Cowpea genotypes responses to salinity stress

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Abstract. Growth and yield of copea can be affected by abiotic stress such as salinity. Aim of this study was to analyze the tolerant level of cowpea againstsalinity. The study was conducted in the greenhouse of Iletri at Malang, East Java during the dry season of 2017. This study conduct using factorial completely randomized design with two replications. The first factor was two environments (non salt stress and salt stress with soil electrical conductivity 9.5 dS m⁻¹). The second factor was 125 cowpea genotypes. Data measurement included plant height, number of branches, number of nodes, pod number, pod length, number of seeds per pods, and seed weight. The soil used in the trial was highly saline, and the stress intensity were ranged from 0.25 to 0.88 for all the observed characters. The genotypes responded variably to salt stress, and there were a very significant effect on all the characters except the number of nodes. Seed yield and pod number per plant experienced the highest stress level with stress intensity above 0.80. Based on seed yield and the tolerance index, six genotypes were tolerant to the high salinity. The six genotypes were MLG 17019, MLG 17055, MLG 17060, MLG 17186, KT 4, and KT 6. Selected genotypes were prospective for development in the improvement of cowpea varieties tolerance to salinity.

Key word: index, tolerance, stress, salinity, Vigna unguiculata L. Walp.

1. Introduction
As the population grows, the demand for food is increasing. Therefore there is no other more effective way to reduce dependency other than diversifying food rationally by optimizing the competitive advantage of local resources to produce nutritious, affordable, and synergetic food with other. On the other hand, with limited optimal land, agricultural food crops in the 21st century penetrated into marginal dry land, including saline land. All potential food crop commodities which are now classified as minor, in the future can be used as alternative commodities in sub-optimal land such as saline land.

Cowpea (Vigna unguiculata) is one of the legume commodities that has long been known and planted by people in Indonesia and is used as food and feed. This plant is part of biodiversity that has not been well utilized in agronomic, genetic, economic and socio-cultural aspects. The dry ingredients of cowpea protein 23-32%, carbohydrates 50-60%, and fat 1%, and have amino acids that are complementary to cereals so it is very important for nutritional food in the human diet [1]. As food is usually used in traditional consumption. Besides being tolerant of drought, cowpea is also tolerant of soil acidity. Of the four bean commodities tested on acidic soils with high Al content, it shows that cowpea is tolerant of soil acidity compared to soybeans, peanuts, and mungbeans [2]. With these characteristics, cowpea is very prospective to be developed in dry land and other sub-optimal lands such as salinity in order to increase land productivity.
Salinity is one of the abiotic stresses that greatly influences plant growth and yield. The area of saline land in Indonesia of about 0.4 million ha is spread including those located along the northern and southern coasts of Java, in the regions of Aceh and Nias due to the tsunami, South Sulawesi and Flores. High salinity is characterized by high salt content in soil or agricultural land so that it can interfere with plant growth and yield. Jones (2002) classified soil salinity into nonsaline (EC <1.0 dS m⁻¹), very slightly saline (EC 1.1-2.0 dS m⁻¹), moderately saline (EC 2.1-4.0 dS m⁻¹), saline (EC 4.0-8.0 dS m⁻¹), strongly saline: (EC 8.1-16.0 dS m⁻¹), and very strongly saline (EC >16.0 dS m⁻¹) [3].

Salinity affects all phases of plant growth, from germination to the end of the reproductive period. Salinity stress in cowpea causes delays in germination, a low percentage of germination, a decrease in the number of plants that can survive, an increase in dead plants, a decrease in plant height, plant fresh weight, root length, GSTI, and increases germination time and the amount of variation between genotypes [5-8]. Seed and pod yields decreased significantly at EC above 9.0 dS m⁻¹ [9], decreased 72% at EC 7 dS m⁻¹, and 100% at EC 10.5 dS m⁻¹ [10]. The magnitude crucial in genotype evaluation. Concentration that can be used for salt toleration testing in cowpea are 150 ml NaCl [11].

Tolerance is defined as the ability of plants to continue to grow in a stress environment. Fernandez (1992) used several criteria for identifying the effectiveness selection of mungbean, Mean Productivity (MP), Tolerance (TOL), Stress Susceptibility Index (SSI), Stress Tolerance Index (STI) [12]. The fourth index is also used by Khodarahmpouret et al. (2011) in maize [13] and Allel et al. (2019) in barley [14]. In cowpea, Chen et al. (2017) used the Salt Response Index (SRI) [6], Ravelomba et al. (2017) used 4 parameters to evaluating cowpea genotype tolerance to salinity stress, Absolute Decrease (AD), Inhibition Index (II), Relative Salt Tolerance (RST), and Salt Tolerance Index (STI) [11], and Bashandy and El-Shaieeny (2016) used stress tolerance index (STI) and stress susceptibility index (SSI) [15]. SNPI (stress-non stress production index) is used to select wheat genotypes that are tolerant to drought stress and high productivity ([16-19]). Whereas Akscura et al. (2011) suggested the use of SSI as an indicator in drought-tolerant local wheat breeding in Turkey [20]. In rice plants, Raman et al. (2012) used MYI (mean yield index) combined with the appearance of genotypic appearance in various environments to help plant breeders get superior rice in various circumstances [21].

In Indonesia, cowpea cultivation area was about 14,000 ha with productivity of 0.6 ton.ha⁻¹ [22]. The yield gap (the difference between potential yield and yield realized at farmer level) in cowpea is relatively high ranging from 0.7-1.5 ton.ha⁻¹. There is little information about cowpea yield in saline. The purpose of this study was to evaluate the response of cowpea genotypes to salinity stress.

2. Material and Methods

Evaluation of cowpea genotypes was conducted in the dry season of 2017 at the ILETRI’s screen house in Malang, East Java using factorial completely randomized design, two replications. Research material consisted of 125 cowpea genotypes included of 18 local varieties, 8 superior varieties, 17 introductions, and 83 breeding lines. The first factor was two environments (non salt stress and salt stress with soil electrical conductivity 9.5 dS m⁻¹), and the second factor was 125 cowpea genotypes. Cowpea was planted in a pot containing 6.5 kg of soil. Before planting, the soil was given Phonska fertilizer (15% N, 15% P2O5, 15% K2O, 10% SO4) at a dose of 0.95 g per pot. Each genotype was planted two seeds per pot. Watering was done with tap water (non salt). Plant maintenance was carried out intensively by controlling pests and weeds. Soil electrical conductivity was measured using Hanna portable EC meter type HI1993310.

Plant data observed in this experiment included: plant height, number of branches, number of nodes, pod number, pod length, number of seeds per pods, and seed weight. Data were subjected to analyses of variance (ANOVA) using MSTAT-C software. Assessment of cowpea genotype tolerance using Stress intensity (SI), Mean productivity (MP), Tolerance (TOL), Stress susceptibility index (SSI), Geometric mean productivity (GMP), and Stress tolerance index (STI) refers to Fernandez (1992). Salt response index (SRI) and plant growth score on salinity using salt injury grading criteria on a 0-4 scale.
[6] salt injury categorize as scale 0 if 100% of plant survive without any damage; categorize as 1 or mild injury when there are small area (approximately 1/5) of leaf apex and leaf margin turning yellow; categorize as moderate (2) when 1/2 leaf apex and leaf margin became chlorotic; severe (3) when majority of apexes and leaf margin turned yellow and categorize as extreme salt injury (4) when leaves fell off, stem became shrunken, and the plant eventually died.

The index formula refers to Fernandez (1992) and (Chen et al. 2007) as follows [6,12]:

\[
\text{Stress intensity (SI)} = 1 - \frac{\bar{Y}_r + \bar{Y}_p}{\bar{Y}_s} \\
\text{Mean productivity (MP)} = \frac{(Y_s + Y_p)}{2} \\
\text{Tolerance (TOL)} = \frac{Y_p - Y_s}{(Y_p - Y_s)} \\
\text{Stress susceptibility index (SSI)} = \frac{1 - (Y_r/Y_p)}{Y_s} \\
\text{Geometric mean productivity (GMP)} = \sqrt[\bar{Y}_s x \bar{Y}_p} \\
\text{Stress tolerance index (STI)} = \frac{Y_p}{(Y_s/Y_p)} \\
\text{Relative salt tolerance (RST)} = \frac{Y_s}{Y_p} \\
\]

3. Results and Discussion

The analysis of variance showed cowpea genotypes varied for all the traits observed except number of branches. The environments affected a very significantly on all characters observed except the number of branches, and the response of various genotypes to saline stress for plant height, number of branches, number of pods. Under non salt conditions plant height was between 20.8-53.5 cm (average 38.4 cm), number of branches 2, number of nodes 7-17 (average 11), number of pods 3-14 pods (average 8), seed weight 4.43-26.1 g (average 8.4 g), pod length 10.2-17.6 cm (average 10.5 cm), and number of seeds per pod 6-14 (average 10) (Table 1).

Saline stress during the trial was considered high as shown by soil EC above 9 dSm⁻¹. The cowpea genotypes were able to germinate, but the germination time were varied, ranging from 10-14 days, longer compared to that in the non salt condition which ranged from 7-10 days. This condition showed stress has occurred since the beginning of growth, characterized by slow growth. Plant height between 5.3-10.8 cm (average 12.6 cm), number of branches 2, number of nodes 2-10 (average 5), number of filled pods per plant 0-4 pods (average 2), seed weight of 0-3.11 g (1.4 g average), pod length of 3.3-17.9 cm (1.5 cm average), and number of seeds per pod 1-12 (average 6) (Table 1).

Stress intensity ranged from 0.25 to 0.88, an indication of moderate to highly stress intensity. Under salt stress conditions cowpea became shorter, number of branches and nodes, pods number, pod length were reduced, as well as the seed weight, and the yield loss reached 84%. Among those traits pod number and seed yield showed the highest stress with stress intensity above 0.80. Seed weight and pod number decreased were 84% and 81%, respectively (Table 1). Longer germination time, lower percentage of germination and number of plants that can survive on cowpea are also reported [4,7-8]. Salinity affected seed germination by generating osmotic potential stresses that limit external osmotic potential that limits water absorption due to the toxic effects of Na + and Cl- ions during seed germination. Salinity also reduces the availability of nutrients and transportation to plant growing areas so that it affects the vegetative and generative phases and also has an effect on decreasing quality at plant height [23]

| Agronomic traits         | Non salt Mean | Non salt Range | Salt stress Mean | Salt stress Range | Stress Intensity (SI) | Yield loss (%) |
|--------------------------|---------------|----------------|------------------|-------------------|----------------------|---------------|
| Germination time (day)   | 7             | 7-9            | 12               | 10-14             | 0.44                 | 44            |
| Plant height (cm)        | 38.4          | 20.8-53.5      | 21.6             | 5.3-10.8          | 0.45                 | 45            |
| Number of branches       | 1.8           | 0.3-3.5        | 1.0              | 1-1.0             |                      |               |
Mild salt injury had been seen since three weeks after planting (WAP) and the damage severity was increase until the sixth week after planting. Cowpea genotype showed varied responses to salinity stress from moderately tolerant to very sensitive, indicated by a score salt injury grading criteria of 2-4 (Table 2). Most genotypes were able to survive until the reproductive stage as shown for its ability to flower, produce pods, and seeds. Cowpea with moderate stress since the beginning of growth produced only a few pods with an average of 2 pods per plant, shorter plants, fewer nodes, shorter pods, fewer number of seeds per pod, and very low seed yield (Table 1). Some studies also report symptoms of salt poisoning in salinity characterized by dwarf plant growth, chlorosis and necrosis in young and old leaves, smaller leaf size with a greener color, leaves quickly fall, and in severe conditions the leaves turn yellow, and edges the leaves dry [24-25]. Salinity stress since the beginning of vegetative growth in cowpea is very influential on plant dry weight, weight and number of young and old pods, and seed yield. The effect would be reduced if the stress occurs after flowering or pod filling phase [26].

### Table 2. Salt injury grading criteria of 125 cowpea genotypes under stress salinity.

| Grading | Cowpea performance | Number of cowpea genotypes |
|---------|--------------------|----------------------------|
|         |                    | 3 WAP | 5 WAP | 6 WAP | 7 WAP |
| 0       | no salt injury, 100% survival without damage | 8     | 4     | 1     | 0     |
| 1       | mild salt injury, indicated by small area (approximately 1/5) of leaf apex and leaf margin turning yellow | 116   | 110   | 2     | 0     |
| 2       | moderate salt injury when 1/2 leaf apex and leaf margin became chlorotic | 1     | 11    | 119   | 121   |
| 3       | severe salt injury when majority of apexes and leaf margin turned sallow | 0     | 0     | 3     | 3     |
| 4       | extreme salt injury when leaves fell off, stem became shrunken, and the plant eventually died | 0     | 0     | 0     | 1     |

WAP= weeks after planting

Analysis of variance showed genotype, environment and the interaction of genotype and environment for seed weight were significant. This indicated that response of cowpea genotypes varied from non salt stress and salt stress. There was no significant correlation between seed weight (yield) under salt stress and non stress environment. The result suggested that high yield in non stress environment and in salt environment were not necessarily found on the same genotype. Based on several tolerance indices there were a high positive correlation between yield under salt stress (Ys) with geometric mean productivity (GMP), stress tolerance index (STI), and salt response index (SRI) and high negative correlation with stress susceptibility index (SSI) (Table 3). This indicated that genotypes with high STI, GMP, SRI, SRI, would have high yields under stress environment. STI had a positive correlation with yield on salt stress (Ys) and non salt stress (Yp), MP, TOL, GMP, and SRI showed that genotypes with high STI were also produced high yield in stress and non-stress conditions. MP was more suitable for yield under non stress conditions, whereas TOL did not correlate with yield under salt stress condition. Positive correlations between yields on stress environment (Ys) with MP, STI, GMP, Y1 and negative correlations with SSI, TOL were also reported in wheat [11-19]. The use of multiple tolerance indices to select the superior genotypes showed that geometric mean
productivity (GMP) and stress tolerance index (STI) were able to discriminate tolerant genotypes under stress conditions and producing high yield in stress and non stress conditions.

Under salt stress environment, most of the genotypes were survived until the end of the reproductive period but not all ones were able to produce mature pods and seeds. Seed weight per plant ranged from 0-3.11 g, an average of 1.38 g. STI values for seed weight ranged from 0-0.39 with an average of 0.17. Based on yield (seed weight) on salt stress (Ys) and using the STI, GMP and SRI indices MLG 17019, MLG 17055, MLG 17060, KT 4, MLG 17186, and KT 6 proved to be the most salt tolerant genotypes. Yield of these genotypes were above average seed weight plus 2 times the standard deviation (≥2.556 g) and were consistently had high STI, SRI, and GMP values (Table 4). Two of them are varieties, namely KT 4 and KT 6. In addition there were 15 genotypes with yield above average plus standard deviations (≥1,976 g), two of which were varieties KT 5 and KT 8. In general, selected genotypes showed higher plants, longer pods, higher number of seeds per pod, and higher seed yields in stress environment (Table 4).

Table 3. Correlation between parameters of cowpea tolerance index to salinity.

|       | Ys    | Yp   | MP   | TOL  | SSI  | GMP  | STI  |
|-------|-------|------|------|------|------|------|------|
| Yp    | -0.09**|      |      |      |      |      |      |
| MP    | 0.33**| 0.97**|      |      |      |      |      |
| TOL   | -0.16**| 0.97**| 0.88**|      |      |      |      |
| SSI   | -0.86**| 0.38**| 0.15**| 0.59**|      |      |      |
| GMP   | 0.85**| 0.56**| 0.74**| 0.34**| -0.50**|      |      |
| STI   | 0.77**| 0.66**| 0.82**| 0.46**| -0.39**| 0.95**|      |
| SRI   | 0.85**| -0.38**| -0.15**| -0.59**| -0.99**| 0.49**| 0.39**|

Ys= Yield on stress environment, Yp= Yield on non stress environment, MP= Mean productivity, TOL= Tolerance, SSI= Stress susceptibility index, GMP= Geometric mean productivity, STI= Stress tolerance index, SRI= Salt response index, ns= non significant, **= significant at P≤0.01

Table 4. The tolerance index parameters of the selected cowpea genotypes.

| No | Genotype | YP  | YS  | SSI | GMP | STI | SRI |
|----|----------|-----|-----|-----|-----|-----|-----|
| 1  | MLG 17055| 8.780| 3.105| 0.78 | 5.22 | 0.39 | 0.35 |
| 2  | P-30 (KT 6) | 8.085| 2.940| 0.77 | 4.88 | 0.34 | 0.36 |
| 3  | MLG 17060 | 9.240| 2.805| 0.84 | 5.09 | 0.37 | 0.30 |
| 4  | MLG 17168 (KT 4) | 8.000| 2.730| 0.79 | 4.67 | 0.31 | 0.34 |
| 5  | MLG 17019 | 8.325| 2.655| 0.82 | 4.70 | 0.31 | 0.32 |
| 6  | MLG 17186 | 8.430| 2.555| 0.84 | 4.64 | 0.31 | 0.30 |
| 7  | MLG 17013 | 8.045| 2.525| 0.83 | 4.51 | 0.29 | 0.31 |
| 8  | MLG 17108 | 8.637| 2.490| 0.86 | 4.64 | 0.30 | 0.29 |
| 9  | MLG 17044 | 7.195| 2.435| 0.80 | 4.19 | 0.25 | 0.34 |
| 10 | MLG 17051 | 6.065| 2.420| 0.72 | 3.83 | 0.21 | 0.40 |
| 11 | MLG 17188 | 9.050| 2.320| 0.90 | 4.58 | 0.30 | 0.26 |
| 12 | MLG 17091 | 9.845| 2.315| 0.92 | 4.77 | 0.32 | 0.24 |
| 13 | MLG 17057 | 7.820| 2.265| 0.86 | 4.21 | 0.25 | 0.29 |
| 14 | MLG 17104 | 9.960| 2.220| 0.94 | 4.70 | 0.31 | 0.22 |
| 15 | MLG 17042 | 8.755| 2.180| 0.90 | 4.37 | 0.27 | 0.25 |
| 16 | P-28 (KT8) | 6.480| 2.175| 0.80 | 3.75 | 0.20 | 0.34 |
| No | Genotype         | Plant height (cm) | Pod number | Pod length (cm) | Number of seeds per pod | Seed weight (g) |
|----|------------------|-------------------|------------|-----------------|-------------------------|-----------------|
|    |                  | N     | S     | N     | S     | N     | S     | N     | S     | N     | S     | N     | S     |
| 1  | MLG 17055        | 43.0  | 21.0 | 8     | 2     | 15.9 | 12.3 | 10    | 6     | 8.780 | 3.105 |
| 2  | P- 30 (KT 6)     | 43.8  | 33.0 | 6     | 2     | 15.9 | 15.3 | 12    | 9     | 8.085 | 2.940 |
| 3  | MLG 17060        | 40.0  | 27.0 | 9     | 2     | 15.1 | 12.8 | 9     | 6     | 9.240 | 2.805 |
| 4  | MLG 17168 (KT 4)| 40.8  | 21.0 | 7     | 1     | 17.9 | 15.7 | 12    | 9     | 8.000 | 2.730 |
| 5  | MLG 17019        | 40.3  | 19.4 | 8     | 2     | 15.3 | 7.4  | 9     | 5     | 8.325 | 2.655 |
| 6  | MLG 17186        | 39.3  | 24.7 | 8     | 2     | 14.2 | 13.3 | 9     | 8     | 8.430 | 2.555 |
| 7  | MLG 17013        | 37.8  | 23.0 | 7     | 2     | 14.4 | 13.8 | 10    | 8     | 8.045 | 2.525 |
| 8  | MLG 17108        | 38.0  | 22.3 | 7     | 2     | 15.6 | 15.2 | 11    | 9     | 8.637 | 2.490 |
| 9  | MLG 17044        | 36.0  | 20.8 | 8     | 2     | 13.1 | 12.9 | 7     | 5     | 7.195 | 2.435 |
| 10 | MLG 17051        | 39.0  | 23.5 | 7     | 2     | 13.7 | 12.5 | 8     | 9     | 6.065 | 2.420 |
| 11 | MLG 17188        | 45.0  | 21.3 | 9     | 2     | 12.6 | 11.6 | 10    | 6     | 9.050 | 2.320 |
| 12 | MLG 17091        | 39.0  | 22.5 | 7     | 2     | 16.0 | 13.7 | 10    | 8     | 9.845 | 2.315 |
| 13 | MLG 17057        | 32.0  | 24.5 | 9     | 2     | 14.0 | 11.8 | 9     | 7     | 7.820 | 2.265 |
| 14 | MLG 17104        | 53.5  | 21.0 | 9     | 2     | 16.1 | 15.2 | 10    | 8     | 9.960 | 2.220 |
| 15 | MLG 17042        | 41.5  | 37.8 | 10    | 2     | 14.3 | 11.1 | 7     | 3     | 8.755 | 2.180 |
| 16 | P- 28 (KT8)      | 39.3  | 32.2 | 8     | 2     | 11.4 | 11.3 | 10    | 10    | 6.480 | 2.175 |
| 17 | MLG 17027        | 34.5  | 20.0 | 8     | 2     | 14.0 | 13.2 | 12    | 9     | 7.665 | 2.125 |
| 18 | MLG 17189        | 20.8  | 18.0 | 6     | 2     | 12.3 | 11.4 | 9     | 5     | 5.190 | 2.065 |
| 19 | P- 02B (KT 5)    | 31.0  | 31.0 | 5     | 2     | 15.2 | 13.0 | 11    | 7     | 5.905 | 2.035 |
| 20 | MLG 17041        | 34.5  | 28.3 | 7     | 2     | 14.5 | 11.1 | 10    | 6     | 10.380| 2.020 |
| 21 | MLG 17030        | 31.8  | 21.5 | 7     | 2     | 15.0 | 13.8 | 9     | 8     | 9.785 | 1.990 |

Average 125 genotypes
Average selected genotype
Minimum
Maximum
Average+standard deviation
Average+2 standard deviation

Table 5. Plant height, pod number, pod length, number of seeds per pod, and seed weight of the selected cowpea genotypes.

Y = Yield on stress environment, Y≠ yield on non stress environment, SSI= Stress susceptibility index, GMP=Geometric mean productivity, STI=Stress tolerance index, SRI=Salt response index.
N=Non stress environment, S=Salt stress environment

4. Conclusion

The study revealed a considerable variation of tolerances among the cowpea genotypes under high salinity stress. Seed weight and number of pods were the most negatively affected by the stress. Based on stress tolerance index and yield of seeds six genotypes were belong to tolerant to high salinity. Those tolerant genotypes were prospective for developing cowpea varieties tolerant to salinity.

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