A Simple and Cost-effective Approach for Salt Tolerance Evaluation in Cowpea (*Vigna unguiculata*) Seedlings

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Abstract. Little has been done with respect to breeding for salt-tolerant cowpea (*Vigna unguiculata*) cultivars despite of salt stress being a growing threat to cowpea production. Seedling stage is one the most susceptible stages to salt stress in cowpea. Establishing a streamlined methodology for rapidly screening a large number of genotypes will significantly contribute toward enhancing cowpea breeding for salt tolerance. Therefore, the objective of this study was to establish and validate a simple approach for salt tolerance evaluation in cowpea seedlings. A total of 30 genotypes including two controls (PI582468, a salt-tolerant genotype, and PI255774, a salt-sensitive genotype) were greenhouse-grown under 0 mM and 200 mM NaCl. A total of 14 above-ground traits were evaluated. Results revealed: (1) significant differences were observed in average number of dead plants per pot, leaf injury scores, relative salt tolerance (RST) for chlorophyll, plant height, and leaf and stem biomass among the 30 genotypes; (2) all PI255774 plants were completely dead, whereas those of PI582468 were fully green after 2 weeks of salt stress, which validated this methodology; (3) RST for chlorophyll content was highly correlated with number of dead plants and leaf injury scores; (4) RST for leaf biomass was moderately correlated with number of dead plants and leaf injury scores; and (5) RST in plant height was poorly correlated with number of dead plants and leaf injury scores. Therefore, less number of dead plants per pot, high chlorophyll content, and less leaf injury scores were good criteria for salt tolerance evaluation in cowpea. This study provided a simple methodology and suggested straightforward criteria to evaluate salt tolerance at seedling stage in cowpea.

Cowpea [*Vigna unguiculata* (L.) Walp.] is a diploid legume species ($2n = 2x = 22$) widely grown in Africa, Asia, the Middle East, southern Europe, southern and western United States, and Central and South America. Worldwide cowpea production is estimated to be 5.4 million tons of cowpea grain annually and Africa is the leading producer (Olufajo 2012). Cowpea is cultivated on more than 14

million hectares (Singh et al., 2003). It provides good-quality nutrition for human consumption (Frota et al., 2008). In addition, cowpea can contribute toward protecting soils from being eroded because it is an excellent cover crop. In the western part of the United States, a growing interest in using cowpea as a cover crop has been noticed since cowpea can tolerate drought conditions (Agbicodo et al., 2009). However, increasing concerns due to salinity in this part of the country can limit the use of cowpea as a cover crop (Wilson et al., 2006). In semiarid regions in which cowpea cultivation is predominant, the low rainfall frequency could lead to salt compounds not being properly leached out, hence accumulating within soils and exacerbating salinity-related issues (Zhang et al., 2012).

Salinity is one of the major limiting factors that have been constraining agricultural production globally (Allakhverdiev et al., 2000). In croplands, salinity is due to an undesirable increase in the concentration of cations such as K^+ , Mg^{2+} , Ca^{2+} , and Na^+ and anions such as NO_3^- , HCO_3^- , SO_4^{2-} , and Cl^- according to Wallender and Tanji (2011). Salinity due to sodium chloride (NaCl) has been predominant (Ayers and Westcot, 1985); hence, tolerance to this type of salt was reported in this current investigation. The estimate of cropland areas facing salinity was more than 19.6 million hectares in the United States (Shannon 1997). Costs related to problems imposed by salinity on agriculture were 12 billion US dollars (Läuchli and Lüttge, 2002). Multiple factors such as rock weathering, deforestation, poor quality of irrigation water, and inadequate fertilization practices can worsen salinity on cultivated lands (Omami and Hammes, 2006).

Studies have shown that salt stress can cause serious concerns to cowpea production. Cowpea germination has been shown to be unfavorably affected by salt stress (Zahedi et al., 2012). Salt-stressed cowpea plants exhibited a reduced plant growth and vigor (Mini et al., 2015). Salt stress can impair plant physiology, photosynthesis, and absolutely important functions such as cell extension and division (Maas and Hoffman, 1977). These aforementioned factors could lead to a significant cowpea yield reduction (Dutta and Bera, 2014). Breeding for cowpea salt-tolerant cultivars is one of the most affordable solutions to tackle these issues. However, few studies have focused on addressing salt stress in cowpea in efforts to adequately providing breeders with critical information on the tolerance of cowpea genotypes to salinity.

Phenotyping is a substantial process in screening genotypes for a particular trait of interest. It is usually a labor-intensive, time-consuming, and a costly task to undertake for plant breeders. The increasing needs for accurate and less expensive phenomics require the establishment of a fast and cost-effective methodology. To the best of our knowledge, there is no reported methodology on salt tolerance phenotyping in cowpea. Salt phenotyping can be carried in fields. However, the uncontrolled factors such as differences in soil fertility, temperature, and transpiration could increase the unexplained part of the variation in salt tolerance among cowpea genotypes, thus leading to biased conclusions (Pathan et al., 2007). Hydroponic system has long been considered the ideal approach for salt tolerance phenotyping in crops. However, this requires adequate facilities and specialized skills (An et al., 2001), which could significantly increase the phenotyping cost.

Since cowpea is cultivated predominantly in developing countries, a methodology that can be applied in these areas in which funds and facilities are very limited would be most helpful. In addition, the screening methodology should allow for a rapid and accurate salt

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tolerance phenotyping of a large number of genotypes to be efficient. Seedling stage is one of the most vulnerable stages to salt stress in cowpea (Win and Oo, 2015). Suggesting a strategy that can help cowpea breeders select for salt-tolerant genotype at seedling stage is therefore important and can also assist with at least narrowing down the number of genotypes for salt tolerance screening at a later stage. Therefore, the objective of this study

was to establish an approach that can be easily applied for salt tolerance phenotyping for cowpea at seedling stage.

Materials and Methods

Plant materials. A total of 30 cowpea accessions originating from 13 countries were used in this study (Table 1). These genotypes were PIs from the United States

Department of Agriculture (USDA) Germplasm Resources Information Network cowpea germplasm accessions. Cowpea seeds were obtained from the USDA Plant Genetic Resources Conservation Unit at Griffin, GA. Seeds were increased in the summer of 2017 at the University of Arkansas, Fayetteville, AR. Of the 30 cowpea genotypes, PI582468 (salt-tolerant) and PI255774 (salt-susceptible) (Ravelombola et al., 2017), were used as control to validate the methodology. At the end of the experiment, the two extreme genotypes from the remaining 28 along with the aforementioned controls were independently repeated from the current investigation to further validate the results.

Growth conditions and experiment design. The experiment was conducted in the greenhouse of Harry R. Rosen Alternative Pest Control of the University of Arkansas, Fayetteville, AR (Fig. 1). Temperatures in the greenhouse were 26 °C/21 °C (day/night) and daylight length was 14 h. Cowpea plants were established in pots previously filled up with 100 g Sunshine Natural & Organic (Agawam, MA).

Holes were designed at the bottom of each pot, and paper was placed at the bottom of each pot as well to prevent soil medium from leaking during irrigation. In each pot, six to eight seeds were sown. When cowpea plants emerged, four vigorous and uniform plants were kept. One week after plant emergence, plants were fertilized with an application of a solution of 50 mL of Miracle-Gro fertilizers (Scotts Miracle-Gro, Detroit, MI) in each pot, and the same fertilizer was weekly applied to all pots until the end of the experiment.

Each genotype was planted in six pots, of which three pots were salt-treated, whereas the remaining three pots were irrigated with deionized water. Pots were placed on rectangular plastic trays to facilitate the irrigation. Salt (NaCl) treatment began when the first trifoliate leaf began to expand (V1 stage) (Fehr et al., 1971). Salt concentration was 200 mM NaCl as described previously (Abeer et al., 2015; Ashebir et al., 2013; Paul et al., 2011; Ravelombola et al., 2017).

We conducted a preliminary test involving only the two accessions used as control under the aforementioned NaCl concentration and used the current screening methodology and found that all plants from the salt-tolerant genotype (PI582468) were fully green and that of from the salt-susceptible genotype (PI255774) were completely dead after 14 d of salt stress (Fig. 2). Salt concentration was obtained by dissolving a total of 11.7 g of NaCl powder of Science Company (Lakewood, CO) in 1 L of deionized water.

Irrigation was performed by supplying either deionized water or salt solution to the plastic trays described previously. Irrigation was achieved such that pots were soaked with solution up to two third of pot height. The solution was kept within the plastic trays for 2 h every day. The treatment was conducted until the susceptible check (PI255774) was completely dead. This irrigation strategy was key, since it assisted cowpea roots with being

Table 1. List of 30 cowpea accessions including two accessions (PI255774 and PI582468) used as control.

Accession	Plant name ^z	Origin ^z
PI190191	TVu 1557	Mexico
PI229734	CHESH BOLBOLI LUBI	Iran
PI255774 ^y	TVu 2428	Nigeria
PI291140	NEGRO	Australia
PI292898	TVu 1890	Hungary
PI293469	BROWN CROWDER	United States
PI293586	WILT RESISTANT BLACKEYE	N/A ^x
PI349674	ALOOMBAA	Australia
PI354832	P 1350	India
PI354835	P 1353	India
PI354860	P 1387	India
PI354864	P 1392	India
PI354865	P 1393	India
PI582352	UCR 154	Saudi Arabia
PI582353	UCR 155	Saudi Arabia
PI582366	UCR 191	India
PI582368	UCR 193	India
PI582402	PITIUBA	Brazil
PI582428	LAURA B	Trinidad and Tobago
PI582468 ^y	UCR 347	N/A ^x
PI582551	UCR 1004	Botswana
PI582573	KVu 23	Kenya
PI582697	UCR 1176	Botswana
PI582812	UCR 794	Botswana
PI582852	UCR 935	Botswana
PI582863	UCR 1017	Botswana
PI583232	UCR 3317	Senegal
PI664515	Bettergreen	United States
PI664517	Bettergro Blackeye	United States
PI664524	Green Dixie Blackeye	United States

^zPlant name and country of origin were based on the information found at <https://npgsweb.ars-grin.gov/gringlobal/descriptors.aspx?>.

^yPI255774 is a salt-sensitive genotype, whereas PI582468 is a salt-tolerant one as previously reported (Ravelombola et al., 2017). These genotypes were used to validate the methodology.

^xInformation was not available.

Fig. 1. Phenotyping of salt tolerance in cowpea at seedling stage 14 d of salt stress. (R) Salt-tolerant genotype, PI582468, and (S) salt-sensitive genotype, PI255774, used as controls. Salt treatment was conducted by irrigating each plastic pot from the bottom.

permanently exposed to salt ions, which could lead to salt stress. In addition, doing so could limit within pot variation due to the differences in soil–root transmission if the rhizosphere was not completely soaked with solution. This irrigation approach has been proven to be efficient in salt tolerance screening in other crops (Ledesma et al., 2016).

The experiment design was completely randomized design with three replications per genotype. Factors include the set of 30 genotypes evaluated for salt tolerance. Genotypes were assumed to have fixed effects whereas the replications a random effect.

Measurements. Measurements were taken when the susceptible check was completely dead. Leaf injury was assessed based on a 1 to 7 scale (Fig. 3) (1 = healthy plants, 2 = first sign of leaf chlorosis, 3 = expansion of chlorosis on leaf surface, 4 = totally chlorotic leaf, 5 = first sign of necrosis, 6 = expansion of

of necrosis on leaf surface, and 7 = completely dead plants).

The number of dead plants per pots was counted. Plant height (from the bottom part to growing point) for both non-stressed and salt-stressed plants was measured on per plant basis. RST for plant height, described as the ratio between plant height under stress and non-stress conditions, was computed (Saad et al., 2014). Data on fresh leaf biomass under non-stress and stress conditions were collected and RST for fresh leaf biomass was calculated. Fresh stem biomass under non-stress and stress conditions along with the RST for fresh stem biomass were assessed as well. Leaf chlorophyll was measured using a chlorophyll SPAD-502 Plus (Spectrum Technologies Inc., Plainfield, IL) for non-stressed and salt-stressed plants and RST for chlorophyll content was computed.

Data analysis. Data were analyzed using PROC MIXED of SAS v.9.4 (SAS Institute Inc., Cary, NC). Mean separation was done using a protected least significant difference (LSD) procedure at $\alpha = 0.05$.

LSD procedure was described as $LSD = t_{\alpha/2} \sqrt{2MSE_{error}/n}$, where $t_{\alpha/2}$ was a critical value from the t-table with $df(SSE_{error}) = \text{Number of observations} - \text{Number of genotypes}$, and $n = \text{number of replications}$. Person's correlation coefficients and descriptive statistics were computed using JMP Genomics 7 (SAS Institute Inc., Cary, NC). Graphs and path analysis were established using the packages 'MASS' and 'Network' of R 3.1.1.

Results

Number of dead plants. The average number of dead plants per pot was evaluated for each genotyped at 14 d of salt stress. At that time, all plants from the susceptible check, PI255774, were completely dead, whereas those from the tolerant check, PI582468, were fully green (Fig. 1). The

number of dead plants varied from 0.00 to 4.00 dead plants per pot, with an average of 3.18 dead plants per pot and an SD of 1.20. Distribution of number of dead plants per pot was left-skewed (Fig. 4). Analysis of variance (ANOVA) revealed significant differences in number of dead plants among the 30 genotypes (F-value = 18.50, P value < 0.0001) (Table 2). The genotypes having less than two dead plants per pot were PI582468 (0.00), PI349674 (0.00), PI582812 (1.00), PI293469 (1.33), and PI190191 (1.67) (Supplemental Table 1). All plants from the genotypes PI664517, PI664515, PI582852, PI582573, PI582551, PI582428, PI582402, PI354860, PI354835, PI293586, PI291140, PI255774, and PI229734 were completely or almost dead at 14 d of salt stress.

To further validate the results, the two checks (PI582468 and PI255774) along with PI349674, having all plants being fully green at 14 d of salt stress, and PI582573, showing severe chlorosis at that time, were independently repeated from the previous trial. The results from the repeated experiment were consistent with the previous one as shown in Fig. 5. The tolerant control was fully green, whereas the susceptible check was completely dead. In addition, none of the plants from the genotypes PI349674 were dead, whereas those of PI582573 were chlorotic (Fig. 5), indicating that this current methodology could provide replicability of salt tolerance or salt susceptibility over time, hence stable and useful for investigating potential major genes affecting salt tolerance in cowpea.

Leaf injury score. Leaf injury was scored based on a 1 to 7 scale depending on leaf greenness and chlorosis. Leaf injury scores were in the range of 1.33 to 7.00, with an average of 5.66 and an SD of 1.52, indicating a large variation of leaf injury score among the genotypes. Distribution of leaf injury scores was left-skewed (Fig. 4). A significant difference in leaf injury scores was found among the 30 genotypes (F-value = 30.58, P value < 0.0001) (Table 2). Leaf score injury for tolerant control was 1.33, whereas the susceptible scored 7.00, suggesting that this methodology permitted a clear distinction between the two controls. In addition to the tolerant check, PI349674 (1.67), PI582812 (3.33), and PI190191 (3.50) scored the least (Supplemental Table 1), suggesting that these genotypes were salt-tolerant. Greatest leaf injury score was recorded for the genotypes PI291140 (6.50), PI582368 (6.50), PI582863 (6.50), PI293586 (6.60), PI354865 (6.67), PI664515 (6.67), PI292898 (6.77), PI664517 (6.83), PI582428 (7.00), PI582573 (7.00), and PI582852 (7.00) (Supplemental Table 1), which suggested that these genotypes were susceptible to salt stress. Leaf scoring was consistent in the repeated trials involving the controls along with PI349674 and PI582573, indicating that the methodology was stable.

Chlorophyll (SPAD). Chlorophyll (SPAD) was assessed in both non-salt-treated and salt-stressed cowpea plants, and RST for chlorophyll content (SPAD) was calculated.

Fig. 2. Differences in aboveground traits between salt-tolerant and salt-sensitive genotypes 14 d of salt stress. R = salt-resistant; S = salt-sensitive.

Fig. 3. Foliar injury due to salt stress: 1 = healthy plants, 2 = first sign of leaf chlorosis, 3 = expansion of chlorosis on leaf surface, 4 = totally chlorotic leaf, 5 = first sign of necrosis, 6 = expansion of necrosis on leaf surface, and 7 = completely dead plants.

Chlorophyll content of plants without salt stress was greater than those under salt stress at 14 d of salt stress, indicating that salt stress significantly affected leaf chlo-

rophyll (Fig. 6). Distributions of chlorophyll content in leaves of salt-stressed and non-stress plants and RST were about normally distributed (Fig. 6). For the salt-

stressed plants, chlorophyll content varied from 2.00 to 26.07, with a mean of 13.07 and an SD of 5.53, at 14 d of salt stress. Significant difference in chlorophyll content was found (F -value = 9.27, P value < 0.0001) (Table 2). Chlorophyll content (SPAD) of the tolerant check (PI582468) was 26.07, whereas that of the susceptible check was 5.83 (Supplemental Table 1). The well-performing genotypes under salt stress in addition to the tolerant check were PI349674 (24.10), PI582812 (21.60), PI293469 (19.43), PI664524 (18.70), and PI190191 (18.43) (Supplemental Table 1), indicating that these genotypes were tolerant to salt stress. The least performers in terms of chlorophyll content besides the susceptible check were PI354835 (9.90), PI293586 (9.77), PI582368 (9.73), PI664517 (9.17), PI292898 (8.67), PI582852 (8.47), PI582573 (4.30), and PI582428 (2.00) (Supplemental Table 1).

RST for chlorophyll content was the ratio between chlorophyll content of salt-stressed and non-stressed plants. The greater the RST was, the more salt-tolerant the genotype was. RST for chlorophyll content ranged from 0.08 to 0.97, with an average of 0.47 and an SD of 0.19. Significant differences in RST among the genotypes were found (F -value = 7.62, P value < 0.0001) (Table 2). The tolerant check had a RST value of 0.97, whereas the susceptible check had a relative salt value of 0.21. The most salt-tolerant genotypes based on RST for chlorophyll content in addition to the tolerant check were PI349674 (0.75), PI293469 (0.75), PI664524 (0.67), and PI582812 (0.67) (Supplemental Table 1). Those having the lowest RST value

Fig. 4. Distributions of the average number of dead plants per pot and leaf injury score.

Table 2. ANOVA table for traits evaluated for salt tolerance phenotyping at seedling stage.

Phenotype ^z	Source	DF	Sum of squares	Mean square	F value	Pr > F
Dead plants	Accession	29	125.16	4.32	18.50	<0.0001
	Error	60	14.00	0.23		
Leaf injury score	Accession	29	201.61	6.95	30.58	<0.0001
	Error	60	13.64	0.23		
Chlorophyll_NonStress	Accession	29	786.68	27.13	4.81	<0.0001
	Error	60	338.59	5.64		
Chlorophyll_Stress	Accession	29	2659.46	91.71	9.27	<0.0001
	Error	60	593.45	9.89		
Chlorophyll_RST ^y	Accession	29	3.15	0.11	7.62	<0.0001
	Error	60	0.86	0.01		
Height_NonStress	Accession	29	636.48	21.95	27.19	<0.0001
	Error	60	48.43	0.81		
Height_Stress	Accession	29	174.36	6.01	11.08	<0.0001
	Error	60	32.56	0.54		
Height_RST	Accession	29	0.39	0.01	4.01	<0.0001
	Error	60	0.20	0.003		
LeafBiomass_NonStress	Accession	29	38.92	1.34	11.99	<0.0001
	Error	60	6.71	0.11		
LeafBiomass_Stress	Accession	29	13.60	0.47	8.74	<0.0001
	Error	60	3.22	0.05		
LeafBiomass_RST	Accession	29	2.79	0.10	5.64	<0.0001
	Error	60	1.02	0.02		
StemBiomass_NonStress	Accession	29	18.58	0.64	15.36	<0.0001
	Error	60	2.50	0.04		
StemBiomass_Stress	Accession	29	5.36	0.18	16.88	<0.0001
	Error	60	0.66	0.01		
StemBiomass_RST	Accession	29	1.42	0.05	5.13	<0.0001
	Error	60	0.57	0.01		

^zPhenotypes were collected at 14 d of salt stress on a per plant basis. The susceptible check was completely dead at 14 d of salt stress.

^yRelative salt tolerance (RST) was the ratio between the phenotypic values under salt stress and without salt stress.

ANOVA = analysis of variance.

Fig. 5. Independent replicated trial involving the tolerant check (Tc: PI582468), the susceptible check (Sc: PI255774), and one of the salt-tolerant genotypes (T: PI349674) and salt-susceptible ones (S: PI582573) as identified in the previous experiment. The results from the independent replicated trials showed that the current methodology was stable.

Fig. 6. Distributions of chlorophyll content of non-salt-stressed and salt-stressed plants, and relative salt tolerance for chlorophyll content.

besides the susceptible check were PI292898 (0.34), PI664515 (0.33), PI664517 (0.33), PI582852 (0.31), PI582573 (0.16), and PI582428 (0.08) (Supplemental Table 1).

Plant height. Plant height of salt-stressed and non-stressed plants was measured at 14 d of salt stress when the susceptible check was completely dead. Salt stress significantly reduced plant height (Fig. 1). Plant height of non-stressed plants varied from 10.43 to 20.00 cm, with an average of 14.70 cm and an SD of 2.70 cm. That of stressed plants ranged between 5.87 and 11.80 cm, with a mean of 8.18 cm and an SD of 1.42 cm. Plant height under both conditions was about normally distributed (Fig. 7). Significant differences in plant height without salt stress (F-value = 27.19, P value < 0.0001) and under salt stress (F-value = 11.08, P value < 0.0001) (Table 2) were identified.

Under salt treatment, the tallest genotypes were PI664524 (11.80 cm), PI582353 (10.50 cm), PI582551 (10.30 cm), PI664517 (9.73 cm), PI354865 (9.60 cm), PI582352 (9.57 cm), and PI293469 (9.47 cm), whereas the shortest ones were PI354860 (6.93 cm), PI582428 (6.83 cm), PI354832 (6.80 cm), PI582573 (6.00 cm), PI582366 (5.93 cm), and PI582812 (5.87 cm) (Supplemental Table 1).

RST was the ratio between plant height under salt stress conditions and plant height without salt stress. RST for plant height varied from 0.37 to 0.70, with an average of 0.59 and an SD of 0.07. RST was significantly different among the genotypes (F-value = 4.01, P value < 0.0001) (Table 2). Interestingly, RST for PI582468 (0.49) (tolerant control) was less than PI255774 (0.59) (susceptible control), suggesting that RST for

plant height could not be accurately evaluated using the current methodology.

Fresh leaf biomass weight. Leaf biomass was measured when the susceptible check was completely dead. Leaf biomass of plants without salt stress was about normally distributed, whereas that of salt-stressed plants was right-skewed (Fig. 8).

Under non-stress conditions, average leaf biomass per plant ranged from 1.51 g to 4.69 g, with an average of 2.55 g and an SD of 0.67 g. Under salt treatment, leaf biomass varied between 0.15 and 1.39 g, with an average of 0.77 g and an SD of 0.40 g. In addition, correlation analysis showed weak association ($r = 0.15$) between leaf biomass under salt stress and non-stress conditions, indicating that the observed variation in leaf biomass under salt stress among the genotypes was more likely to be associated with a genetic response specific to the genotype rather than being correlated with an adaptation due to plant morphology. ANOVA showed significant differences in leaf biomass under salt stress among the genotypes (F-value = 0.47, P value < 0.0001) (Table 2). Genotypes having the heaviest leaf biomass under salt conditions were PI664524 (1.50 g), PI582551 (1.39 g), PI349674 (1.38 g), PI582352 (1.30 g), PI293469 (1.25 g), and PI582468 (1.18 g) (Supplemental Table 2). Those having the lightest leaf biomass under salt stress were PI582368 (0.36 g), PI354865 (0.35 g), PI582428 (0.30 g), PI664515 (0.24 g), PI582573 (0.24 g), PI229734 (0.24 g), and PI255774 (0.15 g) (Supplemental Table 2).

RST for leaf biomass had a right-skewed distribution (Fig. 8). Leaf biomass RST varied from 0.05 to 0.71, with a mean of 0.32 and an SD of 0.18. RST for leaf biomass was statistically significantly different among the genotypes (F-value = 5.64, P value < 0.0001). Genotypes having the greatest RST for leaf biomass were PI293469 (0.71), PI582551 (0.65), PI349674 (0.60), PI354864 (0.54), and PI354860 (0.51) (Supplemental Table 2). The lowest RST for leaf biomass was recorded for PI229734 (0.10), PI582428 (0.09), PI255774 (0.08), and PI664515 (0.05) (Supplemental Table 2).

Fresh stem biomass weight. Fresh stem biomass of plants under salt stress and without salt treatment was recorded on a per plant basis at 14 d of salt stress. At that time, the susceptible check was completely dead. Stem biomass of salt-treated plants was lower than plants without being salt-treated (Fig. 9). Stem biomass was nearly normally distributed for plants without salt stress, whereas distribution was right-skewed for stem biomass of salt-stressed plants (Fig. 9). Stem biomass per plant varied from 0.86 to 2.53 g, with an average of 1.64 g and an SD of 0.46 under non-stress conditions. Under salt treatment, stem biomass was in the range of 0.36 and 1.19 g, with a mean of 0.71 g and an SD of 0.25. Stem biomass was significantly different among the genotypes under salt stress (F-value = 16.88, P value < 0.0001) and without salt stress (F-value = 15.36, P value < 0.0001) (Table 2).

Fig. 7. Distributions of plant height of non-salt-stressed and salt-stressed plants, and relative salt tolerance for plant height.

Fig. 8. Distributions of leaf biomass of non-salt-stressed and salt-stressed plants, and relative salt tolerance for leaf biomass.

RST for stem biomass varied from 0.18 to 0.68, with a mean of 0.45 and an SD of 0.13. Values of RST were about normally distributed (Fig. 9). RST for stem biomass was significantly different among the genotypes (F -value = 5.13, P value < 0.0001) (Table 2). Genotypes having the greatest RST for stem biomass were PI582551 (0.68), PI354865 (0.68), PI293586 (0.64), PI354835 (0.61), PI582368 (0.59), PI664524 (0.59), and PI354860 (0.58) (Supplemental Table 2). Lowest RST was recorded for PI582468 (0.38), PI349674 (0.37), PI229734 (0.35),

PI582366 (0.34), PI582812 (0.30), PI291140 (0.25), PI582428 (0.22), and PI664515 (0.18) (Supplemental Table 2). Similar to plant height, none of the two controls were grouped into these extreme genotypes, indicating that stem biomass was not a good indicator for salt tolerance under this methodology.

Network analysis between traits and correlation analysis. Network analysis revealed existing pathways between number of dead plants, leaf injury scores, RST for chlorophyll content, chlorophyll content un-

der salt stress, RST for leaf biomass, and leaf biomass under salt stress (Fig. 10). Pearson's correlation coefficients between number of dead plants and leaf injury score, number of dead plant and chlorophyll content under salt stress, and number of dead plants and RST for chlorophyll were 0.91, -0.81, and -0.77 (Supplemental Table 3), respectively, indicating that salt stress caused severe leaf chlorosis, which resulted in leaf tissue damage and reduction in leaf matter, thus plant death. Another pathway defined by plant height under salt stress, plant height without salt stress, stem biomass under salt stress, and leaf biomass under salt stress was identified (Fig. 10). All parameters within the second network were related to non-stressed plants except for stem biomass and plant height, suggesting that phenotypic values obtained using these parameters were likely associated with plant morphology rather than response to salt tolerance. Since the second pathway was independent from the first one (Fig. 10), there was almost no correlation between the network defined by number of dead plants, leaf injury scores, RST for chlorophyll content, chlorophyll content under salt stress, RST for leaf biomass, and leaf biomass under salt stress, and that of plant height under salt stress, plant height without salt stress, stem biomass under salt stress, and leaf biomass under salt stress.

Discussion

Salt stress has been increasingly threatening crop production globally (Flowers, 2004). Salinity affects more than 830 million hectares of croplands worldwide (Chaitanya et al., 2014). Shannon (1997) estimated a total of 1 to 60 t of salt compound being annually added to cultivated areas, which has made salinity a growing concern to agriculture. The effects of salinity have been found to be more severe in semiarid regions, where cowpea is widely grown (Zhang et al., 2012). Providing farmers with genotypes that tolerate salt conditions better would be the most affordable way to limit the negative effects of salinity on crop production. Establishing a straightforward phenotyping strategy to select for salt-tolerant genotype will significantly help cowpea breeders to do so.

Since cowpea cultivation is predominant in developing countries in which there is a limited access to funding opportunities and facilities to set up hydroponic system to screen for salt tolerance in cowpea at seedling stage, providing cowpea scientists working in these areas with an easy-to-implement and cost-effective approach would help in enhancing breeding programs aiming at releasing salt-tolerant cowpea cultivars. In this current investigation, we developed a rapid screening methodology that can be followed and used by cowpea breeders when phenotyping for salt tolerance.

This research has been conducted in a controlled condition to limit potential effects of uncontrolled factors such as differences in soil fertility, transpiration, and root-soil

Fig. 9. Distributions of stem biomass of non-salt-stressed and salt-stressed plants, and relative salt tolerance for stem biomass.

Fig. 10. Network analysis between traits evaluated under salt stress and non-salt conditions. Pathways were shown using solid lines when absolute value of Pearson's correlations was greater than 0.65.

transmission that commonly occurred in field phenotyping (Pathan et al., 2007). Therefore, cowpea breeders can rapidly replicate promising investigations identifying good genotypes before conducting a field phenotyping with a fewer number of genotypes to screen, which could significantly limit the unexplained variation due to field conditions as previously stated. In addition, we have established easy-to-track phenotyping traits such as leaf score injury and leaf biomass for

assessing salt tolerance, which does not require substantial costs to record, hence can be easily scaled up.

The current methodology has been validated by the use of two checks, PI582468 (salt-tolerant) and PI255774 (salt-sensitive), as previously reported (Ravelombola et al., 2017). Substantial discrepancy in above-ground traits between these genotypes was found even at 10 d of salt stress, suggesting that this approach can help differentiate a

salt-tolerant genotype from a salt-sensitive one.

Replicating is a critical part of applied sciences and data from investigations that fail to be replicated cannot be used for further experiments in general. Therefore, to further validate our results, the two checks along with the two contrasting genotypes were repeated. Similar results from the previous screening were obtained in the replicated trial. The two salt-tolerant genotypes were fully green, whereas the two sensitive ones were almost dead at 11 d of salt stress as shown in Fig. 4, which further validated the methodology and the data from this investigation.

A total of 14 above-ground parameters was evaluated in this study. Mini et al. (2015) reported a high correlation between accumulation of salt ions and chlorophyll content in leaves of salt-stressed cowpea plants. Therefore, we suggested that chlorophyll content is a good indicator of salt tolerance in cowpea. Since analyzing ion contents within salt-stressed cowpea leaves and roots could be expensive, measuring chlorophyll content could give a good approximation of salt tolerance. In fact, our results suggested that the two controls (PI582468 and PI255774) were significantly contrasting in terms of chlorophyll content under salt stress. In addition, Murillo-Amador et al. (2002) reported that ion exchange mechanisms played an important role in conferring salt tolerance in cowpea. Praxedes et al. (2010) stated that salt-sensitive cowpea plants were not able to limit the uptake of Na^+ and Cl^- under salt stress, which substantially lowered the chlorophyll content in the salt-sensitive genotypes as reported in this investigation.

A scoring-based scale for salt leaf injury (1 = green plant and 7 = completely dead plant) was established to help cowpea scientists quantify the stay-green phenomenon under a prolonged period of salt stress. Establishing a straightforward scoring for salt injury has been proved to allow for a rapid screening for salt tolerance in other crops such as soybean [*Glycine max* (L.) Merr.] (Ledesma et al., 2016). In addition, path analysis from this investigation revealed significant correlations between number of dead plants, leaf injury scores, chlorophyll content of salt-stressed cowpea plants, and leaf biomass of salt stressed cowpea plants. Therefore, leaf injury score also could be used as a good indicator for salt tolerance in cowpea. Based on our experiment, we suggest that the criterion or the most "effective" parameters will depend on specific experiment for the salt treatment duration and salt stress level. At the end of the experiments, if there are large number of plants dead, then biomass may not be the best (accurate) parameter. If the plants are starting to die by the end of the study, visual scoring would be very effective. In this case, SPAD reading would be a great additional parameter to add.

The current methodology also allowed for clear distinction between the salt-tolerant genotype from the salt-sensitive one based on fresh leaf biomass weight under salt stress.

El-Mashad and Mohamed (2012) reported that cowpea plants that were able to keep cell constituents from being extensively damaged by oxidative reaction occurring in leaf cells under salt stress were likely to withstand the stress, whereas those failing to prevent extensive lipid peroxidation occurring in leaves were highly susceptible to salt stress (Cavalcanti et al., 2004).

This research aimed at providing a streamlined protocol for salt tolerance phenotyping, which will have practical applications for cowpea breeding. The cowpea genotypes used as controls in this investigation can be freely accessed through the USDA Germplasm Resources Information Network website (<https://npgsweb.ars-grin.gov/gringlobal/search.aspx>) and available for orders and can be used for further references when selecting for salt-tolerant genotypes. Since the controls were freely available to everyone, we can expect that the present protocol can be used by other cowpea scientists contributing toward unraveling the genetics of salt tolerance in cowpea.

Conclusion

Phenotyping is one of the most challenging tasks in plant breeding. Being provided with a fast and accurate phenotyping strategy will allow for enhanced salt tolerance phenomics-related investigations, which is common in modern breeding. In this study, we conducted 14 above-ground traits for measuring salt tolerance in cowpea and found that the fewer number of dead plants per pot, high chlorophyll content, and less leaf injury scores were highly correlated and that each of the three parameters can be used as the criterion for salt tolerance evaluation in cowpea; thus, we developed a simple and cost-effective salt tolerance methodology in cowpea, which is not yet available despite being important, to the best of our knowledge.

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Supplemental Table 1. LSMMeans of average number of dead plants per pot, leaf injury scores, chlorophyll content under non-salt conditions, chlorophyll content under salt stress, relative salt tolerance for chlorophyll content, plant height under non-salt conditions, plant height under salt stress, and relative salt tolerance for plant height.

Accession	Avg number of dead plants per pot			Leaf injury score			Chlorophyll Non-Stress (SPAD value)			Chlorophyll Stress (SPAD value)			Chlorophyll RST ^z			Height Non-Stress (cm)			Height Stress (cm)			Height_RST		
	Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD		Mean	SD	
PI190191	1.67	0.58	d ^y	3.50	0.50	ij	29.37	0.64	cde	18.43	2.30	cde	0.63	0.08	bcde	14.27	1.36	defg	7.33	0.76	ijk	0.51	0.01	ijk
PI229734	4.00	0.00	a	6.43	0.31	abcd	26.37	3.30	defgh	10.17	0.76	hijkl	0.39	0.04	ghijk	13.53	1.55	fgh	7.87	0.47	hijk	0.59	0.04	bcdefghij
PI255774	4.00	0.00	a	7.00	0.00	a	27.90	0.17	defg	5.83	3.24	lmn	0.21	0.02	klm	13.57	0.55	fgh	7.97	0.45	ghijk	0.59	0.01	bcdefghij
PI291140	4.00	0.00	a	6.50	0.50	abc	26.87	0.78	defgh	10.83	0.49	hijkl	0.40	0.11	ghijk	12.03	1.07	ij	7.47	0.60	hijk	0.62	0.07	abcdef
PI292898	3.67	0.58	ab	6.77	0.40	abc	25.50	2.78	efgh	8.67	1.93	jklm	0.34	0.05	ijkl	13.87	0.72	efgh	7.53	0.55	hijk	0.54	0.02	defghijk
PI293469	1.33	0.58	d	4.17	1.04	hi	25.93	2.46	defgh	19.43	5.72	bcd	0.75	0.18	bc	15.53	0.91	bcd	9.47	0.60	bcde	0.61	0.05	abcdefgh
PI293586	4.00	0.00	a	6.60	0.36	abc	24.93	0.81	fgh	9.77	1.27	ijkl	0.39	0.04	ghijk	19.37	0.35	a	9.13	0.81	cdefg	0.47	0.05	k
PI354832	3.67	0.58	ab	5.60	0.36	efg	27.27	2.72	defg	11.37	3.07	ghijk	0.42	0.10	fghij	16.37	1.72	bc	8.63	0.55	defgh	0.53	0.07	fghijk
PI354835	4.00	0.00	a	6.43	0.12	abcd	26.63	1.56	defgh	9.90	0.50	ijkl	0.37	0.02	ghijk	13.73	1.54	fgh	8.60	0.61	klm	0.50	0.09	jk
PI354860	4.00	0.00	a	6.00	0.00	cdefg	27.07	3.35	defg	11.60	2.29	ghijk	0.44	0.12	efghij	16.03	0.38	bc	8.60	0.40	defgh	0.54	0.02	efghijk
PI354864	3.67	0.58	ab	6.17	0.76	bcdef	28.13	0.87	def	15.20	6.66	defgh	0.53	0.22	defghi	12.87	1.00	ghi	6.93	0.74	jklm	0.54	0.07	defghijk
PI354865	3.67	0.58	ab	6.67	0.58	abc	25.60	4.51	defgh	10.80	4.57	hijkl	0.41	0.12	fghij	13.60	0.79	fgh	9.60	0.46	bcd	0.70	0.02	a
PI582352	2.67	0.58	c	4.50	0.50	h	27.50	1.73	defg	16.37	1.31	defg	0.60	0.08	bcdef	18.57	0.93	a	9.57	0.38	bcd	0.52	0.03	hijk
PI582353	3.00	1.00	bc	4.50	0.50	h	28.20	2.79	def	17.43	4.51	cdef	0.62	0.18	bcde	20.00	0.36	a	10.50	0.95	b	0.53	0.06	ghijk
PI582366	3.67	0.58	ab	6.00	0.00	cdefg	34.07	0.81	ab	12.30	1.76	fghijk	0.36	0.05	hijk	10.57	0.47	jk	5.93	0.49	lm	0.56	0.07	cdefghijk
PI582368	3.67	0.58	ab	6.50	0.87	abc	23.17	1.00	h	9.73	4.97	ijkl	0.42	0.20	fghij	12.67	0.95	hi	8.27	0.31	efghi	0.65	0.03	abc
PI582402	4.00	0.00	a	6.33	0.58	abcde	24.73	0.71	fgh	12.33	2.74	fghijk	0.50	0.11	defghij	16.50	0.20	b	9.27	0.60	cdef	0.56	0.03	cdefghijk
PI582428	4.00	0.00	a	7.00	0.00	a	25.73	4.64	defgh	2.00	0.82	n	0.08	0.04	m	18.70	1.20	a	6.83	0.74	klm	0.37	0.05	l
PI582468	0.00	0.00	e	1.33	0.29	k	27.07	4.01	defg	26.07	0.92	a	0.97	0.12	a	15.67	0.74	bcd	7.67	1.36	hijk	0.49	0.07	k
PI582551	4.00	0.00	a	5.67	0.29	defg	29.40	1.71	cd	16.40	3.02	defg	0.56	0.08	cdefg	16.37	0.83	bc	10.30	0.82	bc	0.63	0.04	abcde
PI582573	4.00	0.00	a	7.00	0.00	a	25.13	2.21	fgh	4.30	2.76	mn	0.16	0.10	lm	10.57	0.31	jk	6.00	0.70	lm	0.57	0.08	bcdefghijk
PI582697	3.33	0.58	abc	6.33	0.29	abcde	26.73	1.50	defgh	10.23	1.93	hijkl	0.38	0.08	ghijk	13.57	0.57	fgh	8.10	0.95	fghij	0.60	0.08	bcdefghij
PI582812	1.00	0.00	d	3.33	0.29	j	32.40	0.79	bc	21.60	2.42	abc	0.67	0.09	bcd	10.43	0.85	k	5.87	0.75	m	0.57	0.10	bcdefghijk
PI582852	4.00	0.00	a	7.00	0.00	a	27.20	0.26	defg	8.47	3.31	klm	0.31	0.12	jkl	14.97	0.70	cdef	7.83	1.40	hijk	0.52	0.08	ghijk
PI582863	3.00	1.00	bc	6.50	0.87	abc	25.03	2.21	fgh	13.70	4.85	efghij	0.55	0.18	defgh	12.73	1.16	hi	7.83	0.80	hijk	0.62	0.03	abcdefg
PI583232	2.67	0.58	c	5.50	0.50	fg	24.20	3.39	gh	14.83	3.93	defghi	0.62	0.22	bcde	16.43	0.95	bc	8.20	0.82	fghi	0.50	0.07	jk
PI664515	4.00	0.00	a	6.67	0.58	abc	36.97	1.17	a	12.30	1.56	fghijk	0.33	0.04	jkl	13.17	0.25	ghi	7.27	0.23	ijk	0.55	0.01	defghijk
PI664517	4.00	0.00	a	6.83	0.29	ab	27.50	1.51	defg	9.17	3.96	jklm	0.33	0.12	jkl	15.30	0.20	bcde	9.73	1.10	bcd	0.63	0.07	abcd
PI664524	2.67	1.15	c	5.33	0.58	g	27.87	2.00	defg	18.70	1.90	cde	0.67	0.09	bcd	19.27	0.91	a	11.80	0.10	a	0.61	0.04	abcdefg

^zRelative salt tolerance (RST) was the ratio between the phenotypic values under salt stress and without salt stress.

^yMeans followed by the same letter are not significantly different at $\alpha=0.05$.

Supplemental Table 2. LSMeans of leaf biomass under non-salt conditions, leaf biomass under salt stress, relative salt tolerance for leaf biomass, stem biomass under non-salt conditions, stem biomass under salt stress, and relative salt tolerance for stem biomass.

Accession	Leaf biomass_Non_Stress (g)			Leaf biomass_Stress (g)			Leaf biomass_RST ^z			Stem biomass_Non_Stress (g)			Stem biomass_Stress (g)			Stem biomass_RST (g)		
	Mean			Mean			Mean			Mean			Mean			Mean		
	SD			SD			SD			SD			SD			SD		
PI190191	2.33	0.24	ghijk ^y	1.04	0.31	bcdefg	0.45	0.17	bcde	1.54	0.20	ijkl	0.66	0.10	ghijk	0.43	0.03	l
PI229734	2.28	0.11	hijkl	0.24	0.09	no	0.10	0.04	hi	1.51	0.02	ijkl	0.53	0.04	ijklm	0.35	0.03	kl
PI255774	1.77	0.16	lm	0.15	0.03	o	0.08	0.03	hi	1.29	0.34	klm	0.52	0.14	jklmn	0.40	0.06	jkl
PI291140	2.49	0.23	fghij	0.83	0.13	efghij	0.34	0.04	cdefg	1.49	0.11	jkl	0.38	0.03	mn	0.25	0.03	ijkl
PI292898	2.63	0.18	efghi	0.56	0.12	ijklmn	0.22	0.06	fghi	1.40	0.28	jklm	0.76	0.15	fg	0.55	0.11	hijk
PI293469	1.78	0.11	lm	1.25	0.40	abcd	0.71	0.22	a	1.31	0.05	klm	0.66	0.04	ghijk	0.51	0.05	ghijk
PI293586	2.53	0.32	fghij	0.84	0.32	efghij	0.35	0.16	cdefg	1.89	0.21	efgh	1.19	0.08	ab	0.64	0.10	ghijk
PI349674	2.33	0.35	ghijk	1.38	0.25	ab	0.60	0.13	ab	1.97	0.10	defg	0.73	0.07	fgh	0.37	0.04	ghijk
PI354832	3.37	0.54	bc	0.88	0.20	defghij	0.26	0.04	efghi	1.84	0.21	fghi	0.71	0.08	fgh	0.40	0.08	ghij
PI354835	1.51	0.36	m	0.51	0.06	jklmno	0.35	0.09	cdefg	1.24	0.19	lm	0.74	0.09	fg	0.61	0.18	fghij
PI354860	2.11	0.30	ijkl	1.09	0.37	bcdef	0.51	0.13	abc	1.24	0.25	lm	0.69	0.07	ghij	0.58	0.18	fghij
PI354864	2.57	0.91	fghij	1.10	0.53	bcdef	0.54	0.46	abc	1.38	0.48	jklm	0.62	0.08	ghijkl	0.50	0.20	fghij
PI354865	2.48	0.26	fghij	0.35	0.11	lmno	0.15	0.06	ghi	1.60	0.20	hijk	1.07	0.13	bc	0.68	0.13	fghi
PI582352	2.83	0.15	cdefg	1.30	0.07	abc	0.46	0.02	bcde	2.28	0.12	abcd	0.88	0.12	def	0.39	0.07	efghi
PI582353	3.26	0.26	bcd	0.95	0.27	cdefgh	0.29	0.07	defgh	2.47	0.27	ab	1.19	0.28	ab	0.48	0.11	efghi
PI582366	2.15	0.26	ijkl	0.40	0.04	klmno	0.18	0.02	fghi	1.10	0.20	mn	0.36	0.07	n	0.34	0.15	defghi
PI582368	2.18	0.15	ijkl	0.36	0.04	lmno	0.17	0.01	ghi	1.11	0.01	mn	0.66	0.10	ghijk	0.59	0.09	defghi
PI582402	2.93	0.47	cdef	0.74	0.09	fghijk	0.25	0.03	efghi	1.66	0.07	ghij	0.73	0.07	fgh	0.44	0.04	defghi
PI582428	3.21	0.42	bcd	0.30	0.02	mno	0.09	0.01	hi	2.49	0.08	ab	0.57	0.05	hijkl	0.22	0.02	cdefgh
PI582468	3.15	0.40	bcde	1.18	0.33	abcde	0.38	0.11	cdef	2.03	0.09	cdef	0.77	0.14	efg	0.38	0.08	bcdefgh
PI582551	2.17	0.16	ijkl	1.39	0.44	ab	0.65	0.24	ab	1.50	0.24	jkl	0.99	0.02	cd	0.68	0.13	bcdefgh
PI582573	1.87	0.17	klm	0.24	0.13	no	0.12	0.07	hi	0.86	0.13	n	0.37	0.07	mn	0.44	0.13	bcdefg
PI582697	1.54	0.14	m	0.69	0.08	ghijkl	0.45	0.07	bcde	1.30	0.25	klm	0.60	0.03	ghijkl	0.47	0.09	abcdef
PI582812	2.05	0.41	jklm	0.93	0.18	cdefghi	0.47	0.15	bcd	1.22	0.16	lm	0.36	0.06	n	0.30	0.05	abcde
PI582852	2.34	0.23	ghijk	0.53	0.20	jklmn	0.22	0.07	fghi	1.60	0.31	hijk	0.70	0.23	ghi	0.44	0.10	abcd
PI582863	2.62	0.13	efghi	0.63	0.31	hijklm	0.24	0.11	fghi	1.23	0.20	lm	0.51	0.02	klmn	0.42	0.08	abcd
PI583232	2.73	0.50	defgh	0.91	0.16	defghi	0.34	0.08	cdefg	1.54	0.13	ijkl	0.64	0.08	ghijk	0.41	0.06	abc
PI664515	4.69	0.09	a	0.24	0.04	no	0.05	0.01	i	2.53	0.16	a	0.46	0.04	lmn	0.18	0.03	ab
PI664517	3.50	0.30	b	0.59	0.23	hijklmn	0.17	0.05	ghi	2.36	0.16	abc	0.94	0.13	cde	0.40	0.08	a
PI664524	3.19	0.39	bcd	1.50	0.16	a	0.48	0.09	bcd	2.19	0.17	bcde	1.28	0.03	a	0.59	0.05	a

^zRelative salt tolerance (RST) was the ratio between the phenotypic values under salt stress and without salt stress.

^yMeans followed by the same letter are not significantly different at $\alpha=0.05$.

Supplemental Table 3. Pearson's correlation coefficients between trait values used for phenotyping salt tolerance at seedling stage in cowpea.

Correlation r	Dead	Leaf_injury	Chlorophyll_NonStress	Chlorophyll_Stress	Chlorophyll_RST	Ht_NonStress	Ht_Stress	Height_RST	LeafBiomass_NonStress	LeafBiomass_Stress	LeafBiomass_RST	StemBiomass_NonStress	StemBiomass_Stress	StemBiomass_RST
Dead	1.00													
Leaf_injury	0.91	1.00												
Chlorophyll_NonStress	-0.22	-0.28	1.00											
Chlorophyll_Stress	-0.81	-0.85	0.32	1.00										
Chlorophyll_RST	-0.77	-0.79	0.07	0.96	1.00									
Height_NonStress	-0.10	-0.18	-0.18	0.13	0.19	1.00								
Height_Stress	-0.04	-0.08	-0.22	0.22	0.30	0.66	1.00							
Height_RST	0.11	0.17	-0.09	0.07	0.10	-0.43	0.38	1.00						
LeafBiomass_NonStress	0.04	-0.02	0.20	0.10	0.08	0.29	0.13	-0.21	1.00					
LeafBiomass_Stress	-0.52	-0.61	0.05	0.68	0.69	0.32	0.37	0.02	0.00	1.00				
LeafBiomass_RST	-0.46	-0.50	0.07	0.56	0.54	0.13	0.23	0.10	-0.36	0.89	1.00			
StemBiomass_NonStress	-0.11	-0.19	0.19	0.17	0.14	0.66	0.38	-0.35	0.79	0.15	-0.16	1.00		
StemBiomass_Stress	-0.03	-0.10	-0.20	0.19	0.25	0.69	0.81	0.11	0.19	0.39	0.21	0.45	1.00	
StemBiomass_RST	0.14	0.12	-0.37	-0.03	0.05	0.15	0.48	0.40	-0.42	0.24	0.36	-0.37	0.62	1.00