showed that with a total variance of 72.8% and an eigenvalue >1.0, four factors might account for the outcome. The variance explained by the first four main components was 24.38%, 20.46%, 13.86%, and 11.5%, respectively. Leaf length, pod count, and seed weight were the characteristics that influenced variation in the first main component. The second major factor has to do with plant height, harvesting day, and flowering day. The number of branches, pod length, and number of seeds per pod were the third primary component, while the weight and yield of hundreds of seeds were the fourth important component (**Table 3**).

Table 3. Main component analysis of eight quantitative morphological characters of cowpea

Characters	PC1	PC2	PC3	PC4
Plant height (PH)	0.165	0.619	0.117	0.263
Number of braches (NB)	0.143	-0.156	0.632	0.498
Leaf Length (LL)	-0.776	-0.305	-0.005	0.179
Leaf width (LW)	0.543	0.704	-0.316	-0.014
Rasio LL/LW (RLW)	-0.661	-0.623	0.233	0.046
Flowering day (FD)	-0.450	0.737	0.123	0.177
Harvesting Day (HD)	-0.184	0.770	0.294	0.117
Number of pod (NP)	0.770	-0.184	0.214	-0.164
Pod length (PL)	-0.019	-0.216	-0.687	0.475
Number of seed per pod (SP)	0.139	-0.145	-0.679	0.482
Seed weight per plant (SW)	0.805	-0.221	0.151	0.190
Wieght of 100 seeds (W100S)	-0.291	0.365	0.121	0.546
Yield (Y)	0.451	-0.429	0.336	0.529
Eigenvalue	3.17	2.99	1.80	1.50
% of variance	24.39	23.03	13.86	11.53
% cumulative of variance	24.39	47.41	61.28	72.80

The use of principal components analysis (PCA) has been carried out on cowpeas. Among the twenty-two cowpea genotypes, Gerrano *et al.* (2019) found four principal components (PCs) that accounted for 73.62% of the total explained variation. Leaf width, pod diameter, pod length, and grain yield were the first major component; leaf length and plant height were the second; number of branches, number of leaves, and number of seeds per plant were the third; and hundred seed weight was the fourth (Gerrano *et al*., 2019). Based on 87 cowpea accessions, (Saidaiah *et al*., 2021); reported that 90.87% of the overall cumulative variability was attributed to three primary factors.. The first main factor associates with flowering and maturity days, the second main factor related to pod length and pod weight, and the third factor related to number of pod and pod yield. Furthermore, on cowpea selection, (Sousa *et al*., 2015) reported that there are two main components which contributed to 61.60% of total cumulative variability, the primary factors associated with life precocity (flowering and maturation) and yield (pod length, beans/pod, and productivity/plot), respectively. From these reports, the characters that contribute to each of the main components varied, this might be due to variation the genotype and environment of the test cowpea.

PCA bi-plots and heatmap were widely used to identify genotypes that have multiple traits. Cowpea genotype distribution according to the primary components 1 and 2 and the characteristics for the observed traits were presented in **Figure 8**.

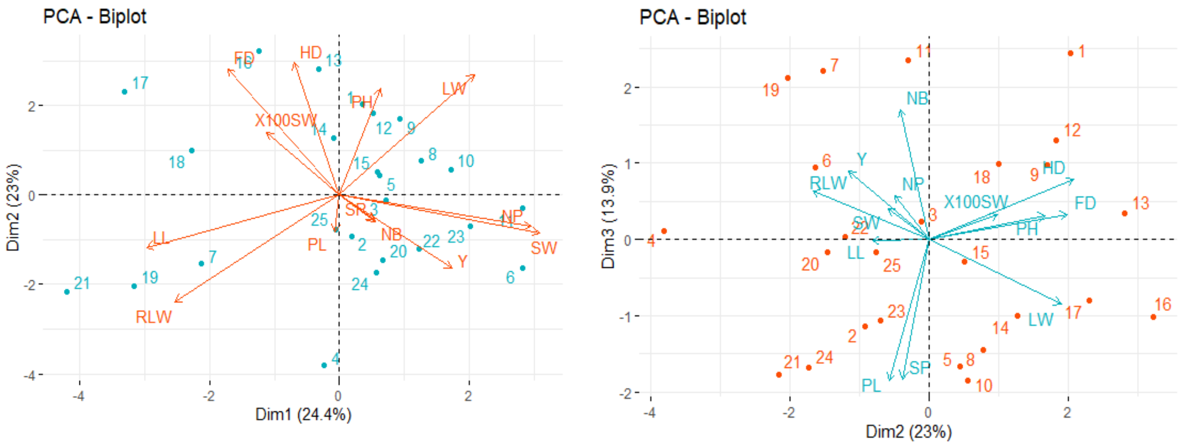


Figure 8. Principal components 1 (PC1) and 2 (PC2) showing the spatial distribution of the 25 cowpea genotypes. plant height (PH), number of branches (NB), leaf Length (LL), leaf width (LW), ratio LL/LW (RLW), flowering day (FD), harvesting Day (HD), number of pod (NP), pod length (PL), number of seed per pod (SP), seed weight per plant (SW), weight of 100 seeds (W100S), yield (Y).

Cowpea genotypes number 7, 9, and 21 were grouped together based on large ratio of leaf length and width and small leaf width. The characteristics of this group are lanceolate-shaped leaves showed in the large ratio of the leaves length and width. Similarly, genotypes number 3, 4, 6, 22 were grouped together based on yield and pod length. This group consists of genotypes that potential for high yields and early maturity useful for breeding of salinity. Genotype number 17 has a large seed size.

The heat map for genotypes and trait in this study was presented in **Figure 9**. Cowpea genotypes showed variability for the observed characters. Based on the heatmap, it can be showed that there were a variation for number of pod (NP), seed weight (SW), hundred seed weight (100 SW). The variation in leaf length (LL) and days to harvesting (FD were smaller compared to the above traits.

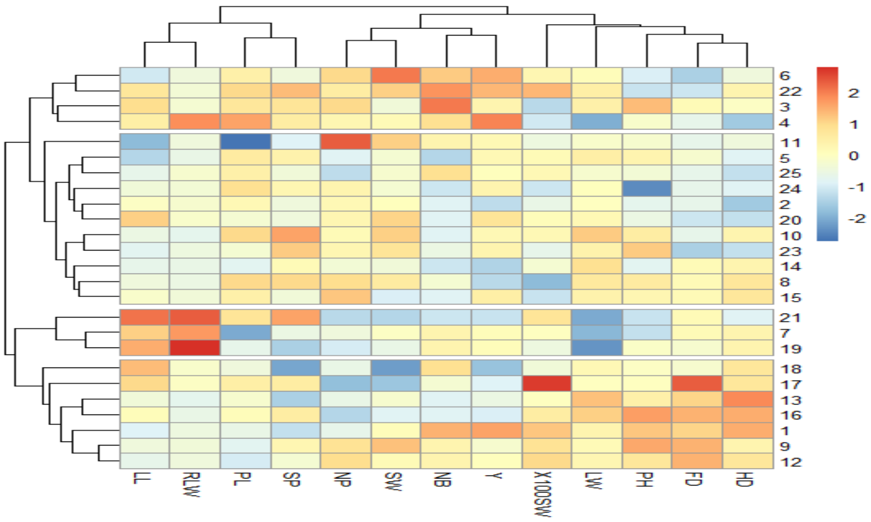


Figure 9. Heatmap of genotypes and morphological characteristics of cowpea.

The genotypes used in this study were classified into 4 clusters (**Table 4**). The first cluster was the largest with 11 genotypes (2, 5, 8, 10, 11, 14, 15, 20, 23, 24, 25) were dominated by genotypes with early maturity and smaller seed size. Because they have the shortest crop length, varieties with early flowering and maturity work well in cropping systems. The second cluster consists of 3 genotypes (7, 19, 21) with lanceolate leaf shape and low yield. The third cluster had 7 genotypes (1, 9, 12, 13, 16, 17, 18) were characterized by shorter pods genotypes, and the fourth cluster consists of 4 genotypes (3, 4, 6, 22) with high yield, long pods, and bigger seeds (**Table 4**).

Table 4. Mean values and standard deviation of four groups of cowpea in saline soil

Characters	Cluster			
	1	2	3	4
Plant height (PH)	56.5±6.15	59.4±3.87	56.2±4.55	57.1±7.15
Number of braches (NB)	1.9±0.24	1.8±0.29	2.1±0.30	2.1±0.18
Leaf Length (LL)	11.80±0.76	12.27±0.50	12.57±0.86	12.76±0.91
Leaf width (LW)	8.29±0.70	8.58±0.40	6.71±0.90	7.26±2.19
Rasio LL/LW (RLW)	1.32±0.17	1.35±0.52	2.17±0.22	1.99±1.04
Flowering day (FD)	38.5±1.29	41.3±0.58	39.8±1.81	40.0±1.71
Harvesting Day (HD)	64.7±2.21	67.1±1.73	65.9±1.46	65.5±2.16
Number of pod (NP)	8.7±1.49	7.3±0.71	7.9±1.58	7.3±0.54
Pod length (PL)	16.0±1.66	15.7±2.15	15.0±0.81	16.7±0.77
Number of seed per pod (SP)	13.4±1.53	12.8±3.08	11.8±2.09	14.0±1.46
Seed weight per plant (SW)	59.3±9.12	46.4±8.70	51.1±15.06	51.6±13.67
Weight of 100 seeds (W100S)	12.6±1.05	13.6±0.97	13.3±1.48	13.9±1.96
Yield (Y)	1.79±0.28	1.48±0.26	1.75±0.36	1.81±0.24
Number of genotypes	11	3	7	4
Genotype number	2, 5, 8, 10, 11, 14, 15, 20, 23, 24, 25	7, 19, 21	1, 9, 12, 13, 16, 17, 18	3, 4, 6, 22
Group characteristics	Early maturity, smaller seed size	Tall plant, lanceolate leaf shape, medium maturity, low yield	Shorter pods	High yield, long pods, bigger seed size

Based on the results of testing in the greenhouse, there were 10 genotypes with STI above average. Three genotypes came from group 1 (2,8,10), 5 genotypes from group 3 (1,9,12,13,17), and two genotypes from group 4 (4,6). These three groups had higher seed yields than group 2 (**Table 4**). A positive correlation was found between seed yield in the saline stress in the greenhouse and seed yield in the saline stress in the field (r=0.38*), indicated that genotypes with high yields in the greenhouse test also showed high yields in the field.

Cowpeas were chosen for salinity tolerance based on their capacity to yield seeds under stressful conditions. The average seed yield of cowpea 1.63 $t.h^{-1}$ ranged from 1.03–2.31 t ha^{-1}. Based on seed yields, there were four cowpea genotypes such as MLG- 17013, MLG- 17042, MLG- 17055, and KT 6 (genotypes number 1, 4, 6,and 22) recorded higher seed yield (above the average plus standard deviation, 1.98 $t.ha^{-1}$) with seed yield 2.18 $t.ha^{-1}$, 2.31 $t.ha^{-1}$, 2.14 $t.ha^{-1}$, 2.12 $t.ha^{-1}$, respectively. Three genotypes from the fourth cluster (genotype number 1,4, 6) and one genotypes (number 22) from the third cluster. Genotypes number 19, 20, 2, 22,23, 24, and 25 were cowpea varieties in Indonesia (KT 1, KT4, KT 5, KT6, KT 7, KT 8, and KT9) with seed yields ranging from 1.12 -2.12 t ha^{-1} (**Table 5, Figure 10**).

Table 5. Yield and yield components of the selected cowpea genotypes

No	Genotype	PH	NB	LL	LW	RLW	FD	HD	PN	PL	SP	SW	100SW	SY
1	MLG- 17013	63.4	2.4	11.7	7.9	1.48	42	69	6.5	15.1	10.8	50.7	14.94	2.18
3	MLG- 17027	63.8	2.6	13.5	8.1	1.66	40	65	9.1	17.1	14.6	44.2	10.99	1.74
4	MLG- 17042	54.4	2.2	13.1	3.6	3.69	38	61	8.0	18.5	14.2	51.0	11.43	2.31
5	MLG- 17051	57.6	1.4	11.2	8.2	1.37	39	63	6.3	16.9	13.8	45.8	13.37	1.71
6	MLG- 17055	49.4	2.3	11.5	7.5	1.53	36	64	9.1	16.7	12.2	75.2	13.48	2.14
7	MLG- 17057	47.8	2.0	13.7	3.8	3.61	40	66	6.9	12.9	12.0	48.5	13.04	1.66
10	MLG- 17104	59.0	1.6	12.0	9.4	1.28	38	66	7.8	17.5	16.2	63.3	13.45	1.70
11	MLG- 17168	54.2	2.0	10.7	7.0	1.53	38	64	11.1	11.8	11.4	63.5	12.28	1.70
15	MLG- 17204	57.2	1.6	12.4	8.0	1.56	40	67	9.4	16.7	12.4	37.1	11.25	1.81
19	KT 1	54.6	2.0	14.1	3.0	4.64	39	66	6.0	15.0	10.2	41.5	12.34	1.67
20	KT 4	52.0	1.6	13.7	7.7	1.79	37	62	8.0	15.6	12.2	62.3	13.26	1.89
21	KT 5	48.0	1.5	14.7	3.5	4.20	40	63	5.5	17.2	16.2	31.2	14.25	1.22

22	KT 6	48.0	2.5	13.3	8.2	1.63	37	66	8.4	17.5	15.7	63.1	15.24	2.12
23	KT 7	63.0	1.7	11.8	8.0	1.49	36	62	8.0	15.6	15.4	58.8	12.07	1.75
24	KT 8	40.0	1.5	12.2	7.4	1.65	38	63	8.1	17.4	13.6	45.8	11.35	1.75
25	KT 9	53.4	2.2	11.9	6.9	1.74	38	62	5.6	16.7	12.4	46.3	13.40	1.65
	Average	55.5	1.9	12.6	7.3	2.00	40	65	7.6	16.0	13.1	49.2	13.1	1.63
	Sd	6.36	0.4	1.01	1.8	0.96	2.35	2.5	1.5	1.52	1.92	12.7	1.5283	0.35

Note: PH = plant height; NB = number of braches; LL = leaf Length; LW = leaf width; RLW = rasio of LL/LW; FD = flowering day; HD = harvesting day; PN =pod number; PL = pod length; SP = seed number per pod; SW = seed weight per plant; 100 SW = 100 seed weight; SY = seed yield

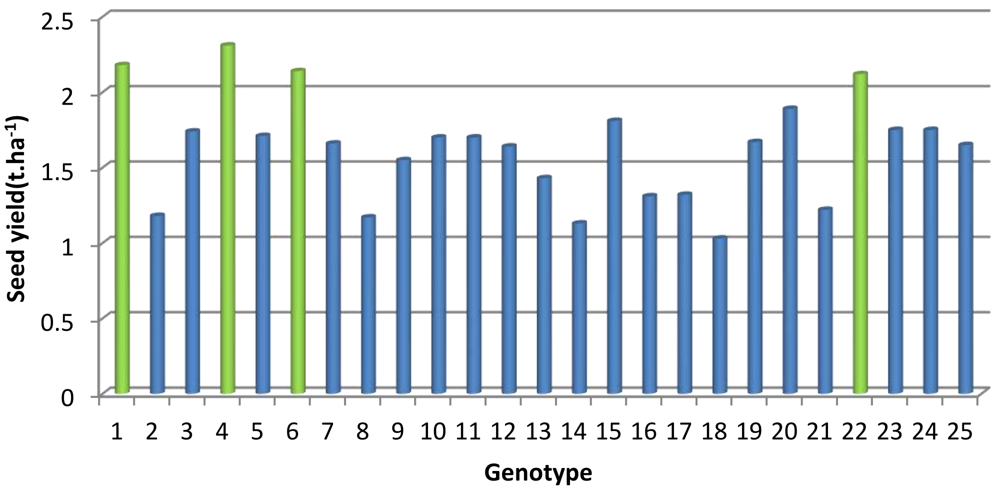


Figure 10. Seed yield of 25 cowpea genotypes.

MLG 17013 is a cross line between KT 9 and TVx 1850-01E, while MLG 17042 is a descendant of a cross between Tvx 4661 and KT 5, and MLG 17055 is a derivative of a cross between Harapan and KT 4. To improve cowpea adaptability in salty soil, these genotypes may be taken into consideration.

4. Conclusion

Different cowpea genotypes responded differently to salt stress compared to non-salt stress. The genotype was not always associated with a high yield in a salt environment or a non-stress environment. The positive correlation was observed between Stress Tolerance Index (STI) and yield on salt stress (Y_s) (0.89**) and yield in non-salt stress (Y_p) (0.54**). In both stress and non-stress situations, genotypes with high STI also generated high yields. Pod number and seed yield exhibited the greatest stress under salt stress conditions, with stress intensity greater than 0.70. Four clusters of cowpea genotypes were identified, with 11, 3, 7, and 4 genotypes in each cluster. A positive association was observed between seed yield in the saline stress in the greenhouse and seed yield in the saline stress in the field (r=0.38*), indicated that genotypes with high yields in the greenhouse test also showed high yields in the field. Based on seed yields, four genotypes (MLG 17013, MLG 17042, MLG 17055, and KT 6) yielded above 2.0 t ha^{-1}. To increase cowpea adaptability in salty soil, these genotypes may be taken into consideration.

Funding

This research received no external funding.

Author Contributions

Conceptualization: T., M. J. M.

Methodology: T., N., R. S., M. J. M.

Investigation: T., N., R. S., P., Y. B., G. W. A. S., A. S., H. K., F. C. I., R. D. P., K. N., S., A. H., Y. P., M. J. M.

Data Curation: T., N., R. S., P., Y. B., G. W. A. S., A. S., H. K., F. C. I., R. D. P., K. N., S., A. H., Y. P.

Formal analysis: T., N., R. S., P., Y. B., G. W. A. S., A. S., H. K., F. C. I., R. D. P., K. N., S., A. H., Y. P.

Writing original draft: T., N., R. S., P., Y. B., G. W. A. S., A. S., H. K., F. C. I., R. D. P., K. N., S., A. H., Y. P., M. J. M.

Review and editing: T., N., R. S., P., Y. B., G. W. A. S., A. S., H. K., F. C. I., R. D. P., K. N., S., A. H., Y. P., M. J. M.

Correspondence: T., M. J. M.

All authors read and approved the final manuscript.

Declarations

Clinical trial registration: Not applicable

Consent to publish: Not applicable.

Ethics approval and consent to participate

The plant materials used in this study were obtained from the Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Republic of Indonesia. Permission for the use of the plant materials in this study was granted by ILETRI. All procedures complied with relevant institutional, national, and international guidelines.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Acknowledgments

The authors would like to thank the Indonesian Legumes and Tuber Crops Research Institute, Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture of the Republic of Indonesia, for providing the breeding materials used in this study.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alharbi, A. 2017. Effect of Mulch on Soil Properties Under Organic Farming Conditions in Center of Saudi Arabia. *Mechanization in Agriculture & Conserving of the Resources*, *63*(4), 161–167.

Allel, D., Benamar, A., Badri, M., Abdelly, C. 2019. Evaluation of salinity tolerance indices in north african barley accessions at reproductive stage. *Czech Journal of Genetics and Plant Breeding*, *55*(2), 61–69. https://doi.org/10.17221/50/2017-CJGPB

Bashandy, T., El-Shaieny, A. 2016. Screening of Cowpea (Vigna unguiculata L. Walp) genotypes for salinity tolerance using field evaluation and molecular analysis. *Journal of Agricultural Chemistry and Biotechnology*, *7*(9), 249–255. https://doi.org/10.21608/jacb.2016.41126

Ceritoğlu, M., Erman, M. 2020. Determination of Some Agronomic Traits and Their Correlation with Yield Components in Cowpea. *Selcuk Journal of Agricultural and Food Sciences*, *34*(2), 154–161. https://doi.org/10.15316/sjafs.2020.210

Chen, C., Tao, C., Peng, H., Ding, Y. 2007. Genetic analysis of salt stress responses in asparagus bean (Vigna unguiculata (L.) ssp. sesquipedalis Verdc.). *Journal of Heredity*, *98*(7), 655–665. https://doi.org/10.1093/jhered/esm084

Ďzdemir, O., Ünlükara, A., Kurunc, A. 2009. Response of cowpea (Vigna unguiculata) to salinity and irrigation regimes. *New Zealand Journal of Crop and Horticultural Science*, *37*(3), 271–280. https://doi.org/10.1080/01140670909510273

Fernandez et al., G. J. 1992. Effective selection criteria for assessing plant stress tolerance. *Proc. Int.*

Symp. Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress, August 13−16, 1992, 257–270.

Gerrano, A. S., Jansen van Rensburg, W. S., Kutu, F. R. 2019. Agronomic evaluation and identification of potential cowpea (Vigna unguiculata L. Walp) genotypes in South Africa. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *69*(4), 295–303. https://doi.org/10.1080/09064710.2018.1562564

Harnowo, D., Ginting, E., Trustinah, Rozi, F. 2016. Recent achievement in research and development of pulses in Indonesia. In S. Dasgupta & I. Roy (Eds.), *Proceedings of the regional consultation on the promotion of pulses in Asia for multiple health benefits. Food and Agriculture Organization of thr United Nations. International Year of Pulses. Monograph (Technical Report)* (pp. 73–83). FAO. http://eprints.icrisat.ac.in/id/eprint/14205

Himaya, S. M. M. S., Prapagar, K., Afreen, S. M. M. S. 2019. The Influence of Salt Stress on Yield of Vegetable Cowpea (Vigna unguiculata). *International Journal of Research Publications*, 1–7.

Islam, M. M., Haque, M. S., Sarwar, A. G. 2019. Salt tolerance of cowpea genotypes during seed germination and seedling growth. *Journal of the Bangladesh Agricultural University*, *17*(1), 39–44. https://doi.org/10.3329/jbau.v17i1.40661

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B. C., Nammi, S., Liyanage, R. 2018. Cowpea: an overview on its nutritional facts and health benefits. *Journal of the Science of Food and Agriculture*, *98*(13), 4793–4806. https://doi.org/10.1002/jsfa.9074

Jones, Jr., J. B.(2003. Agronomic Handbook. In *Agronomic Handbook*. CRC Press. https://doi.org/10.1201/9781420041507

Karolinoerita, V., Annisa, W. 2020. Land Salinization and Its Problems in Indonesia. *Jurnal Sumberdaya Lahan*, *14*(2), 91. https://doi.org/10.21082/jsdl.v14n2.2020.91-99

Karuwal, R. L., Suharsono, Tjahjoleksono, A., Hanif, N. 2021. Short Communication: Characterization and nutrient analysis of seed of local cowpea (Vigna unguiculata) varieties from Southwest Maluku, Indonesia. *Biodiversitas*, *22*(1), 85–91. https://doi.org/10.13057/biodiv/d220112

Kaur, R., Kaur, H., Singh, S., Jagota, N., Bindra, S., Sharma, A. 2023. Optimization of stress tolerance index for screening of multiple abiotic stress tolerant genotype and determination of reliable trait in Cicer arieitnum L. *Total Environment Research Themes*, *8*(August), 100073. https://doi.org/10.1016/j.totert.2023.100073

Lacerda, C. F., Sousa, G. G., Silva, F. L. B., Guimarães, F. V. A., Silva, G. L., Cavalcante, L. F. 2011. Soil Salinization and Maize and Cowpea Yield in the Crop Rotation System Using Saline Waters Salinização do solo e rendimento de milho e feijão-caupi no sistema de rotação de safra usando águas salinas. *Eng. Agríc. Jaboticabal*, *31431*(4), 663–675.

Mofokeng, M. A., Mashilo, J., Rantso, P., Shimelis, H. 2020. Genetic variation and genetic advance in cowpea based on yield and yield-related traits. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *70*(5), 381–391. https://doi.org/10.1080/09064710.2020.1749295

Posit. 2022. *RStudio Desktop Professional Edition Administration Guide* (2002.02.3; pp. 1–22). Posit.

Ravelombola, W. S., Shi, A., Weng, Y., Clark, J., Motes, D., Chen, P., Srivastava, V. 2017. Evaluation of salt tolerance at germination stage in cowpea [Vigna unguiculata (L.) walp]. *HortScience*, *52*(9), 1168–1176. https://doi.org/10.21273/HORTSCI12195-17

Saidaiah, P., Rao, P. S., Geetha, A., Kumar, A. K., Kamala, V. 2021. Assessment of genetic diversity based on principal component analysis in vegetable cowpea (Vigna unguiculata (L.) Walp.) germplasm. *Annals of Phytomedicine: An International Journal*, *10*(1). https://doi.org/10.21276/ap.2021.10.1.22

Salih, H. O., Kia, D. R. 2013. Effect of salinity level of irrigation water on cowpea (Vigna Unguiculata) growth. *IOSR Journal of Agriculture and Veterinary Science*, *6*(3), 37–41. https://doi.org/10.9790/2380-0633741

Sehrawat, N., Yadav, M., Sharma, A. K., Kumar, V., Jaiwal, P. K., Bhat, K. V, Sairam, R. K. 2018. Seedling Vigor : An Important Criterion for the Selection of Salt Tolerant Lines for Mungbean [Vigna radiata (L .) Wilczek]. *Thai Journal of Agricultural Science*, *51*(3), 119–132.

Sousa, C. C., Damasceno-Silva, K. J., Bastos, E. A., Rocha, M. M. 2015. Selection of cowpea progenies with enhanced drought-tolerance traits using principal component analysis. *Genetics and Molecular Research*, *14*(4), 15981–15987. https://doi.org/10.4238/2015.December.7.10

Taufiq, A., Purwaningrahayu, R. D. 2013. Response of mungbean (Vigna radiata L.) varieties to salinity stress. *Food Crops Agric. Res.*, *32*(3), 159–170. https://doi.org/10.21082/jpptp.v32n3.2013.p159-170

Trustinah, Iswanto, R., Hapsari, R. 2016. Tolerance of mungbean F3 lines to salinity stress. In R. AA (Ed.), *Proc. Legume Tuber Technol.* (pp. 489–497). Pusat Penelitian dan Pengembangan Tanaman Pangan.

Trustinah, Kasno, A., Wijanarko, A., Iswanto, R., Kuswantoro, H. 2007. Response of legume crops in acid soil. *Inovasi Teknologi Kacang-Kacangan Dan Umbi-Umbian Mendukung Kemandirian Pangan Dan Kecukupan Energi*, 200–207. https://balitkabi.litbang.pertanian.go.id/wp-content/uploads/2017/11/prosiding-2007-21_trustinah.pdf

Trustinah, Nugrahaeni, N. 2021. Cowpea genotypes responses to salinity stress. *IOP Conference Series: Earth and Environmental Science*, *743*(1). https://doi.org/10.1088/1755-1315/743/1/012036

Trustinah, Taufiq, A., Iswanto, R. 2019. Evaluation of Mungbean Genotypes Tolerance to Salinity Stress. *Jurnal Agronomi Indonesia (Indonesian Journal of Agronomy)*, *46*(3), 269–275. https://doi.org/10.24831/jai.v46i3.16220

Wahid, A., Hameed, M., Rasul, E. 2004. Salt-induced injury symptoms, changes in nutrient and pigment composition, and yield characteristics of mungbean. *International Journal of Agriculture & Biology*, *6*(6). http://www.fspublishers.org/ijab/past-issues/IJABVOL_6_NO_6/49.pdf