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Evaluation and Association Analysis of Cowpea Salt Tolerance

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Evaluation and Association Analysis of Cowpea Salt Tolerance

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by

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Abstract

Cowpea [*Vigna unguiculata* (L.) Walp.] (2n=2x=22) is a leguminous crop providing inexpensive protein for human consumption that can be grown worldwide. Salinity is one of the major threats to cowpea production, particularly in semi-arid regions of Africa. Salinity significantly affects seed germination and decreases pod yield in cowpea. However, little has been done to provide farmers salt-tolerant cowpea cultivars. Knowledge on the genetics of cowpea salt tolerance is very limited. This study aimed to:(1) evaluate cowpea salt tolerance at germination stage, (2) evaluate cowpea salt tolerance at seedling stage, (3) conduct an association study for cowpea salt tolerance at germination and seedling stages and to identify SNP markers associated with salt tolerance in cowpea. A total of 151 cowpea genotypes at germination stage and 203 cowpea genotypes at seedling stage were evaluated in this research. Association analysis was performed on 116 genotypes at germination stage and 155 genotypes at seedling stage. The results indicated that: (1) substantial variability in salt tolerance was found among the tested cowpea genotypes at both germination and seedling stages; (2) three SNPs, Scaffold87490_622, Scaffold87490_630, and C35017374_128 were highly associated with salt tolerance at germination stage; (3) seven SNPs Scaffold93827_270, Scaffold68489_600, Scaffold87490_633, Scaffold87490_640, Scaffold82042_3387, C35069468_1916, and Scaffold93942_1089 were found to be associated with salt tolerance at seedling stage, and (4) PI582422, 09-529, PI293584, and PI582570 were highly salt tolerant at germination stage, and PI293570, PI582812, PI582856, PI180014, PI257463, 09-175, 09-529, PI666260, I582402, and PI582340 were highly salt tolerant at seedling stage. This research will have practical applications in cowpea breeding and genetics. The salt tolerant lines could be used as parents for

breeding programs and the SNP markers could be used as a tools in cowpea molecular breeding through marker-assisted selection.

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Dedication

This thesis is dedicated to:

- My dad: Second Modeste Velombola
- My mom: Francine Razanamalala
- My sister: Francia Seconde Ravelombola

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Chapter 1. Introduction

Cowpea

Cowpea [*Vigna unguiculata* (L.) Walp.] (2n=2x=22) is a leguminous crop, in the Family *Fabaceae* (Verdcourt, 1970). It is usually known as southern pea or blackeye pea in the US, beans, ‘niebe’, ‘wake’ or ‘ewa’ in Africa, and ‘caupi’ in Brazil (Agbicodo, 2009). The center of origin of cowpea is still under debate. Earlier investigations suggested that India is the center of origin for cowpea; however, recent research points out that it likely comes from Africa. In fact, wild cowpea is only found in Africa and Madagascar (Blackhurst and Miller, 1980). Cowpea cultivation is adopted in various agroecological zones such as Asia, Oceania, the Middle East, southern Europe, Africa, southern USA, and Central and South America (Watt et al., 1985; Fery, 1990; Perrino et al., 1993). Furthermore, cowpea is also grown in semiarid areas of the tropics.

Cowpea is cultivated on over an estimated area of 11 million hectares worldwide. Central and West Africa provides the majority of the cowpea production. More than 70 % of the worldwide cowpea production comes from these regions (Singh et al., 2003). The worldwide production is estimated at 5.4 million tons of cowpea dried seeds with Nigeria as the leading producer (Olufajo, 2012).

Utilization of cowpea involves a wide range of sectors from livestock to industrial purposes. Cowpea young leaves, green pods, and seeds with various colors (Figure 1-1) can be used as vegetable. Dried seeds are primarily used for human consumption and livestock fodder. Cowpea can be processed into other destinations such as cowpea flour or cowpea milk (Akpapuimam and Markakis 1981; Caygill et al., 1981).

Figure 1-1. Diversity of seed cowpea genotypes (Photo credit: Dr. Ainong Shi)

Effects of salinity on cowpea

Studies have emphasized the negative effects of salinity on cowpea. Salt stress noticeably undermines cowpea growth. Jacob (2015) found that when the salt concentration increased, cowpea biomass was significantly reduced and cowpea genotypes exhibited lower dry matter than those used as controls after four weeks of salt treatment application of 150 mM NaCl.

Crops which are susceptible to salt stress are unable to exclude Na+ and Cl- ions during the transpiration process and accumulate these ions in leaves and shoot tissues (Harvey, 1985). For cowpea, high ion chloride content stresses the plant. Consequently, proteins located in the leaves and roots are decreased (Calvet et al., 2012). In addition, photosynthesis is considerably affected by the high concentration of chloride in cowpea leaves, and evapotranspiration is reduced (Plaut et al., 1989). All these aforementioned processes lower yield and lead to plant death. For a NaCl concentration higher than 90 Mm, seed and pod yield significantly decreased (Düzdemir et al., 2009).

Responses of crops to salinity stress vary depending upon the plant growth stage (Bernstein and Hayward, 1958). In general, germination and early plant growth are the two most sensitive stages to salinity. Important salinity concentrations during these stages hamper crop establishment, resulting in a drastic yield decrease (Mano and Takeda, 1997).

Major mechanisms of salt tolerance in plants

Salt tolerance is believed to be controlled by multiple genes (Joosen et al., 2010; DeRose-Wilson and Gaut, 2011). Salt tolerance mechanisms are a complex process in plants. Tolerance involves a wide range of factors which can either act independently or interact with each other. The most common salt tolerance mechanisms which have been investigated are ion selectivity, ion accumulation, organic solute production, and growth regulation (Akbar et al., 1977; Tal and Shannon, 1983; Grumet and Hanson, 1986). As described by Shannon (1997), these mechanisms rely on the anatomical structure and the physiological process within plants.

Ion selectivity

Salt-tolerant plants usually select the essential ion nutrients for uptake and exclude those which are toxic (Harvey, 1985; Flowers and Yeo, 1995). The concept of including or excluding ions are used in soybean (*Glycine max* L.) breeding to term whether a genotype is salt tolerant or not. Soybean genotypes excluding ion chloride out of the transpiration stream are termed Cl^- excluders; otherwise, they are includers (Valencia et al., 2008). In addition, the ability to differentiate two chemically similar ions usually enhances adaptability to salinity in plants (Bliss et al., 1984). Salt conditions engender high concentrations of Na^+ and Cl^- in soil medium.

The aptitude of plants to select between Na^+ and K^+, both present in soil medium with Na^+ at a higher concentration than K^+ due to salt stress, is essential for the adaptation to salinity conditions (Shannon, 1997). In this case, plants can limit the uptake of Na^+ and exhibit tolerance to salinity. Ion selectivity in plants is a gene-dependent process (Vose, 1963; Smith and Epstein, 1964).

Ion accumulation

Ion accumulation is another mechanism for salt tolerance in plants. Salt-tolerant plants can accumulate higher concentration of ions such as Na^+ in leaves and store them away from salt-sensitive cellular elements. Such a mechanism has been studied by comparing the responses of a wild tomato species (*Lycorpesicon cheemanii* R.) to a cultivated one (Rush and Epstein, 1980). Their results indicated that the wild tomato species was more tolerant than the cultivated. Rush and Epstein (1980) suggested the wild species had better ion accumulation capacity, making it more tolerant to salinity.

The ion accumulation process is more obvious for halophytes. These types of plants are permanently exposed to salinity conditions. They are able to take up important salt ions and store them into specially designed cellular organs such as salt glands (Levitt, 1980). Afterwards, halophyte plants are able to evacuate the toxic salt ions (Flowers et al., 1977).

Osmotic adjustment

Studies indicated that plants with good osmotic adjustment capabilities were able to maintain their turgor potential under salinity stress (Munns, 1988). The failure to do so results in turgor loss, which undermines cell division and elongation (Harris et al., 1924). During osmotic adjustment in plants, the concentration of solute increases, which results in a decrease in plant osmotic potential (Girma and Krieg, 1992). Such processes help plants better withstand salt stress conditions.

Organic solutes

Organic solutes such as sugars, proline, glycine betaine, and other organic compounds play a major role in salt tolerance. These elements enhance osmotic balance and protect enzyme activity while toxic ions are present (Tal et al., 1979; Greenway and Munns, 1980). Rathert (1984) suggested that the evaluation of the concentration of these elements could be used as a selection criterion to screen salt-tolerant genotypes.

Growth regulation

Under a saline environment, plants showed reduced growth rate. Doing so is an adaptive strategy for salt-tolerant plants to avoid turgor loss and to improve water use efficiency (Zhu, 2000). Inadequate photosynthesis occurs during salt stress due to stomatal closure and resulted in limited carbon dioxide uptake. Regulating these processes enabled plants to cope with salinity.

Genetics and breeding for salt tolerance

Addressing salinity-related issues in agriculture is challenging. Different techniques have been investigated to limit the negative impacts of salinity. One of the strategies is to establish vegetation to prevent groundwater from rising, which is a major source of salinity in agriculture (Pannell, 2001). However, doing so can be expensive and technically challenging. A study carried out by Abeer et al. (2015) showed that arbuscular mycorrhizal fungi could also improve salt tolerance in cowpea. Nevertheless, this approach requires the production of the aforementioned fungi, which will not only increase the cost, but could also be difficult to be scaled up at the smaller farm level.

Breeding is a cost-effective and reasonable way to overcome the effects of salinity in cowpea. This approach proves to be effective in other crops such as soybean. In the last 50 years, conventional breeding positively impacts soybean cultivar improvement (Pathan and Lee, 2007). In addition, breeding considerably contributes to the development of salt-tolerant crops (Bänziger and Araus, 2007; Li and Xu, 2007). Thanks to molecular plant breeding, providing salt tolerant crops could be faster, thus less expensive than other techniques to address the impacts of salinity in agriculture. The use of epigenetics could be also incorporated into a plant breeding platform to optimize the use of plant breeding technologies (Springer, 2013).

Arabidopsis has been used as a model plant to unravel the genetics of salt tolerance in plants (Zhu, 2000). Change in Na^+ and/or K^+ transport engenders salt tolerance in crops. Genes such as *AtHKT1*, *SOS1*, *AtNHX1*, *Rab7*, *DHAR*, *GPX*, *AAO*, *MAPK*, *MAP*, and *MKK2* have been reported to enhance salt tolerance in plants (Zhou et al., 2007).

Molecular breeding in plants

DNA marker technology and next generation sequencing

DNA markers play a major role in plant breeding and genetics by providing key information on the genetic variation among genotypes. RFLP (Restriction fragment length polymorphism), RAPD (Random amplified polymorphism DNA), AFLP (Amplified fragment length polymorphism), SSR (Simple sequence repeat), and SNP (Single nucleotide polymorphism) are the most commonly used DNA markers (Xu, 2010).

RFLP is a Southern blot-based marker. The polymorphism is due to the variation of the recognition sites of the restriction enzymes in the DNA sequence (Botstein et al., 1980). RAPD is a PCR-based marker; the polymorphism is caused by the annealing site between primers in the DNA sequence and the DNA length between the two primer annealing sites (Williams et al., 1990). AFLP is a PCR-based marker relying on the recognition of a particular sequence by restriction enzymes. The restriction sites and the selective PCR primers yield the polymorphism

(Vos et al., 1995). SSR markers are repeat sequence-based markers. The polymorphism is due to the variation of the number of repeats within the DNA target sequence (Tautz and Renz, 1984). SNP is a single variation of nucleotides between DNA sequences which results in polymorphism among genotypes (Batley and Edwards, 2007).

The relatively recent advances in DNA sequencing technology have allowed geneticists to perform high-throughput sequencing. The development of the next generation sequencing (NGS) technologies and platform positively impact genetics, genomics, bioinformatics, plant breeding…being aware and updated on this rapidly changing technology is essential for researchers. Thanks to NGS, performing a whole genome shotgun sequencing, exome sequencing, RNA-sequencing, and ChiP-sequencing (Chromatin Immuno Precipitation) is possible (Liu et al., 2012).

The choice of NGS technologies depends upon the objective and the cost per million bases. Roche 454®, Illumina®, SoliD®, HeliScope®, Ion Torrent®, PacBio®, and Oxford® nanopore are the currently reported NGS platforms (Glenn, 2011) (Figure 1-2). Each platform has its advantages and limitations. The read length of Illumina technology is much shorter than that of PacBio so that using PacBio can solve the problem of repeated sequences during the alignment of reads. However, Illumina has a lower error rate than PacBio (Rhoads and Au, 2015).

Roche 454®
(http://www.ssllc.com/)

Illumina®
(http://biology.as.nyu.edu)

Ion torrent®
(https://www.technologyreview.com)

PacBio®
(http://www.pacb.com)

Figure 1-2. Next generation sequencing technologies

QTL and association mapping

QTL mapping steps consist of developing a mapping population, phenotyping, genotyping, establishing a linkage map, and conducting a marker-trait association analysis (Xu, 2010). Molecular markers, phenotypes, and linkage maps are necessary in a QTL mapping analysis. With respect to salt tolerance, a couple of studies dealt with QTL mapping. In soybean, Lee et al. (2004) reported a major QTL related to salt tolerance in a population consisting of 106 recombinant inbred lines resulting from the cross between soybean S-100 (salt-tolerant) and Tokyo (salt-sensitive). In tomato (*Solanum lycopersicum* L.), QTL analysis pertaining to salt tolerance was performed during seed germination. Foolad and Jones (1993) reported five QTLs associated with tomato-salt tolerance at germination stage on chromosomes 1, 3, 7, 8 and 12.

Chankaew et al. (2014) identified a QTL for salt tolerance in beach cowpea [*Vigna marina* (Burm.) Merrill]. Their results indicated that the QTL accounted for 50% of the phenotypic variance. QTL and association mapping are important parts in molecular breeding as shown in Figure 1-3. Molecular breeding procedures involve donor screening, population development, phenotyping, genotyping, data analysis, and QTL and association analysis. Molecular markers are identified afterwards. The next step is marker (SSR and SNP) implementation, and the use of these markers through marker-assisted selection (MAS).

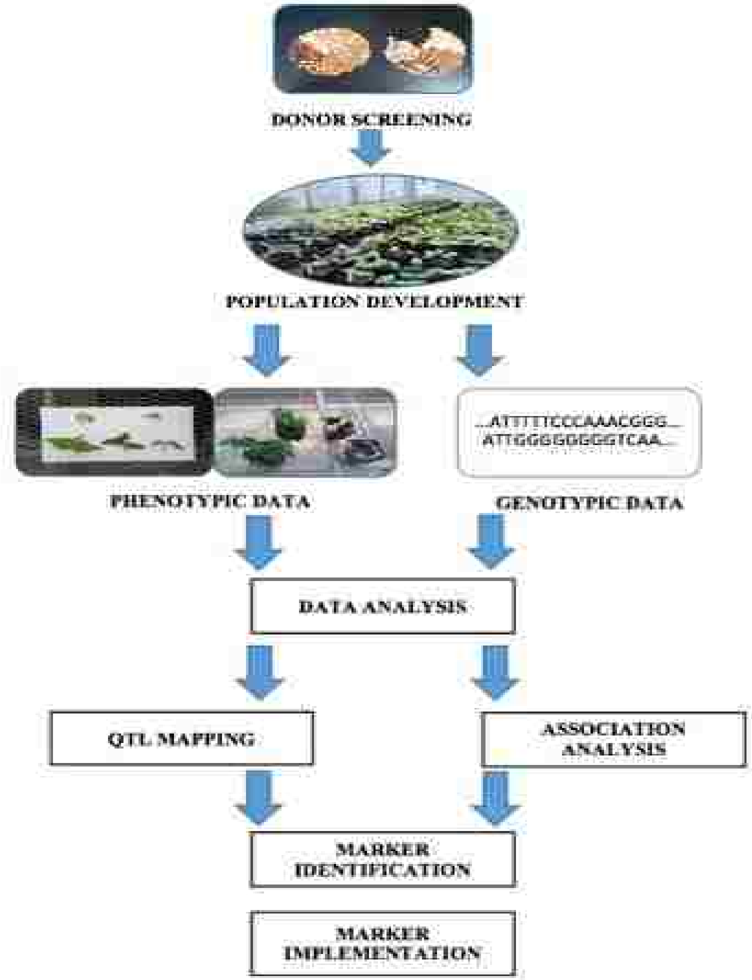

Figure 1-3. Procedure for QTL and association mapping. Adapted from Shi (2014).

Association mapping is an alternative to linkage mapping. It deals with the relationship between phenotype and genotype in unrelated populations (Myles et al., 2009). Association mapping is a rapid and cost-effective way to identify molecular markers, which are associated to traits of interest.

Association mapping has been conducted to find molecular markers associated with important traits in cowpea. Shi et al. (2016) conducted an association analysis study in a total of 249 USDA cowpea lines in order to identify SNP markers associated with bacterial blight (CoBB, *Xanthomonas axonopodis* pv. *Vignicola*, *Xav*). Association mapping was performed using Tassel 5, GAPIT, and Qgene 4. Shi et al. (2016) used 1,031 SNPs, which were postulated from genotyping-by-sequencing (GBS). They identified four SNP markers, C35046071_1260, C35084634_455, scaffold96328_3387, and scaffold96765_4430, which were highly associated with cowpea bacterial blight. SNP markers associated with seed antioxidant and seed coat color were identified from a set of cowpeas consisting of 369 genotypes (Qin et al., 2016). A total of 1,047 SNP markers were used. Three models, single marker regression (SMR), general linear model (GLM_Q), and mixed linear model (MLM_Q+K), were used by Qin et al. (2016). Scaffold7139_14363 and Scaffold29110_4657 were found to be associated with seed antioxidant content in cowpea. Qin et al. (2016) reported that C35063613_1497, Scaffold81493_886, and Scaffold84620_6785 were associated with seed coat color in cowpea. Seed coat color is an important agronomic trait. In addition to conducting an association analysis study, SNP markers were also used to infer population structure and assess genetic diversity in cowpea. Xiong et al. (2016) conducted such studies by utilizing 798 cowpea genotypes, which were originally from 56 countries, with 5,828 SNP markers. Association mapping is a powerful tool to identify SNP markers in cowpea. To date, association mapping for salt tolerance has not yet been achieved for cowpea.

Rationale and significance

Of the top ten largest cities worldwide in 2100, seven will be in Africa (https://www.thechicagocouncil.org/blog/global-food-thought/growing-food-growing-cities-opportunities-agtech). Singh et al. (2003) reported that over 70% of cowpea worldwide production came from Africa. Being one of the most consumed legumes in Africa, an increase in demand in this crop will be expected in the coming years. However, salinity-related issues are acute in semi-arid regions such as Sub-Saharan Africa where cowpea is widely cultivated (Zhang et al., 2012). Therefore, studies contributing toward reducing negative impacts of salinity on cowpea are needed.

This study will have applications in advancing cowpea genetics and breeding. The SNP markers related to salt tolerance can be used as molecular markers for cowpea breeders. Doing so will speed up the release of salt-tolerant cowpea cultivars. Moreover, this research will enhance food security by developing a nutritional crop. The use of salt-tolerant cowpea cultivars will engender economic benefit for farmers where the negative effects of soil salinity are acute. The use of salt-tolerant cultivars is considered as the most cost effective way to address the damaging impacts of salinity.

The objectives of this study are:

- To evaluate cowpea salt tolerance at germination stage
- To evaluate cowpea salt tolerance at seedling stage
- To conduct an association study for cowpea salt tolerance at germination and seedling stages, and
- To identify SNP markers associated with salt tolerance in cowpea.

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Chapter 2. Evaluation of Salt Tolerance at Germination Stage in Cowpea (*Vigna unguiculata* (L.) Walp)

Abstract

Cowpea is a leguminous and versatile crop which provides nutritional food for human consumption. However, salinity unfavorably reduces cowpea seed germination, thus significantly decreasing cowpea production. Little has been done for evaluating and developing salt-tolerant cowpea genotypes at germination stage. The objectives of this research were to evaluate the response of cowpea genotypes to salinity stress through seed germination rate and to select salt-tolerant genotypes. The seed germination rates under non-salt condition and salinity stress were evaluated in 151 cowpea genotypes. Four parameters, absolute decrease (AD), the inhibition index (II), the relative salt tolerance (RST), and the salt tolerance index (STI) were used to measure salt tolerance in cowpea. The results showed that there were significant differences among the 151 cowpea genotypes for all parameters (p-values<.0001). The AD in germination rate was 5.8% to 94.2%; the II varied from 7.7% to 100%; the RST ranged from 0 to 0.92; and STI varied from 0 to 0.92. A high broad sense heritability (H^2) was observed for all four parameters. High correlation coefficients (r) were estimated among the four parameters. PI582422, 09-529, PI293584, and PI582570 were highly salt tolerant at germination stage, thus they could be used in a breeding program to develop cowpea salt-tolerant cultivars.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] (2n=2x=22) is a legume of economic importance worldwide. It is widely grown in Central and West Africa with a production estimated at 5.4 million tons of dry seed (Olufajo, 2012), and also grown in Latin America, Southeast Asia and in the southern United States (Muchero et al., 2009; Tan et al., 2012). Cowpea plays an essential role for food security and ecosystem. Cowpea has high seed protein content and other nutritional components for human benefit. It also supplies nitrogen to soils (Duke, 1990). Mamiro et al. (2011) reported that cowpea dried seeds had a fat concentration ranging from 5.4 to 11.2% depending on cultivar. The calcium content ranged from 360 to 992.4 mg/kg. The concentration of zinc varied between 31.5 to 35.6 mg/kg. The iron content ranged from 27.6 to 28.9 mg/kg. In addition, their results suggested that cowpea leaves are also rich in micronutrients. In the United States, cowpea, also commonly referred as southern pea, blackeye pea, crowder pea, lubia, niebe, coupe, or frijole is usually grown by small-scale farmers (less than 50 ha) mainly in southern states. It is a profitable crop for growers (Davis et al., 1991; Okiror et al., 2008).

Germination is a pivotal step for crop propagation. Studies reported that germination is highly influenced by a large number of environmental factors. Mistura et al. (2011) stated that salinity affected germination of legumes and plant vigor. Dutta and Bera (2014) found a noticeable decrease in seed germination, plant growth, and vigor indexes for mung bean supplied with salt treatment. In addition, Zhang et al. (2013) stated that low temperatures undermine cowpea germination. Their results indicated that germination rate decreases to 18% at 10°C. Reddy and Reddy (2016) reported that important and incessant rainfall decreased the seed germination of soybean in India. Low germination unfavorably affects crop production. Moreover, seed quality is an important required feature by seed testing centers. To ensure a stable market, seed producer companies have to provide good quality seeds exhibiting high germination rate. Moreover, seed is a principal component in agriculture (Ellis, 1993; Covell et al., 1986). It ensures the propagation of plants to properly ensure food supplies for human consumption (Bewley, 2003). Plant seeds consist of three principal components. The embryo becomes the vegetative plant; the endosperm is used as source of nourishment; the testa or seed coat surrounds the embryo and the endosperm (Ohto et al., 2007). The reserves consisting of lipids, proteins, and carbohydrates are stored in the testa of seeds. There is a close interaction between these three seed parts during seed germination processes (Dieter and Bouman, 1995).

Saline soil is characterized by high concentration of cations (K^+, Mg^{2+}, Ca^{2+}, and Na^+) and anions (NO_3^-, HCO_3^-, SO_4^{2-}, and Cl^-) (Wallender and Tanji, 2011). Sodium chloride is the most well-known salt causing damage in agriculture (Ayers and Westcot, 1985). In addition, Shannon (1997) reported that the concentration of salt in cultivated area has been increasing due to inappropriate irrigation, fertilization, or other factors. Such increase is estimated at 1 to 60 metric tons per hectare. Läuchli and Lüttge (2002) pointed out that cost related to salinity issue was 12 billon US dollars. Rock weathering, seawater, rain, deforestation, air pollution, and contamination of river waters by chemical and domestic animals are substantial sources of salinity in agriculture (Rengasamy et al., 2006; Omami and Hammes, 2006).

However, little has been done regarding studies on seed germination in cowpea along with the factors that markedly affect germination rate. A study conducted by Zahedi et al. (2012) reported that salinity decreased germination rate in cowpea, and poor seed germination resulted in a significant reduction in yield. In addition, salinity had negative effects on plant growth, cell extension, cell division, and photosynthesis (Maas and Hoffman, 1977).

The evaluation of salt tolerance requires a controlled environment. Field screening is an available technique to screen plant genotypes for salinity. For this method, seeds are sown directly in soil with high salt concentration. However, due to the variability of salt content in the field and the considerable influence of other factors such as moisture content in soil, soil fertility, temperature, light, transpiration and weather, the results might be highly biased and non-conclusive in a field setting (Parker et al., 1983; Yang and Blanchar, 1993; Pathan et al., 2007). Plant genotypes can be also hydroponically screened by using nutrient solution as a medium growth and adding salt solution after germination and establishment (An et al., 2001). However, such a method is expensive. Therefore, screening salt-tolerant genotypes at germination stage could provide accurate results because the investigation is carried out in a controlled environment.

Screening for salt-tolerant genotypes at germination stage is one of the most cost effective ways to tackle salinity-related issues. In this view, some research has dealt particularly with the effects of salinity on germination rate and emergence (Kent and Lauchli, 1985; Badia and Meiri, 1994; Mauromicale and Licandro, 2002; Carter et al., 2006). In soybean (*Glycine max* L.), Kan et al. (2015) evaluated the effects of salt stress on a panel of 191 soybean genotypes and identified eight SNP markers highly associated with tolerance to salinity at germination. Concerning cowpea, Wests and Francois (1982) suggested that a salt concentration higher than 120 mM would reduce cowpea germination. Salinity engenders osmotic or toxicity effects, which result in low seed germination (Waisel, 1972). In addition, Taffouo et al. (2009) reported that high sodium chloride concentration significantly affected germination rate in cowpea. Ashebir et al., (2013) studied the effects of salinity at germination in cowpea. Their results revealed that there was a significant variability in response to salt stress among cowpea genotypes. They found that the genotype “211557” and “Asebot” were salt tolerant at germination stage. The objectives of this study were to evaluate the responses of cowpea genotypes to salinity (NaCl) at germination stage, to screen salt-tolerant cowpea genotypes, and to select for the most accurate parameter for assessing cowpea salt tolerance at germination stage for a salt-tolerance breeding program.

Materials and Methods

Plant Materials

A total of 151 cowpea genotypes consisting of 116 United States Department of Agriculture (USDA) Germplasm Resources Information Network (GRIN) germplasm accessions and 35 University of Arkansas lines were used in this study (Supplementary Table S2-1). The 116 germplasm lines were originally collected from 31 countries, and classified into 12 regions depending on their origin (Caribbean, Eastern Africa, Southern Africa, Western Africa, Eastern Asia, Southeastern Asia, Southern Asia, Western Asia Europe, Latin America, and North America). All original seeds of the GRIN germplasm were obtained from the USDA Plant Genetic Resources Conservation Unit at Griffin, GA. They were increased at the Research and Extension Station of the University of Arkansas at Fayetteville, AR in summers 2014 and 2015.

Determination of optimal seed germination temperature and salt concentration

Two pilot experiments were conducted to determine the optimal temperature and salt (NaCl) concentration for cowpea seed germination. For the temperature experiment, the cowpea cultivar “Early Scarlet” was used to test seed germination under three thermogradient temperatures (25ºC, 28ºC, and 31ºC). The results showed that Early Scarlet had the highest seed germination rate at 28 ºC, which was the same optimum temperature for cowpea seed germination reported by Souza et al. (2004). In regard to salt concentration, five cowpea genotypes (Early Scarlet, 07-303, 09-655, PI293584, and PI527561) were tested for seed germination under six levels of concentration (0, 50, 100, 150, 200, and 250 mM NaCl) to determine the optimal NaCl concentration for salt stress. The concentrations were obtained by dissolving 2.92, 5.84, 8.77, 11.69, and 14.61 g of sodium chloride powder of Science Company®, Lakewood, CO, respectively.

Germination conditions

The cowpea seeds used in this study were harvested from the field of University of Arkansas Research and Extension Center at Fayetteville, AR during summer 2015. Seeds having uniform size from each cowpea genotype were selected. To avoid any contamination, clean seeds were selected, and petri dishes used for germination were sterilized by washing with bleach 2% followed by ethanol 75%.

Forty seeds from each cowpea genotype were put on paper filter (Lab Nerd), which was previously placed in a petri dish of 9 cm in diameter. The treatment consisted of adding 14 ml of NaCl solution and deionized water for the control (0 mM NaCl) to each dish. After treatments were applied, the petri dishes were placed in an incubator New Brunswick Scientific Innova 4230® (Manasquan, NJ) at 28°C for 48 hrs. Each genotype and salt treatment combination was placed on three different shelves in the incubator with three replicates, and each shelf was treated as a block. The experiment was run in multiple times due to space limitations. After each run, 75% ethanol solution was sprayed into the incubator to limit any microbial growth.

Measurements

The seed germination data were gathered 48 hours after placing the petri dishes in the incubator. The seed germination rate was recorded when the radicle reached one third of the seed length. The performance of the cowpea genotype under salinity stress was evaluated by computing the values of absolute decrease (AD) due to salinity, inhibition index (II) (González, 1996), relative salt tolerance (RST), and salt tolerance index (STI) (Fernandez, 1992; Saad et al., 2014). These parameters were estimated using the following formulas:

$$\text{Absolute Decrease (AD)} = GC - GS;$$

$$\text{Inhibition Index (II)} = 100 * (GC - GS) / (GC);$$

$$\text{Relative Salt Tolerance (RST)} = GS / GC;$$

$$\text{Salt Tolerance Index (STI)} = (GS * GC) / (GC_{av})^2,$$

where GC = Seed germination rate without salt stress, GS = Seed germination under salt stress, and

GC_{av} = Average of the seed germination rate of a cowpea genotype.

Experimental design

Regarding the preliminary test related to the determination of the optimal salt (NaCl) concentration, a two-factor factorial (genotype X salt) organized in a randomized complete block design (RCBD) with three blocks was adopted. The genotype consisted of Early Scarlet, 07-303, 09-655, PI293584, and PI527561, and the salt treatment levels were 0, 50, 100, 150, 200, and 250 mM. Three replications per genotype and salt treatment combination were used.

With respect to the assessment of salt tolerance among the 151 cowpea genotypes, the design was similar to that of the preliminary test using RCBD. However, the salt treatments were the optimal salt concentration from the preliminary test (150 mM NaCl) and the deionized water with 0 mM NaCl. Each salt-genotype combination was assigned to petri dishes, replicated three times, and each replication corresponded to each of the three shelves in the incubator and the shelf was used as a block.

Data analysis

The parameters used for the analysis resulted from pairing data on a genotype under salinity treatment and without salt stress. Therefore, the statistical model for the analysis was as described below.

In the calculations, Y_{ijk} = value of the parameters for the j^{th} cowpea genotype on the i^{th} shelve at the k^{th} replication, for i = 1, 2, 3; j = 1, … , 151, and k = 1, 2, 3.

$$Y_{ijk} = \mu + S_i + G_j + \boldsymbol{\varepsilon}_{ijk}$$

Where μ: constant (overall mean), S_i: Effect of the i^{th} shelf (random effect) on the variability of the response, G_j: Effect of the j^{th} genotype (fixed effect) on the mean response, and $\boldsymbol{\varepsilon}_{ijk}$: experimental error associated with the ijk^{th} observation. In this study, the effects of experiment runs would be assumed as negligible as the germination study was carried out in an incubator. However, the effect of shelves in the incubator should be taken into account because there could be within incubator temperature variability.

The ANOVA test was carried out using the general linear model (GLM) procedure of JMP Genomics 7 (SAS Institute, Cary, NC). The mean separation was performed using the Student T-test at alpha=0.05. The descriptive statistics were generated using ‘Tabulate’; the correlations among the parameters were analyzed using ‘Multivariate Methods” by “Multivariate” function; and the distribution of the data was drawn using ‘Distribution’ in JMP Genomics 7.

The broad sense heritability (H^2) was obtained from

$H^2 = (σ^2_G / σ^2_P)$ x $100= [σ^2_G / (σ^2_G + (σ^2_E / r)+ (σ^2_S/s)]$ x 100 (Hosseini et al., 2012)

where $σ^2_G$: Genotypic variance, $σ^2_P$: Phenotypic variance, $σ^2_E$: Variance associated with the experimental error, σ^2_S: Variance associated with the shelf, s: number of shelves, and r: number of replications per treatment

σ^2_G, σ^2_E, and σ^2_S were obtained using the following formulas:

$\sigma^2_G = (MSG - MSE) / r$, $\sigma^2_E = MSE$, $\sigma^2_S = (MSS - MSE) / n$ where MSG: Mean Square Genotype, MSE: Mean Square Error, and MSS = Mean Square Shelve, r = number of replications, and n = number of genotypes.

In addition, the effects of the origin (region) of the lines on salt tolerance were analyzed using ANOVA. Prior to this analysis, the genotypes without any information on their origin were discarded so that 132 lines were analyzed.

The cluster analysis involved 151 individuals which were measured using six parameters: germination rate without salt stress (Non-stress) and under salt treatment (Stress), absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI). Ward's method was used as a clustering technique (Sahu, 2013). The phylogenetic tree diagram was designed using 'Multivariate Methods" by "Cluster" in JMP Genomics 7 (SAS Institute, Cary, NC).

Results and Discussion

Optimal concentration for the assessment of cowpea salt tolerance at germination stage

From our preliminary experiment, when the NaCl concentration increased, the seed germination rate decreased either pooled across cowpea genotypes or individually (Figure 2-1). ANOVA indicated that germination rate was significantly different at p-value < 0.0001 (Table 2-1) under the six salt concentrations (0, 50, 100, 150, 200, and 250 mM NaCl). The mean germination rate ranged from 2.5 % (250 mM) to 93.5% (0 mM). For salinity level 150 mM, the seeds had minimal germination making it difficult to separate the tolerant from the susceptible genotypes (Table 2-2). At 150 mM, seed germination differed most among the cowpea genotypes (F=27.37, p-value=0.0001). Therefore, the salt concentration of 150 mM NaCl would be a reasonable concentration to perform salt tolerance testing in cowpea. Wests and Francois (1982) suggested that salt concentration greater than 120 mM NaCl significantly affected seed germination in cowpea. In addition, Thiam et al. (2013) pointed out that high salinity decreased cowpea germination. Zahedi et al. (2012) reported that the increase of salt concentration unfavorably impacted cowpea germination. Lobato et al. (2009) used 150 mM NaCl to conduct their study on the effects of salinity on cowpea germination. According to those reports and our own experiment, we selected the salt concentration with 150 mM NaCl to perform the evaluation of cowpea salt tolerance at germination stage.

Germination at non-salt stress and salt stress conditions

The average germination rate among 151 cowpea genotypes under the non-salt stress condition varied from 60.0% to 99.2%, with a mean of 80.2% and a standard deviation of 11.0%. After supplying salt treatment to the cowpea genotypes, the germination dropped down to 0% to 77.5%, with an overall average of 38.7%, and a standard deviation of 18.3%. These results suggested that salinity significantly reduced the germination rate in cowpea. For all parameters, there was no significant shelf effects. The germination rate was significantly different among the cowpea genotypes under non-stress condition (F=7.37, p-value<0.0001) (Table 2-3). Envoy (99.2%), PI583194 (99.2%), PI487518 (98.3%), PI582579 (97.5%), PI218123 (97.5%), PI253428 (97.5%), PI255765 (97.5%), and PI582421 (97.5%) had the highest germination rate, and PI225922 (60.8%), PI339610 (60.8%), and PI347639 (60%) had the lowest germination rate. Under salinity, PI201498 performed well with a germination rate of 77.5%, indicating it is a salt-tolerant cowpea accession at germination stage; however, the lowest germination rate was recorded for PI252665 (1.7%), 09-393 (0.8%), PI582522 (0.8%), and PI582813 (0.0%), indicating that they are very susceptible to salt stress at the germination stage. The germination rate among the cowpea genotypes was significantly different under salt stress (F=16.62, p-value<0.0001) (Table 2-3).

Absolute decrease and inhibition index

The absolute decrease (AD) indicated the decrease of the germination rate between the non-salinity conditions and the salt treatment. In this study, salinity reduced germination rate from 5.8% to 94.2%. The cowpea genotypes responded differently to salinity environment at germination stage in terms of absolute decrease (F=10.1, p-value<0.0001) (Table 2-3). PI582522 (94.2%) exhibited the highest absolute decrease. PI585422 had the lowest absolute decrease, 5.8%. The higher the absolute decrease was, the more salt susceptible the genotype.

The inhibition index (II) is a parameter which is widely used for studies related to plant stress (González, 1996). The inhibition index of the germination ranged from 7.7% to 100.0%, with an average of 51.2% and a standard deviation of 22.6%, indicating a large variability in responses to salinity among the cowpea genotypes. In addition, the inhibition of the germination due to salinity significantly differed among the cowpea genotypes (F=11.6, p-value<.0001) (Table 2-3). PI582813, PI582522, and 09-393 had a very high inhibition index, over 99% under salt stress. These results suggested that these lines are highly salt sensitive at germination stage. PI582422, 09-529, PI293584, PI582570, and PI339611 had the lowest inhibition indexes, which were 7.7%, 12.2%, 13.3%, 13.6%, and 14.6%, respectively. These accessions could be excellent sources for salt tolerance at germination stage. The lower the inhibition index is, the more likely the genotype withstood salt stress (González, 1996).

Relative salt tolerance and salt-tolerance index

The relative salt tolerance (STI) was obtained from the ratio between the germination rate under salt stress and the germination rate without salt treatment. The salt-tolerance index (STI) was calculated by dividing the value of the germination rate under saline conditions by the pooled germination rate under the non-saline environment across replications. The higher the RST or STI parameter was , the more likely the genotype was salt tolerant (Fernandez, 1992; Saad et al., 2014).

The RST varied from 0 to 0.92, with a mean of 0.49 and a standard deviation of 0.23. RST was significantly different among the cowpea genotypes (F=11.99, p-value<.0001) (Table 2-3). PI582422, 09-529, PI293584, and PI582570 had the highest RST scores, indicating that they are salt tolerant based on RST. PI582813, 09-393, PI582522, and PI582665 exhibited the lowest relative salt tolerance scores, suggesting that they are salt-sensitive at germination stage.

The STI ranged from 0 to 0.92, with a mean of 0.48 and a standard deviation of 0.22. Significant differences were observed among the cowpea accessions in terms of STI (F=13.16, p-value<.0001) (Table 2-3). PI582422 (0.92), 09-529 (0.87), PI293584 (0.86), and PI582570 (0.85) exhibited the highest salt tolerance index, suggesting that these lines are highly tolerant to salinity at germination stage. The lowest salt tolerance index was found in PI582813 (0), 09-393 (0.01), PI582522 (0.01), and PI582665 (0.02) (Table 2-3), indicating that these lines are highly salt-sensitive.

The population dynamic of a crop is closely related to its seed germination (TeKrony and Egli, 1991). In this study, large variation of responses to salinity among the cowpea genotypes was observed for all parameters. As expected, there was a significant effect of salinity on the germination rate of the cowpea panel. Similar results have been found in other studies (Wests and Francois, 1982; Taffouo et al., 2009; Ashebir et al., 2013).

Analysis by geographical location

Significant differences were observed in seed germination rate without salt treatment and the germination rate under salt stress, absolute decrease, inhibition index, relative salt tolerance, and salt tolerance index among the 12 different regions of origin of the cowpea genotypes (p-values<.0001) (Table 2-4). Genotypes from the Caribbean and Southern Asia showed better salt tolerance at the germination stage. The inhibition index was smaller than 40%, and both relative salt tolerance and salt tolerance indexes were greater than 0.6 on average. Cowpea lines from Europe and North America were more sensitive to salinity at the germination stage (Table 2-5).

The results suggested that the origins of those genotypes markedly impacted their response to salinity, so the origin should be taken into a consideration when one selects cowpea genotypes for salt tolerance. Little has been done regarding the effect of ecological distribution on plant salt tolerance. However, geographical distribution proved to be a strong driving factor on plant adaptation to stress. Burke (1990) reported that crops grown in semi-arid areas developed mechanisms which enabled them to overcome permanent exposure to high temperature. Those mechanisms involved both cellular adaptation and photosynthetic responses to heat stress. In addition, Stankowski et al. (2015) showed that ecology played a pivotal role in plant adaptation. Their study on *Mimulus aurantiacus* C., a flower plant, revealed that floral trait had evolved according to the local conditions, suggesting that geographical location had shaped the adaptation of some traits to a specific environment. With respect to salt tolerance in cowpea, further investigation is required to unravel the mechanisms behind these significant differences in response to salinity among the genotypes from different countries.

Broad sense heritability

The broad sense heritability (H^2) associated with the seed germination without salt stress was 68%. However, it was 83.9% under saline conditions. H^2 was 75.2% for the absolute decrease in germination rate due to salt stress. The results indicated a high broad sense heritability for the parameters related to the inhibition index, relative salt tolerance, and salt tolerance index, with H^2 equal to 77.9%, 78.2%, and 80.8%, respectively. Foolad and Jones (1992) found high heritability for tomato salt tolerance at seed germination stage. They estimated heritability to be 76.0% for tomato salt tolerance of an $F_{2:3}$ population derived from a cross between PI174263 and UCT5, which was close to that of salt tolerance in cowpea in this current investigation.

Correlation between the parameters

The six parameters, without salt stress (Non-stress), with salt stress (Stress), absolute decrease (AD), relative salt tolerance (RST), and salt tolerance index (STI) involved in this study showed near normal distributions (Figures 2-3 and 2-4). The correlation coefficients among the six parameters were estimated (Table 2-6). There was a relatively low linear correlation between the germination rate under non-salt stress condition and the other five parameters related to salinity stress, with r = 0.20, 0.38, 0.10, -0.10, and 0.08, respectively (Table 2-6), suggesting that salt tolerance at germination stage had a weak association with the germination rate in normal conditions in cowpea. However, the seed germination rate under salt stress had a very high negative linear correlation with the absolute decrease (r=-0.83) and the inhibition index (r= -0.95), but a high positive linear correlation observed between the germination rate under salt treatment and the relative salt tolerance (r=0.95), and the salt tolerance index (r=0.95). These results indicate that salt tolerance at germination stage is highly associated with germination rate under salt stress.

Cluster analysis

Five different groups were identified among the 151 cowpea genotypes involved in this study (Figure S2-1). The three cowpea accessions, PI582422, PI293584, and PI582570 having the highest STI were clustered together and located at the same cluster, and the four lowest STI genotypes, PI582813, 09-393, PI582522, and PI582665 were clustered together and belonged to another cluster.

Conclusion

This study provides data on the responses of cowpea genotype to salinity, which could be used to screen for salt tolerant parents for breeding purposes. To our knowledge, this is one of the first reports dealing with cowpea salt tolerance at germination stage which involved a large number of cowpea genotypes having a wide range of variability in terms of country of origin. The results suggest that PI582422, PI293584, PI582570, and 09-529 have strong salt tolerance at germination stage. In addition, the most salt-sensitive lines PI582813, 09-393, PI582522, and PI582665 could be used as parents for developing populations for QTL mapping for cowpea salt tolerance at germination stage. The salt tolerant lines can be used in breeding programs to develop salt-tolerant cultivars.

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Table 2-1. ANOVA for pooled seed germination at six NaCl concentrations for five cowpea genotypes.

Source	DF	Sum of squares	Mean square	F Ratio	Prob > F
Shelf	2	2.534	1.267	0.5061	0.6175
Concentration	5	23605.7	4721.1472	1885.53	<.0001
Error	10	25.039	2.5		

Table 2-2. ANOVA for seed germination of five cowpea genotypes in six salt concentrations of NaCl.

NaCl concentration	Source	DF	Sum of squares	Mean square	F Ratio	Prob > F
0mM	Shelf	2	7.50	3.75	0.2553	0.7807
	Genotype	4	397.50	99.38	6.766	0.0111
	Error	8	117.50	14.69		
50mM	Shelf	2	22.50	11.25	0.4269	0.6666
	Genotype	4	354.17	88.54	3.3597	0.068
	Error	8	210.83	26.35		
100mM	Shelf	2	40.83	20.42	1.0262	0.4011
	Genotype	4	373.33	93.33	4.6911	0.0304
	Error	8	159.17	19.90		
150mM	Shelf	2	55.83	27.92	1.8741	0.215
	Genotype	4	1630.83	407.71	27.3706	0.0001
	Error	8	119.17	14.90		
200mM	Shelf	2	3.70	1.85	0.2441	0.789
	Genotype	4	458.57	114.64	15.1259	0.0008
	Error	8	60.63	7.58		
250mM	Shelf	2	7.50	3.75	0.7059	0.522
	Genotype	4	137.50	34.38	6.4706	0.0126
	Error	8	42.50	5.31		

Table 2-3. ANOVA for traits related to cowpea seed germination rate.

Parameters	Source	DF	Sum of squares	Mean square	F Ratio	Prob > F	Estimated Mean square
Germination without salt stress	Shelf	2	214.528	107.264	2.163	0.1167	$\sigma^2_e + n*\sigma^2_s$
	Genotype	150	54845.211	365.635	7.374	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	300	14875.217	49.584			σ^2_e
Germination under 150 mM NaCl	Shelf	2	86.865	43.433	0.715	0.4899	$\sigma^2_e + n*\sigma^2_s$
	Genotype	150	151389.901	1009.266	16.621	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	300	18217.301	60.724			σ^2_e
Absolute decrease	Shelf	2	55.025	27.513	0.245	0.7828	$\sigma^2_e + n*\sigma^2_s$
	Genotype	150	169967.007	1133.113	10.093	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	300	33681.420	112.271			σ^2_e
Inhibition index	Shelf	2	40.441	20.221	0.153	0.8581	$\sigma^2_e + n*\sigma^2_s$
	Genotype	150	229783.382	1531.889	11.597	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	300	39629.810	132.099			σ^2_e
Relative salt tolerance	Shelf	2	0.004	0.002	0.139	0.8699	$\sigma^2_e + n*\sigma^2_s$
	Genotype	150	22.947	0.153	11.587	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	300	3.961	0.013			σ^2_e
Salt tolerance index	Shelf	2	0.058	0.029	2.560	0.079	$\sigma^2_e + n*\sigma^2_s$
	Genotype	150	22.465	0.150	13.164	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	300	3.413	0.011			σ^2_e

Table 2-4. ANOVA for traits related to the origin of the cowpea genotypes.

Traits	Source	DF	Sum of squares	Mean square	F Ratio	Prob > F
Non_stress_(%)	Region	11	6038.717	548.974	3.9444	<.0001
	Error	384	53444.976	139.18		
	C. Total	395	59483.693			
Stress_(%)	Region	11	9729.556	884.505	2.4725	0.0053
	Error	384	137371.517	357.738		
	C. Total	395	147101.073			
Absolute_decresease_(%)	Region	11	10023.452	911.223	2.0521	0.0229
	Error	384	170513.885	444.047		
	C. Total	395	180537.337			
Inhibition_Index_(%)	Region	11	14775.172	1343.197	2.3674	0.0077
	Error	384	217873.973	567.38		
	C. Total	395	232649.145			
Relative_Salt_tolerance	Region	11	1.479	0.134	2.3737	0.0076
	Error	384	21.754	0.057		
	C. Total	395	23.233			
Salt_tolerance_index	Region	11	1.411	0.128	2.3359	0.0086
	Error	384	21.081	0.055		
	C. Total	395	22.491			

Table 2-5. Mean separation for salt tolerance parameters of different regions.

Regions	**Relative salt tolerance index**		**Salt tolerance index**		**Absolute decrease (%)**		**Inhibition index (%)**	
Caribbean	0.80	A	0.79	A	15.70	D	19.91	D
Southern_Asia	0.61	AB	0.61	AB	33.02	CD	39.57	CD
Southeastern_Asia	0.53	ABCD	0.53	ABCD	42.92	ABC	47.18	ABC
Western_Africa	0.57	BC	0.56	BC	37.52	BC	43.31	BC
Southern_Africa	0.50	BCD	0.49	CD	38.97	BC	50.45	ABC
Eastern_Africa	0.48	BCD	0.45	BCD	43.56	ABC	51.79	ABC
Latin_America	0.48	BCD	0.47	CD	43.89	ABC	52.27	ABC
Eastern_Asia	0.47	BCD	0.46	BCD	42.50	ABC	53.26	ABC
Oceania	0.44	BCD	0.46	CD	38.33	ABCD	55.66	ABC
Western_Asia	0.46	CD	0.48	CD	47.81	AB	53.82	AB
North_America	0.45	D	0.45	D	45.76	A	54.81	A
Europe	0.40	D	0.41	D	42.50	ABC	59.58	A

*Means followed by the same letter are not significantly different using the Student T-test at alpha=0.05.

Table 2-6. Correlation among six parameters, without salt stress (Non-stress), with salt stress (Stress), absolute decrease (AD), relative salt tolerance index (RST), and salt tolerance index (STI).

Correlation	Non-stress	Stress	AD	II	RST	STI
Non-stress	1	0.20	0.38	0.10	-0.10	-0.08
Stress	0.20	1	-0.83	-0.95	0.95	0.95
AD	0.38	-0.83	1	0.95	-0.95	-0.95
II	0.10	-0.95	0.95	1	-1	-1
RST	-0.10	0.95	-0.95	-1	1	1
STI	-0.08	0.95	-0.95	-1	1	1

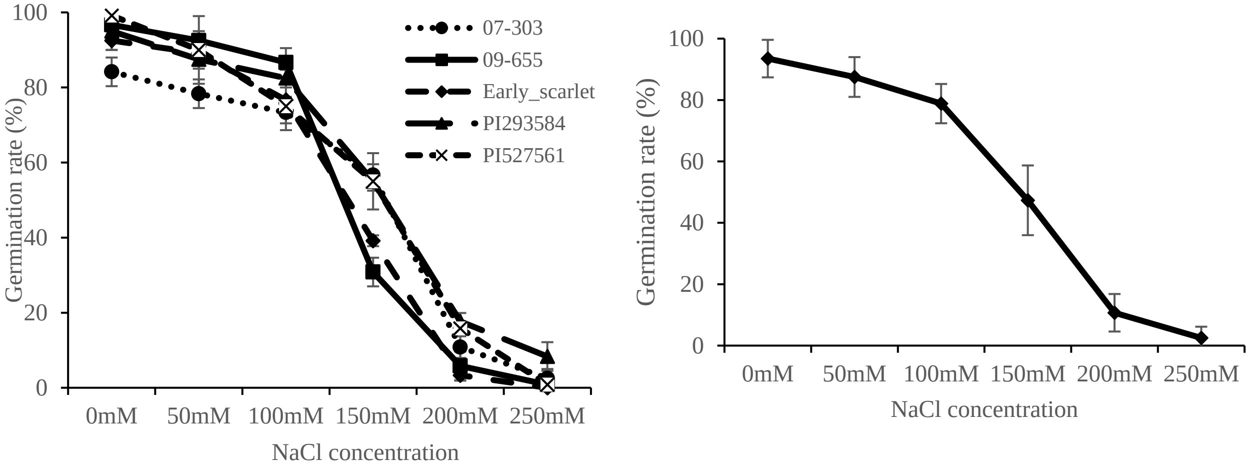

Figure 2-1. Seed germination rate under six salt (NaCl) concentrations in (A) six cowpea genotypes, respectively, and (B) in the pooled five cowpea genotypes.

Figure 2-2. An example of photo for seed germination rate in two cowpea genotypes, ’09-655’ and PI293584 under six salt (NaCl) concentrations in the petri dishes (left) and the radicle length (right) in 48 hours after NaCl treatment at 28 ºC condition.

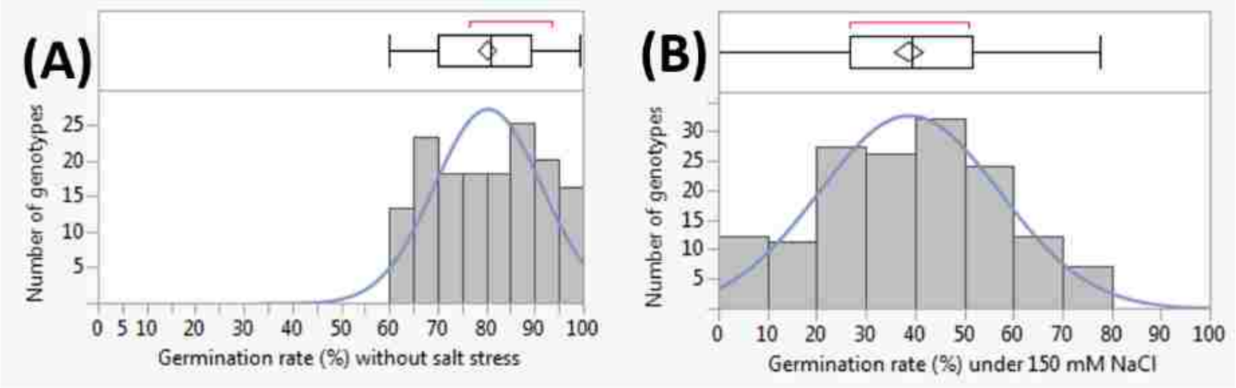

Figure 2-3. Distribution of seed germination rate among 151 cowpea genotypes: (A) without salt stress, and (B) with salt stress.

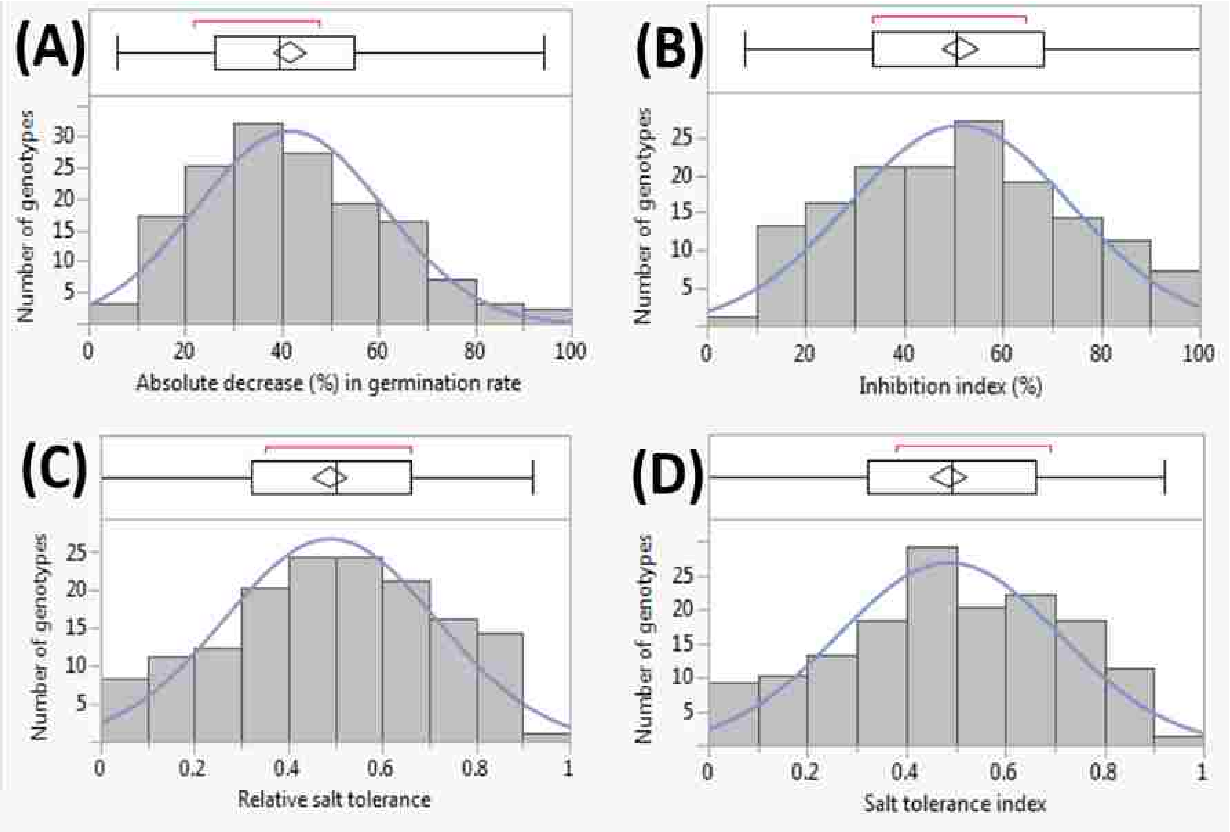

Figure 2-4. Distributions of four parameters among 151 cowpea genotypes: (A) absolute decrease in germination rate, (B) inhibition index, (C) relative salt tolerance, and (D) salt tolerance index.

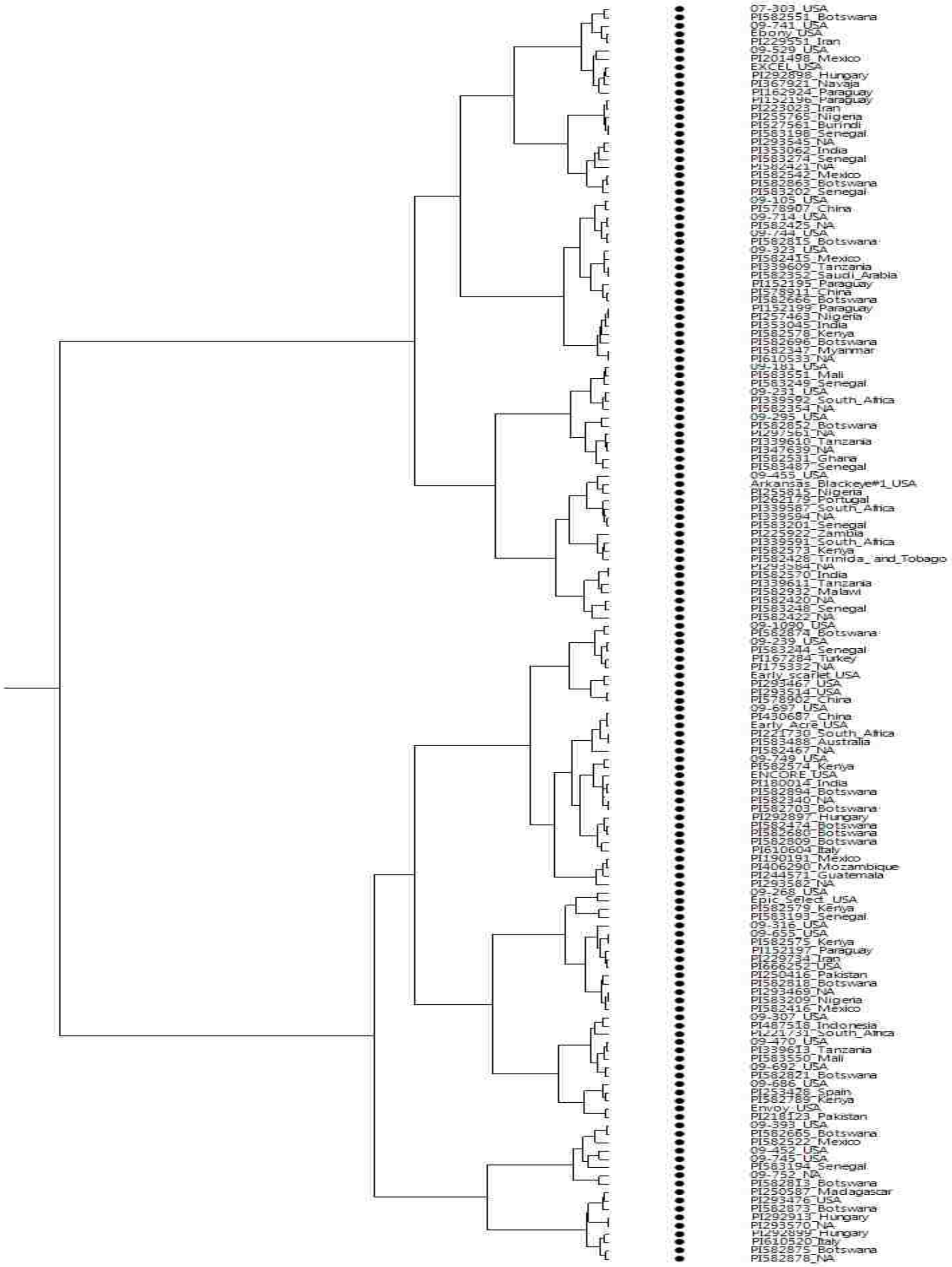

Figure S2-1. Phylogenetic tree diagram among 151 cowpea genotypes based on six salt tolerant parameters.

Table S2-1. Cowpea name (accession number), seed color, origin, germination rates without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI).

Accession_number	Seed_color	Origin	Non-stress_(%)	Stress_(%)	AD_(%)	II_(%)	RST	STI
07-303	Red	AR, USA	82.50	60.83	21.67	25.83	0.74	0.73
09-105	Cream	AR, USA	89.17	46.67	42.50	47.59	0.52	0.52
09-1090	Pinkeye	AR, USA	85.00	35.00	50.00	58.85	0.41	0.41
09-181	Pinkeye	AR, USA	72.50	49.17	23.33	32.09	0.68	0.68
09-231	Pinkeye	AR, USA	68.50	42.50	26.00	37.65	0.62	0.62
09-239	Pinkeye	AR, USA	80.83	34.17	46.67	57.88	0.42	0.43
09-268	Cream	AR, USA	83.33	16.67	66.67	79.92	0.20	0.20
09-295	Pinkeye	AR, USA	64.17	35.83	28.33	44.12	0.56	0.56
09-307	Blackeye	AR, USA	96.67	46.67	50.00	51.71	0.48	0.48
09-316	Black Holstein	AR, USA	90.00	31.67	58.33	64.75	0.35	0.35
09-323	Pinkeye	AR, USA	86.67	50.00	36.67	42.05	0.58	0.58
09-393	Pinkeye	AR, USA	87.50	0.83	86.67	99.02	0.01	0.01
09-452	Black Holstein	AR, USA	88.33	11.67	76.67	86.76	0.13	0.13
09-455	Blackeye	AR, USA	77.50	57.50	20.00	25.89	0.74	0.75
09-470	Pinkeye	AR, USA	91.67	44.17	47.50	51.61	0.48	0.48
09-529	Blackeye	AR, USA	85.50	75.00	10.50	12.16	0.88	0.88
09-655	Pinkeye	AR, USA	92.50	28.33	64.17	69.01	0.31	0.30
09-686	Pinkeye	AR, USA	93.33	37.50	55.83	59.80	0.40	0.40
09-692	Pinkeye	AR, USA	92.50	39.17	53.33	57.33	0.43	0.42
09-697	Pinkeye	AR, USA	68.33	32.50	35.83	52.26	0.48	0.47
09-714	Pinkeye	AR, USA	80.83	40.00	40.83	50.47	0.50	0.50
09-741	Red Holstein	AR, USA	87.50	65.83	21.67	24.41	0.76	0.75
09-744	Cream	AR, USA	85.83	42.50	43.33	50.40	0.50	0.49
09-745	Red Holstein	AR, USA	89.17	6.67	82.50	92.49	0.08	0.07
09-749	Red Holstein	AR, USA	76.67	27.50	49.17	64.21	0.36	0.36
09-752	Black Holstein	AR, USA	81.67	4.17	77.50	94.88	0.05	0.05
Arkansas_Blackeye#1	Blackeye	AR, USA	76.67	50.83	25.83	33.52	0.66	0.66
Early_Acre	Cream	AR, USA	67.50	32.50	35.00	50.92	0.49	0.49
Early_scarlet	Pinkeye	AR, USA	81.67	29.17	52.50	64.39	0.36	0.36
Ebony	Black	AR, USA	90.00	69.17	20.83	23.06	0.77	0.77
ENCORE	Pinkeye	AR, USA	71.67	30.00	41.67	58.10	0.42	0.42
Envoy	Red Holstein	AR, USA	99.17	30.00	69.17	69.72	0.30	0.30
Epic_Select	Tan	AR, USA	81.67	10.83	70.83	86.92	0.13	0.13
EXCEL	Browneye	AR, USA	86.67	70.00	16.67	19.24	0.81	0.81
PI152195	Red	Paraguay	82.50	44.17	38.33	46.71	0.53	0.54
PI152196	Mixed (Cream Brown)	Paraguay	95.00	58.33	36.67	39.42	0.61	0.62
PI152197	Red	Paraguay	88.33	25.00	63.33	71.42	0.29	0.28
PI152199	Mixed (Purple Cream)	Paraguay	79.17	45.83	33.33	42.02	0.58	0.58
PI162924	Mixed (Cream Brown)	Paraguay	83.33	66.67	16.67	18.72	0.81	0.79
PI167284	Mixed (Cream Brown)	Turkey	79.17	32.50	46.67	59.52	0.40	0.42
PI175332	Tan	NA	77.50	31.67	45.83	58.33	0.42	0.41
PI180014	Tan	India	71.67	27.50	44.17	62.76	0.37	0.40
PI190191	Tan	Mexico	63.33	17.50	45.83	72.09	0.28	0.28
PI201498	Blackeye	Mexico	92.50	77.50	15.00	16.24	0.84	0.84
PI218123	Browneye	Pakistan	97.50	30.83	66.67	68.22	0.32	0.32

Table S2-1. Cowpea name (accession number), seed color, origin, germination rates without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed_color	Origin	Non-stress_(%)	Stress_(%)	AD_(%)	II_(%)	RST	STI
PI221730	Red Holstein	South_Africa	69.17	30.83	38.33	55.17	0.45	0.44
PI221731	Red Holstein	South_Africa	95.83	50.83	45.00	46.87	0.53	0.53
PI223023	Browneye	Iran	95.00	56.67	38.33	40.74	0.59	0.60
PI225922	Mixed (Brown Tan)	Zambia	60.83	46.67	14.17	20.25	0.80	0.75
PI229551	Browneye	Iran	90.83	70.83	20.00	21.52	0.78	0.78
PI229734	Blackeye	Iran	88.33	26.67	61.67	70.13	0.30	0.31
PI244571	Mixed (Brown Cream)	Guatemala	66.67	17.50	49.17	73.74	0.26	0.26
PI250416	Cream	Pakistan	88.33	22.50	65.83	73.98	0.26	0.25
PI250587	Dark brown	Madagascar	70.00	5.83	64.17	91.73	0.08	0.08
PI253428	Dark brown	Spain	97.50	38.33	59.17	60.67	0.39	0.39
PI255765	Blackeye	Nigeria	97.50	56.67	40.83	41.85	0.58	0.58
PI255815	Browneye	Nigeria	77.50	54.17	23.33	28.81	0.71	0.69
PI257463	Red Holstein	Nigeria	79.17	46.67	32.50	40.51	0.59	0.59
PI262179	Blackeye	Portugal	73.33	55.00	18.33	24.68	0.75	0.75
PI292897	Grey	Hungary	67.50	28.33	39.17	58.30	0.42	0.42
PI292898	Black	Hungary	86.67	71.67	15.00	17.09	0.83	0.83
PI292899	Tan	Hungary	66.67	11.67	55.00	83.70	0.16	0.19
PI292913	Tan	Hungary	62.50	8.33	54.17	86.71	0.13	0.13
PI293467	Mixed (Tan Brown)	USA	84.17	28.33	55.83	66.45	0.34	0.34
PI293469	Tan	NA	91.67	23.33	68.33	74.72	0.25	0.26
PI293476	Variable (Grey Brown)	USA	70.83	7.50	63.33	87.68	0.12	0.09
PI293514	Grey	USA	78.33	21.67	56.67	72.51	0.27	0.28
PI293545	Mixed (Brown Cream)	NA	93.33	65.00	28.33	30.37	0.70	0.70
PI293570	Mixed (Brown Tan)	NA	62.50	7.50	55.00	87.63	0.12	0.12
PI293582	Grey	NA	70.00	22.50	47.50	68.13	0.32	0.33
PI293584	Variable (Red Brown Cream)	NA	67.50	58.33	9.17	13.31	0.87	0.86
PI297561	Mixed (Brown Grey)	NA	61.67	36.67	25.00	38.75	0.61	0.60
PI339587	Red Holstein	South_Africa	70.00	53.33	16.67	23.46	0.77	0.76
PI339591	Brown Holstein	South_Africa	65.00	51.67	13.33	18.60	0.81	0.79
PI339592	Brown	South_Africa	70.83	44.17	26.67	36.71	0.63	0.62
PI339594	Variable (Red Holstein Red Tan)	NA	71.67	51.67	20.00	23.91	0.76	0.71
PI339609	Tan	Tanzania	89.17	50.00	39.17	43.40	0.57	0.56
PI339610	Variable (Red Brown Cream)	Tanzania	60.83	38.33	22.50	36.71	0.63	0.63
PI339611	Tan	Tanzania	73.33	62.50	10.83	14.60	0.85	0.85
PI339613	Variable (Grey Tan Brown)	Tanzania	93.33	45.00	48.33	51.62	0.48	0.48
PI347639	Tan	NA	60.00	36.67	23.33	38.34	0.62	0.61
PI353045	Red Holstein	India	77.50	45.83	31.67	40.68	0.59	0.59
PI353062	Mixed (Cream Purple)	India	94.17	68.33	25.83	27.51	0.72	0.73
PI367921	Tan	NA	89.17	73.33	15.83	17.68	0.82	0.82
PI406290	Browneye	Mozambique	65.00	18.33	46.67	72.02	0.28	0.28

Table S2-1. Cowpea name (accession number), seed color, origin, germination rates without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed_color	Origin	Non-stress_(%)	Stress_(%)	AD_(%)	II_(%)	RST	STI
PI430687	Red	China	68.33	32.50	35.83	51.75	0.48	0.47
PI487518	Tan	Indonesia	98.33	44.17	54.17	55.26	0.45	0.45
PI527561	Tan	Burindi	96.67	58.33	38.33	39.64	0.60	0.60
PI578902	Red	China	78.33	22.50	55.83	69.99	0.30	0.28
PI578907	Black	China	87.50	44.17	43.33	49.38	0.51	0.51
PI578911	Tan	China	82.50	47.50	35.00	41.91	0.58	0.57
PI582340	Mixed (Brown Pink)	NA	69.17	26.67	42.50	61.38	0.39	0.39
PI582347	Tan	Myanmar	80.83	49.17	31.67	39.10	0.61	0.61
PI582352	Blackeye	Saudi_Arabia	88.33	49.17	39.17	43.35	0.57	0.55
PI582354	Blackeye	NA	69.17	40.83	28.33	39.97	0.60	0.58
PI582415	Tan	Mexico	89.17	52.50	36.67	41.13	0.59	0.59
PI582416	Variable (Red Brown Cream)	Mexico	92.50	25.00	67.50	72.94	0.27	0.27
PI582420	Variable (Red Brown)	NA	77.50	65.00	12.50	15.36	0.85	0.83
PI582421	Grey	NA	97.50	65.00	32.50	33.50	0.66	0.67
PI582422	Blackeye	NA	75.83	70.00	5.83	7.67	0.92	0.92
PI582425	Mixed (Tan Brown)	NA	82.50	40.83	41.67	50.44	0.50	0.49
PI582428	Blackeye	Trinidad_and_Tobago	64.72	49.17	15.56	22.14	0.78	0.76
PI582467	Black Holstein	NA	75.83	37.50	38.33	49.07	0.51	0.49
PI582474	Purple	Botswana	64.17	25.83	38.33	59.41	0.41	0.40
PI582522	Tan	Mexico	95.00	0.83	94.17	99.12	0.01	0.01
PI582531	Variable (Grey Brown Tan)	Ghana	65.83	42.50	23.33	31.66	0.68	0.62
PI582542	Browneye	Mexico	88.33	58.33	30.00	33.67	0.66	0.66
PI582551	Blackeye	Botswana	84.17	64.17	20.00	23.85	0.76	0.76
PI582570	Browneye	India	67.78	58.33	9.44	13.65	0.86	0.86
PI582573	Browneye	Kenya	67.50	53.33	14.17	20.60	0.79	0.79
PI582574	Tan	Kenya	75.00	28.33	46.67	61.01	0.39	0.38
PI582575	Black	Kenya	91.67	28.33	63.33	68.91	0.31	0.31
PI582578	Tan	Kenya	77.50	42.50	35.00	41.86	0.58	0.53
PI582579	Tan	Kenya	97.50	21.67	75.83	77.66	0.22	0.22
PI582665	Variable (Grey Brown Tan)	Botswana	89.17	1.67	87.50	98.20	0.02	0.02
PI582666	Tan	Botswana	83.33	46.67	36.67	44.14	0.56	0.56
PI582680	Browneye	Botswana	65.83	26.67	39.17	59.28	0.41	0.41
PI582696	Brown	Botswana	74.17	44.17	30.00	40.27	0.60	0.59
PI582703	Red Holstein	Botswana	69.17	27.50	41.67	60.29	0.40	0.40
PI582789	Tan	Kenya	95.83	36.67	59.17	61.62	0.38	0.38
PI582809	Tan	Botswana	61.67	23.33	38.33	62.28	0.38	0.38
PI582813	Mixed(Grey Cream Black eye)	Botswana	77.50	0.00	77.50	100.00	0.00	0.00
PI582815	Purple	Botswana	84.17	41.67	42.50	50.45	0.50	0.49
PI582818	Black Holstein	Botswana	90.00	20.00	70.00	77.74	0.22	0.22
PI582821	Mixed (Brown Tan)	Botswana	90.83	40.00	50.83	56.15	0.44	0.44
PI582852	Brown	Botswana	61.67	31.67	30.00	48.31	0.52	0.51

Table S2-1. Cowpea name (accession number), seed color, origin, germination rates without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed_color	Origin	Non-stress_(%)	Stress_(%)	AD_(%)	II_(%)	RST	STI
PI582863	Browneye	Botswana	87.50	59.17	28.33	32.17	0.68	0.68
PI582873	Tan	Botswana	67.50	7.50	60.00	88.53	0.11	0.11
PI582874	Tan	Botswana	85.00	37.50	47.50	55.91	0.44	0.45
PI582875	Tan	Botswana	70.83	11.67	59.17	83.54	0.16	0.17
PI582878	Green	NA	72.50	12.50	60.00	81.76	0.18	0.16
PI582894	Tan	Botswana	71.67	27.50	44.17	61.35	0.39	0.38
PI582932	Browneye	Malawi	70.00	57.50	12.50	17.56	0.82	0.82
PI583193	Variable (Gray Brown)	Senegal	94.17	15.83	78.33	83.11	0.17	0.17
PI583194	Variable (Tan Grey)	Senegal	99.17	9.17	90.00	90.83	0.09	0.09
PI583198	Tan	Senegal	96.67	57.50	39.17	40.82	0.59	0.60
PI583201	Browneye	Senegal	70.83	51.67	19.17	24.75	0.75	0.73
PI583202	Browneye	Senegal	89.17	56.67	32.50	36.56	0.63	0.64
PI583209	Tan	Nigeria	90.83	23.33	67.50	74.27	0.26	0.26
PI583244	Tan	Senegal	80.83	35.83	45.00	55.53	0.44	0.44
PI583248	Browneye	Senegal	77.78	63.33	14.44	18.13	0.82	0.81
PI583249	Browneye	Senegal	69.17	47.50	21.67	30.62	0.69	0.69
PI583274	Browneye	Senegal	88.33	62.50	25.83	28.92	0.71	0.71
PI583487	Tan	Senegal	62.50	39.17	23.33	33.91	0.66	0.61
PI583488	Brown	Australia	69.17	30.83	38.33	55.67	0.44	0.45
PI583550	Purple	Mali	93.33	42.50	50.83	54.27	0.46	0.45
PI583551	Browneye	Mali	70.83	46.67	24.17	33.91	0.66	0.67
PI610520	Black	Italy	69.17	12.50	56.67	81.33	0.19	0.18
PI610533	Blackeye	NA	80.00	49.17	30.83	37.96	0.62	0.61
PI610604	Purple	Italy	65.83	23.33	42.50	64.19	0.36	0.37
PI666252	Tan	USA	90.00	26.67	63.33	70.33	0.30	0.30

Chapter 3. Evaluation of Salt Tolerance at Seedling Stage in Cowpea (*Vigna unguiculata* (L.) Walp)

Abstract

Cowpea is an important legume providing affordable protein and offering ecosystem services through the fixation of atmospheric nitrogen. However, increasing salinity in cultivated areas limits cowpea production. The objective of this study was to evaluate cowpea salt tolerance at seedling stage. A total of 203 cowpea genotypes were used in this research. For each cowpea genotype, we supplied 200 mM NaCl to three plastic pots each containing four cowpea plants, and deionized water to additional three plastic pots containing the same number of plants. The experiment was a completely randomized design (CRD) with three replications per genotype treatment under greenhouse conditions. Results indicated (1) there were significant differences in salt tolerance among the 203 cowpea genotypes based on visual leaf injury, inhibition of the first trifoliate leaf development, plant height reduction, and fresh and dry shoot biomass reduction; (2) a relatively high heritability (>70%) was observed for all traits related to salt tolerance; (3) there were different mechanisms among leaf injury, plant height reduction, and fresh and dry shoot biomass loss in cowpea caused by salt stress; (4) the genetic background promoting cowpea salt tolerance was significantly different between leaf injury and plant height reduction, between leaf injury and fresh shoot biomass, and between leaf injury and dry shoot biomass; (5) the genetic background enhancing salt tolerance had a low association between plant height reduction and fresh shoot biomass, and plant height reduction and dry shoot biomass; (5) the genetic background contributing to cowpea salt tolerance had a strong association with fresh shoot biomass and dry shoot biomass; and (6) PI293570, PI582812, PI582856, PI180014,

PI257463, 09-175, 09-529, PI666260, I582402, and PI582340 were highly salt tolerant and could be used as parents for improving salt tolerance in cowpea.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] (2n=2x=22) is a versatile leguminous crop. It has various names depending upon the region. Cowpea is known as southern pea or blackeye pea in the US, beans, ‘niebe’, ‘wake’ or ‘ewa’ in Africa, and ‘caupi’ in Brazil (Agbicodo, 2009). Some studies stated that cowpea likely originated in India; however, later research indicated Africa as the origin based on the fact that wild cowpea existed only in Africa and Madagascar (Blackhurst and Miller, 1980). Cowpea belongs to the Family *Fabaceae* (Verdcourt, 1970). It is grown in a wide range of agri-ecological zones including Asia, Oceania, the Middle East, Southern Europe, Africa, Southern US, and Central and South America (Fery, 1990; Perrino et al., 1993; Watt et al., 1985). In addition, cowpeas are widely cultivated in semiarid areas of the tropics. An estimated 11 million hectares are annually planted with cowpea in the world. Central and West Africa provide the majority of the cowpea production, accounting for over 70% of the world wide cowpea production (Singh et al., 2003). Worldwide production is estimated at 5.4 million tons of cowpea dried seeds and Nigeria is the leading producer (Olufajo, 2012). Utilization of cowpea involves a wide range of sectors from livestock to industrial purposes. Immature leaves, green pods and seeds with various colors can be used as a vegetable for human consumption. Dried seeds are primarily used for human consumption and livestock fodder. Cowpea can be processed into other products such as cowpea flour or cowpea milk (Akpapuimam and Markakis, 1981; Caygill et al., 1981).

Soil salinity is a limiting factor for cowpea production and is considered a growing threat to food supply (Fery, 1990; Flowers, 2004). In fact, salt stress considerably affects crop growth and productivity (Allakhverdiev et al., 2000; Foolad, 2007; Osmond et al., 1987; Chinnusamy et al., 2005). An estimated area of more than 830 million ha of cultivated land across the world is facing salinity problems (Chaitanya et al., 2014). In addition, salinity affects more than 20% of irrigated crop lands which provide more than 30% of crop production in agriculture (Hillel, 2000; Pitman and Läuchli, 2002). Croplands under salinity are estimated at 19.6 million of ha in the United States (Shannon, 1997). Salt stress is more acute in arid and semi-arid zones (Zhang et al., 2012). In agriculture, salinity is caused by various factors. Noticeable salt level in water irrigation has been pointed out as a source of salinity in croplands. The concentration of salt in cropland continues to increase as an annual salt amount ranging from 1 to 60 metric tons per hectare is added to soil due to poor irrigation methods (Shannon, 1997). Other factors such as rock weathering, seawater, rain, deforestation, air pollution and contamination of river waters by chemical and domestic animals intensify the chloride level of soils, thus threatening agriculture in the world (Omami and Hammes, 2006; Rengasamy et al., 2006). In addition, salinity is a major economic issue in agriculture. Salinity-related issues are estimated to cost the agriculture sector 12 billion US dollars (Läuchli and Lüttge, 2002). This cost is expected to increase as cultivated areas under salinity increase.

Due to the increasing threat of salinity, interest in developing salt-tolerant crops is increasing. Ashraf and McNeilly (1989) showed that maize (*Zea mays* L.) genotypes which were salt-tolerant at early vegetative growth showed good tolerance until maturity. In wheat (*Triticum aestivum* L.), Jafari et al. (1995) reported that salinity was negatively correlated with yield. Such results have been confirmed by Richards (1983). Subsequent investigations have been carried out to develop a quick and accurate methodology to assess tolerance to salinity in soybean at seedling stage (Lee et al., 2008; Valencia et al., 2008). Ledesma et al. (2016) evaluated 92 soybean lines for salinity stress at early vegetative growth, using a salt concentration of 120 mM NaCl. Their results indicated that the soybean genotypes Williams and Clark were salt-sensitive, and the lines HBK R5525 and ‘AG5905 were tolerant to salinity.

Win and Oo (2015) worked on the evaluation of salt tolerance in cowpea at early vegetative growth stage. They assessed 21 genotypes and reported significant differences in growth parameters such as plant height and root length. Based on the salinity tolerance index (STI), they suggested that the cultivars Vita 3, Vu15, and IT-85F-1380 could be used as parents for salt-tolerance breeding. Abeer et al. (2015) used a different approach to enhance salt tolerance in cowpea and indicated that the arbuscular mycorrhizal fungi (AMF) could induce salt tolerance in cowpea. The activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) and glutathione reductase (GR) was enhanced under salt stress after treating cowpea with AMF, which enabled plants to better tolerate salinity. Salt tolerance in cowpea was also evaluated using variables related to nodulation. This method was used by Gómez et al. (2013) to assess salt tolerance in 12 cowpea lines at seedling stage.

The effects of salinity could be insidious at seedling stage in cowpea. The objective of this study was to evaluate the responses of a group of cowpea genotypes to salinity (NaCl) at seedling stage and to select salt-tolerant cowpea genotypes.

Materials and Methods

Plant materials

A total of 203 cowpea genotypes including 164 United States Department of Agriculture (USDA) Germplasm Resources Information Network (GRIN) cowpea germplasm accessions, and 39 University of Arkansas cowpea advanced breeding lines were used for this study (Table S3-1). The 164 USDA cowpea germplasm accessions were originally collected from 32 countries, and the seeds were obtained from USDA Germplasm Resources Information Network (GRIN). USDA accessions were obtained from the USDA Plant Genetic Resources Conservation Unit at Griffin, GA, and were increased at the Research Station and Extension of the University of Arkansas at Fayetteville, AR in summers 2014 and 2015.

Salt tolerance evaluation

The experiment was carried out in the greenhouse of the Rosen Center of the University of Arkansas, Fayetteville, AR. The experimental design was a two-factor completely randomized design (CRD) (Figure S3-1). Cowpea genotype and salt treatment were the two factors in the experiment, with three replicates for each treatment. Salt solution and non-salt solution with deionized water (pure water) were the two treatments. Due to space limitations in the greenhouse, the experiment was conducted in five runs, each consisting of approximately 40 cowpea genotypes. Since the greenhouse conditions across the different experiment runs could have varied, adjustment on measurements was performed during the data analysis. Early Scarlet, a salt susceptible cowpea cultivar, was used as a control.

Forty seeds from each cowpea genotype were germinated in an incubator (New Brunswick Scientific Innova 4230®, Manasquan, NJ). Twenty-four vigor and uniform germinated seeds from each cowpea genotype were selected and planted in six plastic pots containing 85 g of Sunshine® Mix #1 Natural & Organic (Agawan, MA) as growth medium, with four germinated seeds in each pot. To avoid waterlogging in each pot, holes were placed at the bottom. A paper towel placed at the bottom surface of the pots prevented the growth medium from leaking after irrigation with deionized water or salt solution. The pots containing the plants were placed on rectangular plastic trays.

In this study, 200 mM NaCl was used for inducing salinity stress and deionized water for non-salinity stress. Our preliminary experiment with six salt treatments consisting of 0, 100, 150, 200, 250, and 300 mM NaCl showed that 200 mM NaCl was the optimal NaCl concentration to determine salt tolerance in cowpea (data not shown), and this concentration has also been used in other salt tolerance-related studies in cowpea (Ashebir et al., 2013; Abeer et al., 2015).

The salt treatment began when the plants attained the first trifoliate leaf (V1 stage). The saline treatments were performed by adding 100 ml of deionized water to the non-salt-treated pot or 200 mM NaCl solution to the salt-treated one plot. The treatments were applied every two days over two weeks. The medium was kept wet to ensure that the saline treatment would not evaporate, and to avoid the failure of the plants to uptake ions present in the saline solution.

Measurements

The evaluation of plants for salinity effects began 14 days after the first NaCl treatment. At this time, some plants were observed to be dead. The evaluated traits related salt tolerance involved in this study were visual leaf injury, inhibition of first trifoliate leaf development, reduction in plant height, fresh shoot biomass, and dry shoot biomass, which were also used in other investigations to assess plant tolerance to salinity (Zaidem et al., 2004; Gómez Padilla et al., 2013; Win and Oo, 2015).

The visual leaf injury due to salinity was scored for each plant according to the following scale: 1 = no apparent leaf injury; 2 = apparition of leaf injury; 3 = moderate leaf injury; 4 = severe leaf injury; and 5=dead leaves (Figure 3-1). Plant height, fresh shoot biomass, and dry shoot biomass were assessed on a per plant basis and the average of each variable for the four plants was calculated and recorded. Dry shoot biomass was obtained by placing fresh plant matter into a leaf dryer and dried at 105°C for 16 hours.

The following parameters were computed to assess salt tolerance (Fernandez, 1992; González, 1996; Saad et al., 2014).

i. Absolute decrease in height (cm) = (Height at non salt stress) – (Height at salt stress);
ii. Plant height inhibition index (%) = 100 * [(Height at non salt stress) – (Height at salt stress)] / (Height at non salt stress);
iii. Relative salt tolerance for height = (Height at salt stress) / (Height at non salt stress);
iv. Salt tolerance index for height = (Height at non salt stress * Height at salt stress) / (Average of height at non salt stress)^2;
v. Absolute decrease in fresh shoot biomass (g) = (Fresh shoot biomass at non salt stress) – (Fresh shoot biomass at salt stress);
vi. Fresh shoot biomass inhibition index (%) = 100 * [(Fresh shoot biomass at non salt stress) – (Fresh shoot biomass at salt stress)] / (Fresh shoot biomass at non salt stress);
vii. Relative salt tolerance for fresh shoot biomass = (Fresh shoot biomass at salt stress) / (Fresh shoot biomass at non salt stress)
viii. Salt tolerance index for fresh shoot biomass = (Fresh shoot biomass at non salt stress) * Fresh shoot biomass at salt stress) / (Average of fresh shoot biomass at non salt stress)^2

ix. Absolute decrease in dry shoot biomass (g) = (Dry shoot biomass at non salt stress) – (Dry shoot biomass at salt stress);

x. Dry shoot biomass inhibition index (%) = 100 * [(Dry shoot biomass at non salt stress) – (Dry shoot biomass at salt stress)] / (Dry shoot biomass at non salt stress);

xi. Relative salt tolerance for dry shoot biomass = (Dry shoot biomass at salt stress) / (Dry shoot biomass at non salt stress);

xii. Salt tolerance index for dry shoot biomass = (Dry shoot biomass at non salt stress) * (Dry shoot biomass at salt stress) / (Average of dry shoot biomass at non salt stress)^2.

Data analysis

The parameters involved in this study consisted of pairing the growth parameter of a genotype under salinity treatment and without salt stress. The ANOVA test was carried out using the general linear model (GLM) procedure of JMP Genomics 7 (SAS Institute, Cary, NC). The Student T-test was used for the mean separation at alpha = 0.05. The descriptive statistics were generated using ‘Tabulate’; the correlation coefficients were estimated using “Fit Y by X” by “Multivariate Methods”; and the distribution of the data was drawn using ‘Distribution’ in JMP Genomics 7. In the calculations, Y_{ij} = value of the parameters for the ith cowpea genotype at the jth replication, for I = 1, 2, 3…, 203 and j = 1, 2, 3.

The statistical model for the analysis was

$$Y_{ijk} = \mu + G_i + \boldsymbol{\varepsilon}_{ij}$$

Where μ: constant (overall mean), G_i: Effect of the ith genotype (fixed effect) on the mean response, and $\boldsymbol{\varepsilon}_{ij}$: experimental associated with the ijth observation.

The broad sense heritability was calculated using the formula $H^2 = (\sigma^2_G / \sigma^2_P)$ x 100 = $[\sigma^2_G / (\sigma^2_G + (\sigma^2_E / r))]$ x 100 (Hosseini et al., 2012)

Where σ^2_G: Genotypic variance, σ^2_P: Phenotypic variance, and σ^2_E: Variance associated with the experimental error, r: number of replication per treatment

σ^2_G and σ^2_E were obtained using the following formulas.

σ^2_G = (MSG - MSE) / r and σ^2_E = MSE where MSG: Mean square genotype, MSE: Mean square error. The estimates of MSG and MSE were obtained from the ANOVA table.

Results and interpretations

Visual injury on cowpea leaves

The visual injury was evaluated 14 days after the first NaCl treatment when some cowpea genotypes were observed to be dead, defoliating, or leaf chlorosis developed due to salt stress. No such symptoms of poor plant health were exhibited on any plants irrigated with deionized water, suggesting that salinity caused the leaf injury in salt-treated plants. The leaf injury was recorded for each cowpea genotype based on the 1 – 5 scale (Figure 3-1). The scores among the 203 cowpea genotypes showed a right-skewed distribution (Figure 3-2), and ranged from 1.0 to 5.0 with an average of 2.2 and a standard deviation of 0.9 (Table S3-1). The results indicated that there were significant differences for leaf injury among the genotypes (F=11.44, p-value<0.0001); the majority were moderately tolerant to salinity; and the broad sense heritability (H^2) for the visual leaf injury score was 77.7% (Table 3-1 & S3-1). Five cowpea accessions showed high salt tolerance with a visual leaf injury score less than 1.2: PI293570 (1.0), PI582422 (1.1), PI582812 (1.1), PI582863 (1.2), and PI251222 (1.2), and five accessions showed the highest visual leaf injury score greater than 4.7: PI610533 (5.0), PI582665 (4.9), PI255815 (4.9), PI255774 (4.8), and PI527561 (4.7), indicating that these accessions were highly salt-tolerant.

Inhibition of first trifoliate leaf

Salinity inhibited the development of the first trifoliate leaf for 55% of the genotypes subjected to salt treatment (Figure 3-3). The inhibition of the first trifoliate leaf resulted in the inability of the plants to undergo any further growth and development.

Plant height

Non-stress and stress

Plant height showed significant differences among the 203 cowpea genotypes for both treatments (Figure 3-4). Near-normal distribution was observed for both salt stress and non-stress treatments (Figure 3-5). A high broad sense heritability (H^2) was estimated for height parameter under non-salt condition (88.9%) and salt stress for the 200 mM NaCl concentration treatment (74.0%).

Under non-stress, plant height was significantly different among the 203 genotypes (F=25.11, p-value<0.0001) (Table 3-2), and ranged from 10.3 to 23.5 cm, with an average of 15.3 cm and a standard deviation of 2.7 cm (Table S3-2). The tallest accessions were PI250759 (23.5 cm), PI582697 (23.2 cm), PI582530 (23.0 cm), PI582866 (22.0 cm), and PI582575 (21.7 cm); whereas the shortest were 01-1781 (11.1 cm), PI582874 (11.0 cm), 09-529 (10.9 cm), PI293582 (10.5cm), and PI582675 (10.3 cm).

Under saline environment, there were significant differences in plant height among the tested genotypes (F=9.52, p-value<0.0001) (Table 3-2); plant height varied between 4.5 cm and 11.8 cm, with an average of 7.7 cm and a standard deviation of 1.33. The accessions with the tallest plants were PI250587 (11.8 cm), PI582856 (11.7 cm), PI180014 (11.4 cm), PI582575 (11.2 cm), and PI347639 (11.2 cm); whereas the shortest were PI582551 (5.2 cm), PI579909 (5.0

cm), PI582873 (4.8 cm), PI582852 (4.6 cm), and PI339563 (4.5 cm). These results indicated large variations in cowpea height under salt stress and non-stress conditions.

Absolute decrease, inhibition index, relative salt tolerance, and salt tolerance index

Salinity significantly reduced plant height (Table 3-2) and a near normal distribution was observed for absolute decrease in plant height (Figure 3-6A), inhibition index (Figure 3-6B), relative salt tolerance (Figure 3-6C), and salt tolerance index (Figure 3-6D). The four parameters related to plant height reduction under salt condition had relatively high heritability: absolute decrease in height (80.3%), inhibition index (69.4%), relative salt tolerance (69.1%), and salt tolerance index (65.2%).

The absolute decrease in height was significantly different among the genotypes (F=13.2, p-value<0.0001) and varied from 2.7 cm to 16.4 cm 14 days after the first salt treatment (Figure 3-6A, Table 3-2, S3-2). PI582866 (16.4 cm), PI582697 (14.9 cm), PI582530 (14.4 cm), PI250759 (13.8 cm), and PI583274 (13.7 cm) had the highest absolute decrease in height, indicating that these accessions were the most susceptible to salt stress. The smallest absolute decrease was recorded for 09-175 (3.3 cm), PI180014 (3.2 cm), 09-529 (2.9 cm), PI257463 (2.7 cm), and PI582856 (2.7 cm), indicating that these genotypes had the highest salt tolerance based on height absolute decrease.

The inhibition index in height varied from 19 to 74.3%, with an average of 49% and a standard deviation of 9%. Significant differences in inhibition index were observed among the cowpea genotypes (F=7.8, p-value<0.0001) (Tables 3-2 & S3-2). Inhibition index was highest for PI582866 (74.3%), PI582852 (73.5%), PI582812 (65.1%), PI583274 (64.4%), and PI582697 (64.2%), indicating that these accessions were the most susceptible to salt stress; whereas 09-529

(26%), 09-175 (25.7%), PI257463 (24.0%), PI180014 (22.0%), and PI582856 (19.0%) had the smallest inhibition index.

The relative salt tolerance in height ranged between 0.26 and 0.81, with an average of 0.51 and a standard deviation of 0.09. There was statistical evidence to indicate genotype effect on relative salt tolerance (F=7.8, p-value<0.0001) (Table 3-2 & S3-2). PI582856 (0.81), PI180014 (0.78), PI257463 (0.76), 09-175 (0.74), and 09-529 (0.74) had the highest relative salt tolerance, indicating that these genotypes had the highest salt tolerance, whereas, the relative salt tolerance was lowest for PI582697 (0.36), PI583274 (0.36), PI582812 (0.35), PI582852 (0.26), and PI582866 (0.26), indicating that these genotypes had the highest salt susceptible based on the relative salt tolerance in height.

The salt tolerance index in height varied from 0.26 to 0.81, with an average of 0.51 and a standard deviation of 0.09. Significant differences were observed among the genotypes in terms of salt tolerance index (F=6.6, p-value<0.0001) (Tables 3-2 & S3-2). Salt tolerance index was highest for PI582856 (0.81), PI180014 (0.78), PI257463 (0.76), 09-175 (0.74), and 09-529 (0.73), indicating that these genotypes had the highest salt tolerance; whereas the lowest salt tolerance index was recorded for PI582697 (0.36), PI583274 (0.36), PI582812 (0.35), PI582852 (0.26), and PI582866 (0.26), indicating that these genotypes were more salt-susceptible based on salt tolerance index in height.

Fresh shoot biomass

Non-stress and stress

The least square means for the fresh shoot biomass per plant among the 203 cowpea genotypes showed a near normal distribution under salt stress and non-stress conditions (Figure 3-7). Fresh shoot biomass was highly heritable with an H^2 of 74.3% and 80.1% for fresh shoot biomass without salt stress and fresh shoot biomass under salt-stressed with 200 mM NaCl, respectively.

The fresh shoot biomass per plant among the 203 cowpea genotypes under non stress (watered with deionized water) varied from 3.9 to 12.1 g, with an average of 7.6 g and a standard deviation of 1.6 g. There was a significant difference in fresh shoot biomass among the genotypes (F=9.7, p-value<.0001) (Tables 3-3 & S3-3). The highest fresh shoot biomass was recorded for PI582465 (12.1g), PI223023 (12.0 g), PI582825 (11.9 g), PI253428 (11.4 g), and PI255765 (11.3g); PI582823 (4.4 g), PI582402 (4.4 g), PI255815 (4.2 g), PI339563 (4.0 g), and PI583240 (3.9 g) had the lowest fresh shoot biomass.

Under the salt stress condition, the fresh shoot biomass ranged from 1.0 to 7.1 g, with an average of 4.1 g and a standard deviation of 1.2 g. The difference in fresh shoot biomass was significant among the genotypes under salt treatment (F=2.76, p-value=0.002) (Tables 3-3 & S3-3). PI250587 (7.1 g), PI582522 (6.9 g), PI152197 (6.8 g), PI582465 (6.6 g), and PI292891 (6.5 g) had the highest fresh shoot biomass, whereas PI292892 (1.6 g), PI339563 (1.6 g), PI582866 (1.4g), PI255774 (1.3 g), and PI225922 (1.0 g) had the lowest fresh shoot biomass.

Absolute decrease, inhibition index, relative salt tolerance, and salt tolerance index

Salinity significantly reduced fresh shoot biomass (Table 3-3). The four parameters, absolute decrease, inhibition index, relative salt tolerance, and salt tolerance index in fresh shoot biomass were normally distributed (Figure 3-8). In addition, the four parameters were highly heritable. The broad sense heritability (H^2) for each parameter was 71.5, 76.3, 76.1, and 72.2%, respectively.

The absolute decrease in fresh shoot biomass per plant varied from 0.4 to 9.2 g, with an average of 3.5 g and a standard deviation of 1.4 g. The ANOVA indicated a significant difference in absolute decrease in fresh shoot biomass among the cowpea genotypes (F=8.5, p-value<0.0001) (Table 3-3 & S3-3). PI255774 (9.2 g), PI223023 (7.9 g), PI582420 (7.0 g), PI582825 (6.7g), and PI293476 (6.6 g) had the highest absolute decrease, indicating that these accessions had the highest salt sensitivity, whereas the absolute decrease was lowest for PI339602 (1.2 g), PI582340 (1.0 g), Empire (1.0 g), PI582402 (0.7 g), and PI666260 (0.4 g), indicating that these genotypes had the highest salt tolerance based on absolute decrease in fresh shoot biomass.

The inhibition index for fresh shoot biomass ranged between 7.1% and 87.6%, with an average of 45.8% and a standard deviation of 13.9%. Significant differences were observed in inhibition index among the genotypes (F=10.6, p-value <0.0001) (Table 3-3). The highest inhibition index was recorded for PI255774 (87.6%), PI225922 (82.5%), PI582468 (79.4%), PI582852 (78.7%), and PI293476 (75.2%), indicating that the five accessions had the highest salt susceptibility, whereas, Envoy (18.9%), PI339602 (17.6%), PI582402 (15.0%), PI582340 (14.1%), and PI666260 (7.1%) had the lowest inhibition index, indicating that these genotypes had the highest salt tolerance based on the inhibition index in fresh shoot biomass.

The relative salt tolerance varied from 0.12 to 0.93, with an average of 0.54 and a standard deviation of 0.14. The relative salt tolerance was significantly different among the genotypes (F=10.5, p-value <0.0001) (Tables 3-3 & S3-3). PI666260 (0.93), PI582340 (0.86), PI582402 (0.85), PI339602 (0.82), and Envoy (0.81) performed best in terms of relative salt tolerance, indicating that the five genotypes had the highest salt tolerance, whereas, PI293476 (0.25), PI582852 (0.21), PI582468 (0.20), PI225922 (0.17), and PI255774 (0.12) had the lowest relative salt tolerance, indicating that these genotypes had the highest salt susceptibility based on relative salt tolerance in fresh shoot biomass.

The salt tolerance index ranged between 0.17 and 0.99, with an average of 0.54 and a standard deviation of 0.12. The ANOVA analysis indicated significant differences among the genotypes (F=2.1, p-value<0.0001). PI582402 (0.99), PI582340 (0.82), Encore (0.81), PI152195 (0.79), and PI666252 (0.77) showed the highest salt tolerance index, indicating that the five genotypes had the highest salt tolerance, whereas, this parameter was lowest for PI293476 (0.3), PI223023 (0.28), PI582852 (0.28), PI582468 (0.17), and PI255774 (0.17), indicating that these genotypes had the highest salt susceptibility based on the salt tolerance index in fresh shoot biomass.

Dry biomass

Non-stress and stress

The dry shoot biomass under deionized water and NaCl treatments showed a near normal distribution (Figure 3-9), and the broad sense heritability (H^2) was relatively high under both conditions, equaled to 82.3% under non-salt condition and 78.3% under salt treatment.

Under non-salt condition, the dry shoot biomass per plant 14 days after the first NaCl treatment ranged from 0.6 g to 2.2 g, with an average of 1.3 g and a standard deviation of 0.3g. The ANOVA revealed a significant difference in dry shoot biomass per plant among the genotypes (F=14.9, p-value<0.0001) (Tables 3-4 & S3-4). PI582465 (2.21 g), PI253428 (2.15 g), PI293467 (2.14 g), PI223023 (2.13 g), and PI582576 (2.13 g) had the highest dry shoot biomass. PI582823 (0.66 g), PI583240 (0.66 g), PI339563 (0.64 g), Early acre (0.61 g), and PI582402 (0.57g) showed the lowest dry shoot biomass.

Regarding the plants under salt stress, the dry shoot biomass varied from 0.14 g to 1.2 g, with an average of 0.6 g and a standard deviation of 0.2 g. The dry shoot biomass under saline conditions was significantly different among the genotypes (F= 11.8, p-value<0.0001) (Table 3-4 & S3-4). PI582650 (1.2 g), PI152197 (1.2 g), PI582821 (1.1 g), PI582874 (1.1 g), and PI582465 (1.1 g) exhibited the highest dry shoot biomass. The lightest genotypes were PI339563 (0.2 g), PI582852 (0.2 g), PI583241 (0.2 g), PI582866 (0.2 g), and PI225922 (0.1 g).

Absolute decrease, inhibition index, relative salt tolerance, and salt tolerance index

Salinity significantly reduced the dry shoot biomass (Table 3-4) and the four parameters, absolute decrease in dry shoot biomass, of inhibition index, relative salt tolerance, and salt tolerance index showed a near normal distribution (Figure 3-10). The broad sense heritability (H^2) for absolute decrease, inhibition index, relative salt tolerance, and salt tolerance index was 77.6, 75.2, 75, and 70.7%, respectively.

The absolute decrease in dry shoot biomass due to salinity ranged between 0.1 g and 1.8 g, with an average of 0.6 g and a standard deviation of 0.3 g. Statistically, there was a significant difference in absolute decrease among the genotypes (F=11.4, p-value<0.0001) (Table 3-4 & S3-4). PI255774 (1.8 g), PI582468 (1.3 g), PI293476 (1.3 g), PI223023 (1.3 g), and PI255765 (1.2 g) showed the highest absolute decrease in dry shoot biomass per plant, indicating that the five genotypes had the highest salt susceptibility, whereas, the lowest absolute decrease was recorded for Empire (0.2 g), PI582340 (0.2 g), PI666260 (0.1 g), Early Acre (0.1 g), and PI582402 (0.1 g), indicating that these five genotypes had the highest salt tolerance based on absolute decrease in dry shoot biomass.

The inhibition index in dry shoot biomass ranged from 12.0 to 88.1%, with an average of 49.0% and a standard deviation of 13.8%. The inhibition index among the cowpea genotypes was significantly different (F=10.1, p-value<0.0001) (Tables 3-4 & S3-4). PI255774 (88.1%), PI225922 (83.5%), PI582468 (82.2%), PI582852 (79.4%), and PI293476 (76.7%) had the highest inhibition index, indicating that the five genotypes had the highest salt susceptibility; however, Envoy (19.4%), PI293469 (19.4%), PI582402 (17.2%), PI582340 (14.4%), and PI666260 (12%) showed the lowest inhibition index, indicating that these five genotypes had the highest salt tolerance based on inhibition index in dry shoot biomass.

The relative salt tolerance in dry biomass varied between 0.12 and 0.88, with an average of 0.51 and a standard deviation of 0.14. Significant differences in relative salt tolerance were observed among the cowpea genotypes (F=10, p-value<0.0001). PI666260 (0.88), PI582340 (0.86), PI582402 (0.83), Envoy (0.81), and PI293469 (0.81) had the highest relative salt tolerance, indicating that the five genotypes had the highest salt tolerance; whereas, PI293476 (0.23), PI582852 (0.21), PI582468 (0.17), PI225922 (0.16), and PI255774 (0.12), indicating that these five genotypes had the highest salt susceptibility based on the relative salt tolerance in dry shoot biomass.

The salt tolerance index in dry shoot biomass ranged from 0.13 to 0.86, with an average of 0.50 and a standard deviation of 0.14. The ANOVA test indicated significant differences in salt tolerance index among the genotypes (F=3.6, p-value<0.0001). PI582340 (0.86), PI666260 (0.81), Envoy (0.81), PI293469 (0.81), and PI582402 (0.79) had the highest salt tolerance index, indicating that the five genotypes had the highest salt tolerance, whereasPI293476 (0.22), PI582852 (0.21), PI225922 (0.18), PI582468 (0.18), and PI255774 (0.13) showed the lowest salt tolerance index; indicating that these five genotypes had the highest salt susceptibility based on salt tolerance index in dry shoot biomass.

Correlation analysis of the salt tolerant-related traits

In this study, leaf injury, plant height, fresh shoot biomass and dry shoot biomass were used as the salt-tolerant traits for assessing salt tolerance in cowpea. The correlation coefficients among the four traits were estimated using six parameters involving non-stress (deionized water), salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) among the 203 cowpea genotypes (Table 3-5). Leaf injury was not significantly correlated with plant height, fresh shoot biomass nor dry shoot biomass for all six aforementioned parameters, and with an absolute (r) < 0.232. Plant height was associated with fresh and dry shoot biomass with a low absolute (r) value from 0.293 between plant height and dry shoot biomass under non-stress condition to 0.419 between plant height and fresh shoot biomass under absolute decrease. Both fresh and dry shoot biomass had a strong association with a high absolute (r) value from 0.737 to 0.925 (Table 3-5). These results indicate that: (1) there were different mechanisms among the leaf injury, plant height reduction, and fresh and dry shoot biomass loss in cowpea caused by salt stress; (2) the genetic background contributing to cowpea salt tolerance was very different between leaf injury and plant height reduction, between leaf injury and fresh shoot biomass, and between leaf injury and dry shoot biomass; (3) the genetic background for salt tolerance had a low association between plant height reduction and fresh shoot biomass, and plant height reduction and dry shoot biomass; and (4) the genetic background of salt tolerance had a strong association between fresh shoot biomass and dry shoot biomass. In addition, under non saline condition, the plant height had a low association with fresh shoot biomass (r = 0.346) and dry shoot biomass (r = 0.293) (Table 3-5), indicating other factors such as number of plant branches and leaf number and size can affect plant height.

The correlations among absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) were estimated for plant height, fresh shoot biomass, and dry shoot biomass among 203 cowpea genotypes (Table 3-6). All correlations showed a very strong association among all combinations with very high absolute r values ranging from 0.755 to 1.000 (Table 3-6), indicating that the four parameters for evaluating cowpea salt tolerance showed similar results, thus validating the reliability of the results from this study.

Discussion

In this study, we observed cowpea leaves which were damaged due to the salt treatment. The severity of the leaf injury depended on genotypes. For some genotypes, leaf injury led to plant death. Lenis et al. (2011) reported variability of foliar leaf injury in soybean genotypes after salt treatment. The accumulation of Na^+ and Cl^- ions in leaves reduced the chlorophyll content, thus unfavorably affecting plant photosynthesis (Yeo and Flowers, 1983). In addition, Na^+ competes with K^+ in different cellular metabolism. The activation of some enzymes within cells are potassium-specific; high Na^+ concentration deprives K^+ from binding to those enzymes, which results in the failure of cells to properly undergo metabolic processes (Helal and Mengel, 1981; Tester and Davenport, 2003).

Plant growth parameters such as height, fresh and dry shoot biomass were analyzed. Salinity limits plant growth. We found substantial variability in plant height, fresh and dry shoot biomass reduction among the 203 cowpea genotypes, indicating that each genotype has a specific level of tolerance to salinity. Greenway and Munns (1980) reported that high Na+ and Cl-concentrations in plant leaves contributed to reduction in plant growth, suggesting that growth is a good indication to assess salt tolerance. Different formulas such as inhibition index, relative salt tolerance, and salt tolerance index were used in this study to evaluate cowpea salt tolerance. A low inhibition index suggested a better tolerance to salinity; a high relative salt tolerance and salt tolerance index indicated that the genotype is salt tolerant. Significant differences were observed in inhibition index, relative salt tolerance, and salt tolerance index among the 203 genotypes. The plant height reduction or inhibition index in plant height varied from 19.0 to 74.3%. There is evidence to indicate variability in plant height reduction due to salinity among these genotypes. Significant reduction in height was also found in pigeon pea (*Cajanus cajan* L.) under salt treatment. Ahmed and Ahmad (2016) reported a plant height reduction up to 88% in pigeon pea. In rice (*Oryza sativa* L.), Zaidem et al. (2004) reported a plant height reduction ranging from 11.9 to 56.5% due to salt treatment. The relative salt tolerance and salt tolerance index were significantly different for the fresh and dry shoot biomass. Similar results were obtained by Win and Oo (2015) in studying the responses of the cowpea genotypes to salinity.

Numerous processes have been hypothesized to cause plant growth inhibition under salinity stress. Neumann (1997) described a two-phase process which resulted in reducing plant growth after salt treatment. The first phase consisted of the cell responses due to osmotic effect resulting from a high external salt concentration. The second phase involved the accumulation of salt ions within plants. A continuous supply of salt ions caused toxic effects, which resulted in leaf senescence and necrosis. Munns et al. (1995) associated the first phase to the osmotic inhibition of growth. Differences in the osmotic inhibition of growth could result from the differences in genetic background between genotypes. Additional investigations also reported the unfavorable impact of salinity on the physiology and cellular metabolic processes within plants (Delgado et al., 1994; Amor et al., 2001; Akhtar et al., 2013).

Broad-sense heritability is a key and widely used parameter in plant breeding and genetics. In this study, we found a relatively high heritability for all traits related to salt tolerance. A high broad sense heritability (H^2) in plant height, fresh and dry shoot biomass ranging from 80 to 83% was found in barley (*Hordeum vulgare* L.)under salt stress (Long et al., 2013). The results from this current study indicated that salt tolerance in cowpea can be heritable from generation to generation, and salt tolerant cowpea germplasm can be used in a breeding incorporate salt tolerance in new cowpea cultivars.

Based on the correlation analysis, the four parameters consisting of absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) gave similar results; however, II, RST, and STI showed stronger associations among plant height, fresh shoot biomass, and dry shoot biomass for the 203 cowpea genotypes (Tables 3-5 & 3-6), which were similar to that of found by Win and Oo (2015).

In this study, four traits (leaf injury, plant height, fresh shoot biomass, and dry shoot biomass) were used to evaluate salt tolerance in cowpea. Except for leaf injury, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) were calculated to evaluate salt tolerance in the 203 cowpea genotypes. By selecting the top five cowpea genotypes from each of the 13 combinations (three traits x four parameters plus the leaf injury score), a total of 24 cowpea genotypes were recommended as having some degree of salt tolerance (Table 3-7). Some cowpea genotypes were listed in more than one combination. Among the 24 cowpea genotypes, nine were from USA including six Arkansas lines, four from Botswana, one from each of eight countries, Afghanistan, Brazil, India, Nigeria, Paraguay, Senegal, South Africa, and Tanzania, and three from unknown countries (Table 3-7). Based on leaf injury, PI293570 was the most salt tolerant, and PI582812 was the second one. PI582856, PI180014, PI257463, 09-175, and 09-529 were listed as top five for salt tolerance based on plant height reduction. Nine cowpea genotypes, PI582856, PI582402, PI180014, PI257463, PI582340, PI666260, 09-175, 09-529, and Envoy had the highest salt tolerance based on both fresh and dry shoot biomass reduction. Therefore, ten cowpea genotypes were recommended to be used as salt-tolerant parents in a breeding program: PI293570 and PI582812 were selected based on leaf injury score; PI582856, PI180014, PI257463, 09-175, and 09-529 based on plant height; and PI666260, I582402, and PI582340 based on both fresh and dry shoot biomass.

Conclusion

The evaluation of salt tolerance in cowpea was performed by analyzing 203 cowpea genotypes. Foliar injury, inhibition index, plant height, and fresh and dry biomass reduction were used as phenotypic traits to assess cowpea salt tolerance. Significant differences were identified in these traits for the 203 cowpea genotypes, indicating that genetic background contributed to differences in responses to salinity. Absence of foliar injury was noticed in PI293570, PI582812, and PI582422. PI255774, PI255815, PI582665, and PI610533 exhibited severe leaf damage. The development of the first trifoliate leaf was recorded in a large number of genotypes. PI293570, PI582812, PI582856, PI180014, PI257463, 09-175, 09-529, PI666260, PI582402, and PI582340 were highly salt tolerant and could be used as parents for improving salt tolerance in cowpea.

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Table 3-1. ANOVA for visual leaf injury score

Source	**DF**	**Sum of squares**	**Mean square**	**F Ratio**	**Prob > F**	**Estimated mean square**
Genotype	202	444.690	2.201	11.438	<.0001	$\sigma^2_e + r*\sigma^2_g$
Error	406	78.140	0.192			σ^2_e

Table 3-2. ANOVA related to plant height parameters

Parameters	**Source**	**DF**	**Sum of squares**	**Mean square**	**F Ratio**	**Prob > F**	**Estimated mean square**
Height without salt stress	Genotype	202	4369.329	21.630	25.111	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	349.725	0.861			σ^2_e
Height under 200 mM NaCl	Genotype	202	1075.944	5.327	9.518	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	227.208	0.560			σ^2_e
Absolute decrease	Genotype	202	3459.540	17.126	13.237	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	525.285	1.294			σ^2_e
Inhibition index	Genotype	202	49573.957	245.416	7.790	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	12791.364	31.506			σ^2_e
Relative salt tolerance	Genotype	202	4.987	0.025	7.796	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	1.286	0.003			σ^2_e
Salt tolerance index	Genotype	202	4.956	0.025	6.583	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	1.513	0.004			σ^2_e

Table 3-3. ANOVA related to fresh shoot biomass parameters

Parameters	Source	DF	Sum of squares	Mean square	F Ratio	Prob > F	Estimated mean square
Fresh shoot biomass without salt stress	Genotype	202	1525.158	7.550	9.6626	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	317.244	0.781			σ^2_e
Fresh shoot biomass under 200 mM NaCl	Genotype	202	840.960	4.163	13.1068	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	128.959	0.318			σ^2_e
Absolute decrease	Genotype	202	1219.239	6.036	8.5163	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	287.748	0.709			σ^2_e
Inhibition index	Genotype	202	117589.976	582.129	10.658	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	22175.233	54.619			σ^2_e
Relative salt tolerance	Genotype	202	11.761	0.058	10.5555	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	2.240	0.006			σ^2_e
Salt tolerance index	Genotype	202	8.809	0.044	2.0629	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	8.583	0.021			σ^2_e

Table 3-4. ANOVA related to dry shoot biomass parameters

Parameters	Source	DF	Sum of squares	Mean square	F Ratio	Prob > F	Estimated mean square
Dry shoot biomass without salt stress	Genotype	202	69.446	0.344	14.962	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	9.329	0.023			σ^2_e
Dry shoot biomass under 200 mM NaCl	Genotype	202	29.824	0.148	11.837	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	5.064	0.012			σ^2_e
Absolute decrease	Genotype	202	43.111	0.213	11.367	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	7.623	0.019			σ^2_e
Inhibition index	Genotype	202	115603.090	572.293	10.113	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	22975.718	56.590			σ^2_e
Relative salt tolerance	Genotype	202	11.567	0.057	10.072	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	2.308	0.006			σ^2_e
Salt tolerance index	Genotype	202	11.231	0.056	3.626	<.0001	$\sigma^2_e + r*\sigma^2_g$
	Error	406	6.226	0.015			σ^2_e

Table 3-5. Correlation among plant height, fresh shoot biomass, and dry shoot biomass in different parameters of absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) among 203 cowpea genotypes.

Correlation between	Non_stress	Stress	AD	II	RST	STI
leaf_injury : Plant_height	-0.090	-0.231	0.028	0.156	-0.159	-0.148
leaf_injury : Fresh_shoot_biomass	-0.076	-0.205	0.085	0.150	-0.150	-0.104
leaf_injury : Dry_shoot_biomass	-0.027	-0.096	0.045	0.067	-0.067	-0.066
Plant_height : Fresh_shoot_biomass	0.346	0.399	0.419	0.415	0.418	0.360
Plant_height : Dry_shoot_biomass	0.293	0.313	0.368	0.376	0.376	0.370
Fresh_shoot_biomass : Dry_shoot_biomass	0.796	0.829	0.881	0.925	0.925	0.737

Table 3-6. Correlation among absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in plant height, fresh shoot biomass, and dry shoot biomass among 203 cowpea genotypes.

Correlation	**II**	**RST**	**STI**	**Traits**
AD	0.859	-0.857	0.999	Plant height
II		-0.999	-0.999	
RST			0.999	
Correlation	**II**	**RST**	**STI**	
AD	0.835	-0.835	0.811	Fresh shoot biomass
II		-1.000	-0.810	
RST			0.811	
Correlation	**II**	**RST**	**STI**	
AD	0.756	-0.755	0.998	Dry shoot biomass
II		-1.000	-0.998	
RST			0.998	

Table 3-7. List of top five cowpea genotypes from each of the 13 combinations between four parameters (absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI)) and three salt-related traits (plant height (H), fresh shoot biomass (F), and dry shoot biomass (D)) plus the leaf injury.

Accession number	Parameter_trait_top5†	Seed color	Origin
PI251222	LIS_5†	Tan	Afghanistan
PI582812	LIS_2,STI_H_3†	Brown_Holstein	Botswana
PI582852	STI_H_2	Brown	Botswana
PI582856	AD_H_1,II_H_1,RST_H_1	Variable_(Red_Brown_Grey)	Botswana
PI582866	STI_H_1	Brown	Botswana
PI582402	AD_F_2,II_F_3,RST_F_3,STI_F_1, AD_D_1,II_D_3,RST_D_3,STI_D_3	Tan	Brazil
PI180014	AD_H_4,II_H_2,RST_H_2	Tan	India
PI293469	RST_D_5,STI_D_4	Tan	NA
PI293570	LIS_1	Mixed_(Brown_Tan)	NA
PI582422	LIS_3	Blackeye	NA
PI257463	LIS_4,AD_H_2,II_H_3,RST_H_3	Red_Holstein	Nigeria
PI152195	RST_F_5,STI_F_4	Red	Paraguay
PI583274	STI_H_4	Browneye	Senegal
PI339602	AD_F_5,II_F_4,RST_F_4,II_D_4	Mixed_(Grey_Brown)	South_Africa
PI339610	STI_H_5	Variable_(Red_Brown_Cream)	Tanzania
PI582340	AD_F_4,II_F_2,RST_F_2,STI_F_2, AD_D_4,II_D_2,RST_D_2,STI_D_2	Mixed_(Brown_Pink)	USA
PI666252	STI_F_5	Tan	USA
PI666260	AD_F_1,II_F_1,RST_F_1,AD_D_3, II_D_1,RST_D_1,STI_D_1	Pinkeye	USA
Early_acre	AD_D_2	Cream	USA.AR
Empire	AD_F_3, AD_D_5	Pinkeye	USA.AR
09-175	AD_H_5,II_H_4,RST_H_5	Pinkeye	USA.AR
09-529	AD_H_3,II_H_5,RST_H_4	Blackeye	USA.AR
Encore	STI_F_3	Pinkeye	USA.AR
Envoy	II_F_5,II_D_5,RST_D_4,STI_D_5	Red_Holstein	USA.AR

† LIS_5 means the cowpea genotype PI251222 was listed as showing the fifth best salt tolerance based on leaf injury score (LIS), and STI_H_3 means the cowpea line PI582812 listed the third best salt tolerance based on salt tolerance index (STI) in plant height. The "Parameter_trait_top5" consists of three parts for each cowpea line, where "Parameter" signifies that each cowpea genotype has one of four parameters: absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI); "trait" one of three salt-related traits: plant height (H), fresh shoot biomass (F), and dry shoot biomass (D) plus the leaf injury score (LIS); and "top 5" for number one to five.

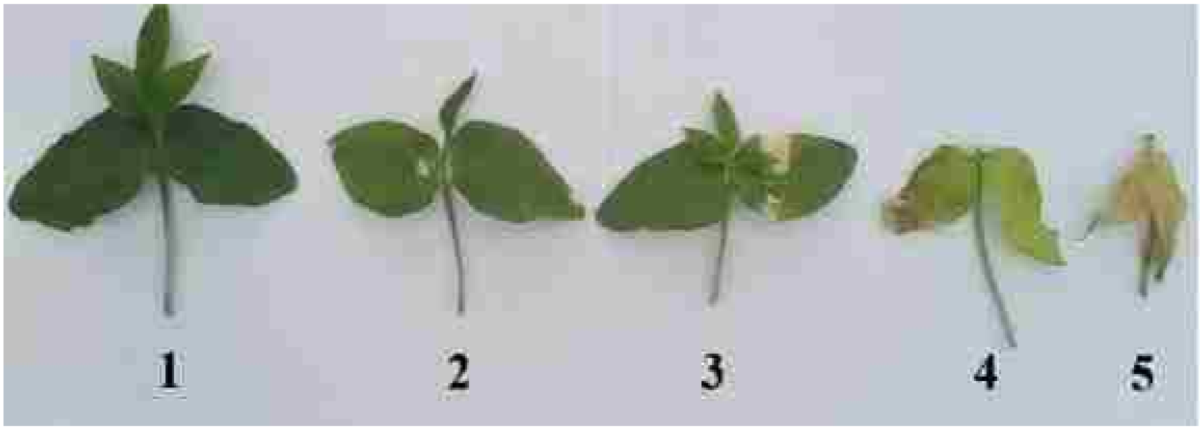

Figure 3-1. Visual leaf injury scored from 1 to 5.

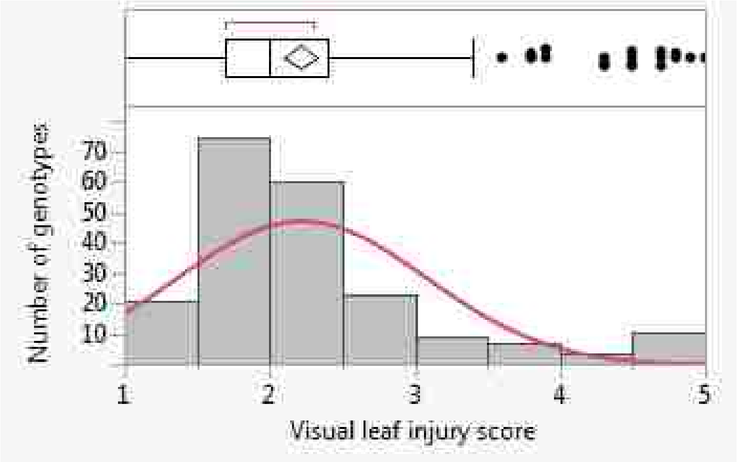

Figure 3-2. Distribution of the scores attributed to the visual injury among 203 cowpea genotypes**.**

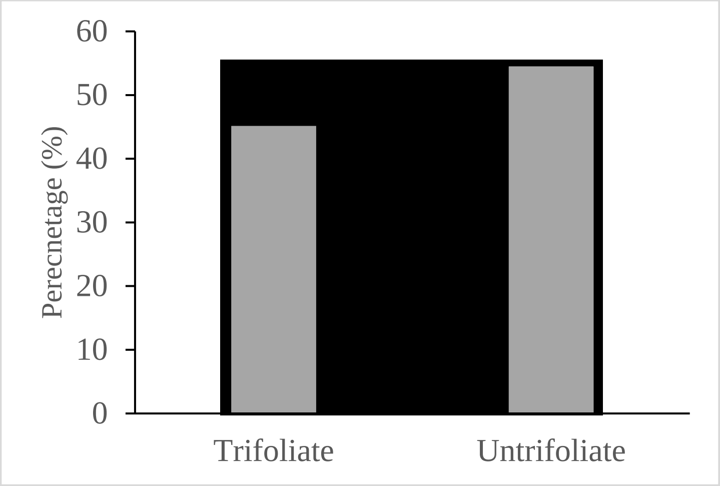

Figure 3-3. Inhibition of the trifoliate leaf development under 200 mM NaCl among 203 cowpea genotypes.

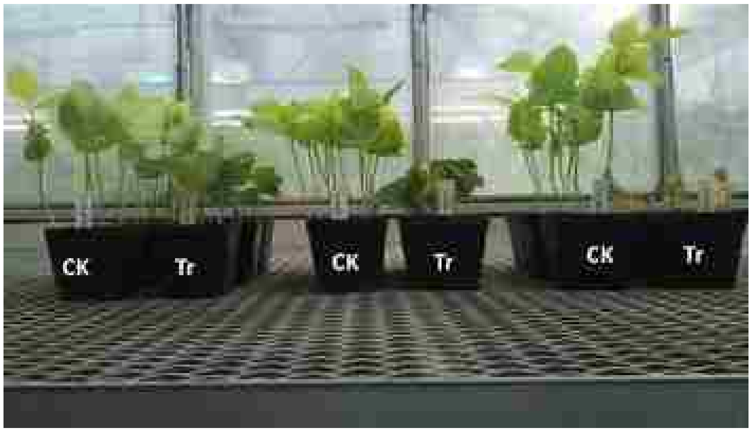

Figure 3-4. Plant height reduction due to salinity (CK: without salt stress, Tr: with salt stress).

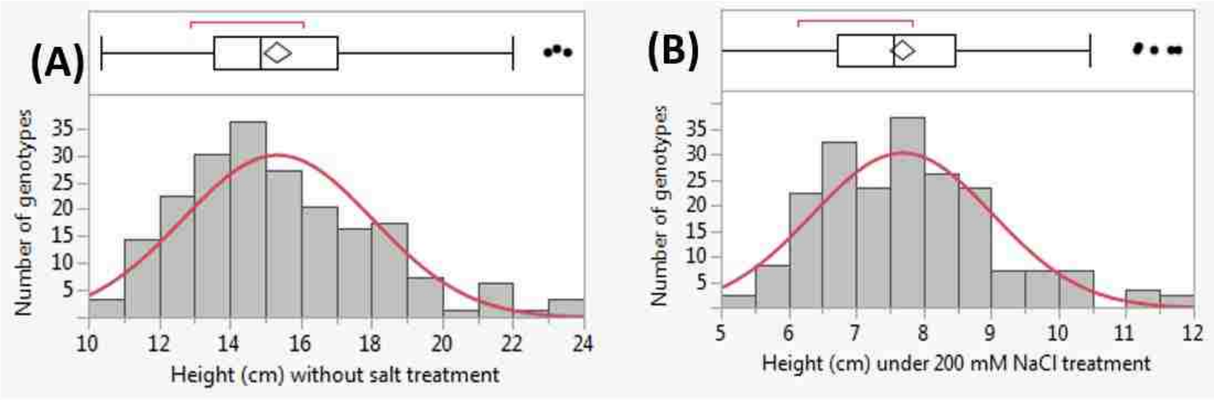

Figure 3-5. Distributions of plant height in 14 days after the first salt treatment among 203 cowpea genotypes: (A) without salt stress, and (B) with salt stress.

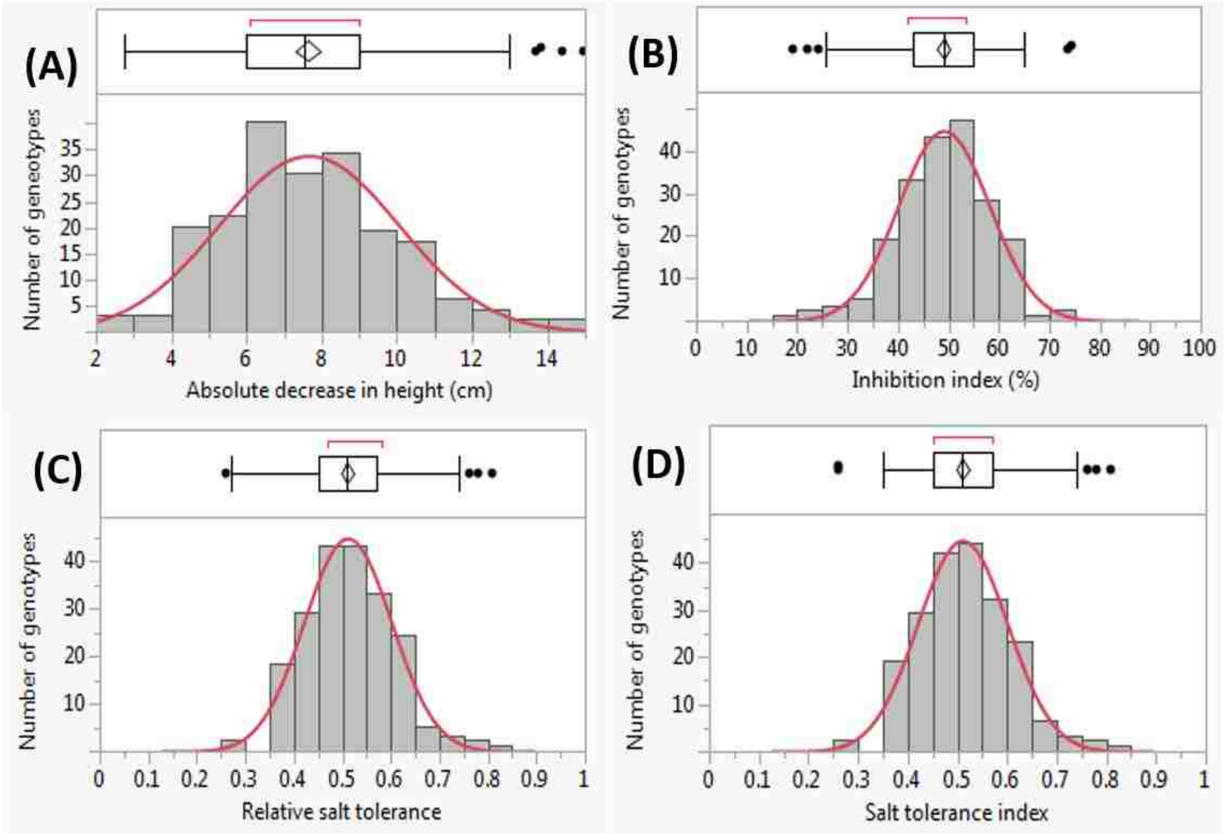

Figure 3-6. Distributions of four parameters in plant height reduction among 203 cowpea genotypes: (A) absolute decrease, (B) inhibition index, (C) relative salt tolerance, and (D) salt tolerance index.

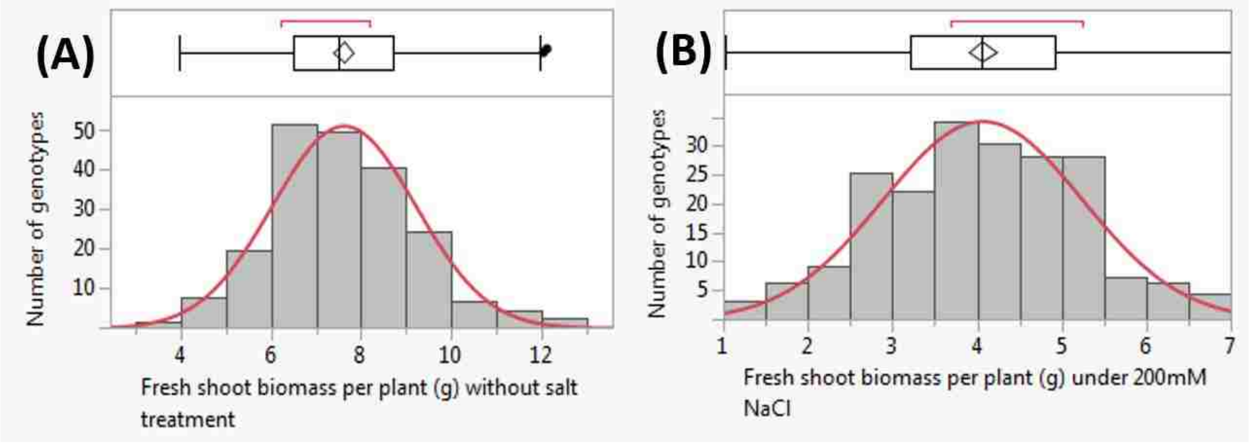

Figure 3-7. Distributions of fresh shoot biomass per plant in 14 days after the first salt treatment among 203 cowpea genotypes: (A) without salt stress, and (B) with salt stress.

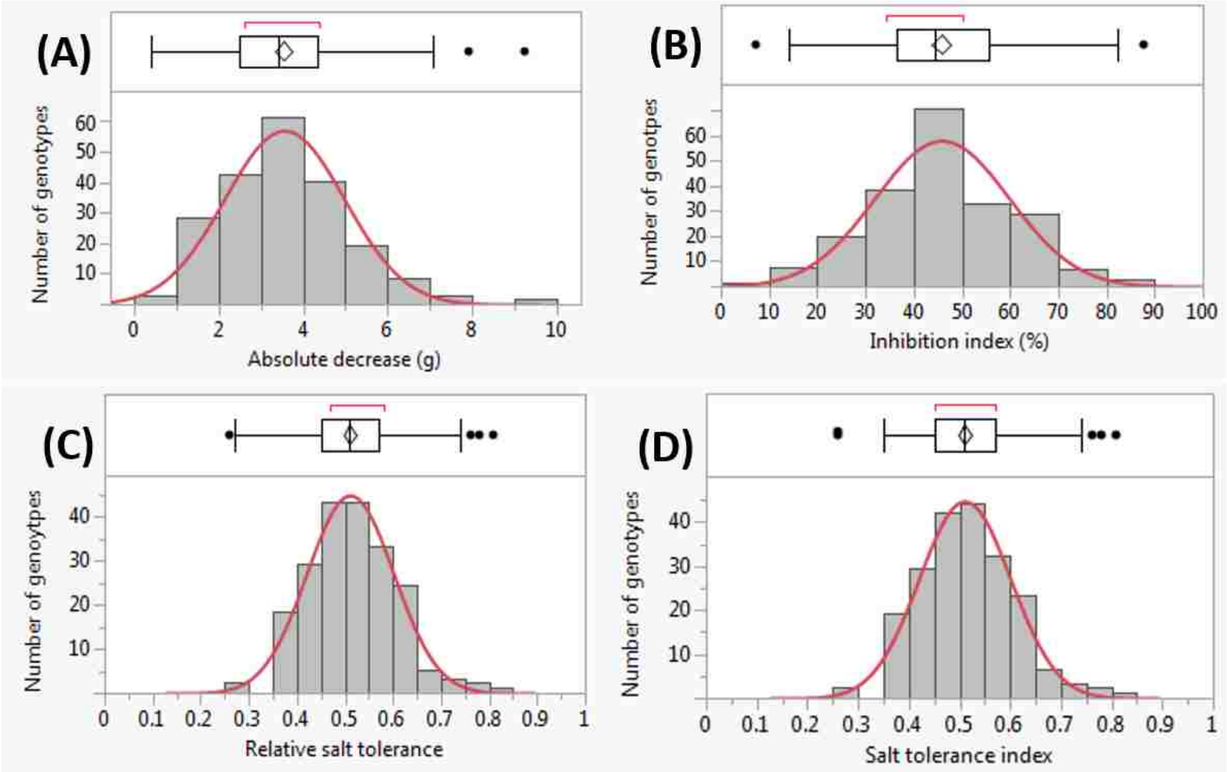

Figure 3-8. Distributions of four parameters in fresh shoot biomass per plant among 203 cowpea genotypes: (A) absolute decrease, (B) inhibition index, (C) relative salt tolerance, and (D) salt tolerance index.

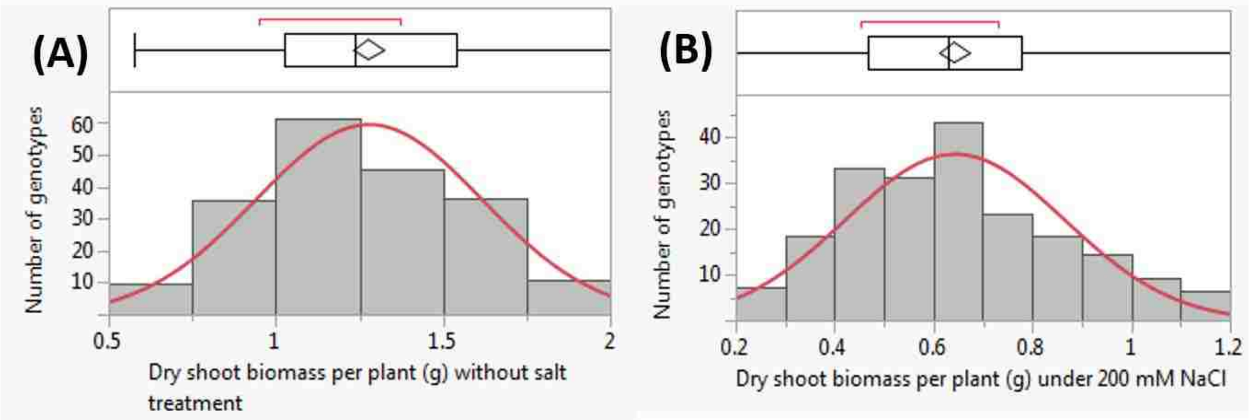

Figure 3-9. Distributions of dry shoot biomass per plant in 14 days after the first salt treatment among 203 cowpea genotypes: (A) without salt stress, and (B) with salt stress.

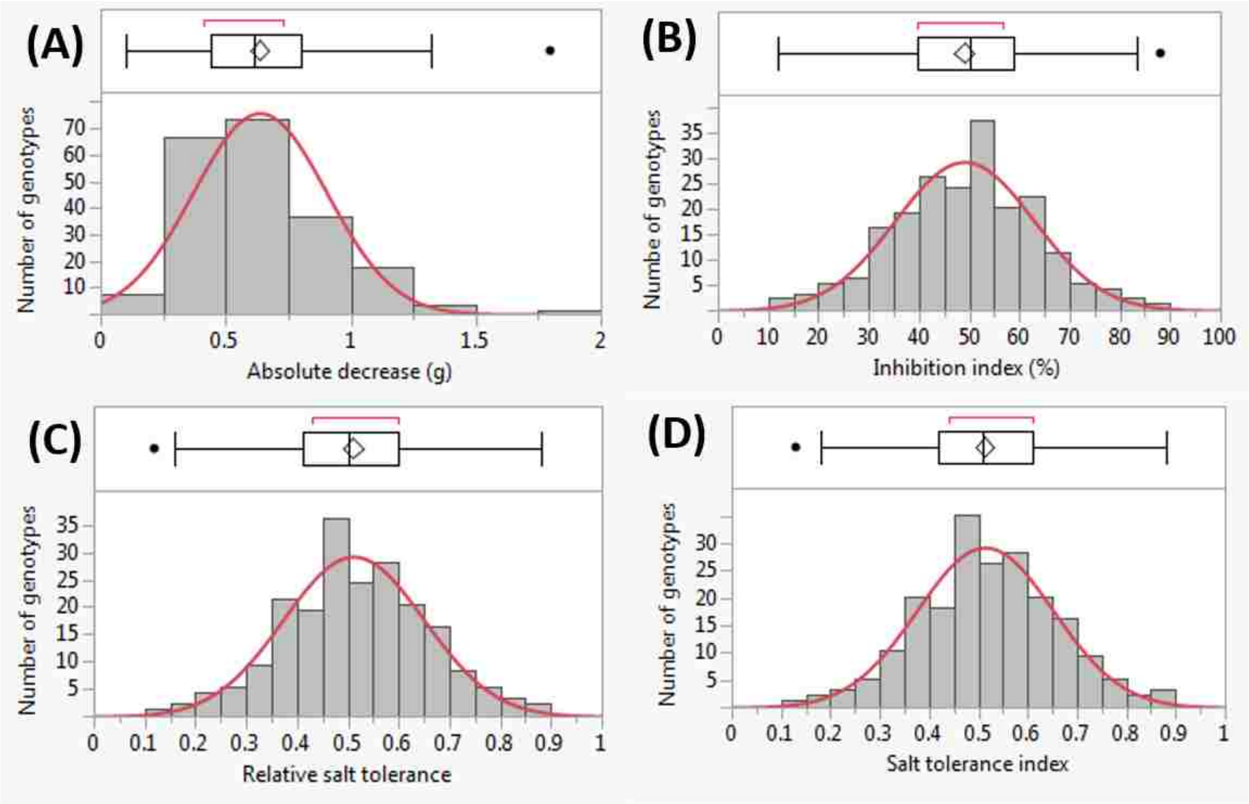

Figure 3-10. Distributions of four parameters in dry shoot biomass per plant among 203 cowpea genotypes: (A) absolute decrease, (B) inhibition index, (C) relative salt tolerance, and (D) salt tolerance index.

Figure S3-1. Greenhouse evaluation of salt tolerance in cowpea.

Table S3-1. Cowpea accession number, seed color, origin, and visual leaf injury score (LIS)

Accession number	Seed color	Origin	LIS
01-1781	Cream	USA	2
07-303	Red	USA	1.7
09-105	Cream	USA	2.4
09-1090	Pinkeye	USA	1.7
09-175	Pinkeye	USA	1.8
09-181	Pinkeye	USA	2.4
09-204	Brown	USA	1.8
09-208	Pinkeye	USA	1.9
09-211	Pinkeye	USA	1.7
09-239	Pinkeye	USA	1.8
09-268	Cream	USA	1.3
09-295	Pinkeye	USA	2
09-307	Blackeye	USA	3
09-316	Black_Holstein	USA	2
09-323	Pinkeye	USA	2.2
09-393	Pinkeye	USA	1.5
09-452	Black_Holstein	USA	1.8
09-455	Blackeye	USA	2.8
09-462	Pinkeye	USA	1.8
09-470	Pinkeye	USA	1.9
09-529	Blackeye	USA	2.1
09-655	Pinkeye	USA	1.9
09-671	Blackeye	USA	2.4
09-686	Pinkeye	USA	1.9
09-692	Pinkeye	USA	3.9
09-697	Pinkeye	USA	2
09-714	Pinkeye	USA	3
09-741	Red_Holstein	USA	1.8
09-745	Red_Holstein	USA	1.8
09-749	Red_Holstein	USA	2.5
09-752	Black_Holstein	NA	3.6
Arkansas_Blackeye#1	Blackeye	USA	2.9
Early_acre	Cream	USA	2.3
Early_scarlet	Pinkeye	USA	2.5
Ebony	Black	USA	2.9
Empire	Pinkeye	USA	1.8
ENCORE	Pinkeye	USA	2
Envoy	Red_Holstein	USA	2.5
EXCEL	Browneye	USA	1.9
PI152195	Red	Paraguay	1.8

Table S3-1. Cowpea accession number, seed color, origin, and visual leaf injury score (LIS) (Cont'd).

Accession number	**Seed color**	**Origin**	**LIS**
PI152196	Mixed_(Cream_Brown)	Paraguay	2.1
PI152197	Red	Paraguay	1.3
PI152199	Mixed_(Purple_Cream)	Paraguay	2.2
PI162924	Mixed_(Cream_Brown)	Paraguay	3.4
PI167284	Mixed_(Cream_Brown)	Turkey	3.1
PI175332	Tan	NA	3.2
PI180014	Tan	India	1.9
PI190191	Tan	Mexico	1.9
PI201024	Purple	Guatemala	1.8
PI201498	Blackeye	Mexico	2.1
PI218123	Browneye	Pakistan	1.5
PI221730	Red_Holstein	South_Africa	1.8
PI221731	Red_Holstein	South_Africa	1.7
PI223023	Browneye	Iran	1.7
PI225922	Mixed_(Brown_Tan)	Zambia	2.3
PI227829	Mixed_(Brown_Cream)	Guatemala	1.5
PI227830	Tan	Guatemala	2
PI229551	Browneye	Iran	1.3
PI229734	Blackeye	Iran	1.6
PI244571	Mixed_(Brown_Cream)	Guatemala	1.5
PI250416	Cream	Pakistan	1.7
PI250587	Darkbrown	Egypt	1.3
PI250759	Tan	Iran	1.7
PI251222	Tan	Afghanistan	1.2
PI253428	Darkbrown	Spain	2
PI255765	Blackeye	Nigeria	2.1
PI255774	Browneye	Nigeria	4.8
PI255815	Browneye	Nigeria	4.8
PI256342	Brown	Pakistan	2.1
PI257463	Red_Holstein	Nigeria	1.2
PI262179	Blackeye	Portugal	4.3
PI292891	Tan	South_Africa	1.5
PI292892	Brown_Holstein	South_Africa	3.8
PI292894	Tan	Zimbabwe	3.9
PI292897	Red_Holstein	Hungary	2.6
PI292898	Black	Hungary	2.8
PI292899	Tan	Hungary	2.7
PI292913	Tan	Hungary	2.3
PI293467	Mixed_(Tan_Brown)	USA	2.3
PI293469	Tan	NA	2.1

Table S3-1. Cowpea accession number, seed color, origin, and visual leaf injury score (LIS) (Cont'd).

Accession number	Seed color	Origin	LIS
PI293476	Variable_(Grey_Brown)	USA	1.7
PI293514	Red_Holstein	USA	1.6
PI293545	Mixed_(Brown_Cream)	NA	1.6
PI293568	Tan	NA	1.8
PI293569	Variable_(Red_Tan)	NA	1.6
PI293570	Mixed_(Brown_Tan)	NA	1
PI293582	Red_Holstein	NA	2.1
PI293584	Variable_(Red_Brown_Cream)	NA	1.8
PI297561	Mixed_(Brown_Grey)	NA	1.9
PI339563	Tan	Australia	2.4
PI339587	Red_Holstein	South_Africa	1.8
PI339590	Brown_Holstein	South_Africa	1.3
PI339591	Brown_Holstein	South_Africa	2.6
PI339592	Brown	South_Africa	2.3
PI339594	Variable_(Red Holstein_Red_Tan)	NA	3.8
PI339598	Tan	South_Africa	2.4
PI339600	Tan	South_Africa	1.6
PI339602	Mixed_(Grey_Brown)	South_Africa	2.3
PI339609	Tan	Tanzania	2.3
PI339610	Variable_(Red_Brown_Cream)	Tanzania	1.6
PI339611	Tan	Tanzania	1.4
PI339613	Variable_(Grey_Tan_Brown)	Tanzania	1.6
PI347639	Tan	NA	2.3
PI349674	Black	Australia	2.7
PI353045	Red_Holstein	India	2.3
PI353062	Mixed_(Cream_Purple)	India	4.3
PI354580	Tan	India	1.4
PI367921	Tan	NA	3.8
PI406290	Browneye	Mozambique	2
PI430687	Red	China	2.2
PI487518	Tan	Indonesia	3.3
PI527561	Tan	Burundi	4.7
PI578902	Red	China	2
PI578911	Tan	China	1.8
PI579909	Blackeye	Nigeria	1.5
PI582340	Mixed_(Brown_Pink)	USA	1.5
PI582347	Tan	NA	1.5
PI582354	Blackeye	USA	1.5
PI582366	Tan	India	1.5
PI582368	Black_Holstein	India	1.5

Table S3-1. Cowpea accession number, seed color, origin, and visual leaf injury score (LIS) (Cont'd).

Accession number	Seed color	Origin	LIS
PI582402	Tan	Brazil	3
PI582415	Tan	Mexico	1.3
PI582416	Variable_(Red_Brown_Cream)	Mexico	2
PI582420	Variable_(Red_Brown)	NA	1.2
PI582421	Red_Holstein	NA	1.7
PI582422	Blackeye	NA	1.1
PI582423	Brown_Holstein	NA	2.1
PI582425	Mixed_(Tan_Brown)	NA	1.8
PI582428	Blackeye	Trinidad_ and_Tobago	1.2
PI582465	Tan	Mexico	1.3
PI582467	Black_Holstein	NA	1.8
PI582468	Tan_Holstein	NA	2
PI582469	Brown_Holstein	Philippines	2
PI582474	Purple	Botswana	1.7
PI582522	Tan	Mexico	1.6
PI582530	Mixed_(Brown_Tan)	Ghana	2.3
PI582531	Variable_(Grey_Brown_Tan)	NA	2.7
PI582542	Browneye	Mexico	2.7
PI582551	Blackeye	Botswana	2.8
PI582554	Tan	NA	2.2
PI582570	Browneye	India	2.6
PI582572	Tan	Kenya	1.7
PI582573	Browneye	Kenya	1.7
PI582574	Tan	Kenya	2.3
PI582575	Black	Kenya	1.8
PI582576	Tan	NA	2.8
PI582578	Tan	Kenya	2
PI582579	Tan	Kenya	2.7
PI582650	Tan	Botswana	1.8
PI582665	Variable_(Grey_Brown_Tan)	Botswana	4.9
PI582666	Tan	Botswana	2.8
PI582675	Tan	Botswana	1.8
PI582680	Browneye	Botswana	2
PI582696	Brown	Botswana	2.2
PI582697	Tan	Botswana	1.8
PI582703	Red_Holstein	Botswana	2.1
PI582809	Tan	Botswana	2
PI582812	Brown_Holstein	Botswana	1.1
PI582813	Mixed_(Grey_Cream_Black eye)	Botswana	1.7
PI582818	Black_Holstein	Botswana	4.5

Table S3-1. Cowpea accession number, seed color, origin, and visual leaf injury score (LIS) (Cont'd).

Accession number	Seed color	Origin	LIS
PI582821	Mixed_(Brown_Tan)	Botswana	1.3
PI582822	Red_Holstein	Botswana	2.4
PI582823	Red_Holstein	Botswana	1.7
PI582824	Red_Holstein	Botswana	1.8
PI582825	Tan	Botswana	2.7
PI582850	Brown_Holstein	Botswana	2
PI582852	Brown	Botswana	1.8
PI582854	Greeneye	Botswana	2.7
PI582856	Variable_(Red_Brown_Grey)	Botswana	2
PI582857	Browneye	Botswana	2.6
PI582863	Browneye	Botswana	1.2
PI582866	Brown	Botswana	1.7
PI582873	Tan	Botswana	2
PI582874	Tan	Botswana	1.8
PI582875	Tan	Botswana	2.2
PI582878	Green	NA	1.4
PI582894	Tan	Botswana	1.9
PI582912	Black_Holstein	Kenya	2
PI583193	Variable_(Gray_Brown)	Senegal	2.2
PI583194	Variable_(Tan_Grey)	Senegal	1.9
PI583195	Red	Senegal	2
PI583198	Tan	Senegal	2.5
PI583201	Browneye	Senegal	4.7
PI583202	Browneye	Senegal	1.6
PI583209	Tan	Nigeria	1.8
PI583240	Tan	Senegal	2.1
PI583241	Red	Senegal	2
PI583244	Tan	Senegal	2
PI583248	Browneye	Senegal	1.7
PI583249	Browneye	Senegal	1.6
PI583250	Browneye	Senegal	1.5
PI583251	Tan	Senegal	2
PI583274	Browneye	Senegal	1.3
PI583487	Tan	NA	4.5
PI583488	Brown	NA	4.7
PI583550	Purple	Mali	2.1
PI583551	Browneye	Mali	1.6
PI610520	Black	NA	4.5
PI610533	Blackeye	NA	5
PI610604	Purple	NA	4.3

Table S3-1. Cowpea accession number, seed color, origin, and visual leaf injury score (LIS) (Cont'd).

Accession number	Seed color	Origin	LIS
PI666252	Tan	USA	3
PI666260	Pinkeye	USA	2.3
PI666262	Browneye	USA	4.5

Table S3-2. Cowpea accession number, seed color, origin, height without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI).

Accession_number	Seed color	Origin	Non_stress (cm)	Stress (cm)	AD (cm)	II (%)	RST	STI
01-1781	Cream	USA	11.13	6.31	4.82	43.26	0.57	0.57
07-303	Red	USA	13.97	7.9	6.07	43.37	0.57	0.57
09-105	Cream	USA	11.72	6.75	4.97	42.38	0.58	0.58
09-1090	Pinkeye	USA	12.6	7.91	4.69	36.99	0.63	0.63
09-175	Pinkeye	USA	12.67	9.36	3.31	25.7	0.74	0.74
09-181	Pinkeye	USA	13.14	8.71	4.43	33.52	0.66	0.66
09-204	Brown	USA	13.58	8.56	5.03	36.92	0.63	0.63
09-208	Pinkeye	USA	15	8.89	6.1	40.45	0.6	0.59
09-211	Pinkeye	USA	15.7	8.15	7.55	48.07	0.52	0.52
09-239	Pinkeye	USA	14.26	7.5	6.76	47.3	0.53	0.53
09-268	Cream	USA	13.21	6.13	7.09	53.57	0.46	0.46
09-295	Pinkeye	USA	14.18	10.07	4.11	28.9	0.71	0.71
09-307	Blackeye	USA	11.31	6.57	4.74	41.91	0.58	0.58
09-316	Black_Holstein	USA	15.35	7.52	7.83	51.02	0.49	0.49
09-323	Pinkeye	USA	12.25	6.66	5.59	45.47	0.55	0.54
09-393	Pinkeye	USA	18.35	10.05	8.29	45.17	0.55	0.55
09-452	Black_Holstein	USA	14.06	6.37	7.69	54.8	0.45	0.46
09-455	Blackeye	USA	14.19	7.81	6.38	44.93	0.55	0.55
09-462	Pinkeye	USA	14.78	8.26	6.52	43.98	0.56	0.56
09-470	Pinkeye	USA	13.79	8.61	5.17	37.51	0.62	0.62
09-529	Blackeye	USA	10.89	8.01	2.88	25.96	0.74	0.73
09-655	Pinkeye	USA	13.88	9.33	4.55	32.72	0.67	0.67
09-671	Blackeye	USA	13.89	7.56	6.33	45.64	0.54	0.55
09-686	Pinkeye	USA	13.65	8.94	4.71	34.2	0.66	0.65
09-692	Pinkeye	USA	13.84	7.09	6.74	48.57	0.51	0.51
09-697	Pinkeye	USA	16.75	8.25	8.5	50.69	0.49	0.49
09-714	Pinkeye	USA	13.39	8.04	5.35	39.77	0.6	0.6
09-741	Red_Holstein	USA	21.58	8.89	12.69	58.56	0.41	0.41
09-745	Red_Holstein	USA	12.78	7	5.78	45.19	0.55	0.55
09-749	Red_Holstein	USA	12.65	7.56	5.09	40.15	0.6	0.6
09-752	Black_Holstein	NA	11.23	7.23	4	35.68	0.64	0.65
Arkansas_Blackeye#1	Blackeye	USA	13.94	6.5	7.44	53.39	0.47	0.47
Early_acre	Cream	USA	14.15	7.67	6.48	45.69	0.54	0.54
Early_scarlet	Pinkeye	USA	18.29	9.29	9	48.62	0.51	0.51
Ebony	Black	USA	15.43	8.44	6.98	45.16	0.55	0.55
Empire	Pinkeye	USA	12.9	8.46	4.44	34.23	0.66	0.66
ENCORE	Pinkeye	USA	17.34	9.02	8.33	47.99	0.52	0.52

Table S3-2. Cowpea accession number, seed color, origin, height without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed color	Origin	Non_stress (cm)	Stress (cm)	AD (cm)	II (%)	RST	STI
Envoy	Red_Holstein	USA	15.23	8.18	7.05	46.31	0.54	0.54
EXCEL	Browneye	USA	14.45	7.22	7.23	49.89	0.5	0.5
PI152195	Red	Paraguay	15.62	6.52	9.1	58.22	0.42	0.42
PI152196	Mixed_(Cream_Brown)	Paraguay	17.73	8.63	9.1	51.13	0.49	0.49
PI152197	Red	Paraguay	16.74	9.69	7.04	42.17	0.58	0.58
PI152199	Mixed_(Purple_Cream)	Paraguay	17.6	8.73	8.87	50.43	0.5	0.5
PI162924	Mixed_(Cream_Brown)	Paraguay	15.19	6.18	9.01	59.29	0.41	0.41
PI167284	Mixed_(Cream_Brown)	Turkey	16.06	6.19	9.87	61.48	0.39	0.39
PI175332	Tan	NA	15	7.73	7.27	48.41	0.52	0.52
PI180014	Tan	India	14.65	11.41	3.24	22.08	0.78	0.78
PI190191	Tan	Mexico	11.53	6.99	4.54	38.82	0.61	0.6
PI201024	Purple	Guatemala	18.68	8.29	10.39	55.61	0.44	0.44
PI201498	Blackeye	Mexico	19.6	8.72	10.89	55.44	0.45	0.44
PI218123	Browneye	Pakistan	17.01	8.48	8.53	50.27	0.5	0.5
PI221730	Red_Holstein	South_Africa	13.52	7.67	5.85	43.12	0.57	0.57
PI221731	Red_Holstein	South_Africa	15.21	8.75	6.46	42.23	0.58	0.57
PI223023	Browneye	Iran	21.49	10.47	11.02	51.4	0.49	0.49
PI225922	Mixed_(Brown_Tan)	Zambia	15.61	6.75	8.86	56.64	0.43	0.43
PI227829	Mixed_(Brown_Cream)	Guatemala	14.13	7.5	6.63	46.62	0.53	0.53
PI227830	Tan	Guatemala	14.33	7.42	6.92	47.99	0.52	0.52
PI229551	Browneye	Iran	13.9	8.47	5.43	38.76	0.61	0.61
PI229734	Blackeye	Iran	15.62	7.56	8.06	51.63	0.48	0.48
PI244571	Mixed_(Brown_Cream)	Guatemala	16.61	7.92	8.69	52.15	0.48	0.48
PI250416	Cream	Pakistan	16.22	6.25	9.97	61.41	0.39	0.39
PI250587	Darkbrown	Egypt	21	11.77	9.23	43.94	0.56	0.56
PI250759	Tan	Iran	23.55	9.72	13.83	58.67	0.41	0.41
PI251222	Tan	Afghanistan	19.08	8.83	10.25	53.8	0.46	0.46
PI253428	Darkbrown	Spain	19.5	9.33	10.18	52.19	0.48	0.48
PI255765	Blackeye	Nigeria	21.22	9.99	11.23	52.85	0.47	0.47
PI255774	Browneye	Nigeria	20.42	7.43	12.99	63.74	0.36	0.37
PI255815	Browneye	Nigeria	13.35	5.76	7.59	56.7	0.43	0.43
PI256342	Brown	Pakistan	19.67	10	9.67	49	0.51	0.51
PI257463	Red_Holstein	Nigeria	11.39	8.64	2.75	24.01	0.76	0.76
PI262179	Blackeye	Portugal	14.85	7.51	7.34	49.43	0.51	0.51
PI292891	Tan	South_Africa	16.43	9.83	6.6	40.1	0.6	0.6
PI292892	Brown_Holstein	South_Africa	12	5.49	6.51	54.51	0.45	0.46
PI292894	Tan	Zimbabwe	12.67	6.21	6.45	50.53	0.49	0.49
PI292897	Red_Holstein	Hungary	15.13	8.66	6.47	42.61	0.57	0.57
PI292898	Black	Hungary	12.43	7.89	4.54	36.11	0.64	0.63

Table S3-2. Cowpea accession number, seed color, origin, height without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed color	Origin	Non_stress (cm)	Stress (cm)	AD (cm)	II (%)	RST	STI
PI292899	Tan	Hungary	18.72	8.98	9.74	51.95	0.48	0.48
PI292913	Tan	Hungary	14.53	7.81	6.72	46.41	0.54	0.54
PI293467	Mixed_(Tan_Brown)	USA	16.02	7.93	8.08	50.44	0.5	0.5
PI293469	Tan	NA	18.12	9.73	8.38	46.21	0.54	0.54
PI293476	Variable_(Grey_Brown)	USA	15.56	6.15	9.41	60.09	0.4	0.39
PI293514	Red_Holstein	USA	11.22	6.93	4.28	38.04	0.62	0.62
PI293545	Mixed_(Brown_Cream)	NA	15.79	7.78	8.01	50.29	0.5	0.49
PI293568	Tan	NA	15.31	5.71	9.61	62.37	0.38	0.37
PI293569	Variable_(Red_Tan)	NA	15.63	7.11	8.52	53.38	0.47	0.45
PI293570	Mixed_(Brown_Tan)	NA	14.21	7.41	6.8	47.37	0.53	0.52
PI293582	Red_Holstein	NA	10.51	6.58	3.94	36.73	0.63	0.62
PI293584	Variable_(Red_Brown_Cream)	NA	16.03	7.66	8.38	51.73	0.48	0.48
PI297561	Mixed_(Brown_Grey)	NA	13.47	7.2	6.27	46.58	0.53	0.53
PI339563	Tan	Australia	12.16	4.55	7.61	62.68	0.37	0.38
PI339587	Red_Holstein	South_Africa	12.23	5.53	6.71	54.79	0.45	0.45
PI339590	Brown_Holstein	South_Africa	14.08	6.36	7.72	54.61	0.45	0.45
PI339591	Brown_Holstein	South_Africa	14.46	6.64	7.81	53.99	0.46	0.46
PI339592	Brown	South_Africa	13.77	8.21	5.56	40.38	0.6	0.6
PI339594	Variable_(Red Holstein_Red_Tan)	NA	15.03	6.2	8.83	58.69	0.41	0.41
PI339598	Tan	South_Africa	13.54	7.68	5.86	43.29	0.57	0.57
PI339600	Tan	South_Africa	14.61	5.68	8.93	61.28	0.39	0.39
PI339602	Mixed_(Grey_Brown)	South_Africa	14.39	6.26	8.13	56.52	0.43	0.44
PI339609	Tan	Tanzania	18.61	7.02	11.6	62.34	0.38	0.38
PI339610	Variable_(Red_Brown_Cream)	Tanzania	17.21	6.28	10.93	63.47	0.37	0.36
PI339611	Tan	Tanzania	16.86	9.88	6.98	41.35	0.59	0.59
PI339613	Variable_(Grey_Tan_Brown)	Tanzania	15.59	7.19	8.4	53.89	0.46	0.46
PI347639	Tan	NA	18.57	11.17	7.4	39.65	0.6	0.6
PI349674	Black	Australia	12.37	7.18	5.19	42.05	0.58	0.58
PI353045	Red_Holstein	India	16.8	8.17	8.63	51.38	0.49	0.49
PI353062	Mixed_(Cream_Purple)	India	13.82	5.63	8.19	59.32	0.41	0.41
PI354580	Tan	India	14.6	6.13	8.48	58.05	0.42	0.42
PI367921	Tan	NA	15.83	8.4	7.43	46.96	0.53	0.53
PI406290	Browneye	Mozambique	15.38	8.84	6.54	42.46	0.58	0.58
PI430687	Red	China	16.97	8.67	8.3	48.84	0.51	0.51
PI487518	Tan	Indonesia	15.92	6.97	8.95	56.28	0.44	0.44
PI527561	Tan	Burundi	16.41	6.45	9.96	60.83	0.39	0.39
PI578902	Red	China	16.57	8.47	8.1	48.79	0.51	0.51
PI578911	Tan	China	19.28	8.06	11.23	58.05	0.42	0.42

Table S3-2. Cowpea accession number, seed color, origin, height without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed color	Origin	Non_stress (cm)	Stress (cm)	AD (cm)	II (%)	RST	STI
PI582340	Mixed_(Brown_Pink)	USA	14.23	9.47	4.77	33.47	0.67	0.67
PI582347	Tan	NA	11.83	6.83	5	42.33	0.58	0.58
PI582354	Blackeye	USA	17.83	7.83	10	56.03	0.44	0.44
PI582366	Tan	India	12	6.33	5.67	47.15	0.53	0.53
PI582368	Black_Holstein	India	12.5	7.83	4.67	37.32	0.63	0.63
PI582402	Tan	Brazil	13.22	7.08	6.13	46.43	0.54	0.54
PI582415	Tan	Mexico	13.67	8.3	5.37	39.23	0.61	0.61
PI582416	Variable_(Red_Brown_Cream)	Mexico	14.83	8.83	6	40.58	0.59	0.6
PI582420	Variable_(Red_Brown)	NA	18.7	8.89	9.81	52.39	0.48	0.48
PI582421	Red_Holstein	NA	19.14	8.94	10.2	53.2	0.47	0.47
PI582422	Blackeye	NA	15.09	8.2	6.89	45.56	0.54	0.54
PI582423	Brown_Holstein	NA	16.52	10.12	6.41	38.77	0.61	0.61
PI582425	Mixed_(Tan_Brown)	NA	13.71	8.33	5.37	38.99	0.61	0.61
PI582428	Blackeye	Trinidad_ and_Tobago	14.75	7.95	6.8	45.72	0.54	0.54
PI582465	Tan	Mexico	17.88	9.38	8.5	47.54	0.52	0.52
PI582467	Black_Holstein	NA	13.23	7.7	5.53	41.8	0.58	0.58
PI582468	Tan_Holstein	NA	13.67	7.24	6.43	47.23	0.53	0.53
PI582469	Brown_Holstein	Philippines	17.07	6.58	10.48	61.4	0.39	0.39
PI582474	Purple	Botswana	12.7	7.37	5.33	42.07	0.58	0.58
PI582522	Tan	Mexico	14.6	8.25	6.35	43.18	0.57	0.56
PI582530	Mixed_(Brown_Tan)	Ghana	23	8.63	14.37	62.4	0.38	0.37
PI582531	Variable_(Grey_Brown_Tan)	NA	14.47	8.33	6.13	42.29	0.58	0.58
PI582542	Browneye	Mexico	12.2	6.2	6	49.16	0.51	0.51
PI582551	Blackeye	Botswana	14.14	5.18	8.96	63.21	0.37	0.37
PI582554	Tan	NA	13.67	7.33	6.33	46.16	0.54	0.54
PI582570	Browneye	India	18.84	8.84	9.99	53.01	0.47	0.47
PI582572	Tan	Kenya	12.73	6.5	6.23	48.76	0.51	0.51
PI582573	Browneye	Kenya	15.93	7.93	8	50.2	0.5	0.5
PI582574	Tan	Kenya	12.33	7.4	4.93	39.86	0.6	0.6
PI582575	Black	Kenya	21.76	11.19	10.57	48.5	0.51	0.51
PI582576	Tan	NA	17.45	7.96	9.49	54.04	0.46	0.46
PI582578	Tan	Kenya	12.88	6.38	6.5	50.58	0.49	0.5
PI582579	Tan	Kenya	13.07	6.87	6.2	47.46	0.53	0.53
PI582650	Tan	Botswana	11.28	6.12	5.16	45.67	0.54	0.54
PI582665	Variable_(Grey_Brown_Tan)	Botswana	16.03	6.58	9.45	58.83	0.41	0.41
PI582666	Tan	Botswana	15.33	7.5	7.83	51.11	0.49	0.49
PI582675	Tan	Botswana	10.33	6.17	4.17	39.96	0.6	0.6
PI582680	Browneye	Botswana	13.17	7.17	6	45.49	0.55	0.54

Table S3-2. Cowpea accession number, seed color, origin, height without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed color	Origin	Non_stress (cm)	Stress (cm)	AD (cm)	II (%)	RST	STI
PI582696	Brown	Botswana	13.63	7.1	6.53	47.89	0.52	0.52
PI582697	Tan	Botswana	23.26	8.32	14.94	64.24	0.36	0.36
PI582703	Red_Holstein	Botswana	18.5	10.2	8.3	44.58	0.55	0.55
PI582809	Tan	Botswana	14.1	8.64	5.46	38.55	0.61	0.61
PI582812	Brown_Holstein	Botswana	18.6	6.5	12.1	65.04	0.35	0.35
PI582813	Mixed_(Grey_Cream_Black eye)	Botswana	16.2	7.74	8.46	52.41	0.48	0.48
PI582818	Black_Holstein	Botswana	11.5	6.1	5.4	46.93	0.53	0.53
PI582821	Mixed_(Brown_Tan)	Botswana	16.3	8.8	7.5	46.05	0.54	0.54
PI582822	Red_Holstein	Botswana	17.42	9.67	7.75	44.36	0.56	0.55
PI582823	Red_Holstein	Botswana	15.8	6.9	8.9	56.3	0.44	0.44
PI582824	Red_Holstein	Botswana	13.37	6.35	7.02	52.51	0.47	0.47
PI582825	Tan	Botswana	16.08	6.23	9.85	60.99	0.39	0.39
PI582850	Brown_Holstein	Botswana	14.14	7.97	6.17	43.57	0.56	0.56
PI582852	Brown	Botswana	17.6	4.63	12.97	73.5	0.27	0.26
PI582854	Greeneye	Botswana	12.97	6.73	6.23	48	0.52	0.52
PI582856	Variable_(Red_Brown_Grey)	Botswana	14.4	11.67	2.73	18.99	0.81	0.81
PI582857	Browneye	Botswana	11.75	6.71	5.04	42.9	0.57	0.57
PI582863	Browneye	Botswana	17.41	8.31	9.1	51.91	0.48	0.47
PI582866	Brown	Botswana	22	5.63	16.37	74.26	0.26	0.26
PI582873	Tan	Botswana	11.58	4.78	6.81	58.55	0.41	0.41
PI582874	Tan	Botswana	11	6.5	4.5	40.83	0.59	0.59
PI582875	Tan	Botswana	11.57	6.93	4.63	40.03	0.6	0.6
PI582878	Green	NA	14.35	5.84	8.51	58.71	0.41	0.41
PI582894	Tan	Botswana	12.65	7.71	4.94	38.87	0.61	0.61
PI582912	Black_Holstein	Kenya	13.08	7.56	5.53	42.4	0.58	0.58
PI583193	Variable_(Gray_Brown)	Senegal	14.82	6.71	8.11	54.65	0.45	0.45
PI583194	Variable_(Tan_Grey)	Senegal	14.79	6.96	7.84	52.16	0.48	0.47
PI583195	Red	Senegal	14.92	6.83	8.08	54.05	0.46	0.46
PI583198	Tan	Senegal	14.56	6.89	7.66	52.31	0.48	0.48
PI583201	Browneye	Senegal	17.33	6.97	10.37	59.84	0.4	0.4
PI583202	Browneye	Senegal	19.54	7.96	11.58	59.26	0.41	0.41
PI583209	Tan	Nigeria	18.58	8.39	10.19	54.78	0.45	0.45
PI583240	Tan	Senegal	17.7	7.47	10.23	57.79	0.42	0.42
PI583241	Red	Senegal	14.82	7.27	7.55	50.88	0.49	0.49
PI583244	Tan	Senegal	15.36	7.57	7.79	50.51	0.49	0.49
PI583248	Browneye	Senegal	17.84	6.64	11.2	62.71	0.37	0.37
PI583249	Browneye	Senegal	16	7.52	8.48	52.81	0.47	0.47
PI583250	Browneye	Senegal	18.14	7.34	10.8	59.49	0.41	0.4

Table S3-2. Cowpea accession number, seed color, origin, height without salt treatment and under salt stress, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) (Cont'd).

Accession_number	Seed color	Origin	Non_stress (cm)	Stress (cm)	AD (cm)	II (%)	RST	STI
PI583251	Tan	Senegal	18.57	8.07	10.5	56.54	0.43	0.43
PI583274	Browneye	Senegal	21.25	7.56	13.69	64.43	0.36	0.36
PI583487	Tan	NA	14.87	6.97	7.9	53.12	0.47	0.47
PI583488	Brown	NA	16.87	6.97	9.9	58.7	0.41	0.41
PI583550	Purple	Mali	14.57	5.73	8.83	60.64	0.39	0.39
PI583551	Browneye	Mali	18.13	7.25	10.88	60.04	0.4	0.4
PI610520	Black	NA	18.67	8	10.67	57	0.43	0.43
PI610533	Blackeye	NA	15.43	7.83	7.6	49.12	0.51	0.51
PI610604	Purple	NA	14.3	6.5	7.8	54.51	0.45	0.46
PI666252	Tan	USA	17.71	10	7.71	43.53	0.56	0.56
PI666260	Pinkeye	USA	18.3	7.67	10.63	58.02	0.42	0.42
PI666262	Browneye	USA	13.83	6.63	7.2	51.94	0.48	0.48

Table S3-3. Cowpea accession number, seed color, origin, fresh shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in fresh shoot biomass.

Accession number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
01-1781	Cream	USA	7.34	4.13	3.21	43.08	0.57	0.59
07-303	Red	USA	6.67	3.7	2.97	44.42	0.56	0.53
09-105	Cream	USA	5.65	3.58	2.07	36.09	0.64	0.77
09-1090	Pinkeye	USA	9.86	5.8	4.06	40.72	0.59	0.52
09-175	Pinkeye	USA	7.39	2.94	4.45	60.08	0.4	0.43
09-181	Pinkeye	USA	6.85	4.25	2.6	37.96	0.62	0.68
09-204	Brown	USA	9.48	4.78	4.7	49.35	0.51	0.5
09-208	Pinkeye	USA	8.55	4.82	3.73	43.49	0.57	0.55
09-211	Pinkeye	USA	9.42	5.23	4.19	44.4	0.56	0.56
09-239	Pinkeye	USA	7.59	4.9	2.69	34.53	0.65	0.61
09-268	Cream	USA	6.81	3.94	2.87	41.71	0.58	0.6
09-295	Pinkeye	USA	6.09	4.49	1.61	26.08	0.74	0.68
09-307	Blackeye	USA	6.38	3.41	2.97	46.54	0.53	0.63
09-316	Black_Holstein	USA	7.54	4.25	3.29	43.99	0.56	0.45
09-323	Pinkeye	USA	8.33	2.64	5.69	68.31	0.32	0.43
09-393	Pinkeye	USA	9.78	4.64	5.14	52.58	0.47	0.4
09-452	Black_Holstein	USA	7.2	2.93	4.27	59.21	0.41	0.47
09-455	Blackeye	USA	8.71	4.65	4.06	46.66	0.53	0.58
09-462	Pinkeye	USA	8.95	5.26	3.7	40.54	0.59	0.52
09-470	Pinkeye	USA	6.69	4.34	2.36	35.17	0.65	0.72
09-529	Blackeye	USA	8.75	5.19	3.57	40.35	0.6	0.61
09-655	Pinkeye	USA	7.79	5.93	1.85	23.59	0.76	0.71
09-671	Blackeye	USA	8.78	4.53	4.25	48.04	0.52	0.49
09-686	Pinkeye	USA	8.01	4.63	3.38	42.17	0.58	0.55
09-692	Pinkeye	USA	6.73	2.97	3.77	56.42	0.44	0.51
09-697	Pinkeye	USA	8.97	4.86	4.11	45.79	0.54	0.51
09-714	Pinkeye	USA	7.45	3.92	3.53	47.32	0.53	0.53
09-741	Red_Holstein	USA	7.78	3.94	3.84	49.31	0.51	0.54
09-745	Red_Holstein	USA	9.15	5.24	3.9	42.49	0.58	0.62
09-749	Red_Holstein	USA	9.55	6.24	3.3	34.26	0.66	0.53
09-752	Black_Holstein	NA	7.15	2.67	4.48	63.08	0.37	0.48
Arkansas_Blackeye#1	Blackeye	USA	9.19	3.69	5.5	59.89	0.4	0.38
Early_acre	Cream	USA	5.17	3.6	1.57	29.73	0.7	0.74
Early_scarlet	Pinkeye	USA	9.42	5.21	4.21	44.4	0.56	0.59
Ebony	Black	USA	8.58	5.16	3.42	39.57	0.6	0.54
Empire	Pinkeye	USA	5.19	4.18	1.01	19.51	0.8	0.77
ENCORE	Pinkeye	USA	6.26	4.19	2.07	33.1	0.67	0.81

Table S3-3. Cowpea accession number, seed color, origin, fresh shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in fresh shoot biomass (Cont'd).

Accession number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
EXCEL	Browneye	USA	8.06	2.63	5.43	67.16	0.33	0.41
PI152195	Red	Paraguay	6.56	5.25	1.32	19.45	0.81	0.79
PI152196	Mixed_(Cream_Brown)	Paraguay	8.56	4.81	3.75	43.13	0.57	0.65
PI152197	Red	Paraguay	9.62	6.84	2.78	27.47	0.73	0.62
PI152199	Mixed_(Purple_Cream)	Paraguay	8.67	4.99	3.68	42.72	0.57	0.49
PI162924	Mixed_(Cream_Brown)	Paraguay	7.39	2.43	4.96	66.14	0.34	0.43
PI167284	Mixed_(Cream_Brown)	Turkey	8.85	3.1	5.75	65.33	0.35	0.31
PI175332	Tan	NA	6.49	3.42	3.06	47.02	0.53	0.68
PI180014	Tan	India	8.5	5.5	3	34.87	0.65	0.57
PI190191	Tan	Mexico	6.3	3.3	3.01	47.83	0.52	0.57
PI201024	Purple	Guatemala	8.99	4.36	4.63	51.58	0.48	0.51
PI201498	Blackeye	Mexico	8.84	5.02	3.82	42.33	0.58	0.46
PI218123	Browneye	Pakistan	8.89	2.97	5.93	66.91	0.33	0.4
PI221730	Red_Holstein	South_Africa	5.95	4.19	1.76	28.66	0.71	0.72
PI221731	Red_Holstein	South_Africa	6.58	3.35	3.23	48.9	0.51	0.48
PI223023	Browneye	Iran	12.05	4.13	7.92	65.97	0.34	0.28
PI225922	Mixed_(Brown_Tan)	Zambia	5.82	1.03	4.8	82.49	0.18	0.42
PI227829	Mixed_(Brown_Cream)	Guatemala	7.76	4.57	3.19	39.97	0.6	0.58
PI227830	Tan	Guatemala	6.26	4.35	1.9	30.19	0.7	0.66
PI229551	Browneye	Iran	6.78	2.66	4.12	60.85	0.39	0.42
PI229734	Blackeye	Iran	8.56	4.22	4.34	50.51	0.49	0.5
PI244571	Mixed_(Brown_Cream)	Guatemala	7.86	3.78	4.08	51.48	0.49	0.54
PI250416	Cream	Pakistan	11.25	4.93	6.32	54.97	0.45	0.53
PI250587	Darkbrown	Egypt	10.77	7.08	3.69	34.59	0.65	0.49
PI250759	Tan	Iran	9.27	3.42	5.85	62.94	0.37	0.47
PI251222	Tan	Afghanistan	9.99	5.63	4.36	42.74	0.57	0.54
PI253428	Darkbrown	Spain	11.45	6.03	5.42	47.33	0.53	0.5
PI255765	Blackeye	Nigeria	11.28	4.99	6.29	55.68	0.44	0.37
PI255774	Browneye	Nigeria	10.58	1.35	9.23	87.57	0.12	0.17
PI255815	Browneye	Nigeria	4.19	2.64	1.55	36.72	0.63	0.55
PI256342	Brown	Pakistan	8.22	4.82	3.4	41.56	0.58	0.57
PI257463	Red_Holstein	Nigeria	6.03	3.88	2.15	35.27	0.65	0.65
PI262179	Blackeye	Portugal	8.55	5.19	3.35	39.42	0.61	0.61
PI292891	Tan	South_Africa	8.81	6.55	2.26	25.28	0.75	0.56
PI292892	Brown_Holstein	South_Africa	5.6	1.64	3.96	70.55	0.29	0.36
PI292894	Tan	Zimbabwe	7.09	2.47	4.61	64.32	0.36	0.48
PI292897	Red_Holstein	Hungary	7	5.21	1.79	25.33	0.75	0.65
PI292898	Black	Hungary	6.42	3.98	2.44	38.04	0.62	0.6

Table S3-3. Cowpea accession number, seed color, origin, fresh shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in fresh shoot biomass (Cont'd).

Accession number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
PI292913	Tan	Hungary	7.5	5.41	2.09	27.08	0.73	0.69
PI293467	Mixed_(Tan_Brown)	USA	8.18	4.66	3.52	41.87	0.58	0.63
PI293469	Tan	NA	6.94	5.33	1.61	23.26	0.77	0.61
PI293476	Variable_(Grey_Brown)	USA	8.74	2.09	6.65	75.21	0.25	0.3
PI293514	Red_Holstein	USA	6.71	4.95	1.76	26.07	0.74	0.75
PI293545	Mixed_(Brown_Cream)	NA	8.92	5.06	3.86	43.14	0.57	0.53
PI293568	Tan	NA	8.1	2.92	5.18	63.63	0.36	0.47
PI293569	Variable_(Red_Tan)	NA	9.37	5.07	4.3	46.04	0.54	0.58
PI293570	Mixed_(Brown_Tan)	NA	7.96	6.3	1.66	19.56	0.8	0.61
PI293582	Red_Holstein	NA	6.63	4.84	1.79	26.49	0.74	0.72
PI293584	Variable_(Red_Brown_Cream)	NA	7.48	5.38	2.1	27.49	0.73	0.71
PI297561	Mixed_(Brown_Grey)	NA	7.62	4.27	3.35	43.01	0.57	0.45
PI339563	Tan	Australia	4	1.56	2.44	60.96	0.39	0.32
PI339587	Red_Holstein	South_Africa	6.25	3.77	2.48	39.18	0.61	0.52
PI339590	Brown_Holstein	South_Africa	7.33	2.72	4.6	61.71	0.38	0.38
PI339591	Brown_Holstein	South_Africa	7.75	4.3	3.45	44.82	0.55	0.63
PI339592	Brown	South_Africa	7.59	4.49	3.1	40.83	0.59	0.57
PI339594	Variable_(Red Holstein_Red_Tan)	NA	5.63	3.65	1.98	34.43	0.66	0.69
PI339598	Tan	South_Africa	8.65	4.31	4.34	49.76	0.5	0.42
PI339600	Tan	South_Africa	6.39	3.48	2.91	45.91	0.54	0.68
PI339602	Mixed_(Grey_Brown)	South_Africa	6.35	5.17	1.18	17.61	0.82	0.62
PI339609	Tan	Tanzania	6.4	3.91	2.49	38.61	0.61	0.51
PI339610	Variable_(Red_Brown_Cream)	Tanzania	6.35	2.49	3.85	60.8	0.39	0.5
PI339611	Tan	Tanzania	7.65	4.84	2.81	36.3	0.64	0.63
PI339613	Variable_(Grey_Tan_Brown)	Tanzania	7.98	3.76	4.21	52.06	0.48	0.49
PI347639	Tan	NA	6.29	4.09	2.2	35.04	0.65	0.64
PI349674	Black	Australia	6.25	4.1	2.15	34.2	0.66	0.7
PI353045	Red_Holstein	India	6.72	5.01	1.71	25.23	0.75	0.69
PI353062	Mixed_(Cream_Purple)	India	7.32	3.84	3.48	47.41	0.53	0.51
PI354580	Tan	India	7.58	3.22	4.35	57.39	0.43	0.41
PI367921	Tan	NA	7.53	2.75	4.78	63.47	0.37	0.43
PI406290	Browneye	Mozambique	8.57	4	4.57	52.93	0.47	0.5
PI430687	Red	China	8.33	5.05	3.27	38.94	0.61	0.46
PI487518	Tan	Indonesia	7.99	2.56	5.43	68.64	0.31	0.4
PI527561	Tan	Burundi	8.42	2.82	5.6	66.6	0.33	0.34
PI578902	Red	China	7.55	4.16	3.39	43.58	0.56	0.49
PI578911	Tan	China	9.19	3.19	6	65.01	0.35	0.41
PI579909	Blackeye	Nigeria	7.18	4.01	3.17	44.2	0.56	0.64

Table S3-3. Cowpea accession number, seed color, origin, fresh shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in fresh shoot biomass (Cont'd).

Accession number	**Seed color**	**Origin**	**Non_stress (g)**	**Stress (g)**	**AD (g)**	**II (%)**	**RST**	**STI**
PI582347	Tan	NA	7.24	4.78	2.46	34.03	0.66	0.61
PI582354	Blackeye	USA	7.01	4.21	2.8	39.63	0.6	0.62
PI582366	Tan	India	6.84	3.79	3.05	44.79	0.55	0.44
PI582368	Black_Holstein	India	5.83	2.73	3.1	53.33	0.47	0.49
PI582402	Tan	Brazil	4.37	3.71	0.66	15.05	0.85	0.99
PI582415	Tan	Mexico	7.77	5.85	1.92	24.72	0.75	0.72
PI582416	Variable_(Red_Brown_Cream)	Mexico	6.88	4.8	2.07	30.03	0.7	0.58
PI582420	Variable_(Red_Brown)	NA	10.05	3	7.05	70.1	0.3	0.32
PI582421	Red_Holstein	NA	5.22	3.35	1.87	35.85	0.64	0.66
PI582422	Blackeye	NA	7.25	3.49	3.76	51.78	0.48	0.48
PI582423	Brown_Holstein	NA	6.8	3.65	3.15	46.13	0.54	0.6
PI582425	Mixed_(Tan_Brown)	NA	9.01	5.04	3.97	44.07	0.56	0.5
PI582428	Blackeye	Trinidad_ and_Tobago	7.57	3.67	3.9	50.13	0.5	0.63
PI582465	Tan	Mexico	12.1	6.56	5.54	45.69	0.54	0.45
PI582467	Black_Holstein	NA	7.28	4.16	3.12	42.98	0.57	0.47
PI582468	Tan_Holstein	NA	8.26	1.7	6.56	79.4	0.21	0.17
PI582469	Brown_Holstein	Philippines	5.22	2.07	3.16	61.49	0.39	0.57
PI582474	Purple	Botswana	6.89	3.96	2.93	42.14	0.58	0.65
PI582522	Tan	Mexico	9.28	6.89	2.39	25.44	0.75	0.59
PI582530	Mixed_(Brown_Tan)	Ghana	5.93	2.43	3.5	57.54	0.42	0.51
PI582531	Variable_(Grey_Brown_Tan)	NA	6.21	3.75	2.46	39.28	0.61	0.61
PI582542	Browneye	Mexico	6.71	3.84	2.86	42.6	0.57	0.53
PI582551	Blackeye	Botswana	6.78	2.64	4.13	61.08	0.39	0.51
PI582554	Tan	NA	7.27	5.44	1.83	25.16	0.75	0.67
PI582570	Browneye	India	9.72	3.93	5.79	59.51	0.4	0.37
PI582572	Tan	Kenya	5.18	1.97	3.21	62.68	0.37	0.39
PI582573	Browneye	Kenya	6.46	2.64	3.82	59.07	0.41	0.46
PI582574	Tan	Kenya	6.21	3.22	2.99	48.18	0.52	0.62
PI582575	Black	Kenya	9.65	5.23	4.42	45.78	0.54	0.53
PI582576	Tan	NA	8.68	4.75	3.93	43.69	0.56	0.47
PI582578	Tan	Kenya	6.27	3.29	2.98	47.03	0.53	0.56
PI582579	Tan	Kenya	6.17	3.74	2.43	39.28	0.61	0.59
PI582650	Tan	Botswana	9.09	5.83	3.25	35.6	0.64	0.63
PI582665	Variable_(Grey_Brown_Tan)	Botswana	10.6	5.39	5.22	49.68	0.5	0.46
PI582666	Tan	Botswana	6.43	3.74	2.69	41.97	0.58	0.54
PI582675	Tan	Botswana	5.63	3.22	2.41	42.86	0.57	0.53
PI582680	Browneye	Botswana	6.63	3.15	3.48	52.7	0.47	0.54
PI582696	Brown	Botswana	7.3	3.8	3.51	48.11	0.52	0.48

Table S3-3. Cowpea accession number, seed color, origin, fresh shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in fresh shoot biomass (Cont'd).

Accession number	**Seed color**	**Origin**	**Non_stress (g)**	**Stress (g)**	**AD (g)**	**II (%)**	**RST**	**STI**
PI582697	Tan	Botswana	9.25	3.41	5.84	62.91	0.37	0.41
PI582703	Red_Holstein	Botswana	9.69	4.59	5.1	52.5	0.48	0.45
PI582809	Tan	Botswana	6.57	3.7	2.87	42.76	0.57	0.55
PI582812	Brown_Holstein	Botswana	6.51	3.45	3.07	46.9	0.53	0.64
PI582813	Mixed_(Grey_Cream_Black eye)	Botswana	10.03	5.1	4.93	48.8	0.51	0.47
PI582818	Black_Holstein	Botswana	6.98	4.5	2.49	35.6	0.64	0.7
PI582821	Mixed_(Brown_Tan)	Botswana	8.16	5.48	2.68	32.81	0.67	0.61
PI582822	Red_Holstein	Botswana	5.62	3.69	1.93	33.54	0.66	0.56
PI582823	Red_Holstein	Botswana	4.43	2.96	1.47	33.86	0.66	0.57
PI582824	Red_Holstein	Botswana	4.62	2.87	1.75	37.94	0.62	0.59
PI582825	Tan	Botswana	11.95	5.26	6.69	55.47	0.45	0.39
PI582850	Brown_Holstein	Botswana	5.53	3.58	1.95	35.3	0.65	0.52
PI582852	Brown	Botswana	8.1	1.73	6.37	78.75	0.21	0.28
PI582854	Greeneye	Botswana	9.17	3.94	5.23	57.11	0.43	0.39
PI582856	Variable_(Red_Brown_Grey)	Botswana	5.3	2.13	3.17	59.73	0.4	0.51
PI582857	Browneye	Botswana	5.61	4.19	1.42	25.1	0.75	0.63
PI582863	Browneye	Botswana	7.13	3.2	3.94	55.07	0.45	0.33
PI582866	Brown	Botswana	5.32	1.4	3.92	73.67	0.26	0.5
PI582873	Tan	Botswana	8.78	4.76	4.02	45.52	0.54	0.57
PI582874	Tan	Botswana	8.63	6.01	2.63	30.44	0.7	0.7
PI582875	Tan	Botswana	7.74	5.45	2.29	29.67	0.7	0.61
PI582878	Green	NA	9.81	3.81	6	61.12	0.39	0.41
PI582894	Tan	Botswana	8.65	4.56	4.09	46.49	0.54	0.46
PI582912	Black_Holstein	Kenya	7.07	3.29	3.78	53.16	0.47	0.54
PI583193	Variable_(Gray_Brown)	Senegal	6.6	4.3	2.3	34.6	0.65	0.65
PI583194	Variable_(Tan_Grey)	Senegal	7.55	4.35	3.2	42.22	0.58	0.53
PI583195	Red	Senegal	7.23	3.62	3.61	49.86	0.5	0.47
PI583198	Tan	Senegal	6.08	2.97	3.11	51.16	0.49	0.49
PI583201	Browneye	Senegal	6.82	2.47	4.35	63.85	0.36	0.4
PI583202	Browneye	Senegal	7.68	4.06	3.61	46.47	0.54	0.58
PI583209	Tan	Nigeria	9.9	4.91	4.99	50.11	0.5	0.39
PI583240	Tan	Senegal	3.95	2.32	1.63	41.43	0.59	0.61
PI583241	Red	Senegal	4.45	1.87	2.58	57.68	0.42	0.56
PI583244	Tan	Senegal	6.95	3.91	3.05	43.38	0.57	0.68
PI583248	Browneye	Senegal	8.55	5.16	3.39	39.87	0.6	0.44
PI583249	Browneye	Senegal	6.58	2.58	4	60.88	0.39	0.39
PI583250	Browneye	Senegal	7.94	2.83	5.11	63.38	0.37	0.45
PI583251	Tan	Senegal	8.77	4.38	4.39	49.37	0.51	0.47

Table S3-3. Cowpea accession number, seed color, origin, fresh shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in fresh shoot biomass (Cont'd).

Accession number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
PI583487	Tan	NA	6.74	2.67	4.07	59.96	0.4	0.42
PI583488	Brown	NA	8.7	4.48	4.22	48.39	0.52	0.48
PI583550	Purple	Mali	7.41	3.05	4.36	58.97	0.41	0.45
PI583551	Browneye	Mali	8.3	3.43	4.87	58.08	0.42	0.39
PI610520	Black	NA	7.39	2.69	4.7	63.73	0.36	0.43
PI610533	Blackeye	NA	9.58	4.71	4.86	50.6	0.49	0.44
PI610604	Purple	NA	4.97	2.98	2	40.29	0.6	0.72
PI666252	Tan	USA	6.62	4.74	1.88	28.33	0.72	0.78
PI666260	Pinkeye	USA	5.8	5.38	0.42	7.08	0.93	0.65
PI666262	Browneye	USA	7.58	4.28	3.29	43.51	0.56	0.4

Table S3-4. Cowpea accession number, seed color, origin, dry shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in in dry shoot biomass.

Accession_number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
01-1781	Cream	USA	1.1839	0.5834	0.601	50.82	0.49	0.5
07-303	Red	USA	1.2173	0.6199	0.597	49.48	0.51	0.52
09-105	Cream	USA	0.7436	0.4593	0.284	37.46	0.63	0.62
09-1090	Pinkeye	USA	1.7485	1.0456	0.703	39.84	0.6	0.6
09-175	Pinkeye	USA	1.2346	0.3424	0.892	72.35	0.28	0.28
09-181	Pinkeye	USA	1.1959	0.6799	0.516	43.5	0.56	0.57
09-204	Brown	USA	1.5819	0.8609	0.721	45.5	0.55	0.54
09-208	Pinkeye	USA	1.4564	0.8587	0.598	40.81	0.59	0.59
09-211	Pinkeye	USA	1.8748	0.9033	0.971	52.08	0.48	0.49
09-239	Pinkeye	USA	0.8967	0.4097	0.487	54.1	0.46	0.46
09-268	Cream	USA	0.855	0.4051	0.45	52.82	0.47	0.48
09-295	Pinkeye	USA	0.9414	0.626	0.315	32.88	0.67	0.66
09-307	Blackeye	USA	0.8766	0.4467	0.43	49.09	0.51	0.51
09-316	Black_Holstein	USA	1.8319	1.1067	0.725	38.93	0.61	0.61
09-323	Pinkeye	USA	1.6038	0.4671	1.137	71	0.29	0.29
09-393	Pinkeye	USA	1.7297	0.8441	0.886	50.97	0.49	0.49
09-452	Black_Holstein	USA	1.1471	0.4358	0.711	61.81	0.38	0.38
09-455	Blackeye	USA	1.2601	0.7148	0.545	43.06	0.57	0.57
09-462	Pinkeye	USA	1.6348	0.9767	0.658	39.62	0.6	0.6
09-470	Pinkeye	USA	1.1211	0.6568	0.464	41.05	0.59	0.58
09-529	Blackeye	USA	1.5403	0.8958	0.645	41.36	0.59	0.58
09-655	Pinkeye	USA	1.464	0.9867	0.477	32.75	0.67	0.68
09-671	Blackeye	USA	1.6051	0.7635	0.842	52.44	0.48	0.48
09-686	Pinkeye	USA	1.5537	0.8481	0.706	45.2	0.55	0.55
09-692	Pinkeye	USA	1.3047	0.6677	0.637	48.79	0.51	0.51
09-697	Pinkeye	USA	1.3287	0.6268	0.702	53.48	0.47	0.48
09-714	Pinkeye	USA	1.376	0.6945	0.682	49.54	0.5	0.51
09-741	Red_Holstein	USA	1.6111	0.7655	0.846	52.49	0.48	0.48
09-745	Red_Holstein	USA	1.422	0.809	0.613	42.93	0.57	0.57
09-749	Red_Holstein	USA	1.629	0.9912	0.638	38.96	0.61	0.61
09-752	Black_Holstein	NA	0.9812	0.3579	0.623	64.05	0.36	0.37
Arkansas_Blackeye#1	Blackeye	USA	1.6039	0.5962	1.008	62.86	0.37	0.37
Early_acre	Cream	USA	0.6107	0.4652	0.146	23.81	0.76	0.77
Early_scarlet	Pinkeye	USA	1.2395	0.6676	0.572	45.88	0.54	0.54
Ebony	Black	USA	1.0773	0.6374	0.44	40.47	0.6	0.59
Empire	Pinkeye	USA	0.7126	0.545	0.168	23.43	0.77	0.76
ENCORE	Pinkeye	USA	1.019	0.6016	0.417	41.06	0.59	0.59

Table S3-4. Cowpea accession number, seed color, origin, dry shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in in dry shoot biomass (Cont'd).

Accession_number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
Envoy	Red_Holstein	USA	1.0274	0.8267	0.201	19.39	0.81	0.8
EXCEL	Browneye	USA	1.2017	0.3759	0.826	68.49	0.32	0.31
PI152195	Red	Paraguay	1.2984	0.8965	0.402	30.84	0.69	0.69
PI152196	Mixed_(Cream_Brown)	Paraguay	1.4338	0.5349	0.899	62.62	0.37	0.38
PI152197	Red	Paraguay	1.6934	1.1822	0.511	29.08	0.71	0.7
PI152199	Mixed_(Purple_Cream)	Paraguay	1.6307	0.9853	0.645	39.55	0.6	0.61
PI162924	Mixed_(Cream_Brown)	Paraguay	1.3505	0.4142	0.936	69.13	0.31	0.32
PI167284	Mixed_(Cream_Brown)	Turkey	1.3672	0.4429	0.924	68.16	0.32	0.33
PI175332	Tan	NA	1.2582	0.5515	0.707	56.37	0.44	0.44
PI180014	Tan	India	1.3543	0.6558	0.699	51.5	0.48	0.49
PI190191	Tan	Mexico	0.8661	0.4334	0.433	50.11	0.5	0.5
PI201024	Purple	Guatemala	1.7431	0.8685	0.875	50.17	0.5	0.5
PI201498	Blackeye	Mexico	1.564	0.7508	0.813	52.6	0.47	0.49
PI218123	Browneye	Pakistan	1.5143	0.4383	1.076	71.54	0.28	0.3
PI221730	Red_Holstein	South_Africa	1.1838	0.7219	0.462	39.56	0.6	0.62
PI221731	Red_Holstein	South_Africa	1.0165	0.4154	0.601	59.47	0.41	0.42
PI223023	Browneye	Iran	2.1325	0.8416	1.291	60.66	0.39	0.4
PI225922	Mixed_(Brown_Tan)	Zambia	0.8676	0.1434	0.724	83.53	0.16	0.18
PI227829	Mixed_(Brown_Cream)	Guatemala	1.2059	0.5459	0.66	54.63	0.45	0.46
PI227830	Tan	Guatemala	1.0676	0.6832	0.384	35.71	0.64	0.64
PI229551	Browneye	Iran	1.0802	0.4022	0.678	62.84	0.37	0.37
PI229734	Blackeye	Iran	1.2388	0.5116	0.727	58.83	0.41	0.42
PI244571	Mixed_(Brown_Cream)	Guatemala	1.4352	0.688	0.747	51.58	0.48	0.48
PI250416	Cream	Pakistan	1.6771	0.7026	0.975	57.36	0.43	0.42
PI250587	Darkbrown	Egypt	1.7172	1.0692	0.648	38.26	0.62	0.63
PI250759	Tan	Iran	1.5533	0.522	1.031	66.06	0.34	0.33
PI251222	Tan	Afghanistan	1.7585	0.9714	0.787	43.69	0.56	0.55
PI253428	Darkbrown	Spain	2.1527	0.9982	1.155	53.9	0.46	0.47
PI255765	Blackeye	Nigeria	2.0614	0.8365	1.225	59.47	0.41	0.41
PI255774	Browneye	Nigeria	2.0522	0.2532	1.799	88.06	0.12	0.13
PI255815	Browneye	Nigeria	0.8119	0.456	0.356	44.14	0.56	0.57
PI256342	Brown	Pakistan	1.128	0.6398	0.488	43.38	0.57	0.57
PI257463	Red_Holstein	Nigeria	0.894	0.4922	0.402	45.31	0.55	0.56
PI262179	Blackeye	Portugal	1.3288	0.6533	0.675	50.76	0.49	0.5
PI292891	Tan	South_Africa	1.313	0.9339	0.379	28.25	0.72	0.71
PI292892	Brown_Holstein	South_Africa	1.0343	0.3137	0.721	69.62	0.3	0.3
PI292894	Tan	Zimbabwe	1.4669	0.4862	0.981	65.91	0.34	0.33
PI292897	Red_Holstein	Hungary	1.2221	0.8384	0.384	32.26	0.68	0.7

Table S3-4. Cowpea accession number, seed color, origin, dry shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in in dry shoot biomass (Cont'd).

Accession_number	**Seed color**	**Origin**	**Non_stress (g)**	**Stress (g)**	**AD (g)**	**II (%)**	**RST**	**STI**
PI292898	Black	Hungary	0.7584	0.4397	0.319	42.11	0.58	0.58
PI292899	Tan	Hungary	1.3455	0.6874	0.658	49.03	0.51	0.51
PI292913	Tan	Hungary	1.03	0.7064	0.324	30.5	0.7	0.68
PI293467	Mixed_(Tan_Brown)	USA	2.1419	1.0573	1.085	50.46	0.5	0.49
PI293469	Tan	NA	1.3447	1.0844	0.26	19.38	0.81	0.81
PI293476	Variable_(Grey_Brown)	USA	1.6828	0.3719	1.311	76.66	0.23	0.22
PI293514	Red_Holstein	USA	1.069	0.7389	0.33	31.06	0.69	0.7
PI293545	Mixed_(Brown_Cream)	NA	1.3299	0.7191	0.611	45.92	0.54	0.54
PI293568	Tan	NA	1.2593	0.3935	0.866	67.95	0.32	0.31
PI293569	Variable_(Red_Tan)	NA	1.8149	0.8981	0.917	51.4	0.49	0.51
PI293570	Mixed_(Brown_Tan)	NA	1.3583	0.9199	0.438	32.54	0.67	0.7
PI293582	Red_Holstein	NA	0.9098	0.6455	0.264	28.29	0.72	0.71
PI293584	Variable_(Red_Brown_Cream)	NA	1.1223	0.7815	0.341	29.86	0.7	0.7
PI297561	Mixed_(Brown_Grey)	NA	1.0002	0.5579	0.442	43.31	0.57	0.56
PI339563	Tan	Australia	0.645	0.2303	0.415	64.19	0.36	0.36
PI339587	Red_Holstein	South_Africa	1.1401	0.6221	0.518	45.35	0.55	0.55
PI339590	Brown_Holstein	South_Africa	0.9637	0.3491	0.615	62.63	0.37	0.36
PI339591	Brown_Holstein	South_Africa	1.3738	0.8228	0.551	40.93	0.59	0.62
PI339592	Brown	South_Africa	1.2686	0.6638	0.605	47.46	0.53	0.52
PI339594	Variable_(Red Holstein_Red_Tan)	NA	1.0392	0.6635	0.376	35.37	0.65	0.65
PI339598	Tan	South_Africa	1.4439	0.7833	0.661	45.58	0.54	0.54
PI339600	Tan	South_Africa	1.0881	0.4995	0.589	54.89	0.45	0.48
PI339602	Mixed_(Grey_Brown)	South_Africa	1.4137	1.0662	0.348	23.97	0.76	0.75
PI339609	Tan	Tanzania	0.9892	0.5516	0.438	44.44	0.56	0.56
PI339610	Variable_(Red_Brown_Cream)	Tanzania	1.1229	0.479	0.644	57.33	0.43	0.43
PI339611	Tan	Tanzania	1.0051	0.6326	0.373	36.69	0.63	0.63
PI339613	Variable_(Grey_Tan_Brown)	Tanzania	1.6511	0.7424	0.909	54.38	0.46	0.45
PI347639	Tan	NA	1.2109	0.728	0.483	40.2	0.6	0.61
PI349674	Black	Australia	1.0665	0.6513	0.415	39.1	0.61	0.61
PI353045	Red_Holstein	India	0.9962	0.6986	0.298	29.78	0.7	0.7
PI353062	Mixed_(Cream_Purple)	India	1.2235	0.5699	0.654	52.92	0.47	0.46
PI354580	Tan	India	1.8416	0.6697	1.172	64.35	0.36	0.37
PI367921	Tan	NA	1.2564	0.5016	0.755	59.98	0.4	0.4
PI406290	Browneye	Mozambique	1.6513	0.6046	1.047	62.96	0.37	0.36
PI430687	Red	China	1.1426	0.6755	0.467	40.8	0.59	0.6
PI487518	Tan	Indonesia	1.3605	0.3334	1.027	75.63	0.24	0.25
PI527561	Tan	Burundi	1.6758	0.7956	0.88	52.36	0.48	0.48
PI578902	Red	China	1.2291	0.5429	0.686	55.57	0.44	0.45

Table S3-4. Cowpea accession number, seed color, origin, dry shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in in dry shoot biomass (Cont'd).

Accession_number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
PI578911	Tan	China	1.33	0.4835	0.847	63.05	0.37	0.36
PI579909	Blackeye	Nigeria	1.2733	0.6787	0.595	46.5	0.53	0.54
PI582340	Mixed_(Brown_Pink)	USA	1.1204	0.9583	0.162	14.4	0.86	0.86
PI582347	Tan	NA	1.236	0.7564	0.48	38.74	0.61	0.61
PI582354	Blackeye	USA	0.9619	0.5602	0.402	41.3	0.59	0.58
PI582366	Tan	India	1.1146	0.5607	0.554	50.28	0.5	0.51
PI582368	Black_Holstein	India	0.7652	0.356	0.409	53.58	0.46	0.47
PI582402	Tan	Brazil	0.5744	0.4748	0.1	17.16	0.83	0.85
PI582415	Tan	Mexico	1.2544	0.9506	0.304	24.2	0.76	0.76
PI582416	Variable_(Red_Brown_Cream)	Mexico	1.2544	0.8037	0.451	36.32	0.64	0.65
PI582420	Variable_(Red_Brown)	NA	1.454	0.4265	1.028	70.65	0.29	0.29
PI582421	Red_Holstein	NA	0.9538	0.6054	0.348	36.58	0.63	0.64
PI582422	Blackeye	NA	1.2663	0.553	0.713	55.75	0.44	0.43
PI582423	Brown_Holstein	NA	1.139	0.554	0.585	51.45	0.49	0.49
PI582425	Mixed_(Tan_Brown)	NA	1.5861	0.8715	0.715	45.05	0.55	0.55
PI582428	Blackeye	Trinidad_ and_Tobago	1.4232	0.6048	0.818	56.16	0.44	0.43
PI582465	Tan	Mexico	2.2112	1.1196	1.092	49.41	0.51	0.51
PI582467	Black_Holstein	NA	0.9576	0.5369	0.421	44.13	0.56	0.56
PI582468	Tan_Holstein	NA	1.6028	0.2833	1.32	82.24	0.18	0.18
PI582469	Brown_Holstein	Philippines	1.0121	0.3435	0.669	65.32	0.35	0.37
PI582474	Purple	Botswana	1.2224	0.4848	0.738	60.21	0.4	0.4
PI582522	Tan	Mexico	1.0964	0.7538	0.343	30.8	0.69	0.69
PI582530	Mixed_(Brown_Tan)	Ghana	0.8787	0.4062	0.473	53.61	0.46	0.48
PI582531	Variable_(Grey_Brown_Tan)	NA	1.0369	0.4844	0.553	53.38	0.47	0.47
PI582542	Browneye	Mexico	0.8807	0.502	0.379	42.93	0.57	0.57
PI582551	Blackeye	Botswana	0.8508	0.3869	0.464	54.68	0.45	0.46
PI582554	Tan	NA	1.2135	0.9126	0.301	24.76	0.75	0.75
PI582570	Browneye	India	1.7946	0.7134	1.081	60.18	0.4	0.4
PI582572	Tan	Kenya	0.8815	0.3964	0.485	55.23	0.45	0.46
PI582573	Browneye	Kenya	1.3372	0.5139	0.823	61.61	0.38	0.38
PI582574	Tan	Kenya	1.2355	0.6293	0.606	49.08	0.51	0.51
PI582575	Black	Kenya	1.6848	0.8338	0.851	50.57	0.49	0.5
PI582576	Tan	NA	2.109	1.0646	1.044	48.18	0.52	0.52
PI582578	Tan	Kenya	1.0113	0.5016	0.51	50.47	0.5	0.51
PI582579	Tan	Kenya	0.9527	0.6354	0.317	33.25	0.67	0.67
PI582650	Tan	Botswana	1.7502	1.1987	0.552	31.58	0.68	0.69
PI582665	Variable_(Grey_Brown_Tan)	Botswana	1.6901	0.9888	0.701	40.78	0.59	0.59
PI582666	Tan	Botswana	1.1376	0.6803	0.457	40.19	0.6	0.6

Table S3-4. Cowpea accession number, seed color, origin, dry shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in in dry shoot biomass (Cont'd).

Accession_number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
PI582680	Browneye	Botswana	1.2112	0.5433	0.668	55.38	0.45	0.45
PI582696	Brown	Botswana	1.2233	0.5763	0.647	52.73	0.47	0.47
PI582697	Tan	Botswana	1.0937	0.3785	0.715	65.03	0.35	0.35
PI582703	Red_Holstein	Botswana	1.217	0.6236	0.594	48.72	0.51	0.51
PI582809	Tan	Botswana	0.9032	0.5867	0.317	35.11	0.65	0.66
PI582812	Brown_Holstein	Botswana	1.5831	0.6894	0.894	56.55	0.43	0.44
PI582813	Mixed_(Grey_Cream_Black eye)	Botswana	1.4953	0.7279	0.767	50.74	0.49	0.48
PI582818	Black_Holstein	Botswana	1.2289	0.7768	0.452	36.78	0.63	0.63
PI582821	Mixed_(Brown_Tan)	Botswana	1.5717	1.1502	0.422	26.45	0.74	0.74
PI582822	Red_Holstein	Botswana	0.8958	0.5547	0.341	38.07	0.62	0.63
PI582823	Red_Holstein	Botswana	0.6602	0.4166	0.244	37.05	0.63	0.64
PI582824	Red_Holstein	Botswana	0.7183	0.3891	0.329	46.15	0.54	0.55
PI582825	Tan	Botswana	1.8579	0.697	1.161	62.5	0.38	0.38
PI582850	Brown_Holstein	Botswana	1.0391	0.7074	0.332	31.94	0.68	0.68
PI582852	Brown	Botswana	1.1115	0.2291	0.882	79.4	0.21	0.21
PI582854	Greeneye	Botswana	1.6758	0.6657	1.01	60.49	0.4	0.4
PI582856	Variable_(Red_Brown_Grey)	Botswana	0.8632	0.3138	0.549	63.74	0.36	0.36
PI582857	Browneye	Botswana	0.9573	0.6546	0.303	30.32	0.7	0.69
PI582863	Browneye	Botswana	0.9368	0.4181	0.519	55.25	0.45	0.45
PI582866	Brown	Botswana	0.859	0.2038	0.655	76.01	0.24	0.24
PI582873	Tan	Botswana	1.2044	0.6332	0.571	47.24	0.53	0.52
PI582874	Tan	Botswana	1.6746	1.1348	0.54	32.21	0.68	0.68
PI582875	Tan	Botswana	1.4997	1.0129	0.487	32.51	0.67	0.68
PI582878	Green	NA	1.7889	0.6291	1.16	64.68	0.35	0.35
PI582894	Tan	Botswana	1.578	0.6217	0.956	59.92	0.4	0.39
PI582912	Black_Holstein	Kenya	1.0471	0.4118	0.635	60.96	0.39	0.4
PI583193	Variable_(Gray_Brown)	Senegal	1.0749	0.725	0.35	32.44	0.68	0.67
PI583194	Variable_(Tan_Grey)	Senegal	0.9915	0.5682	0.423	42.5	0.57	0.57
PI583195	Red	Senegal	0.9506	0.4655	0.485	51.07	0.49	0.49
PI583198	Tan	Senegal	0.9817	0.4326	0.549	56.06	0.44	0.44
PI583201	Browneye	Senegal	1.2596	0.4567	0.803	63.85	0.36	0.36
PI583202	Browneye	Senegal	1.3614	0.613	0.748	55.21	0.45	0.46
PI583209	Tan	Nigeria	1.6539	0.7321	0.922	56.37	0.44	0.45
PI583240	Tan	Senegal	0.6599	0.358	0.302	46.84	0.53	0.56
PI583241	Red	Senegal	0.7572	0.2041	0.553	72.97	0.27	0.27
PI583244	Tan	Senegal	1.3834	0.6644	0.719	51.95	0.48	0.48
PI583248	Browneye	Senegal	1.3204	0.5096	0.811	61.44	0.39	0.39
PI583249	Browneye	Senegal	1.1636	0.3752	0.788	67.43	0.33	0.32

Table S3-4. Cowpea accession number, seed color, origin, dry shoot biomass without salt treatment and under salt stress 14 days after the first treatment, absolute decrease (AD), inhibition index (II), relative salt tolerance (RST), and salt tolerance index (STI) in in dry shoot biomass (Cont'd).

Accession_number	Seed color	Origin	Non_stress (g)	Stress (g)	AD (g)	II (%)	RST	STI
PI583251	Tan	Senegal	1.6014	0.8477	0.754	46.78	0.53	0.53
PI583274	Browneye	Senegal	1.0725	0.577	0.496	46.33	0.54	0.54
PI583487	Tan	NA	1.3951	0.6854	0.71	50.82	0.49	0.49
PI583488	Brown	NA	1.5195	0.7105	0.809	53.51	0.46	0.47
PI583550	Purple	Mali	1.3039	0.5275	0.777	59.71	0.4	0.41
PI583551	Browneye	Mali	1.5605	0.5652	0.995	63.58	0.36	0.36
PI610520	Black	NA	0.8734	0.2975	0.576	66.02	0.34	0.34
PI610533	Blackeye	NA	1.2024	0.584	0.618	51.18	0.49	0.49
PI610604	Purple	NA	0.6833	0.3918	0.292	42.94	0.57	0.58
PI666252	Tan	USA	1.371	0.9308	0.44	32.07	0.68	0.68
PI666260	Pinkeye	USA	1.2385	1.0897	0.149	11.99	0.88	0.88
PI666262	Browneye	USA	1.8416	1.0225	0.819	44.54	0.55	0.56

Chapter 4. Association analysis of salt tolerance in cowpea (*Vigna unguiculata* (L.) Walp) at germination and seedling stages

Abstract

Cowpea is one of the most important cultivated legumes in Africa. The worldwide annual production in cowpea dry seed is 5.4 million metric tons. However, cowpea is unfavorably affected by salinity stress at germination and seedling stages, which is exacerbated by the effects of climate change. The lack of knowledge on the genetic underlying salt tolerance in cowpea limits the establishment of a breeding strategy for developing salt tolerant cowpea cultivars. The objectives of this study were to conduct association mapping for salt tolerance at germination and seedling stages and to identify SNP markers associated with salt tolerance in cowpea. We analyzed the salt tolerance index of 116 and 155 cowpea accessions at germination and seedling stages, respectively. A total of 1,049 SNPs postulated from genotyping-by-sequencing were used for association analysis. Population structure was inferred using Structure 2.3.4; K optimal was determined using Structure Harvester. TASSEL 5, GAPIT, and FarmCPU involving three models such as single marker regression (SMR), general linear model (GLM), and mixed linear model (MLM) were used for the association study. Substantial variation in salt tolerance index for germination rate, plant height reduction, fresh and dry shoot biomass reduction, foliar leaf injury, and inhibition of the first trifoliate leaf was observed. The cowpea accessions were structured into two subpopulations. Three SNPs, Scaffold87490_622, Scaffold87490_630, and C35017374_128 were highly associated with salt tolerance at germination stage. Seven SNPs Scaffold93827_270, Scaffold68489_600, Scaffold87490_633, Scaffold87490_640, Scaffold82042_3387, C35069468_1916, and Scaffold93942_1089 were found to be associated with salt tolerance at seedling stage. The SNP markers were consistent across the three models

and could be used as a tool to select salt-tolerant lines for breeding improved cowpea tolerance to salinity.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] (2n=2x=22) is one of the most consumed legumes worldwide, especially in Africa. It is a mandated crop for the International Institute of Agriculture Tropical (IITA) (www.iita.org/crop/cowpea.htm). Cowpea is a protein-rich crop, which constitutes an affordable source of protein in developing countries. Worldwide cowpea production is 5.4 million metric tons (Olufajo, 2012). However, salinity is a growing threat to cowpea production, and most other crops, in semi-arid regions (Zhang et al., 2012). In the USA, 19.6 million hectares of cultivated areas are under salinity threat (Shannon, 1997).

Strategies for addressing the negative impacts of salinity need to be developed. Providing salt tolerant cowpea cultivars through breeding could be the most cost effective way to lessen the impacts of salinity. Conventional cowpea breeding has been extensively undertaken by different national and international research programs to strengthen the crop from biotic and abiotic stresses. Nevertheless, such a process is time consuming, labor intensive, and expensive. Molecular plant breeding is a successfully demonstrated approach to pyramiding desired traits in crops (Moose and Mumm, 2008; Collard and Mackill, 2008; Xu and Crouch, 2008). DNA markers have been proven to be an effective screening method used by plant breeders to screen for salt-tolerant genotypes (Foolad, 2007). Despite the fact that these molecular markers will speed up the screening process for salt tolerance, genetic research related to salt tolerance on cowpea is very limited.

The single nucleotide polymorphism (SNP) marker is a potential and cost efficient breakthrough for molecular plant breeding. SNP is defined as the individual nucleotide base difference between two DNA sequences (Xu, 2010). SNP discovery is essential for research related to genetic variation, genome mapping, association analysis, and gene isolation (Ganal et al., 2009; Varshney et al., 2009). Regarding cowpea, SNP markers have been used for different purposes. Muchero et al. (2009) were able to design a consensus map for cowpea using EST-derived SNPs. In addition, these SNPs and the genetic map were used to conduct a study of synteny between cowpea, soybean (*Glycine max* L.), and arabidopsis (*Arabidopsis thaliana* L.). Another study conducted by Egbadzor et al. (2013) reported SNP makers associated with seed size in cowpea. Eighteen SNPs were found after conducting an association analysis involving 78 cowpea genotypes. These SNPs were distributed across the cowpea chromosomes. Such results are of interest because they provide substantial information on SNP markers, which can be used for marker assisted selection for seed size-related traits in cowpea. Egbadzor et al. (2014) analyzed the diversity of 113 cowpea accessions using SNPs. They reported 477 SNPs, and 458 of them showed polymorphisms. Their results suggested that these markers were efficient to discriminate the accessions in their study. Shi et al. (2016) conducted an association analysis to study the bacterial blight resistance in cowpea using 1,031 SNP markers. They worked on a panel of 400 cowpea accessions and identified four SNP markers (C35046071_ 1260, C35084634_455, scaffold96328_3387, and scaffold96765_4430) to be highly associated with bacterial blight resistance in cowpea.

Genotyping by sequencing (GBS) is a next generation DNA sequencing approach and can be used to identify SNP markers associated with important traits in plants (Elshire et al., 2011; Sonah et al., 2013; Bastien et al., 2014). GBS is a cost effective and fast way for conducting an association analysis. Such a method deals with a reduced library representation of the genome; GBS provides a high throughout genotyping of populations using a large number of SNP markers (Bradbury et al., 2007).

Association mapping and QTL analysis-based studies have been important components in molecular plant breeding. They have been extensively used to genetically unravel traits related to salt and drought tolerance in different crops (Li and Xu, 2007; Li et al., 2007; Ben-Hayyim and Moore, 2007). In tomato (*Solanum lycorpesicum* L.), QTL analysis pertaining to salt tolerance was performed during seed germination. Foolad and Jones (1993) identified five QTLs located on chromosomes 1, 3, 7, 8 and 12 linked to salt tolerance in tomato. They worked on an F_2 population resulting from a cross between salt-sensitive and salt-tolerant parents. Subsequent investigations have been conducted to validate these aforementioned QTLs (Foolad et al., 1997; Foolad et al., 1998). At the tomato seedling stage, Foolad (1999) identified five QTLs associated with salt tolerance, which were located on chromosomes 1, 3, 5, and 9. In soybean, Kan et al. (2015) reported eight SNPs highly correlated to the ratio between the germination index under salinity stress and the germination index under no-salt conditions, and the ratio of the germination rate under salinity conditions to the germination rate under a non-saline environment. They identified eight candidate genes associated with these SNPs markers, and five of them (Glyma08g12400.1, Glyma08g09730.1, Glyma18g47140.1, Glyma09g00460.1, and Glyma09g00490.3) controlled tolerance to salinity at germination stage in soybean.

Despite the fact that SNP markers are useful in plant breeding, no molecular markers associated with salt tolerance have been reported in cowpea. Discovering SNP markers for salt tolerance will help plant breeders select cowpea lines which are tolerant to salinity. The objectives of this study were to conduct an association analysis for cowpea salt tolerance at germination and seedling stages, and to identify salt-tolerant-related SNP markers in cowpea.

Materials and Methods

Plant materials

A total of 116 cowpea genotypes were used for salt tolerance evaluations at germination stage and 155 cowpea genotypes at seedling stage. The 116 cowpea genotypes consisted of 30 University of Arkansas cowpea advanced lines and 86 USDA cowpea germplasm accessions, which were from 22 countries (Table S4-1). The 155 genotypes for assessing cowpea salt tolerance at seedling stage consisted of 37 cowpea advanced lines from the University of Arkansas, and 118 USDA cowpea germplasm accessions from 25 different countries (Table S4-2). Among the cowpea genotypes involved in this study, 111 genotypes overlapped between seedling and germination stages; among which 29 genotypes were from the University of Arkansas and 82 from USDA germplasm. USDA Germplasm Resources Information Network (GRIN) cowpea accessions were obtained from the USDA Plant Genetic Resources Conservation Unit at Griffin, GA, and were increased at the Research and Extension Station of the University of Arkansas at Fayetteville, AR in summers 2014 and 2015.

Phenotyping of salt tolerance

Forty seeds from each cowpea genotype were germinated on a 9-cm diameter petri dish in an incubator New Brunswick Scientific Innova 4230® (Manasquan, NJ) set at 28℃ (Souza et al., 2004). Seed germination was performed over 48 hours. Controls consisted of adding 14 ml of distilled water to each dish. 150 mM NaCl was added to each salt-treated dish (Lobato et al., 2009). For each genotype, control and salt treatment were replicated three times. The experiment design was a randomized complete block design (RCBD) with three replicates. The experiments were conducted in multiple runs. Data on seed germination rate for genotype without salt treatment and under salt stress were collected.

With respect to salt tolerance phenotyping at seedling stage, 24 germinated seeds from each cowpea were planted in six plastic pots, each containing four germinated seeds. Three of the six pots were water with deionized water and the other three irrigated with 200 mM NaCl solution (Abeer et al., 2015). The experimental design was a completely randomized one with three replicates. The experiments were carried out in five runs due to space limitations. Each run approximately involved 40 genotypes. When the plants reached V1 stage (exhibition of the first trifoliate leaf), the salt treatment was initiated. To each pot, a 100 ml solution of deionized water or NaCl was added each two days over two weeks. Measurements were done 14 days after the first treatment application. Some plants were completely dead after 14 days. Visual leaf injury due to salinity was rated (1 = no apparent leaf injury; 2 = apparition of leaf injury; 3 = moderate leaf injury; 4 = severe leaf injury; and 5=dead leaves), inhibition of first trifoliate leaf development (1=Untrifoliate, 9=Trifoliate), plant height reduction, and fresh and dry biomass reduction due to salt stress. Data were collected on a per plant basis.

Salt tolerance index (STI) was calculated for germination rate, plant height, and fresh and dry shoot biomass. The STI was obtained using the following formula (Fernandez, 1992; Saad et al., 2014).

$$\mathrm{STI} = (Y_{\text{non salt stress}} * Y_{\text{salt stress}}) / (\bar{Y}_{\text{non salt stress}})^2$$

where $Y_{\text{non salt stress}}$: Seed germination rate without salt stress/plant height, fresh and dry shoot biomass without salt stress

$Y_{\text{salt stress}}$: Seed germination rate under salt stress/plant height, fresh and dry shoot biomass under salt stress

$\bar{Y}_{\text{non salt stress}}$: Average of seed germination rate without salt stress/plant height, fresh and dry shoot biomass without salt stress

Descriptive statistics were generate using “Tabulate” function of JMP Genomics 7. Data distribution was drawn using “Distribution” function in JMP Genomics 7.

Genotyping for association analysis

DNA extraction, library preparation, and genotyping-by-sequencing (GBS)

Genomic DNA was extracted from young fresh leaves when the cowpea plants reached V1 stage (first trifoliate). These leaves were stored at -80ºC overnight and then dried in a lypophilizer® (Salt Lake City, UT). A CTAB (hexadecyltrimethyl ammonium bromide) protocol described by Kisha et al. (1997) was used for DNA extraction. Cowpea leaves were ground using Mixer Mill MM 400® (Haan, Germany). The DNA extraction buffer was added to each sample, which was centrifuged at 13,000 rpm for 10 minutes. Then, the aqueous solution was transferred to 2 ml tubes. Then 1 ml of chloroform-isoamyl alcohol (24:1) was added to each sample to subtract proteins. The samples were centrifuged at 10,000 rpm for 5 minutes, and the supernatant liquid was transferred to additional 2 ml tubes. The use of 1 ml of isopropanol added to each tube helped the DNA precipitate from the solution. The samples were then stored over night at -20 ºC. DNA pellets were subsequently washed with 70% and 90% ethanol and dried for 30 minutes. A total of 200 µl of 0.1X TE per tube were used to solubilize the DNA. Afterwards, 3 µl of RNAse was added to each tube.

DNA was quantified using a NanoDrop 200c spectrophotometer (Thermo SCIENTIFIC, Wilmington, DE). 1% agarose gel with ethidium bromide gel stain permitted to check on DNA qualities. Genotyping-by-sequencing libraries were constructed according to the *ApeKI* protocol described by Elshire et al. (2011). DNA sequencing was performed using GBS (Elshire et al., 2011; Bastien et al., 2014) by HiSeq series in Beijing Genome Institute (BGI). The GBS protocol of BGI consisted of several steps. Briefly, DNA was digested using the restriction enzyme *ApeK1*. Adaptors were ligated to the restriction fragments. In situ PCR was conducted using two specific primers. Prior to sequencing, quality check on GBS libraries was performed. The data was filtered afterwards (GBS protocol of BGI).

SNP assembly, mapping, discovery, and filtering

SNP assembly, mapping, and discovery were conducted by BGI using SOAP family software (http:/soap.genomics.org.cn/). The short-reads were aligned to a cowpea genome reference (cowpea_Genome_0.03.fa) by SOAPalinger/soap2 (http://soap.genomics.org.cn/). Initial SNP calls were performed using SAOPsnp v 1.05 (Li et al., 2009). Dr.T imothy J. Close from the University of California Riverside, CA, provided the cowpea_Genome_0.03.fa (644,126 scaffolds or contigs).

Cowpea genotypes having more than 35% missing SNP data was discarded from the analysis. For each SNP, if its heterozygous calls were greater than 35%, the SNP was considered as missing data; the minor allele frequency threshold was set at 0.05 for SNP callings. SNPs containing more than 35% missing data were also removed from the analysis. Finally, 1,049 SNPs postulated from GBS were used for association analysis after SNP filtering.

Population structure analysis

STRUCTURE 2.3.4 was used to infer the population structure of the cowpea accession panel (Pritchard et al., 2000). An admixture model along with a correlated allele frequency model, independent for each run, were involved in the analysis to assess the population structure (K). The use of such models have been shown to provide conclusive results for cowpea association mapping related-studies (Shi et al., 2016).

For each estimated K value, 10 runs were performed. Markov Chain Monte Carlo (MCMC) length of the burn-in period was 30,000. After the burn-in period, the number of MCMC iterations was adjusted to 50,000. In order to screen appropriate K-values, values of delta K and optimal K were computed using STRUCTURE Harvester (Earl and VonHoldt, 2011; http://taylor0.biology.ucla.edu/structureHarvester/) based on the formula developed by Evanno et al. (2005).

After defining K optimal, Q-matrix with the K vectors were generated and used for association analysis using TASSEL 5. Each cowpea genotype was allocated to each cluster (Q). The cut-off probability for assigning an accession to a Q cluster was 0.5. In order to visualize the structure among the cowpea panel, bar plots using STRUCTURE PLOT with the option “Sort by Q” and based on K optimal were designed (Ramasamy et al., 2014).

Genetic diversity

The analysis of genetic diversity and the drawing of phylogenetic trees were performed using MEGA 7 (Kumar et al., 2016). The Maximum Likelihood tree was adopted as statistical method. The following parameters were set in MEGA 7:

Analysis: Phylogeny Reconstruction;

Statistical method: Maximum Likelihood;

Test of phylogeny: None;

Substitutions type: Nucleotide;

Model/Method: Tamura-Nei Model;

Rates among sites: Gamma distributed with Invariant sites (G+I);

No of Discrete Gamma Categories: 5;

Gaps/Missing Data treatment;

ML Heuristic Method: Nearest-Neighbor-Interchange (NNI);

Initial Tree for ML: Make initial tree automatically (Default - NJ/BioNJ);

Branch Swap Filter: Moderate;

Number of threads: 1;

Test of Phylogeny: None;

No. of Bootstrap Replications: 500;

Model/Method: General Time Reversible Model;

Rates among Sites: Gamma distributed with invariant sites (G+1);

Number of discrete gamma categories: 5;

Gaps/Missing data treatment: use of all sites;

ML Heuristic method: Subtree-Pruning-Regrafting-Extensive (SPR level 5);

Initial tree for ML: Make initial tree automatically (Neighbor Joining); and

Branch swap filter: Moderate.

The population structure along with the outputs containing the Q clusters were imported to MEGA 7 for combined analysis of genetic diversity while drawing the phylogenetic trees. For the sub-trees for each cluster (Q), the shape of “Node/Subtree Marker” and the “Branch Line” were colored similarly as the bar plots displayed by STUCTURE PLOTS.

Association analysis

Association analysis was conducted using TASSEL 5 (Bradbury et al., 2007) and R package (Liu et al., 2016). Four types of models were used. These models consisted of a single marker regression (SMR) for all SNPs without structure and kinship, a general linear model (GLM) using structure outputs, a mixed linear model (MLM) involving structure and kinship from TASSEL 5, and a fixed and random model Circulating Probability Unification (FarmCPU) using R. The LOD threshold for significant SNPs was between 2 and 3 (Lander and Botsteins ’b, 1989).

Results and Interpretations

Phenotyping of salt tolerance

Evaluation of cowpea salt tolerance was performed on 116 genotypes at germination stage and 155 genotypes at seedling stage. Data on germination rate under normal condition, salt tolerance index for seed germination, foliar leaf injury, inhibition of the development of first trifoliate leaf, salt tolerance index for plant height, fresh and dry shoot biomass were collected 14 days after the first salt treatment (Tables S4-1, S4-2, and S4-3). At this time, some plants were completely dead.

Salt tolerance index for seed germination, plant height, fresh and dry shoot were calculated (Table S4-1, S4-2, and S4-3). Data were normally distributed (Figure 4-1) expect for visual leaf injury, which showed a right-skewed distribution (Figure 4-2). Germination rate under non-saline condition varied from 60.8% to 99.2%, with a mean of 81.1% and a standard deviation of 11.1%. Envoy (99.2%), PI583194 (99.2%), PI487518 (98.3%), PI218123 (97.5%), and PI255765 (97.5%) had the highest germination rate, whereas PI293570 (62.5%), PI582809 (61.7%), PI582852 (61.7%), PI225922 (60.8%), and PI339610 (60.8%) showed the lowest germination rate (Table S4-1). Salt tolerance index for seed germination ranged between 0.00 and 0.92, with a mean of 0.47 and a standard deviation of 0.23. Highest salt tolerance index for germination was recorded for PI582422 (0.92), 09-529 (0.88), PI293584 (0.86), PI201498 (0.84), and PI582420 (0.83), indicating that these genotypes were salt-tolerant. 09-745 (0.07), PI582665 (0.02), 09-393 (0.01), PI582522 (0.01), and PI582813 (0.00) had the lowest salt tolerance index for germination rate, suggesting that they were salt-sensitive at germination stage.

The development of first trifoliate leaf was inhibited for 54% of the genotypes tested at seedling stage. Foliar leaf injury scores ranged between 1.0 and 4.9, with a mean of 2.2 and a standard deviation of 0.8. Leaf damage was substantial for PI582665 (4.9), PI255815 (4.8), PI255774 (4.7), PI583201 (4.7), and PI527561 (4.6), indicating that these genotypes were salt-susceptible. Leaf injury was almost absent for PI582420 (1.2), PI582428 (1.2), PI582422 (1.1), PI582812 (1.1), and PI293570 (1.0) (Table S4-2), indicating that these accessions were salt-tolerant based on leaf injury at seedling stage.

Salt tolerance index for plant height varied from 0.26 to 0.76, with a mean of 0.52 and a standard deviation of 0.09. PI257463 (0.76), 09-175 (0.74), 09-529 (0.73), 09-295 (0.71), and 09-655 (0.67) had the highest salt tolerance index for plant height, indicating that these genotypes were salt-tolerant based on plant height reduction. Lowest salt tolerance index for plant height was found for PI339610 (0.36), PI582697 (0.36), PI582812 (0.35), PI582852 (0.26), and PI582866 (0.26), indicating that these genotypes were salt-tolerant base on plant height reduction (Table S4-3).

Fresh shoot biomass salt tolerance index ranged between 0.17 and 0.82, with a mean of 0.54 and a standard deviation of 0.12. PI582340 (0.82), ENCORE (0.81), PI152195 (0.79), PI666252 (0.78), and 09-105 (0.77) had the highest salt tolerance index for fresh shoot biomass, indicating that they were salt salt-tolerant based on fresh shoot biomass; whereas PI293476 (0.30), PI223023 (0.28), PI582852 (0.28), PI582468 (0.17), and PI255774 (0.17) showed the lowest fresh biomass salt tolerance index, suggesting that these genotypes were highly susceptible to salt stress.

Salt tolerance index for dry shoot biomass varied from 0.15 to 0.86, with a mean of 0.54 and a standard deviation of 0.15. Highest dry shoot biomass salt tolerance index was recorded for PI666260 (0.86), PI293469 (0.84), PI293582 (0.82), PI582340 (0.81), and PI292891 (0.81), indicating that these genotypes were salt-tolerant based on dry shoot biomass; whereas PI487518 (0.25), PI582468 (0.22), PI582852 (0.20), PI225922 (0.19), and PI255774 (0.15) had the lowest salt tolerance index for dry shoot biomass, indicating that these lines were salt-sensitive (Table S4-3).

Genetic diversity and population structure

The population structure was inferred using STRUCTURE 2.3.4 in 116 cowpea accessions at germination stage and 155 cowpea genotypes at seedling stage. For both stages, Structure Harvester indicated an optimal delta K for K equal to 2, suggesting that two main populations were found in the cowpea accession panel (Figures 4-3 and 4-4). Each population cluster was termed as Q1 or Q2. In regard to the germination stage, Q1 made up 58% (67 cowpea accessions) of the total cowpea accessions; the remaining 42 % fell under Q2 (49 cowpea accessions). With respect to the seedling stage, Q1 accounted for 45 % (70 cowpea accessions) of total cowpea accessions, and Q2 represented 55% (85 cowpea accessions).

The phylogenetic trees were drawn using the Maximum Likelihood (ML) method in MEGA 7 (Figures 4-3 and 4-4). Two main populations were identified for the cowpea accessions at germination and seedling stages. The first cluster Q1 was represented in red, and the second cluster Q2 in green (Figures 4-3 and 4-4). The same colors were used in the phylogenetic trees where the red circles show the accessions belonging to Q1 and the green ones for those representing Q2. The option radiation was used to draw both genetic trees. The accessions under each cluster tended to be clustered together in the phylogenetic tree (Figures 4-3 and 4-4), which indicated that the results from the structure analysis were consistent with that of the genetic diversity. Therefore, two distinct populations were found within the cowpea accession panel.

Association analysis

Association analysis was conducted using TASSEL 5 and R package. Four different models, single marker regression (SMR) without structure and kinship, general linear model (GLM) with structure, mixed linear model (MLM) using structure and kinship, and Fixed and random model Circulating Probability Unification (FarmCPU) were used in this study. SNPs having an LOD (= -log (p-value)) higher than 2 were selected. SNPs related to cowpea germination rate under normal condition, salt tolerance index for germination, inhibition of the development of first trifoliate leaf, visual leaf injury, salt tolerance index pertaining to plant height, and fresh and dry biomass were identified.

C35042053_245, Scaffold27032_5665, and Scaffold94454_419 were found to be highly associated with cowpea germination. A range of 8.43 to 10.97% of the variation in cowpea germination were attributed to these SNPs. C35042053_245 had an LOD greater than 2.5 across the four different models (Table 4-1). Five SNPs, C35017374_128, Scaffold36825_365, Scaffold51130_55, Scaffold87490_622, and Scaffold87490_630 were associated with germination salt tolerance index in cowpea. The highest LOD was recorded for Scaffold87490_630, having an R-square value up to 15% (Table 4-1).

At seedling stage, Scaffold68489_600 and Scaffold93827_270 were associated with the inhibition of first trifoliate leaf development after salt stress in cowpea. R-square values pertaining to Scaffold68489_600 were 13.35%, 13.09%, and 7.74% in SMR, GLM, and MLM, respectively. R-square values were 6.45%, 10.06%, and 8.30% for Scaffold93827_270 in SMR, GLM, and MLM, respectively. Five SNPs C35051519_114, C35070194_1643, Scaffold87490_633, Scaffold87490_640, and Scaffold87665_2770 were highly associated with visual leaf injury due to salinity. LOD values related to these SNPs decreased from SMR to FarmCPU expect for C35051519_114 whose LOD was 3.21 in MLM. Scaffold87490_640 had the highest LOD value (2.02) using FarmCPU. Values for R-square varied from 9.50 to 11.05% for foliar leaf injury-related SNPs (Table 4-1).

Eight SNPs, C35043181_374, Scaffold22931_1172, Scaffold6972_9093, Scaffold70430_30, Scaffold70430_31, Scaffold82042_3387, Scaffold87665_2770, and Scaffold9649_28 were identified to be associated with plant height salt tolerance index in cowpea. C35043181_374 had the highest LOD value, ranging from 2.15 to 4.10 across the four models. The second highest LODs were recorded for Scaffold82042_3387, varying between 2.44 to 3.03 for four models. Values for R-square ranged from 4.64% to 11.84% (Table 4-1), indicating that SNPs could have effects on plant height salt tolerance index in cowpea. Scaffold87665_2770 was associated to both visual leaf injury and plant height salt tolerance index in cowpea.

Regarding salt tolerance index for fresh shoot biomass, six SNPs, C35069468_1916, Scaffold2771_4351, Scaffold3150_538, Scaffold32980_10968, Scaffold77319_293, and

Scaffold93942_1089 were identified. LOD values varied from 1.71 to 2.88 under four different models (Table 4-1). Higher LOD values were observed using SMR. R-square values ranged between 4.17% and 12.64%. Highest R-square values were recorded for C35069468_1916.

C35069468_1916, Scaffold2771_4351, Scaffold32980_10968, Scaffold61254_2828, and Scaffold93942_1089 were associated with dry shoot biomass salt tolerance index (Table 4-1). LOD values ranged between 1.60 and 3.00, and R-square values varied from 7.58% to 13.15%. C35069468_1916, Scaffold2771_4351, Scaffold32980_10968, and Scaffold93942_1089 overlapped between salt tolerance index for fresh shoot biomass and that of dry shoot biomass, indicating that these SNPs can be used to screen for cowpea salt tolerance at seedling stage using fresh and dry shoot biomass as phenotypic markers.

Discussion

Large variability in salt tolerance-related traits among cowpea genotypes was observed in this study. Traits included germination under normal condition, salt tolerance at germination stage, visual leaf injury due to salinity, inhibition of first trifoliate leaf development, salt tolerance index related to plant height, and fresh and dry shoot biomass. Trait distribution was approximately normal expect for visual leaf injury. These phenotypic results indicated that cowpea salt tolerance could be controlled by QTLs. Zhang et al. (2014) reported a normal distribution of salt tolerance index related to main root length, fresh and dry root biomass, and hypocotyl length in soybean.

Values of R-square associated with SNPs varied from 4.17% to 15% for germination, plant height, and fresh and dry shoot biomass salt tolerance indexes. These findings were similar to those of Xu et al. (2013) who reported QTLs explaining 3.06% to 12.98% of salt tolerance in rice. Overall, cowpea salt tolerance at germination and seedling stages seem to quantitative traits controlled by multiple loci. In this study, several SNP markers were found to be associated with each of the salt stress-related traits, indicating that cowpea salt tolerance could be controlled by QTLs, which were consistent with the fact that traits were approximately normally distributed. In addition, these results were in agreement with that of found in sorghum (*Sorghum bicolor* L.) salt tolerance. Wang et al. (2014) reported multiple QTLs controlling sorghum salt tolerance at germination and seedling stages. In soybean, Kan et al. (2016) reported 11 QTLs associated with salt tolerance. Elakhdar et al. (2016) identified 46 QTLs associated with salt tolerance in a barley population consisting of 60 progenies, suggesting that salt tolerance is a complex trait controlled by multiple loci.

In this study, we found that Scaffold87665_2770 was associated with both visual leaf injury and plant height salt tolerance index in cowpea. Foliar injury results from the accumulation of Na+ and Cl- (Yeo and Flowers, 1983), whereas plant height reduction is due the osmotic inhibition of growth and the accumulation of salt ion in plants (Munns et al., 1995; Neumann, 1997). Therefore, we could suggest that Scaffold87665_2770 is likely associated with salt ion regulation in cowpea since salt ions are common features in foliar leaf injury and plant growth inhibition.

Scaffold87490_622 and Scaffold87490_630 were associated with salt tolerance index for germination and Scaffold87490_633 and Scaffold87490_640 were associated with foliar leaf injury due to salinity. Since these SNPs belong to the same contig/scaffold and only had 19 bp range, we suggest that there could be a QTL on Scaffold87490 which controls salt tolerance at both germination and seedling stages in cowpea.

Significant overlapping SNPs were found between salt tolerance index for fresh shoot biomass and dry shoot biomass, which supports our results since fresh shoot biomass and dry shoot biomass are two highly correlated traits. In barley, overlapping SNPs for fresh and dry shoot biomass were identified by Long et al. (2013). Kordrostami et al. (2016) also reported overlapping SNPs for salt tolerance index related to shoot dry biomass and shoot fresh biomass in rice (*Oryza sativa* L.).

To date, few studies have dealt with association mapping analysis for important traits in cowpea (Agbicodo et al., 2010; Qin et al., 2016; Shi et al., 2016; Xiong et al., 2016). Cowpea salt tolerance research at the genetic level is very limited. So far, this present report could be one of the first investigations to do so. The salt-tolerant SNP markers identified in this study have practical applications. Cowpea breeders can use these SNPs as a tool to select cowpea salt-tolerant plants. Breeding programs could benefit from these molecular markers to rapidly identify salt tolerant plants. In addition, the results could significantly advance knowledge on the genetic architecture governing salt tolerance in cowpea. However, more studies are required to unravel the genetic mechanisms underlying salt tolerance in cowpea.

Conclusion

The phenotypic evaluation of salt tolerance indicated large variation among the cowpea accessions t in this study. Association analysis was conducted to identify SNPs associated with salt tolerance at both germination and seedling stages in cowpea. Scaffold87490_622, Scaffold87490_630, and C35017374_128 were highly associated with salt tolerance at germination stage. Scaffold93827_270, Scaffold68489_600, Scaffold87490_633, Scaffold87490_640, Scaffold82042_3387, C35069468_1916, and Scaffold93942_1089 were associated with salt tolerance at seedling stage. To our knowledge, this is one of the first investigations reporting SNP markers for salt tolerance in cowpea. These SNPs could be used as a tool in marker-assisted selection for improving salt tolerance of cowpea genotypes.

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Table 4-1. SNP markers associated with cowpea germination rate, foliar leaf injury, inhibition of the development of the first trifoliate leaf, salt tolerance index* using germination, plant height, and fresh and dry shoot biomass as phenotypic markers.

Trait	**SNP marker****	**SNP type**	**Contig/scaffold**	**SNP position**	**LOD(-log(P)) value**				**R-square (%) value**		
					SMR	GLM (Q)	MLM (Q+K)	FarmCPU	SMR	GML (Q)	MLM (Q+K)
Germination rate under normal condition	C35042053_245	C/T	C35042053	245	2.51	2.80	2.58	2.88	9.61	10.97	10.31
	Scaffold27032_5665	A/G	Scaffold27032	5665	2.18	2.52	2.17	2.28	8.43	10.13	9.54
	Scaffold94454_419	A/T	Scaffold94454	419	2.15	2.34	2.03	1.77	8.84	9.82	8.88
Germination	C35017374_128	G/T	C35017374	128	2.80	2.58	1.86	1.90	15.24	14.14	12.24
	Scaffold36825_365	A/G	Scaffold36825	365	2.37	2.57	2.63	1.96	7.58	8.39	9.22
	Scaffold51130_55	A/G	Scaffold51130	55	2.26	2.39	2.25	2.21	8.83	9.52	9.31
	Scaffold87490_622	C/T	Scaffold87490	622	2.48	2.47	2.36	1.91	13.46	13.54	14.12
	Scaffold87490_630	G/T	Scaffold87490	630	2.56	2.78	1.94	3.17	13.84	14.99	13.31
First trifoliate leaf development	Scaffold68489_600	G/T	Scaffold68489	600	3.36	3.27	1.63	2.72	13.35	13.09	7.74
	Scaffold93827_270	A/G	Scaffold93827	270	2.18	3.23	2.46	1.66	6.45	10.06	8.30
Foliar injury	C35051519_114	A/T	C35051519	114	2.94	2.92	3.21	1.94	10.07	10.11	11.05
	C35070194_1643	A/C	C35070194	1643	2.64	2.58	2.34	1.58	9.94	9.81	9.84
	Scaffold87490_633	C/T	Scaffold87490	633	2.53	2.58	2.36	1.92	10.06	10.28	9.50
	Scaffold87490_640	A/T	Scaffold87490	640	2.55	2.45	2.35	2.02	9.94	9.65	9.52
	Scaffold87665_2770	A/C	Scaffold87665	2770	2.57	2.56	2.54	1.76	10.28	10.33	9.53
Plant height	C35043181_374	C/T	C35043181	374	3.10	2.91	2.15	4.10	9.94	9.26	7.72
	Scaffold22931_1172	C/T	Scaffold22931	1172	2.20	2.23	2.06	2.93	7.03	7.17	7.09
	Scaffold6972_9093	C/T	Scaffold6972	9093	2.63	3.01	2.39	1.78	10.34	11.84	11.48
	Scaffold70430_30	C/G	Scaffold70430	30	2.37	2.19	1.88	2.48	5.81	5.29	4.67

[redacted]

Table 4-1. SNP markers associated with cowpea germination rate, foliar leaf injury, inhibition of the development of the first trifoliate leaf, salt tolerance index* using germination, plant height, and fresh and dry shoot biomass as phenotypic markers (Cont'd).

Trait	SNP marker**	SNP type	Contig/scaffold	SNP position	LOD(-log(P)) value				R-square (%) value		
					SMR	GLM (Q)	MLM (Q+K)	FarmCPU	SMR	GML (Q)	MLM (Q+K)
Plant height	Scaffold70430_31	G/T	Scaffold70430	31	2.42	2.24	1.87	2.52	5.90	5.38	4.64
	Scaffold82042_3387	C/T	Scaffold82042	3387	3.03	2.87	2.44	2.46	11.16	10.56	10.31
	Scaffold87665_2770	A/C	Scaffold87665	2770	2.37	2.52	1.86	1.91	9.53	10.09	9.44
	Scaffold9649_28	C/T	Scaffold9649	28	3.22	2.99	2.59	1.65	9.69	8.93	8.44
Fresh shoot biomass	C35069468_1916	A/T	C35069468	1916	2.88	2.85	2.28	1.82	12.64	12.49	12.25
	Scaffold2771_4351	A/C	Scaffold2771	4351	2.22	2.19	1.83	1.72	9.10	9.04	8.45
	Scaffold3150_538	A/T	Scaffold3150	538	2.74	2.81	2.53	1.97	11.12	11.36	11.76
	Scaffold32980_10968	C/T	Scaffold32980	10968	2.33	2.21	1.75	2.27	9.70	9.26	8.05
	Scaffold77319_293	A/T	Scaffold77319	293	2.03	2.21	1.71	2.25	4.76	5.31	4.17
	Scaffold93942_1089	C/T	Scaffold93942	1089	2.31	2.22	1.78	2.63	9.44	9.16	8.27
Dry shoot biomass	C35069468_1916	A/T	C35069468	1916	3.00	2.96	2.16	1.73	13.15	13.09	12.08
	Scaffold2771_4351	A/C	Scaffold2771	4351	2.26	2.24	1.77	1.60	9.27	9.27	8.66
	Scaffold32980_10968	C/T	Scaffold32980	10968	2.09	2.05	1.56	1.68	8.78	8.69	7.58
	Scaffold61254_2828	C/G	Scaffold61254	2828	2.39	2.41	1.71	1.94	9.61	9.77	7.89
	Scaffold93942_1089	C/T	Scaffold93942	1089	3.00	2.96	2.38	2.71	12.12	12.05	11.86

*Salt tolerance index is obtained by multiplying the value of the phenotypic marker (germination, plant height, and fresh and dry shoot biomass) by under non-stress and salt-stress conditions for each replication, which is divided by the average value of the phenotypic marker at non-stress condition.

**SNP marker refers to the contig/scaffold name along with the SNP position. LOD is obtained by computing -log(p-value), and p-value corresponds to the p-value associated with the SNP marker. P-value and R-square were from TASSEL 5 (Bradbury et al., 2007) using three models. SMR (Single model regression)= Phenotype + Genotype,

GLM (General linear model)= Phenotype + Genotype+ Q_matrix, Q_matrix is obtained from STRUCTURE (Pritchard et al., 2000), MLM (Mixed linear model)= Phenotype Genotype + Q_matrix + Kinship (K), K is obtained from TASSEL 5. FarmCPU (Fixed and random model circulating probability unification) is a method usingGAPIT (Liu et al., 2016)

[redacted] .

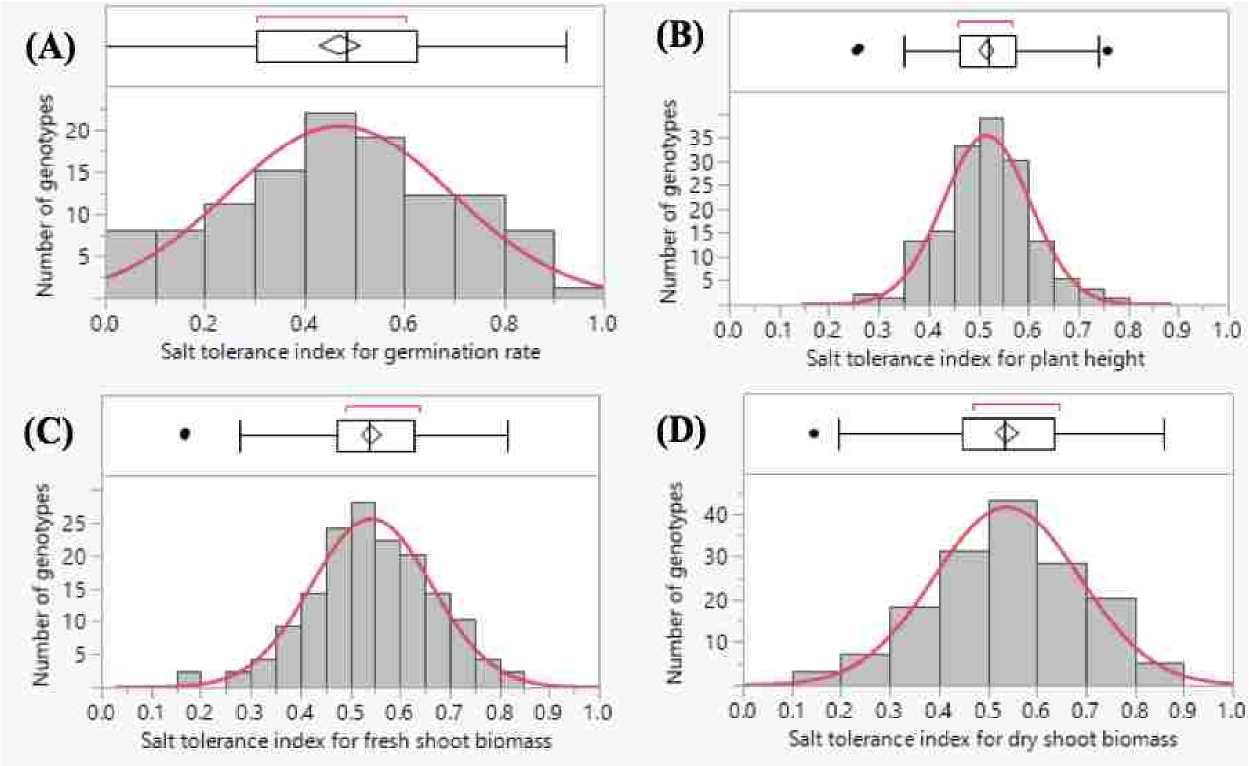

Figure 4-1. Distributions of salt tolerance index: (A) seed germination, (B) plant height, (C) fresh shoot biomass, and (D) dry shoot biomass in cowpea.

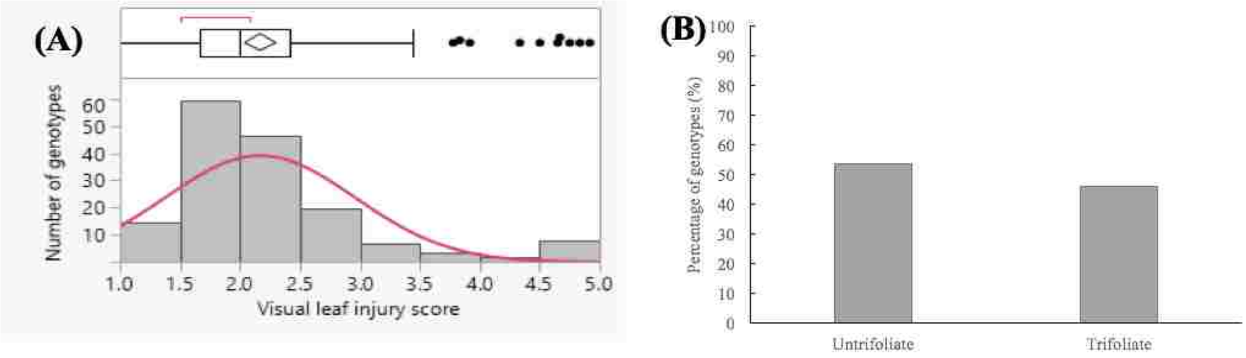

Figure 4-2. Distribution of the visual leaf injury scores (A) and inhibition of first trifoliate leaf development (B) at seedling stage.

[redacted]

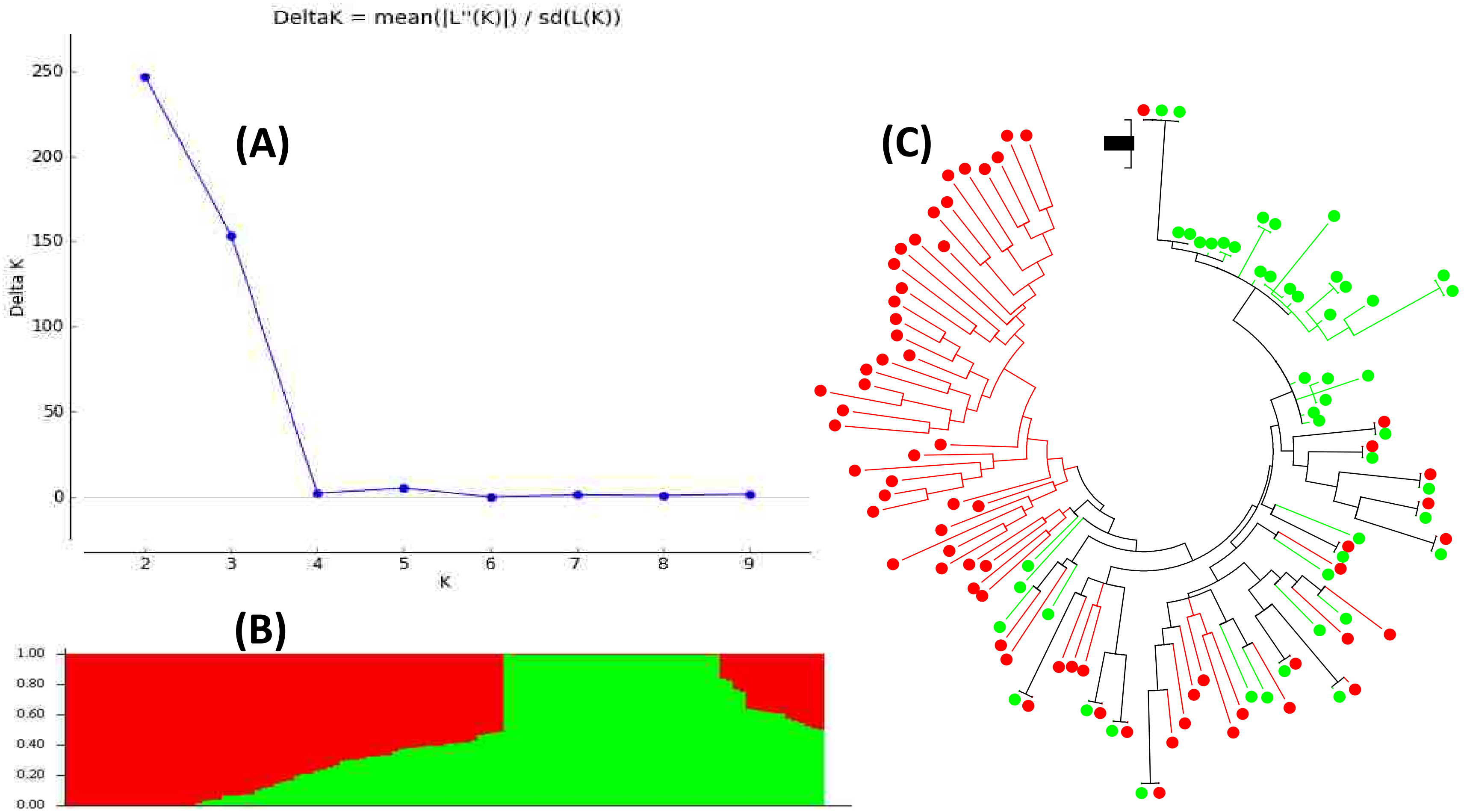

Figure 4-3 . Model-based populations of 116 cowpea genotypes at germination stage: (A) Delta K values for different numbers of populations assumed (K) in STRUCTURE analysis drawn by STRUCTURE HARVESTER, (B) Classification of the 116 cowpea accessions into two populations using STRUCTURE 2.3.4, where the subgroup membership is presented on the y-axis, and each accession on the x-axis. The color code shows the distribution of the different cowpea accessions (Q1 or cluster1: green, and Q2 or cluster2: red), and (C) Maximum Likelihood (ML) tree of the 116 cowpea accessions drawn using MEGA 7. Color coding in B and C are consistent.

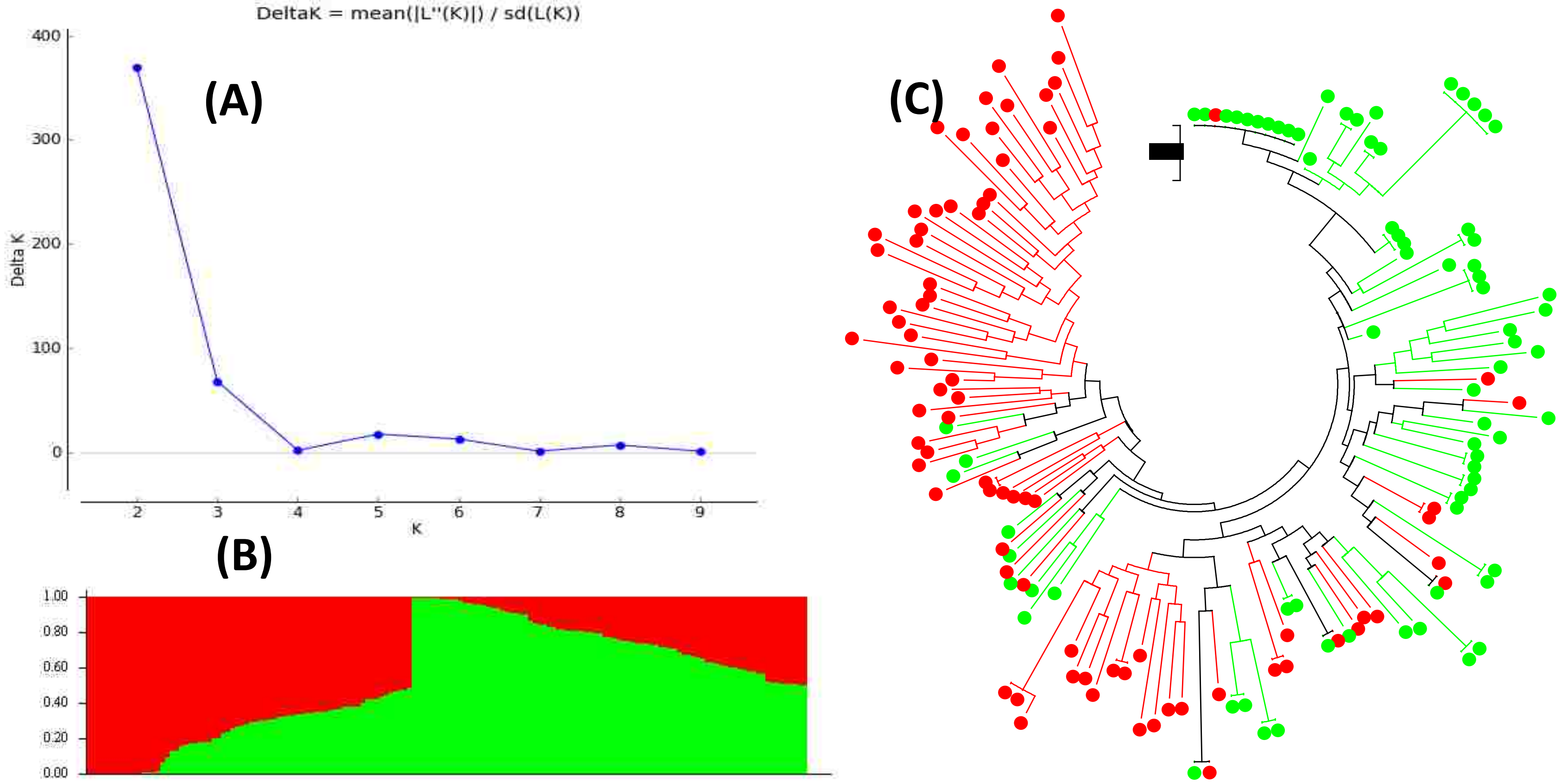

Figure 4-4. Model-based populations of 155 cowpea genotypes at seedling stage: (A) Delta K values for different numbers of populations assumed (K) in STRUCTURE analysis drawn by STRUCTURE HARVESTER, (B) Classification of the 155 cowpea accessions into two populations using STRUCTURE 2.3.4, where the subgroup membership is presented on the y-axis, and each accession on the x-axis. The color code shows the distribution of the different cowpea accessions (Q1 or cluster1: red, and Q2 or cluster2: green), and (C) Maximum Likelihood (ML) tree of the 155 cowpea accessions drawn using MEGA 7. The color codes are consistent in B and C.

Table S4-1. Cowpea accession number, seed color, origin, germination rate without salt treatment and under salt stress, and salt tolerance index (STI).

Accession	Seed_color	Origin	Germination_ non_stress_(%)	Germination_ stress_(%)	Germination_ STI
07-303	Red	USA	82.50	60.83	0.73
09-105	Cream	USA	89.17	46.67	0.52
09-1090	Pinkeye	USA	85.00	35.00	0.41
09-181	Pinkeye	USA	72.50	49.17	0.68
09-231	Pinkeye	USA	68.50	42.50	0.62
09-239	Pinkeye	USA	80.83	34.17	0.43
09-268	Cream	USA	83.33	16.67	0.20
09-295	Pinkeye	USA	64.17	35.83	0.56
09-307	Blackeye	USA	96.67	46.67	0.48
09-316	Black Holstein	USA	90.00	31.67	0.35
09-323	Pinkeye	USA	86.67	50.00	0.58
09-393	Pinkeye	USA	87.50	0.83	0.01
09-452	Black Holstein	USA	88.33	11.67	0.13
09-455	Blackeye	USA	77.50	57.50	0.75
09-470	Pinkeye	USA	91.67	44.17	0.48
09-529	Blackeye	USA	85.50	75.00	0.88
09-655	Pinkeye	USA	92.50	28.33	0.30
09-686	Pinkeye	USA	93.33	37.50	0.40
09-692	Pinkeye	USA	92.50	39.17	0.42
09-697	Pinkeye	USA	68.33	32.50	0.47
09-714	Pinkeye	USA	80.83	40.00	0.50
09-745	Red Holstein	USA	89.17	6.67	0.07
09-749	Red Holstein	USA	76.67	27.50	0.36
AR_BE_#1	Blackeye	USA	76.67	50.83	0.66
EARLY_ACRE	Cream	USA	67.50	32.50	0.49
EARLY_SCARLET	Pinkeye	USA	81.67	29.17	0.36
EBONY	Black	USA	90.00	69.17	0.77
ENCORE	Pinkeye	USA	71.67	30.00	0.42
Envoy	Red Holstein	USA	99.17	30.00	0.30
EXCEL	Browneye	USA	86.67	70.00	0.81

Table S4-1. Cowpea accession number, seed color, origin, germination rate without salt treatment and under salt stress, and salt tolerance index (STI) (Cont'd).

Accession	Seed_color	Origin	Germination_ non_stress_(%)	Germination_ stress_(%)	Germination_ STI
PI152195	Red	Paraguay	82.50	44.17	0.54
PI152196	Mixed (Cream Brown)	Paraguay	95.00	58.33	0.62
PI152197	Red	Paraguay	88.33	25.00	0.28
PI152199	Mixed (Purple Cream)	Paraguay	79.17	45.83	0.58
PI162924	Mixed (Cream Brown)	Paraguay	83.33	66.67	0.79
PI175332	Tan	NA	77.50	31.67	0.41
PI190191	Tan	Mexico	63.33	17.50	0.28
PI201498	Blackeye	Mexico	92.50	77.50	0.84
PI218123	Browneye	Pakistan	97.50	30.83	0.32
PI221730	Red Holstein	South_Africa	69.17	30.83	0.44
PI221731	Red Holstein	South_Africa	95.83	50.83	0.53
PI223023	Browneye	Iran	95.00	56.67	0.60
PI225922	Mixed (Brown Tan)	Zambia	60.83	46.67	0.75
PI229551	Browneye	Iran	90.83	70.83	0.78
PI229734	Blackeye	Iran	88.33	26.67	0.31
PI244571	Mixed (Brown Cream)	Guatemala	66.67	17.50	0.26
PI250416	Cream	Pakistan	88.33	22.50	0.25
PI250587	Dark brown	Madagascar	70.00	5.83	0.08
PI255765	Blackeye	Nigeria	97.50	56.67	0.58
PI257463	Red Holstein	Nigeria	79.17	46.67	0.59
PI262179	Blackeye	Portugal	73.33	55.00	0.75
PI292897	Grey	Hungary	67.50	28.33	0.42
PI292898	Black	Hungary	86.67	71.67	0.83
PI292899	Tan	Hungary	66.67	11.67	0.19
PI292913	Tan	Hungary	62.50	8.33	0.13
PI293467	Mixed (Tan Brown)	USA	84.17	28.33	0.34

Table S4-1. Cowpea accession number, seed color, origin, germination rate without salt treatment and under salt stress, and salt tolerance index (STI) (Cont'd).

Accession	Seed_color	Origin	Germination_ non_stress_(%)	Germination_ stress_(%)	Germination_ STI
PI293469	Tan	NA	91.67	23.33	0.26
PI293476	Variable (Grey Brown)	USA	70.83	7.50	0.09
PI293514	Grey	USA	78.33	21.67	0.28
PI293545	Mixed (Brown Cream)	NA	93.33	65.00	0.70
PI293570	Mixed (Brown Tan)	NA	62.50	7.50	0.12
PI293582	Grey	NA	70.00	22.50	0.33
PI293584	Variable (Red Brown Cream)	NA	67.50	58.33	0.86
PI339587	Red Holstein	South_Africa	70.00	53.33	0.76
PI339591	Brown Holstein	South_Africa	65.00	51.67	0.79
PI339592	Brown	South_Africa	70.83	44.17	0.62
PI339609	Tan	Tanzania	89.17	50.00	0.56
PI339610	Variable (Red Brown Cream)	Tanzania	60.83	38.33	0.63
PI339613	Variable (Grey Tan Brown)	Tanzania	93.33	45.00	0.48
PI353045	Red Holstein	India	77.50	45.83	0.59
PI367921	Tan	NA	89.17	73.33	0.82
PI430687	Red	China	68.33	32.50	0.47
PI487518	Tan	Indonesia	98.33	44.17	0.45
PI527561	Tan	Burundi	96.67	58.33	0.60
PI578902	Red	China	78.33	22.50	0.28
PI578907	Black	China	87.50	44.17	0.51
PI578911	Tan	China	82.50	47.50	0.57
PI582340	Mixed (Brown Pink)	NA	69.17	26.67	0.39
PI582354	Blackeye	NA	69.17	40.83	0.58
PI582415	Tan	Mexico	89.17	52.50	0.59

Table S4-1. Cowpea accession number, seed color, origin, germination rate without salt treatment and under salt stress, and salt tolerance index (STI) (Cont'd).

Accession	**Seed_color**	**Origin**	**Germination_ non_stress_(%)**	**Germination_ stress_(%)**	**Germination_ STI**
PI582416	Variable (Red Brown Cream)	Mexico	92.50	25.00	0.27
PI582420	Variable (Red Brown)	NA	77.50	65.00	0.83
PI582421	Grey	NA	97.50	65.00	0.67
PI582422	Blackeye	NA	75.83	70.00	0.92
PI582425	Mixed (Tan Brown)	NA	82.50	40.83	0.49
PI582428	Blackeye	Trinidad_ and_Tobago	64.72	49.17	0.76
PI582474	Purple	Botswana	64.17	25.83	0.40
PI582522	Tan	Mexico	95.00	0.83	0.01
PI582542	Browneye	Mexico	88.33	58.33	0.66
PI582551	Blackeye	Botswana	84.17	64.17	0.76
PI582574	Tan	Kenya	75.00	28.33	0.38
PI582575	Black	Kenya	91.67	28.33	0.31
PI582578	Tan	Kenya	77.50	42.50	0.53
PI582579	Tan	Kenya	97.50	21.67	0.22
PI582665	Variable (Grey Brown Tan)	Botswana	89.17	1.67	0.02
PI582666	Tan	Botswana	83.33	46.67	0.56
PI582680	Browneye	Botswana	65.83	26.67	0.41
PI582696	Brown	Botswana	74.17	44.17	0.59
PI582789	Tan	Kenya	95.83	36.67	0.38
PI582809	Tan	Botswana	61.67	23.33	0.38
PI582813	Mixed(Grey Cream Black eye)	Botswana	77.50	0.00	0.00
PI582815	Purple	Botswana	84.17	41.67	0.49
PI582818	Black Holstein	Botswana	90.00	20.00	0.22
PI582821	Mixed (Brown Tan)	Botswana	90.83	40.00	0.44
PI582852	Brown	Botswana	61.67	31.67	0.51

Table S4-1. Cowpea accession number, seed color, origin, germination rate without salt treatment and under salt stress, and salt tolerance index (STI) (Cont'd).

Accession	**Seed_color**	**Origin**	**Germination_ non_stress_(%)**	**Germination_ stress_(%)**	**Germination_ STI**
PI582873	Tan	Botswana	67.50	7.50	0.11
PI582874	Tan	Botswana	85.00	37.50	0.45
PI582875	Tan	Botswana	70.83	11.67	0.17
PI582894	Tan	Botswana	71.67	27.50	0.38
PI582932	Browneye	Malawi	70.00	57.50	0.82
PI583193	Variable (Gray Brown)	Senegal	94.17	15.83	0.17
PI583194	Variable (Tan Grey)	Senegal	99.17	9.17	0.09
PI583198	Tan	Senegal	96.67	57.50	0.60
PI583201	Browneye	Senegal	70.83	51.67	0.73
PI583202	Browneye	Senegal	89.17	56.67	0.64
PI583209	Tan	Nigeria	90.83	23.33	0.26

Table S4-2. Cowpea accession number, seed color, origin, foliar leaf injury, untrifoliate (1)/trifoliate (9).

Accession	**Seed_color**	**Origin**	**LIS**	**Trifoliate(9)_ untrifoliate(1)**
01-1781	Cream	USA	2.00	9
07-303	Red	USA	1.67	1
09-105	Cream	USA	2.44	1
09-1090	Pinkeye	USA	1.69	9
09-175	Pinkeye	USA	1.83	1
09-181	Pinkeye	USA	2.44	1
09-204	Brown	USA	1.83	1
09-208	Pinkeye	USA	1.92	1
09-211	Pinkeye	USA	1.68	1
09-239	Pinkeye	USA	1.83	1
09-268	Cream	USA	1.25	9
09-295	Pinkeye	USA	2.00	1
09-307	Blackeye	USA	3.00	1
09-316	Blackholstein	USA	2.00	1
09-323	Pinkeye	USA	2.19	9
09-393	Pinkeye	USA	1.53	1
09-452	Blackholstein	USA	1.75	9
09-455	Blackeye	USA	2.81	1
09-462	Pinkeye	USA	1.83	1
09-470	Pinkeye	USA	1.92	1
09-529	Blackeye	USA	2.08	1
09-655	Pinkeye	USA	1.92	1
09-671	Blackeye	USA	2.39	1
09-686	Pinkeye	USA	1.89	1
09-692	Pinkeye	USA	3.92	1
09-697	Pinkeye	USA	2.00	1
09-714	Pinkeye	USA	3.00	1
09-745	Redholstein	USA	1.83	1
09-749	Redholstein	USA	2.53	1
AR_BE_#1	Blackeye	USA	2.92	9
EARLY_ACRE	Cream	USA	2.25	1

Table S4-2. Cowpea accession number, seed color, origin, foliar leaf injury, untrifoliate (1)/trifoliate (9) (Cont'd).

Accession	Seed_color	Origin	LIS	Trifoliate(9)_ untrifoliate(1)
EARLY_SCARLET	Pinkeye	USA	2.50	1
EBONY	Balck	USA	2.92	1
EMPIRE	Pinkeye	USA	1.83	1
ENCORE	Pinkeye	USA	2.00	9
Envoy	Redholstein	USA	2.50	1
EXCEL	Broweye	USA	1.89	1
PI152195	Red	Paraguay	1.83	9
PI152196	Mixed_(Cream_Brown)	Paraguay	2.08	9
PI152197	Red	Paraguay	1.25	9
PI152199	Mixed_(Purple_Cream)	Paraguay	2.17	1
PI162924	Mixed_(Cream_Brown)	Paraguay	3.44	9
PI175332	Tan	NA	3.17	9
PI190191	Tan	Mexico	1.90	1
PI201024	Purple	Guatemala	1.75	1
PI201498	Blackeye	Mexico	2.08	9
PI218123	Browneye	Pakistan	1.50	1
PI221730	Red_Holstein	South_Africa	1.83	9
PI221731	Red_Holstein	South_Africa	1.67	9
PI223023	Browneye	Iran	1.67	1
PI225922	Mixed_(Brown_Tan)	Zambia	2.30	9
PI227829	Mixed_(Brown_Cream)	Guatemala	1.53	9
PI227830	Tan	Guatemala	2.00	9
PI229551	Browneye	Iran	1.33	9
PI229734	Blackeye	Iran	1.58	9
PI244571	Mixed_(Brown_Cream)	Guatemala	1.50	9
PI250416	Cream	Pakistan	1.67	9
PI250587	Darkbrown	Egypt	1.33	1
PI251222	Tan	Afghanistan	1.17	9
PI255765	Blackeye	Nigeria	2.08	9
PI255774	Browneye	Nigeria	4.75	9
PI255815	Browneye	Nigeria	4.83	9

Table S4-2. Cowpea accession number, seed color, origin, foliar leaf injury, untrifoliate (1)/trifoliate (9) (Cont'd).

Accession	Seed_color	Origin	LIS	Trifoliate(9)_ untrifoliate(1)
PI256342	Brown	Pakistan	2.07	9
PI257463	Red_Holstein	Nigeria	1.22	1
PI262179	Blackeye	Portugal	4.33	9
PI292891	Tan	South_Africa	1.50	1
PI292892	Brown_Holstein	South_Africa	3.78	9
PI292897	Grey	Hungary	2.58	9
PI292898	Black	Hungary	2.83	1
PI292899	Tan	Hungary	2.67	9
PI292913	Tan	Hungary	2.33	1
PI293467	Mixed_(Tan_Brown)	USA	2.33	9
PI293469	Tan	NA	2.08	9
PI293476	Variable_(Grey_Brown)	USA	1.67	9
PI293514	Grey	USA	1.58	1
PI293545	Mixed_(Brown_Cream)	NA	1.58	9
PI293570	Mixed_(Brown_Tan)	NA	1.00	1
PI293582	Grey	NA	2.08	9
PI293584	Variable_(Red_Brown_Cream)	NA	1.81	1
PI339563	Tan	Australia	2.42	9
PI339587	Red_Holstein	South_Africa	1.75	9
PI339591	Brown_Holstein	South_Africa	2.58	9
PI339592	Brown	South_Africa	2.28	9
PI339598	Tan	South_Africa	2.42	9
PI339600	Tan	South_Africa	1.60	9
PI339609	Tan	Tanzania	2.32	9
PI339610	Variable_(Red_Brown_Cream)	Tanzania	1.56	9
PI339613	Variable_(Grey_Tan_Brown)	Tanzania	1.58	9
PI349674	Black	Australia	2.73	9
PI353045	Redholstein	India	2.33	1
PI367921	Tan	NA	3.83	1
PI430687	Red	China	2.17	9
PI487518	Tan	Indonesia	3.33	9

Table S4-2. Cowpea accession number, seed color, origin, foliar leaf injury, untrifoliate (1)/trifoliate (9) (Cont'd).

Accession	Seed_color	Origin	LIS	Trifoliate(9)_ untrifoliate(1)
PI527561	Tan	Burundi	4.65	1
PI578902	Red	China	2.00	1
PI578911	Tan	China	1.75	9
PI582340	Mixed_(Brown_Pink)	USA	1.50	1
PI582354	Balckeye	USA	1.50	1
PI582366	Tan	India	1.50	9
PI582368	Black_Holstein	India	1.50	9
PI582415	Tan	Mexico	1.33	9
PI582416	Variable_(Red_Brown_Cream)	Mexico	2.00	1
PI582420	Variable_(Red_Brown)	NA	1.17	1
PI582421	Grey	NA	1.67	1
PI582422	Blackeye	NA	1.11	1
PI582425	Mixed_(Tan_Brown)	NA	1.81	1
PI582428	Blackeye	Trinidad_ and_Tobago	1.17	9
PI582465	Tan	Mexico	1.33	9
PI582468	Tan_Holstein	NA	2.00	9
PI582469	Brown_Holstein	Philippines	2.00	9
PI582474	Purple	Botswana	1.67	9
PI582522	Tan	Mexico	1.58	1
PI582530	Mixed_(Brown_Tan)	Ghana	2.33	1
PI582542	Browneye	Mexico	2.67	1
PI582551	Blackeye	Botswana	2.83	1
PI582572	Tan	Kenya	1.67	9
PI582574	Tan	Kenya	2.33	9
PI582575	Black	Kenya	1.83	1
PI582578	Tan	Kenya	2.00	9
PI582579	Tan	Kenya	2.67	9
PI582650	Tan	Botswana	1.75	9
PI582665	Variable_(Grey_Brown_Tan)	Botswana	4.92	9
PI582666	Tan	Botswana	2.83	9
PI582675	Tan	Botswana	1.83	9

Table S4-2. Cowpea accession number, seed color, origin, foliar leaf injury, untrifoliate (1)/trifoliate (9) (Cont'd).

Accession	Seed_color	Origin	LIS	Trifoliate(9)_ untrifoliate(1)
PI582680	Browneye	Botswana	2.00	9
PI582696	Brown	Botswana	2.17	9
PI582697	Tan	Botswana	1.75	1
PI582809	Tan	Botswana	2.00	1
PI582812	Brown_Holstein	Botswana	1.10	1
PI582813	Mixed_(Grey_Cream_Black eye)	Botswana	1.67	1
PI582818	Black Holstein	Botswana	4.50	9
PI582821	Mixed_(Brown_Tan)	Botswana	1.25	1
PI582822	Red_Holstein	Botswana	2.39	1
PI582823	Red_Holstein	Botswana	1.67	1
PI582825	Tan	Botswana	2.67	1
PI582850	Brown_Holstein	Botswana	2.00	9
PI582852	Brown	Botswana	1.78	9
PI582854	Greeneye	Botswana	2.67	9
PI582857	Browneye	Botswana	2.58	1
PI582866	Brown	Botswana	1.67	1
PI582873	Tan	Botswana	2.00	9
PI582874	Tan	Botswana	1.83	9
PI582875	Tan	Botswana	2.17	9
PI582894	Tan	Botswana	1.93	9
PI582912	Black_Holstein	Kenya	2.00	9
PI583193	Variable_(Gray_Brown)	Senegal	2.22	9
PI583194	Variable_(Tan_Grey)	Senegal	1.94	9
PI583195	Red	Senegal	2.02	9
PI583198	Tan	Senegal	2.53	1
PI583201	Broweye	Senegal	4.67	9
PI583202	Browneye	Senegal	1.58	1
PI583209	Tan	Nigeria	1.75	9
PI666252	Tan	USA	3.00	9
PI666260	Pinkeye	USA	2.33	1
PI666262	Browneye	USA	4.50	9

Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass.

Accession	**Seed_color**	**Origin**	**Plant height non_stress (cm)**	**Plant height stress (cm)**	**Plant height STI**	**Fresh biomass non_stress (g)**	**Fresh biomass stress (g)**	**Fresh biomass STI**	**Dry biomass non_stress (g)**	**Dry biomass stress (g)**	**Dry biomass STI**
01-1781	Cream	USA	11.13	6.31	0.57	7.34	4.13	0.59	1.24	0.61	0.46
07-303	Red	USA	13.97	7.90	0.57	6.67	3.70	0.53	1.28	0.71	0.53
09-105	Cream	USA	11.72	6.75	0.58	5.65	3.58	0.77	0.68	0.45	0.62
09-1090	Pinkeye	USA	12.60	7.91	0.63	9.86	5.80	0.52	1.65	1.04	0.64
09-175	Pinkeye	USA	12.67	9.36	0.74	7.39	2.94	0.43	1.24	0.38	0.30
09-181	Pinkeye	USA	13.14	8.71	0.66	6.85	4.25	0.68	1.24	0.76	0.63
09-204	Brown	USA	13.58	8.56	0.63	9.48	4.78	0.50	1.55	0.83	0.52
09-208	Pinkeye	USA	15.00	8.89	0.59	8.55	4.82	0.55	1.48	0.89	0.56
09-211	Pinkeye	USA	15.70	8.15	0.52	9.42	5.23	0.56	1.91	1.03	0.49
09-239	Pinkeye	USA	14.26	7.50	0.53	7.59	4.90	0.61	0.85	0.40	0.40
09-268	Cream	USA	13.21	6.13	0.46	6.81	3.94	0.60	0.88	0.47	0.50
09-295	Pinkeye	USA	14.18	10.07	0.71	6.09	4.49	0.68	0.92	0.66	0.78
09-307	Blackeye	USA	11.31	6.57	0.58	6.38	3.41	0.63	0.89	0.48	0.50
09-316	Blackholstein	USA	15.35	7.52	0.49	7.54	4.25	0.45	2.02	1.16	0.62
09-323	Pinkeye	USA	12.25	6.66	0.54	8.33	2.64	0.43	1.64	0.52	0.32
09-393	Pinkeye	USA	18.35	10.05	0.55	9.78	4.64	0.40	1.86	0.87	0.48
09-452	Blackholstein	USA	14.06	6.37	0.46	7.20	2.93	0.47	1.13	0.45	0.39
09-455	Blackeye	USA	14.19	7.81	0.55	8.71	4.65	0.58	1.30	0.70	0.52
09-462	Pinkeye	USA	14.78	8.26	0.56	8.95	5.26	0.52	1.53	0.97	0.72

Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	**Seed_color**	**Origin**	**Plant height non_stress (cm)**	**Plant height stress (cm)**	**Plant height STI**	**Fresh biomass non_stress (g)**	**Fresh biomass stress (g)**	**Fresh biomass STI**	**Dry biomass non_stress (g)**	**Dry biomass stress (g)**	**Dry biomass STI**
09-470	Pinkeye	USA	13.79	8.61	0.62	6.69	4.34	0.72	1.09	0.69	0.66
09-529	Blackeye	USA	10.89	8.01	0.73	8.75	5.19	0.61	1.47	0.93	0.67
09-655	Pinkeye	USA	13.88	9.33	0.67	7.79	5.93	0.71	1.49	1.10	0.77
09-671	Blackeye	USA	13.89	7.56	0.55	8.78	4.53	0.49	1.68	0.79	0.51
09-686	Pinkeye	USA	13.65	8.94	0.65	8.01	4.63	0.55	1.51	0.85	0.59
09-692	Pinkeye	USA	13.84	7.09	0.51	6.73	2.97	0.51	1.35	0.69	0.50
09-697	Pinkeye	USA	16.75	8.25	0.49	8.97	4.86	0.51	1.39	0.75	0.54
09-714	Pinkeye	USA	13.39	8.04	0.60	7.45	3.92	0.53	1.41	0.72	0.51
09-745	Redholstein	USA	12.78	7.00	0.55	9.15	5.24	0.62	1.43	0.81	0.60
09-749	Redholstein	USA	12.65	7.56	0.60	9.55	6.24	0.53	1.65	1.08	0.59
AR_BE_#1	Blackeye	USA	13.94	6.50	0.47	9.19	3.69	0.38	1.61	0.70	0.43
EARLY_ACRE	Cream	USA	14.15	7.67	0.54	5.17	3.60	0.74	0.58	0.44	0.79
EARLY_SCARLET	Pinkeye	USA	18.29	9.29	0.51	9.42	5.21	0.59	1.24	0.65	0.55
EBONY	Balck	USA	15.43	8.44	0.55	8.58	5.16	0.54	1.06	0.64	0.69
EMPIRE	Pinkeye	USA	12.90	8.46	0.66	5.19	4.18	0.77	0.70	0.56	0.78
ENCORE	Pinkeye	USA	17.34	9.02	0.52	6.26	4.19	0.81	1.02	0.63	0.61
Envoy	Redholstein	USA	15.23	8.18	0.54	7.82	6.33	0.67	1.05	0.81	0.75
EXCEL	Broweye	USA	14.45	7.22	0.50	8.06	2.63	0.41	1.20	0.40	0.31
PI152195	Red	Paraguay	15.62	6.52	0.42	6.56	5.25	0.79	1.29	0.93	0.70

Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	Seed_color	Origin	Plant height non_stress (cm)	Plant height stress (cm)	Plant height STI	Fresh biomass non_stress (g)	Fresh biomass stress (g)	Fresh biomass STI	Dry biomass non_stress (g)	Dry biomass stress (g)	Dry biomass STI
PI152196	Mixed_(Cream_Brown)	Paraguay	17.73	8.63	0.49	8.56	4.81	0.65	1.34	0.50	0.41
PI152197	Red	Paraguay	16.74	9.69	0.58	9.62	6.84	0.62	1.85	1.23	0.73
PI152199	Mixed_(Purple_Cream)	Paraguay	17.60	8.73	0.50	8.67	4.99	0.49	1.71	1.02	0.54
PI162924	Mixed_(Cream_Brown)	Paraguay	15.19	6.18	0.41	7.39	2.43	0.43	1.54	0.46	0.28
PI175332	Tan	NA	15.00	7.73	0.52	6.49	3.42	0.68	1.29	0.62	0.48
PI190191	Tan	Mexico	11.53	6.99	0.60	6.30	3.30	0.57	0.87	0.48	0.56
PI201024	Purple	Guatemala	18.68	8.29	0.44	8.99	4.36	0.51	1.81	0.90	0.49
PI201498	Blackeye	Mexico	19.60	8.72	0.44	8.84	5.02	0.46	1.67	0.89	0.50
PI218123	Browneye	Pakistan	17.01	8.48	0.50	8.89	2.97	0.40	1.55	0.52	0.31
PI221730	Red_Holstein	South_Africa	13.52	7.67	0.57	5.95	4.19	0.72	1.26	0.82	0.66
PI221731	Red_Holstein	South_Africa	15.21	8.75	0.57	6.58	3.35	0.48	1.07	0.47	0.41
PI223023	Browneye	Iran	21.49	10.47	0.49	12.05	4.13	0.28	2.16	0.81	0.35
PI225922	Mixed_(Brown_Tan)	Zambia	15.61	6.75	0.43	5.82	1.03	0.42	0.85	0.12	0.19
PI227829	Mixed_(Brown_Cream)	Guatemala	14.13	7.50	0.53	7.76	4.57	0.58	1.23	0.59	0.54
PI227830	Tan	Guatemala	14.33	7.42	0.52	6.26	4.35	0.66	1.06	0.70	0.70
PI229551	Browneye	Iran	13.90	8.47	0.61	6.78	2.66	0.42	1.07	0.46	0.45
PI229734	Blackeye	Iran	15.62	7.56	0.48	8.56	4.22	0.50	1.26	0.56	0.42
PI244571	Mixed_(Brown_Cream)	Guatemala	16.61	7.92	0.48	7.86	3.78	0.54	1.33	0.69	0.51

Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	**Seed_color**	**Origin**	**Plant height non_stress (cm)**	**Plant height stress (cm)**	**Plant height STI**	**Fresh biomass non_stress (g)**	**Fresh biomass stress (g)**	**Fresh biomass STI**	**Dry biomass non_stress (g)**	**Dry biomass stress (g)**	**Dry biomass STI**
PI250416	Cream	Pakistan	16.22	6.25	0.39	11.25	4.93	0.53	1.84	0.73	0.38
PI250587	Darkbrown	Egypt	21.00	11.77	0.56	10.77	7.08	0.49	1.77	1.21	0.74
PI251222	Tan	Afghanistan	19.08	8.83	0.46	9.99	5.63	0.54	1.59	0.97	0.59
PI255765	Blackeye	Nigeria	21.22	9.99	0.47	11.28	4.99	0.37	2.10	0.84	0.38
PI255774	Browneye	Nigeria	20.42	7.43	0.37	10.58	1.35	0.17	2.13	0.36	0.15
PI255815	Browneye	Nigeria	13.35	5.76	0.43	4.19	2.64	0.55	0.85	0.51	0.64
PI256342	Brown	Pakistan	19.67	10.00	0.51	8.22	4.82	0.57	1.10	0.61	0.54
PI257463	Red_Holstein	Nigeria	11.39	8.64	0.76	6.03	3.88	0.65	0.93	0.56	0.63
PI262179	Blackeye	Portugal	14.85	7.51	0.51	8.55	5.19	0.61	1.42	0.69	0.47
PI292891	Tan	South_Africa	16.43	9.83	0.60	8.81	6.55	0.56	1.26	0.97	0.81
PI292892	Brown_Holstein	South_Africa	12.00	5.49	0.46	5.60	1.64	0.36	1.01	0.33	0.31
PI292897	Grey	Hungary	15.13	8.66	0.57	7.00	5.21	0.65	1.32	0.96	0.75
PI292898	Black	Hungary	12.43	7.89	0.63	6.42	3.98	0.60	0.77	0.48	0.63
PI292899	Tan	Hungary	18.72	8.98	0.48	10.72	5.61	0.49	1.34	0.70	0.57
PI292913	Tan	Hungary	14.53	7.81	0.54	7.50	5.41	0.69	1.01	0.73	0.80
PI293467	Mixed_(Tan_Brown)	USA	16.02	7.93	0.50	8.18	4.66	0.63	2.05	1.05	0.51
PI293469	Tan	NA	18.12	9.73	0.54	6.94	5.33	0.61	1.34	1.10	0.84
PI293476	Variable_(Grey_Brown)	USA	15.56	6.15	0.39	8.74	2.09	0.30	1.49	0.43	0.35

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Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	**Seed_color**	**Origin**	**Plant height non_stress (cm)**	**Plant height stress (cm)**	**Plant height STI**	**Fresh biomass non_stress (g)**	**Fresh biomass stress (g)**	**Fresh biomass STI**	**Dry biomass non_stress (g)**	**Dry biomass stress (g)**	**Dry biomass STI**
PI293514	Grey	USA	11.22	6.93	0.62	6.71	4.95	0.75	1.11	0.78	0.61
PI293545	Mixed_(Brown_Cream)	NA	15.79	7.78	0.49	8.92	5.06	0.53	1.35	0.72	0.53
PI293570	Mixed_(Brown_Tan)	NA	14.21	7.41	0.52	7.96	6.30	0.61	1.44	1.02	0.59
PI293582	Grey	NA	10.51	6.58	0.62	6.63	4.84	0.72	0.86	0.67	0.82
PI293584	Variable_(Red_Brown_Cream)	NA	16.03	7.66	0.48	7.48	5.38	0.71	1.05	0.78	0.74
PI339563	Tan	Australia	12.16	4.55	0.38	4.00	1.56	0.32	0.64	0.28	0.48
PI339587	Red_Holstein	South_Africa	12.23	5.53	0.45	6.25	3.77	0.52	1.18	0.67	0.55
PI339591	Brown_Holstein	South_Africa	14.46	6.64	0.46	7.75	4.30	0.63	1.29	0.75	0.50
PI339592	Brown	South_Africa	13.77	8.21	0.60	7.59	4.49	0.57	1.25	0.75	0.63
PI339598	Tan	South_Africa	13.54	7.68	0.57	8.65	4.31	0.42	1.41	0.81	0.54
PI339600	Tan	South_Africa	14.61	5.68	0.39	6.39	3.48	0.68	1.12	0.56	0.42
PI339609	Tan	Tanzania	18.61	7.02	0.38	6.40	3.91	0.51	1.03	0.61	0.61
PI339610	Variable_(Red_Brown_Cream)	Tanzania	17.21	6.28	0.36	6.35	2.49	0.50	1.14	0.46	0.40
PI339613	Variable_(Grey_Tan_Brown)	Tanzania	15.59	7.19	0.46	7.98	3.76	0.49	1.69	0.83	0.41
PI349674	Black	Australia	12.37	7.18	0.58	6.25	4.10	0.70	1.11	0.71	0.62
PI353045	Redholstein	India	16.80	8.17	0.49	6.72	5.01	0.69	0.94	0.67	0.69

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Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	Seed_color	Origin	Plant height non_stress (cm)	Plant height stress (cm)	Plant height STI	Fresh biomass non_stress (g)	Fresh biomass stress (g)	Fresh biomass STI	Dry biomass non_stress (g)	Dry biomass stress (g)	Dry biomass STI
PI367921	Tan	NA	15.83	8.40	0.53	7.53	2.75	0.43	1.27	0.54	0.40
PI430687	Red	China	16.97	8.67	0.51	8.33	5.05	0.46	1.23	0.72	0.63
PI487518	Tan	Indonesia	15.92	6.97	0.44	7.99	2.56	0.40	1.50	0.38	0.25
PI527561	Tan	Burundi	16.41	6.45	0.39	8.42	2.82	0.34	1.60	0.76	0.52
PI578902	Red	China	16.57	8.47	0.51	7.55	4.16	0.49	1.25	0.59	0.54
PI578911	Tan	China	19.28	8.06	0.42	9.19	3.19	0.41	1.43	0.48	0.34
PI582340	Mixed_(Brown_Pink)	USA	14.23	9.47	0.67	7.21	6.19	0.82	1.15	0.98	0.81
PI582354	Balckeye	USA	17.83	7.83	0.44	7.01	4.21	0.62	0.93	0.56	0.54
PI582366	Tan	India	12.00	6.33	0.53	6.84	3.79	0.44	1.15	0.65	0.59
PI582368	Black_Holstein	India	12.50	7.83	0.63	5.83	2.73	0.49	0.74	0.34	0.43
PI582415	Tan	Mexico	13.67	8.30	0.61	7.77	5.85	0.72	1.25	0.97	0.79
PI582416	Variable_(Red_Brown_Cream)	Mexico	14.83	8.83	0.60	6.88	4.80	0.58	1.31	0.91	0.76
PI582420	Variable_(Red_Brown)	NA	18.70	8.89	0.48	10.05	3.00	0.32	1.49	0.43	0.30
PI582421	Grey	NA	19.14	8.94	0.47	5.22	3.35	0.66	1.00	0.64	0.67
PI582422	Blackeye	NA	15.09	8.20	0.54	7.25	3.49	0.48	1.19	0.59	0.52
PI582425	Mixed_(Tan_Brown)	NA	13.71	8.33	0.61	9.01	5.04	0.50	1.60	0.93	0.59
PI582428	Blackeye	Trinidad_ and_Tobago	14.75	7.95	0.54	7.57	3.67	0.63	1.43	0.66	0.33
PI582465	Tan	Mexico	17.88	9.38	0.52	12.10	6.56	0.45	2.26	1.25	0.53

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Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	Seed_color	Origin	Plant height non_stress (cm)	Plant height stress (cm)	Plant height STI	Fresh biomass non_stress (g)	Fresh biomass stress (g)	Fresh biomass STI	Dry biomass non_stress (g)	Dry biomass stress (g)	Dry biomass STI
PI582468	Tan_Holstein	NA	13.67	7.24	0.53	8.26	1.70	0.17	1.59	0.34	0.22
PI582469	Brown_Holstein	Philippines	17.07	6.58	0.39	5.22	2.07	0.57	0.78	0.33	0.28
PI582474	Purple	Botswana	12.70	7.37	0.58	6.89	3.96	0.65	1.24	0.49	0.36
PI582522	Tan	Mexico	14.60	8.25	0.56	9.28	6.89	0.59	1.08	0.76	0.75
PI582530	Mixed_(Brown_Tan)	Ghana	23.00	8.63	0.37	5.93	2.43	0.51	0.74	0.35	0.47
PI582542	Browneye	Mexico	12.20	6.20	0.51	6.71	3.84	0.53	0.91	0.50	0.56
PI582551	Blackeye	Botswana	14.14	5.18	0.37	6.78	2.64	0.51	0.85	0.36	0.45
PI582572	Tan	Kenya	12.73	6.50	0.51	5.18	1.97	0.39	0.91	0.41	0.51
PI582574	Tan	Kenya	12.33	7.40	0.60	6.21	3.22	0.62	1.26	0.66	0.52
PI582575	Black	Kenya	21.76	11.19	0.51	9.65	5.23	0.53	1.71	0.92	0.58
PI582578	Tan	Kenya	12.88	6.38	0.50	6.27	3.29	0.56	0.92	0.45	0.47
PI582579	Tan	Kenya	13.07	6.87	0.53	6.17	3.74	0.59	0.95	0.63	0.64
PI582650	Tan	Botswana	11.28	6.12	0.54	9.09	5.83	0.63	1.69	1.16	0.71
PI582665	Variable_(Grey_Brown_Tan)	Botswana	16.03	6.58	0.41	10.60	5.39	0.46	1.80	0.99	0.50
PI582666	Tan	Botswana	15.33	7.50	0.49	6.43	3.74	0.54	1.18	0.71	0.60
PI582675	Tan	Botswana	10.33	6.17	0.60	5.63	3.22	0.53	0.84	0.48	0.56
PI582680	Browneye	Botswana	13.17	7.17	0.54	6.63	3.15	0.54	1.22	0.56	0.42
PI582696	Brown	Botswana	13.63	7.10	0.52	7.30	3.80	0.48	1.19	0.61	0.55
PI582697	Tan	Botswana	23.26	8.32	0.36	9.25	3.41	0.41	1.06	0.42	0.34

Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	**Seed_color**	**Origin**	**Plant height non_stress (cm)**	**Plant height stress (cm)**	**Plant height STI**	**Fresh biomass non_stress (g)**	**Fresh biomass stress (g)**	**Fresh biomass STI**	**Dry biomass non_stress (g)**	**Dry biomass stress (g)**	**Dry biomass STI**
PI582809	Tan	Botswana	14.10	8.64	0.61	6.57	3.70	0.55	0.85	0.53	0.60
PI582812	Brown_Holstein	Botswana	18.60	6.50	0.35	6.51	3.45	0.64	1.61	0.79	0.48
PI582813	Mixed_(Grey_Cream_Black eye)	Botswana	16.20	7.74	0.48	10.03	5.10	0.47	1.41	0.76	0.53
PI582818	Black Holstein	Botswana	11.50	6.10	0.53	6.98	4.50	0.70	1.23	0.81	0.64
PI582821	Mixed_(Brown_Tan)	Botswana	16.30	8.80	0.54	8.16	5.48	0.61	1.61	1.17	0.64
PI582822	Red_Holstein	Botswana	17.42	9.67	0.55	5.62	3.69	0.56	0.98	0.62	0.59
PI582825	Tan	Botswana	16.08	6.23	0.39	11.95	5.26	0.39	1.92	0.76	0.38
PI582850	Brown_Holstein	Botswana	14.14	7.97	0.56	5.53	3.58	0.52	1.05	0.72	0.67
PI582852	Brown	Botswana	17.60	4.63	0.26	8.10	1.73	0.28	1.07	0.22	0.20
PI582854	Greeneye	Botswana	12.97	6.73	0.52	9.17	3.94	0.39	1.70	0.70	0.39
PI582857	Browneye	Botswana	11.75	6.71	0.57	5.61	4.19	0.63	0.86	0.65	0.72
PI582866	Brown	Botswana	22.00	5.63	0.26	5.32	1.40	0.50	0.81	0.23	0.27
PI582873	Tan	Botswana	11.58	4.78	0.41	8.78	4.76	0.57	1.24	0.63	0.50
PI582874	Tan	Botswana	11.00	6.50	0.59	8.63	6.01	0.70	1.66	1.09	0.71
PI582875	Tan	Botswana	11.57	6.93	0.60	7.74	5.45	0.61	1.49	1.07	0.75
PI582894	Tan	Botswana	12.65	7.71	0.61	8.65	4.56	0.46	1.49	0.62	0.47
PI582912	Black_Holstein	Kenya	13.08	7.56	0.58	7.07	3.29	0.54	1.09	0.46	0.41
PI583193	Variable_(Gray_Brown)	Senegal	14.82	6.71	0.45	6.60	4.30	0.65	1.04	0.72	0.69

[redacted]

Table S4-3. Cowpea accession number, seed color, origin, plant height, fresh and dry shoot rate without salt treatment and under salt stress, and salt tolerance index (STI) for plant height, and dry shoot biomass (Cont'd).

Accession	**Seed_color**	**Origin**	**Plant height non_stress (cm)**	**Plant height stress (cm)**	**Plant height STI**	**Fresh biomass non_stress (g)**	**Fresh biomass stress (g)**	**Fresh biomass STI**	**Dry biomass non_stress (g)**	**Dry biomass stress (g)**	**Dry biomass STI**
PI583194	Variable_(Tan_Grey)	Senegal	14.79	6.96	0.47	7.55	4.35	0.53	0.94	0.56	0.63
PI583195	Red	Senegal	14.92	6.83	0.46	7.23	3.62	0.47	0.98	0.49	0.50
PI583198	Tan	Senegal	14.56	6.89	0.48	6.08	2.97	0.49	0.99	0.50	0.50
PI583201	Broweye	Senegal	17.33	6.97	0.40	6.82	2.47	0.40	1.28	0.48	0.38
PI583202	Browneye	Senegal	19.54	7.96	0.41	7.68	4.06	0.58	1.43	0.68	0.45
PI583209	Tan	Nigeria	18.58	8.39	0.45	9.90	4.91	0.39	1.78	0.85	0.46
PI666252	Tan	USA	17.71	10.00	0.56	6.62	4.74	0.78	1.38	1.00	0.68
PI666260	Pinkeye	USA	18.30	7.67	0.42	5.80	5.38	0.65	1.19	1.05	0.86
PI666262	Browneye	USA	13.83	6.63	0.48	7.58	4.28	0.40	1.86	1.06	0.53

Chapter 5. Evaluation of Total Seed Protein Content in Eleven Arkansas Cowpea (*Vigna unguiculata* (L.) Walp.) Lines

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Abstract

Cowpea is cultivated on more than 11 million hectares with a worldwide production of 5.4 million tons of dried seeds. Cowpea is an affordable source of protein, which is used as an alternative to soybean for people who are allergic to soybean protein. The aim of this research was to assess the variability of the total seed protein content in cowpea. Eleven Arkansas lines were used in this study. Field experiment design was a randomized complete block design (RCBD) with 2 blocks, and conducted in three different locations within Arkansas (Fayetteville, Alma, and Hope) in 2015. A standard chemical protocol was performed involving an analysis of the total nitrogen by combustion using an Elementar Rapid N III instrument to estimate the protein content. The average protein content was 25.4%, and ranged from 23.7 to 27.4% with a standard deviation of 1.9%. The significant effects of genotype, environment (location), and genotype by environment were observed for the total seed protein content in cowpea. The broad sense heritability (H^2) for cowpea seed protein was estimated to be 57.8% based on the eleven cowpea genotypes studied. The cowpea lines, ‘Early Scarlet’ and 09-204 had the highest seed protein content with 27.4% and 26.9% dried seed weight, respectively. This study provides valuable information on cowpea protein content for breeders to select and utilize those lines with high seed protein content to develop new high protein cowpea cultivars.

Keywords

Cowpea, *Vigna unguiculata,* seed protein content, gemplasm

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] serves multiple purposes. It can be consumed as dry seeds and a vegetable, or used as a cover crop (Nielsen et al., 1997; Fang et al., 2006). Industries process cowpeas by both canning and freezing (Fery, 1993). Cowpea seed grain is used for human consumption as an affordable source of protein and constitute a supplement fodder to cereal for livestock (Carnovale et al., 1990; Pedalino et al., 1990; Singh et al., 2003). In addition, a study carried out by Kushwaha and Kumar (2014) stated that cowpea flour can be used to develop high protein biscuits, which would help enhance the nutritional quality of food. The good functional properties of cowpea protein provide plant protein source for people who are suffering allergies to soybean protein.

Cowpea probably helps address the issue of food security, which is a great concern. Food insecurity is accelerated by the rapidly growing world population and the visible effects of climate change, which hamper the livelihood of farmers worldwide (Lobell et al., 2008). It has been shown that cowpea consumption could have a positive impact on human health (Itatat et al., 2013). Sreerama et al. (2012) reported that cowpea could be used as an ingredient to develop healthy food. Moreover, an increased consumption in cowpea has decreased severe malnutrition up to 100% in children (Nnanyelugo and Ngoddy, 1997).

The assessment of protein content in cowpea is of interest in order to identify genotypes with high protein content. Itatat et al. (2013) assessed eleven cowpea genotypes for seed protein content. Their results revealed that the seed protein content of those cowpea lines ranged from 20.57% to 24.95%. Research performed by Afiukwa et al. (2013) on 110 cowpea genotypes exhibited a greater variability than Itatat et al. (2013) reported. Afiukwa et al. (2013) have found that the total seed protein content for their genotypes varied from 15.06% to 38.5%, with a mean of 25.99 ± 4.82% in dry seeds. Oke et al. (2015) analyzed the protein content in five varieties of cowpea and found that the protein content ranged from 25.80% to 28.95%. Moreover, protein fractions viz. albumins, globulins, prolamins and glutelins of cowpea genotypes showed significant differences according to a study by Gupta et al. (2014). Their analysis on molecular weights of protein bands from 11 cowpea genotypes using SDS-polyacrylamide gel electrophoresis displayed a variation between 10 to 141.3, 15.85 to 147.9, 10 to 125.9, 7.94 to 56.23 and 10 to 79.43 kDa for total proteins, albumins, globulins, prolamins, and glutelins, respectively. Cowpea leaves are also an important source of protein. Okonya and Maass (2014) used Near Infrared Spectrometry (NIRS) to determine the crude protein content of leaves from six cowpea genotypes, and found that the protein content in leaves in these genotypes averaged 30%.

However, the total seed protein contents in cowpea significantly differed among cowpea cultivars (Afiukwa, 2013; Itatat et al., 2013). Fernandes et al. (2012) reported that seed protein content was controlled by three to seven genes with very high narrow-sense heritability (h^2) 87.6% in the P1, P2, F1, F2, and backcross populations derived from the cross IT97K-1042-3 × Canap and with a moderate h^2 (47.7%) in another cross IT97K-1042-3 × BRS Tapaihum. In addition to the genetic background, location can engender such variability in seed protein content in cowpea (Fernandes et al., 2012). This variability will impact food quality involving cowpea in a way that the physical properties of food such as firmness, springiness, cohesiveness and chewiness of gluten-free rice muffins, for instance, depend on seed protein content (Shevkani et al., 2015). Because cowpea seed protein content has high heritability with few genes, it provides the possibility of developing new high protein cowpea cultivars. Seed protein content is an important parameter in cowpea. Gathering data on this nutritional parameter is crucial, because doing so will help plant breeders choose cowpea breeding lines with high protein contents. The objective of this study was to assess the seed protein content in eleven cowpea genotypes.

Materials and Methods

Plant materials, field experiment, and seed sample preparation

Eleven cowpea genotypes with different seed colors developed by the University of Arkansas were used in this study for evaluation of total seed protein content. Included were 01—1781 (seed color: cream), 07—303 (red), 09—204 (brown eye), 09—208 (pink eye), 09—393 (pink eye), 09—655 (pink eye), 09—714 (pink eye), 09—741 (red Holstein), 'AR Blackeye #1' (black eye), Early Scarlet (pink eye), and 'Ebony' (black) (Table 1).

The field experiment was conducted in three different locations within Arkansas (Fayetteville, Alma, and Hope) in 2015. A randomized complete block design (RCBD) with two blocks was used for the experiment in each location.

In each plot, cowpea genotypes were planted in four rows 15 feet long, with three feet between rows. Plant spacing within row was four inches. During the growing season, no pesticides or herbicides were sprayed to control pests, diseases, and weeds. The irrigation was rain fed.

The cowpeas were harvested when 90% of pods were dried. The seeds of each cowpea genotype were bulk harvested. The pods were harvested and kept in clean and previously labeled paper bags. A total of 66 samples were collected from the 11 cowpea genotypes from three locations, with two blocks from each location. The pods of the 66 samples were dried and cleaned, then stored in a refrigerator. Before measuring seed protein content, each cowpea genotype was further selected for seeds with uniform size and without any insect damage. In order to have a sufficient quantity of seed for the protein analysis, approximately 100 g of seed from each sample were prepared.

Seed protein content evaluation

Cowpea seed content was measured by analyzing the percentage of Nitrogen by combustion using an Elementar Rapid N III instrument. The cowpea seeds were ground and the flour sifted using a sieve of 850 µm, and each sample was measured for protein content.

At high temperature and in presence of pure oxygen, nitrogen is liberated by combustion. The nitrogen is then isolated from other combustion products. A thermal conductivity detector measures the nitrogen content in the sample (Horneck and Miller, 1998). The percentage of nitrogen in each sample was provided, and the total protein content for each sample was estimated by times 6.25 % nitrogen (Moore et al., 2010).

Data analysis

Analysis of cowpea seed protein data was performed by analysis variance (ANOVA) using the general linear models (GLM) procedure of JMP Genomics 7 (SAS Institute, Cary, NC). For comparisons among genotypes, the student T-test was used to perform multiple comparisons for least square mean (LSM) protein content at $P = 0.05$. The mean, range, standard deviation (SD), standard error (SE) and coefficient of variation (CV) were estimated for seed protein content using ‘Tabulate’; and the distributions of protein content was also performed using ‘Distribution’ in JMP Genomics 7.

In the calculations, Y_{ijk}= value of the total seed protein content in the i^{th} location and the j^{th} block for the k^{th} cowpea genotype, for $i=1,2,3$ $j=1,2$ and $k=1,2,\ldots,11$. Because there were no replicates in each block, the block was treated as replicates in model for analysis.

The statistical model for the analysis was the following:

$$Y_{ijk}= \mu + L_i + G_k + LG_{ik} + \boldsymbol{\varepsilon}_{ijk}$$

Where μ: constant (overall mean), L_i: Effect of the i^{th} location (fixed effect) on the mean protein content, G_k: Effect of the k^{th} genotype (fixed effect) on the mean response, LG_{ik}: potential joint effect of the i^{th} location and the k^{th} genotype on the mean response, and $\boldsymbol{\varepsilon}_{ijk}$: experimental associated with the ijk^{th} observation.

The broad-sense heritability (H^2) was estimated using the formula.

$$H^2= (\sigma^2_G/ \sigma^2_P) \text{ x } 100= (\sigma^2_G/(\sigma^2_G+(\sigma^2_{GE}/l)+(\sigma^2_E/bl))) \text{ x } 100 \text{ (Holland, 2003)}$$

Where σ^2_G: Genotypic variance, σ^2_P: Phenotypic variance, σ^2_{GE}: Genotype X Location variance, σ^2_E: Variance associated with the experimental error, b: number of blocks within each location
l: number of locations

σ^2_G, σ^2_{GE}, and σ^2_E were obtained using the following formulas:

σ^2_G= (MSG-MSG x E)/bl, σ^2_{GE}= (MSG x E-MSE)/b, σ^2_E=MSE where MSG: Mean Square Genotype, MSG x E: Mean Square Genotype X Location, and MSE: Mean Square Error.

The estimates of MSG, MSG X E, and MSE were derived from the ANOVA table.

Results and Discussion

In this study, protein content was estimated by using the percentage of total nitrogen after combustion. However, there is evidence that not all nitrogenous compounds are protein so that the estimated protein would not correspond to the true protein content (Mariotti et al., 2008). Despite this limitation, the evaluation of crude protein in legume seeds has proven to be effective in germplasm evaluation, and breeding and genetics studies pertaining to protein content. In soybean, thanks to such a methodology, Warrington et al. (2015) reported four QTLs associated with crude protein. Similarly, Jadhav et al. (2015) identified 4 QTLS associated with crude protein in chickpea (*Cicer arietinum* L.) using NIR SpectraAlyzer® after calibrating the system with 30 genotypes using the combustion method.

The seed protein content averaged 25.4%, with a range from 23.7% to 27.4%, and had a standard deviation (Std) of 1.94% with 0.24% Std Error, indicating the seed protein content had large variation in the 11 cowpea genotypes (Figure .1).

Significant differences in protein content were observed among the 11 cowpea genotypes (Table 1). Early Scarlet had the highest seed protein content, with 27.4% dried seed weight; 09-204 was second highest (26.9%); and the two were not significantly different each other, but they had total protein content significantly higher than the other genotypes. 01-1781 was third highest in protein content with 25.9% dry seed weight and it was not significantly different from 09-393 (25.9%), 09-208 (25.5%), and 07-303 (25.2%). AR Blackeye #1, 09-714, and Ebony had the same total protein content, with 24.9% dry seed weight, which is significantly different from others but not from 09-655 (24.0%). 09-741 had the lowest seed protein content with 23.7% dried seed weight, but was not significantly different from 09-655 (24.0%) (Table 1).

Location effect was detected among the three locations (Table 2 and 3). Alma exhibited the highest seed protein content; Hope second; and Fayetteville the lowest, indicating the environment (Location) affected seed protein content in cowpea.

Significant differences were detected among the cowpea genotypes, location, and the interaction of genotype x location (Table 2), suggesting that significant genotype effects existed and genotype by environment effect existed.

From Table 2, MSE (Least Mean Square of Error) was 0.73; MSG X E (Least Mean Square Genotype by Location) was estimated 3.04; and the estimate of MSG (Least Mean Square Genotype) was 7.21. The estimate of the broad sense heritability H^2 for cowpea seed protein was 57.8% based on the eleven cowpea genotypes. This relatively low estimate of H^2 indicated that factors such as the location (environment) significantly affected seed protein in cowpea.

The genotypes Early Scarlet (27.4%) and 09-204 (26.9%) had the highest protein content among the eleven cowpea lines involved in this study. A significant difference was found in terms of total seed protein content between those lines (p-value < 0.0001). A study performed by Itatat et al. (2013) revealed that the genotype ‘Ife Bimpe’ had the highest seed protein content (24.95%) among the cowpea genotypes in their study. In addition, Boukar et al. (2011) evaluated protein content in cowpea germplasm from IITA (International Center of Tropical Agriculture) genetic resources unit and found an average of 24.7%. Therefore, Early Scarlet and 09-204 could be good parents for breeding purposes for high protein content in cowpea. With respect to the relatively narrow variability in crude protein content among the 11 cowpea genotypes, future study involving a large number genotypes would provide more consistent data for selecting parents for high protein content in cowpea. In addition, further study is needed in order to unravel the protein fractions existing within the cowpea seeds. In addition to the variability in protein content among cowpea cultivars, Gupta et al. (2014) showed that the prevalence of different protein fractions, mainly consisting of albumins, globulins, prolamins and glutelins, was variable among cowpea lines.

A significant genotype by location interaction (p-value = 0.0002) was found in this study. According to a study by Oluwatosin (1997), the environment accounts for 71 % of the variability in protein in cowpea. The results from the research performed by Bliss et al. (1973) also indicated a significant genotype by location interaction effect on protein content in cowpea. In addition, Ddamulira and Santos (2015) found that protein content in cowpea was significantly affected by the genotype X environment interaction. The environmental factor mainly consists of the soil type and the climate under which cowpea genotypes are planted. In addition, research pointed out that soil fertility along with fertilization have significantly affected the total protein content in cowpea. Sebetha and Modi (2015) reported that nitrogen fertilization have enhanced the crude protein content in cowpea. Farouk and Amany (2012) stated that cowpea crude protein was influenced by an increase in phosphorus supply.

Broad sense heritability is a commonly used parameter in plant breeding (Holland, 2003). The estimate of H^2 represents the proportion of phenotypic variance which is due to genetic effects (Nyquist and Baker, 1991). In this study, the estimate of such a parameter was 57.8%, which was medium, indicating that protein content can be inherited and can be selected for in the progeny. Inheritance was also affected by environment, which could be explained by the significant genotype X environment interaction. In addition, Noubissie et al. (2012) found that the broad sense heritability for seed protein content in common beans varied from 46 to 78%. A study conducted by Ajeigbe et al. (2008) indicated a broad sense heritability in cowpea ranging from 56 to 95%. Those results indicate that the estimate of the broad sense heritability dramatically varies among cowpea genotypes and the 57.8% protein content from the present study was reasonable.

Conclusion

Cowpea provides cheap protein for human consumption. Dried seeds can be cooked or transformed into flour for multiple purposes. In this study, significant genotype, location, genotype x location effects were found. The results indicate that protein content was significantly different among cowpea genotypes, and the environment also had significant effect on the total seed protein content in cowpea. The genotypes Early Scarlet (27.4%) and 09-204 (26.9%) had the highest protein content and can be used for breeding to enhance high protein content in cowpea. Further studies will be carried out to determine the different protein fractions in cowpea, and more lines will be evaluated for protein content.

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Table 5-1. Eleven cowpea lines and their multiple comparisons.

Cowpea genotype	**LSMean* protein content (%)**	**Significant at P=0.05**
Early Scarlet	27.4	A[#]
09-204	26.9	A
01-1781	25.9	B
09-393	25.9	BC
09-208	25.6	BCD
07-303	25.2	BCD
AR Blackeye #1	24.9	CDE
09-714	24.9	DE
Ebony	24.9	DE
09-655	24.0	EF
09-741	23.7	F

*LSMean signifies the Least Square Mean for each of the 11 cowpea genotypes, estimated from JMP Genomics. [#]Significant test of seed protein content of the 11 cowpea genotypes across three locations two replicates (blocks). The capital letters represent the statistical significance at P = 0.05 level.

Table 5-2. ANOVA for the total seed protein content among 11 cowpea genotypes.

Source	**DF**	**Sum of squares**	**Mean squares**	**F ratio**	**Prob > F**
Location	2	90.75	45.38	62.2753	<.0001
Genotype	10	72.11	7.21	9.897	<.0001
Genotype*Location	20	60.73	3.04	4.1674	0.0002
Error	30	21.86	0.73	--	--

Table 5-3. Multiple comparisons of the location effect.

Location	**LSMean* protein content (%)**	**Significant at P=0.05**
Alma	26.6	A[#]
Hope	25.8	B
Fayetteville	23.8	C

*LSMean signifies the Least Square Mean for each of the 11 cowpea genotypes, estimated from JMP Genomics. [#]Significant test of seed protein content of the 11 cowpea genotypes across three locations two replicates (blocks). The capital letters represent the statistical significance at P = 0.05 level.

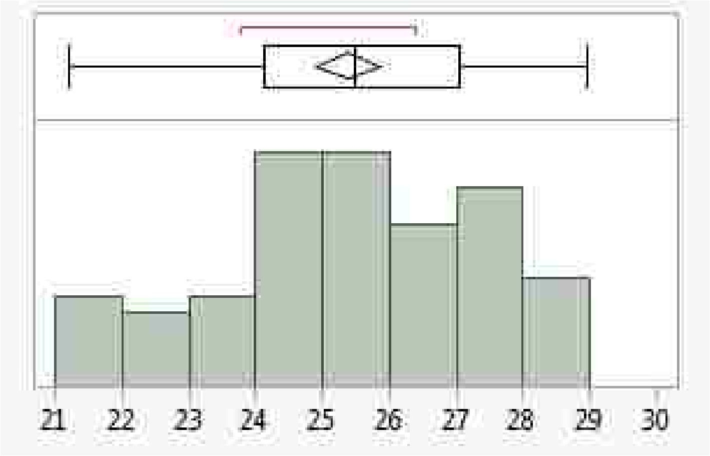

Figure 5-1. Distribution of the total seed protein content among 11 cowpea lines. X-axis presents the seed protein content percentage and the y-axis represents the number of observations.