



Drought tolerance indices and variability in leaf abscisic acid accumulation among accessions of cowpea (*Vigna unguiculata* L. Walp) under drought stress

A. T. AJAYI

¹Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria
Corresponding e-mail: toyin.ajayi@aaau.edu.ng

Abstract

Keywords:

ABA accumulation, tolerance indices, cowpea, variability

This study was done to assess the level of variability for drought tolerance in accessions of cowpea based on tolerance indices of plant height, dry root weight and accumulation of abscisic acid (ABA). The 23 accessions of cowpea from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were grown under well watered and drought stressed conditions at seedling stage for three weeks in a Completely Randomized Design (CRD) with three replicates. Drought was imposed at two weeks after planting for three weeks in the screen house. At 21 days after imposition of stress, terminal leaves of each plant from each pot were excised for ABA extraction and quantification. Data were also recorded for plant height and dry root weight and were used for drought tolerance indices estimation. Significant variability was observed among accessions under both conditions. Plant height (4.61 to 55.87%) and dry root weight (3.33 to 82.47%) were significantly reduced by drought stress in cowpea. Tolerance index based on plant height and dry root weight separated the 23 accessions into 5 groups of tolerance. There were contradictory responses among accessions for leaf ABA content under both conditions. Generally, accessions with leaf ABA of up to 15mol and above under control condition were all susceptible accessions except AC15, while the accessions with leaf ABA content of up to 15mol and above under drought stressed condition were mostly tolerant accessions except AC09. These contradictory responses suggest that the constitutive nature of ABA biosynthesis and the genes involved in the pathway of its synthesis in cowpea genotypes may depend on other factors present in the soil to be up regulated or down regulated and also on all traits which confer drought tolerance on plants which are also constitutive in nature rather than stress induced

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INTRODUCTION

Cowpea is an important food legume with protein content of between 20 – 25% making it the choice for quality nourishment for the people of the tropical and subtropical countries. It is also an essential component of the cropping system in these regions for its tremendous ability to suppress erosion and fix atmospheric nitrogen into the soil (Ajayi, *et al.*, 2018). It has a level of drought tolerance and more adapted to soils with diverse pH range compared to other legume crops. Despite these tremendous attributes of cowpea, its productivity can be severely limited by drought (Abdu Razakou *et al.*, 2013).

Drought is a condition of sustained moisture deficit in the soil significantly reducing crop growth, development and yield and; its occurrence has become more unpredictable due to the erratic nature of the present climatic condition (Ajayi *et al.*, 2018). Drought stress in cowpea has been found to trigger various physiological, biochemical and morphological changes in an attempt to avoid, tolerate or to escape its devastating effects. In many cases, combination of different tolerance mechanisms are adopted by crop plants at a time in order to cope with drought (Mitra, 2001; Agbicodo *et al.*, 2009). Still, there are many disadvantages associated with

all the mechanisms of coping with drought stress in plants as regards yield potential. Therefore, crop adaptation to water deficit must show a balance among avoidance, escape and tolerance while maintaining adequate yield (Agbicodo *et al.*, 2009). One of the first responses of crop to moisture deficit is stomata closure. Stomatal closure can limit water loss, unfortunately, this also restricts photosynthetic rate in crop plants.

Abscisic acid (ABA) accumulation in plant leaves has been implicated to be responsible for rapid stomatal closure under drought stress, thereby facilitating reduced transpiration, enhancement of water potential and prevention of turgor loss in leaf tissues (Wang and Huang, 2003). Abscisic acid is a phytohormone (though found in fungi and other organisms) that regulates physiological processes such as seed maturation, seed dormancy, and stress adaptation (Seo and Kochiba, 2002; Endo *et al.*, 2014). These physiological responses are triggered by the fluctuation of endogenous ABA levels in accordance with changing surroundings or developmental stimuli. Endogenous ABA levels are largely controlled by the balance between biosynthesis and catabolism (Endo *et al.*, 2014). The major role of ABA in plants is to alleviate stress, as it accumulates more when plants wilt and closing stomata in this regard.

The pathway for ABA biosynthesis in plant is now well understood and all the major genes involved in the biosynthesis pathway of the enzyme identified (Xiong and Zhu, 2003). Some of the genes involved in ABA biosynthesis and confer drought tolerance in cowpea including other genes outside ABA systems have been reported (Iuchi *et al.*, 1996b; Iuchi *et al.*, 2000; Matoset *et al.*, 2001). Iuchi *et al.* (2000) isolated two cDNA by differential screening, and discovered that these genes were significantly induced by drought stress and their mRNAs decreased significantly by rehydration after 10 hours. One of the cDNAs (CPRD65) had sequence homology with 9-cis-epoxycarotenoid dioxygenase found to be involved in ABA biosynthesis. Endogenous ABA was also induced by drought stress and results indicated that the gene responsible for 9-cis-epoxycarotenoid dioxygenase was mainly responsible for ABA biosynthesis in cowpea under drought stress.

Genotypic differences in drought tolerance and ABA concentration have been found to be correlated in

many annual crops and drought susceptible varieties have been reported to accumulate higher levels of ABA than drought resistant varieties in maize, spring wheat, and sorghum, while in soybean and millet, positive correlation between drought tolerance and ABA accumulation has been reported (Wang and Huang, 2003). In pearl millet, ABA accumulation was found to be higher under well watered condition in drought tolerant genotypes than in sensitive ones and the levels of ABA did not increase by drought stress. Transpiration rate was also found to be lower among the tolerant genotypes compared to the sensitive one (Kholova *et al.*, 2010). Despite this conflicting reports, accumulation of leaf ABA has been found to be effective in selection of genotypes for drought tolerance in crop species especially in cereals, meanwhile, not much information regarding this has been reported in cowpea. Screening that combine abscisic acid content with tolerance indices of quantitative traits in cowpea genotypes would be more effective for effective cowpea selection under drought stress.

The objectives of the study were to assess the relationships between leaf abscisic acid content and drought tolerance indices of plant height and root weight in different accessions of cowpea under drought stress.

MATERIALS AND METHODS

The experiments were conducted at the screen house of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko between Latitude 7.2° N, Longitude 5.44° E and Altitude 423M above sea level, Ondo State, Nigeria. The list of accessions are presented in Table 1.

Table 1: Names and codes of the 23 accessions of cowpea

S/N	ACCESSION	CODE
1	TVu-7362	AC01
2	TVu-185	AC02
3	TVu-199	AC03

4	Tvu-207	AC04
5	Tvu-224	AC06
6	Tvu-235	AC07
7	Tvu-236	AC08
8	Tvu-239	AC09
9	Tvu-241	AC10
10	IT98K-205-8	AC11
11	IT98K-555-1	AC12
12	Tvu-4886	AC13
13	Tvu-4866	AC14
14	Tvu-8660	AC15
15	Tvu-9225	AC16
16	Tvu-11986	AC17
17	Tvu-9256	AC18
18	Tvu-11979	AC20
19	IT97K-568-18	AC21
20	IT89K-288	AC22
21	IT96-610	AC23
22	IT81-994	AC24
23	IT89K-391	AC25

Drought treatment

The seeds of 23 accessions of cowpea were planted in plastic pots filled with 3.5kg of sandy loam soil without fertilizer in the screen house. After emergence, plants were thinned to three fairly uniform plants per pot with 3 pots per treatment (well watered and drought stressed) and three replications for each accession in a Completely Randomized Design (CRD). Each pot was watered with 250ml of water per day for two weeks, after which watering was stopped for the drought stressed condition for three weeks. At 21 days of drought stress, leaves were isolated for leaf abscisic acid (ABA) analysis. Plant height and root weight of each accession at 21 days of drought stress were also used for determination of drought tolerance index as described by (Fernandez, 1992; Al-Rawi, 2016): $(Y_s)(Y_p)/(\text{Grand mean of } Y_p)^2$; where Y_s = mean value under stressed condition and Y_p = mean value under well watered condition and higher values of the index indicated tolerant to drought stress. Based on the indices, accessions were ranked into different classes of tolerance. Plant height was measured with a meter rule. Roots of each accession were carefully removed from soil and rinsed carefully in water after which they were thoroughly air dried for two weeks. A sensitive weighing balance was used to determine the dry root weight. Data were subjected to statistical analysis

Abscisic acid (ABA) isolation and quantification

ABA was isolated from the fresh leaves of accessions following the procedure of Iuchi *et al.* (2000) with some modifications at the Center for Biocomputing and Drug Development Laboratory, Adekunle Ajasin University, Akungba-Akoko. The leaves from each accession were homogenized with 800 μ l of 80% aqueous methanol for 30 min at 4^oC across the 23 accessions per treatment. The mixture was centrifuged for 30 min at 16,000 x g. The supernatant was aspirated into new sterile 1ml Eppendorf tubes across board. Samples were analyzed using UV wavelength at 220 nm. ABA standard solution was determined photometrically using the mathematical model Absorption maximum (220 nm): extinction coefficient (25200) x I x C, where I = length in cm⁻¹, and C = concentration in mol⁻¹. Data were analyzed and the bar chart showing the concentration of leaf ABA was done on Graph pad prism platform (version 7.04, for Mac).

RESULTS AND DISCUSSION

The mean square values of plant height and root weight of accessions are presented in Table 2. Analysis of Variance (ANOVA) showed the existence of high significant differences ($P \leq 0.05$) among accessions for plant height and root weight under well watered and drought stress conditions. High significant differences among accessions for plant height and root weight in both conditions indicates the existence of sufficient level of genetic variability among accessions and possibility of selection for favourable accessions for drought tolerance.

Table 2: Mean square values of plant height and root weight of accessions of cowpea under drought stress

Source of variation	Accession	Error	CV (%)
	DF = 22	DF =46	
PH WW	57.49**	6.44	11.19
PH DS	32.76**	2.82	10.07
RTW WW	0.08**	0.01	22.22
RTW DS	0.13**	0.003	28.83

** : Significant at $P \leq 0.05$; DF: Degree of freedom; CV: Coefficient of variation; PH: Plant height; RTW: Root weight; WW: Well watered; DS: Drought stressed.

Variability for plant height and root weight

The mean values of plant height and root weight of the 23 accessions of cowpea under seedling are presented in Table 3. The highest plant height (31.33cm) under well watered condition (control) was obtained in AC22, while the lowest (13.67cm) was obtained in AC24. The highest plant height (24.93cm) under drought stressed condition was obtained in AC15 while the lowest (11.77cm) was obtained in AC17. Dry root weight was highest (0.97g) in AC13 under well watered condition (control), while the lowest value (0.20g) was obtained in AC01. The highest dry root weight (0.32g) under drought stressed condition was

obtained in AC21, while the lowest (0.07g) was obtained in AC10. Plant height among the accessions was significantly higher under the well watered condition compared to drought stressed condition except in AC15 and AC24 which had higher values for plant height under the drought stressed condition. Reduction of plant height by drought stress ranged from 4.61% in AC21 to 55.87% in AC17. Drought stress also significantly reduced root dry weight from 3.33% in AC24 to 82.47% in AC13. All accessions had reduced root dry weight under drought stress compared to well water condition. These results agree with the findings of Abdou Razakou *et al.* (2013) and Farnia and Tork (2015) who reported that drought stress significantly reduced plant height and root mass in cowpea and wheat respectively. Drought stress is considered as one of the most important growth-limiting factors that decrease plant growth (Tatrai *et al.*, 2016).

Table 3: Mean values for plant height and root weight of accessions of cowpea under well watered (WW) and drought stressed (DS) conditions

ACCESSION	PH WW (cm)	PH DS (cm)	REDUCTION (%)	RTW WW (g)	RTW DS (g)	REDUCTION (%)
AC01	20.00±1.53 ^{cde}	16.67±1.45 ^{de}	16.65	0.20±0.05 ^a	0.15±0.02 ^{abcd}	25.00
AC02	15.10±0.06 ^{ab}	14.07±0.07 ^{abc}	6.82	0.35±0.00 ^{abcd}	0.22±0.00 ^{cdef}	37.14
AC03	19.67±2.18 ^{bcde}	13.07±0.07 ^{ab}	33.55	0.43±0.37 ^{bcdef}	0.18±0.00 ^{bcde}	58.14
AC04	25.33±0.67 ^{ghi}	14.00±0.00 ^{abc}	44.73	0.42±0.58 ^{bcdef}	0.13±0.00 ^{abc}	69.05
AC06	21.67±1.20 ^{cdefg}	17.00±1.00 ^{cde}	21.55	0.33±0.01 ^{abc}	0.24±0.03 ^{defg}	27.27
AC07	25.33±0.67 ^{ghi}	19.17±0.60 ^{efg}	24.32	0.49±0.03 ^{def}	0.26±0.06 ^{efg}	46.94
AC08	27.33±0.88 ^{ij}	18.00±0.58 ^{def}	34.14	0.69±0.07 ^g	0.14±0.02 ^{abc}	79.71
AC09	23.67±2.40 ^{defghi}	13.33±1.45 ^{ab}	43.68	0.31±0.07 ^{ab}	0.09±0.00 ^{ab}	70.97
AC10	24.33±1.20 ^{efghi}	20.60±0.56 ^{fg}	15.33	0.43±0.06 ^{bcdef}	0.18±0.02 ^{bcde}	58.14
AC11	22.33±0.67 ^{cdefgh}	19.47±0.73 ^{efg}	12.81	0.68±0.01 ^g	0.15±0.03 ^{abcd}	77.94
AC12	19.00±0.58 ^{bcd}	15.33±0.67 ^{abc}	19.32	0.33±0.03 ^{abc}	0.22±0.02 ^{cdefg}	33.33
AC13	20.33±2.00 ^{cdef}	15.50±0.60 ^{abc}	23.76	0.97±0.01 ^h	0.17±0.04 ^{abcde}	82.47
AC14	25.00±0.57 ^{fghi}	13.33±1.47 ^{ab}	46.68	0.40±0.08 ^{bcde}	0.13±0.01 ^{abc}	67.5
AC15	19.00±1.00 ^{bcd}	24.93±1.83 ^h	-31.21	0.38±0.04 ^{bcde}	0.30±0.08 ^{fg}	21.05
AC16	28.00±2.65 ^{ij}	14.63±0.35 ^{abc}	40.61	0.42±0.05 ^{bcdef}	0.15±0.01 ^{abcd}	64.29
AC17	26.67±1.20 ^{hi}	11.77±0.27 ^a	55.87	0.49±0.02 ^{def}	0.16±0.00 ^{abcde}	67.35
AC18	26.67±1.33 ^{hi}	15.07±0.64 ^{abc}	43.49	0.32±0.05 ^{ab}	0.07±0.01 ^a	78.13
AC20	26.33±0.33 ^{ghi}	16.83±0.73 ^{cde}	36.08	0.56±0.06 ^{fg}	0.16±0.02 ^{abcde}	71.43
AC21	18.00±0.58 ^{abc}	17.17±0.09 ^{cde}	4.61	0.42±0.01 ^{bcdef}	0.32±0.00 ^g	23.81
AC22	31.33±2.40 ^j	21.03±1.02 ^{fg}	32.88	0.38±0.04 ^{bcde}	0.16±0.03 ^{abcde}	57.89
AC23	24.00±2.08 ^{efghi}	21.33±2.40 ^g	11.13	0.49±0.06 ^{cdef}	0.21±0.05 ^{cdef}	57.14
AC24	13.67±0.33 ^a	18.17±0.09 ^{def}	-32.92	0.30±0.03 ^{ab}	0.29±0.00 ^{fg}	3.33
AC25	18.67±0.33 ^{bc}	12.90±0.74 ^{ab}	30.91	0.54±0.07 ^{ef}	0.19±0.01 ^{bcde}	64.81

Means with the same superscript within a column are not significantly different from one another at $P < 0.05$ using Duncan Multiple Range Test (DMRT). PH: Plant height; RTW: Root weight; WW: Well watered; DS: Drought stressed.

Variability for leaf abscisic acid accumulation

The results of abscisic acid accumulation for the 23 accessions under drought stress for 21 days are presented in Figure 1. Across the two conditions (well watered and drought stressed), four of the tolerant accessions (AC10, AC23, AC15 and AC21) had higher leaf ABA under well watered condition (control) compared to drought stressed condition, which is in line with the findings of Kholova *et al.* (2010) in pearl millet, and on the contrary, the remaining five tolerant accessions (AC20, AC11, AC08, AC07 and AC13) had lower leaf ABA under control compared to drought stressed condition and one accession (AC22) was the same for leaf ABA content in both conditions. All accessions with higher leaf ABA content under control had mostly moderate tolerant index for root weight. Five of the susceptible accessions (AC02, AC03, AC09, AC18 and AC25) had higher leaf ABA content under drought stressed condition compared to their control, which is also in line with the findings of Wang and Huang (2003) in Kentucky bluegrass and Kholova *et al.* (2010) in pearl millet, while the four remaining susceptible

accessions (AC24, AC01, AC14 and AC04) had higher leaf ABA content under control condition compared to the drought stressed condition in contradiction to the findings of Wang and Huang (2003) and Kholova *et al.* (2010), whereas AC12 was the same for leaf ABA content under both conditions. All moderately tolerant accessions (AC06, AC16 and AC17) had higher leaf ABA under drought stressed condition compared to their respective control. Generally, accessions with the highest leaf ABA of up to 15mol and above under control condition were all susceptible accessions except AC15, while the accessions with leaf ABA content of up to 15mol and above under drought stressed condition were mostly tolerant accessions except AC09. Previous reports in cowpea (Iuchi *et al.*, 2000) and other species (Asch *et al.*, 1995; Chandrasekar *et al.*, 2000; Li and Wang, 2003; Yin *et al.*, 2005; Zhang *et al.*, 2005) indicated that ABA content was significantly increased under drought stress conditions seem to contradict the responses of AC22 and AC12, which were the same for leaf ABA content under both control and drought stressed conditions in this study.

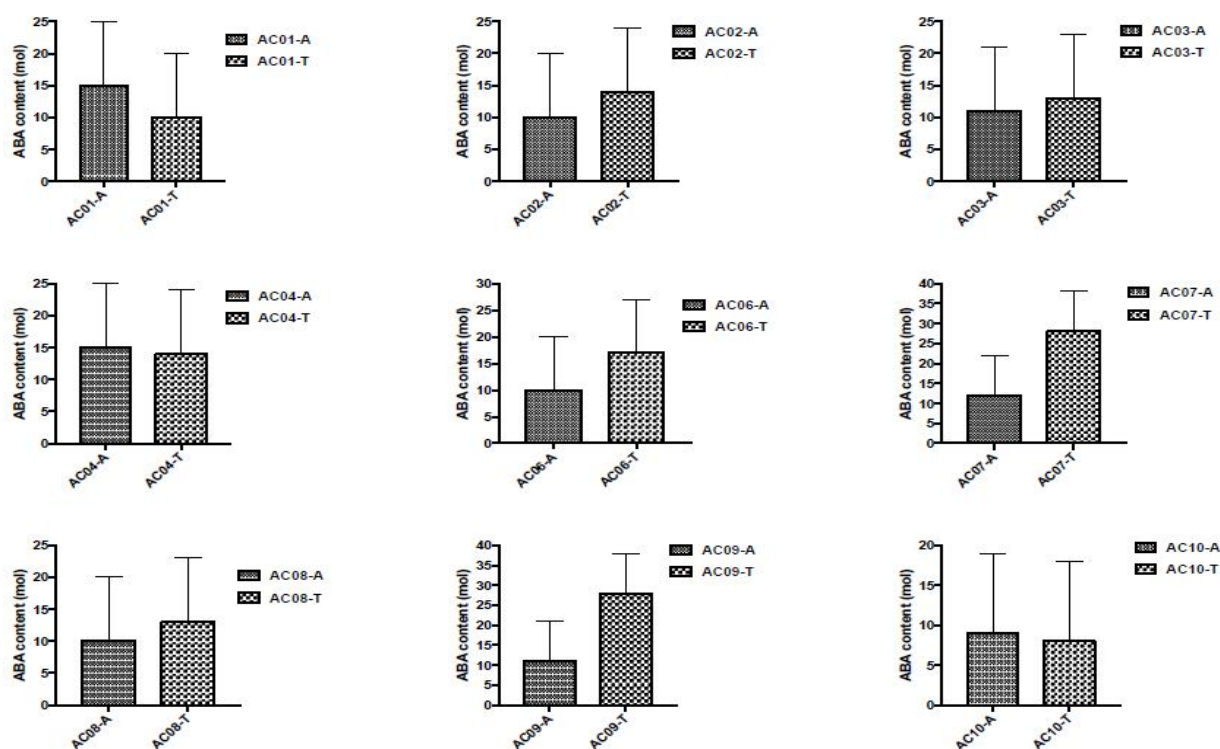


Figure 1: Variability among accessions of cowpea for leaf ABA content under well watered (A) and drought stressed (T) conditions

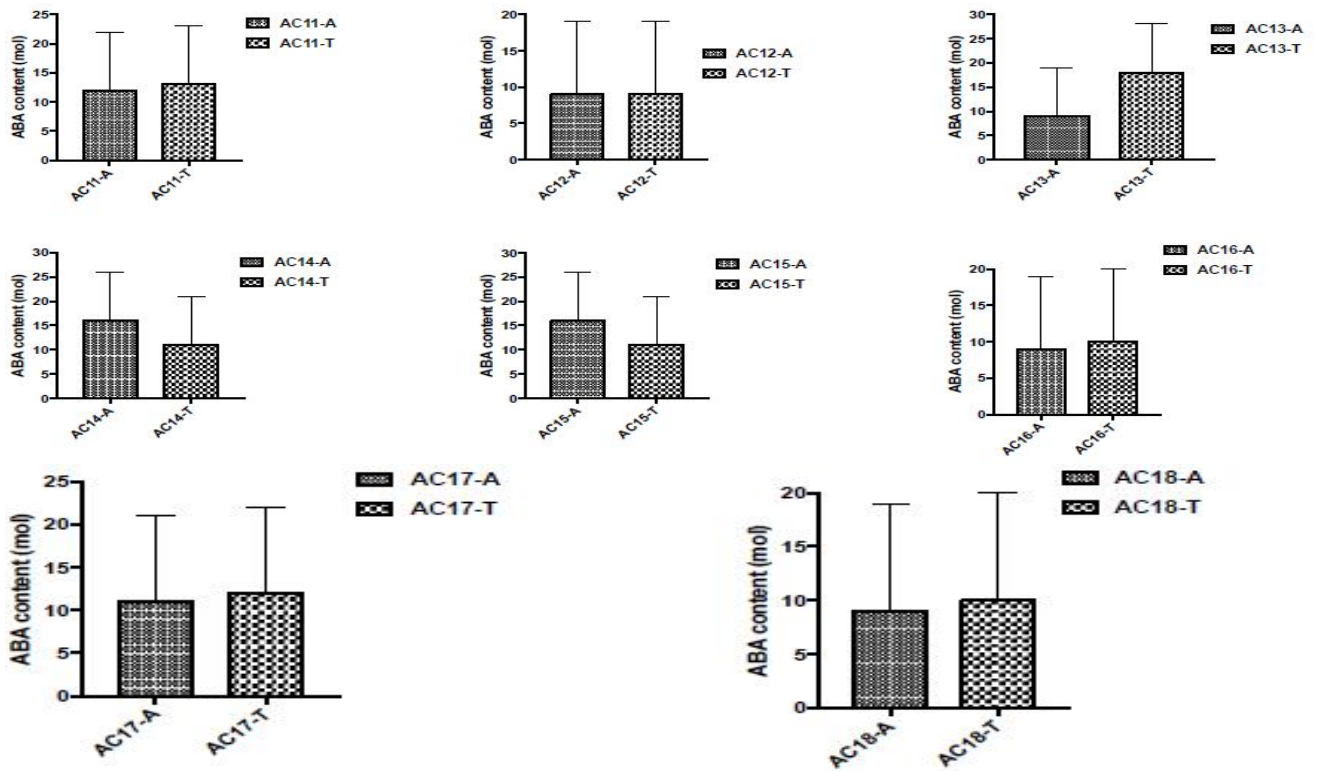


Figure 1 cont'd.

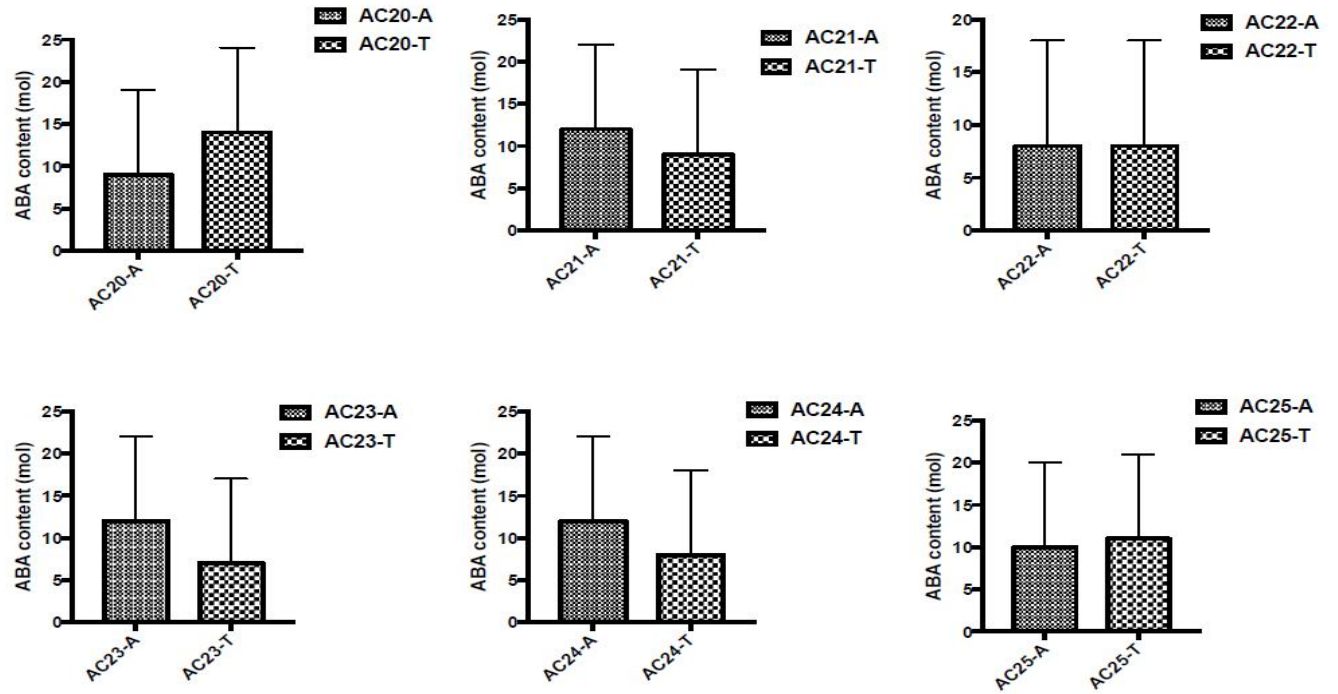


Figure 1 cont'd.

Contradictory responses of the cowpea accessions to accumulation of leaf ABA under control and drought stressed conditions suggest that the constitutive nature of ABA biosynthesis and the genes involved in the pathway of its synthesis in cowpea genotypes demands that their up regulation or down regulation may depend on other factors present in the soil (salinity or low temperature) (Xiong and Zhu, 2003) and also on many traits that confer drought tolerance on plants which include phenology, root size and depth, hydraulic conductivity and storage of reserves, which are associated with plant development and also constitutive in nature rather than stress induced support the findings of this research as suggested by Chaves *et al.* (2002) and Otowe (2007). Also, since the leaf water potential of these accessions was not taken into consideration during the findings, assessment of the leaf ABA across accessions could have been at different leaf water potentials, hence the contradictory responses of accessions even though they were subjected to the same level of stress under the same condition as suggested by Kholova *et al.* (2010).

The results of this study showed that relationships exist between drought tolerance indices and leaf ABA content and the genotypic differences among accessions for ABA could be used for selection under drought stress. The response of agricultural crops to drought stress, and the relationship between drought tolerance and levels of endogenous ABA in these crops have not been studied extensively, hence further attention is needed in this regard (Wan and Li, 2006), especially in cowpea.

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