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Variable Expression of the Candidate Gene *NCED1* Among Cowpea Accessions under Different Drought Stress Conditions

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ABSTRACT

Drought significantly reduces cowpea productivity. Information on genetic variation for differential expression of candidate genes for drought tolerance among cowpea genotypes, from which improvement plan could be drawn is limited in Nigeria. Variability of expression of the candidate gene *NCED1* in cowpea was analyzed under different drought stress conditions. Primers based on *NCED1* and *P-Actin* (used as an internal control) successfully amplified products from both stressed and unstressed accessions of cowpea. Contradictory responses were observed among drought-tolerant (mean STI > 0.57) and susceptible accessions (mean STI < 0.57). *NCED1* was significantly repressed by drought stress in all accessions, except in AC10, AC11, AC13 (tolerant accessions), and AC12 (susceptible accession). The results from stressed and unstressed conditions confirmed that the gene is expressed in both conditions. Biplot divided the accessions into four major groups, with most of the tolerant accessions in groups I and II, while most of the susceptible accessions occupied III and IV. Tolerant accessions such as AC22, AC15, AC23, AC13, AC10, AC11, and AC21 that combined higher plant height and dry root weight under drought stress with stress tolerance indices (STIs) possessed higher gene expression under both control and drought stress conditions. Therefore, positive correlations between the expression of the gene in both conditions and plant height under stress, on one hand, dry root weight under stress on the other hand, and the STIs confirm that its expression may be involved in drought tolerance of cowpea. Hence, the selection of cowpea based on higher levels of gene expression among accessions under both conditions may be effective for breeding drought-tolerant cowpea.

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Introduction

Cowpea plays a key role in both the cropping systems and the nutrition of the tropical and sub-tropical regions of the world (Ajayi *et al*., 2018). This fact has made it very popular among the people of these regions (Ajayi *et al*., 2017a). Drought is one of the factors stunting the productivity of cowpea, hence reducing yield significantly in the crop. Various stages of growth and metabolic processes are affected by drought; and this has become a major concern to plant breeders (Ishiyaku and Yilwa, 2009; Sabiel *et al*., 2014; Ajayi *et al*., 2017b). Extent and degree of drought occurrence in crop species coupled with the developmental stage of occurrence have a devastating effect on growth and yield; and more destructive would effect of drought be if its occurrence coincides with seed germination, seedling growth, root growth, development, and flowering (Ajayi *et al*., 2017a).

Several methods have been adopted to measure the level of drought tolerance in cowpea and

other crop species. Traits such as morphological, physiological, biochemical, and molecular (Ajayi *et al*., 2018) have been adopted and genotypic differences for their expression among genotypes used for selection under drought stress. Differential expression of candidate genes under drought stress has also been one of the techniques adopted to study the mechanism of tolerance in cowpea and in other crop species (Iuchi *et al*., 2000; Diop *et al*., 2004; Agbicodo *et al*., 2009; Contour-Ansel *et al*., 2010; Muchero *et al*., 2010; Leite *et al*., 2014), unfortunately, this information among cowpea accessions is limited in Nigeria. However, more information on the genetic diversity of cowpea based on differential expression of candidate genes under drought stress and unstressed conditions will contribute significantly to effective cowpea selection for drought tolerance.

Certain of the genes involved in ABA biosynthesis and confer drought tolerance in cowpea including other genes external to ABA schemes have been reported (Iuchi *et al*., 1996; Maarouf *et al*., 1999; Iuchi *et al*., 2000; Matos *et al*., 2001; Diop *et al*., 2004; Gazendam and Oelofse, 2007). Iuchi *et al*. (2000) discovered two complementary DNA by differential screening and discovered that these genes were significantly drought stress-induced and their mRNAs decreased significantly after 10 hours of rehydration. One of the cDNAs (*NCED1*) named *VuNCED1* (CPRD65) had sequence homology with 9-cis-epoxycarotenoid dioxygenase found to be involved in ABA biosynthesis (Carvalho *et al*., 2017). Endogenous ABA was correspondingly induced by dehydration pressure and results indicated that the gene responsible for 9-cis-epoxycarotenoid dioxygenase was mainly responsible for ABA biosynthesis in cowpea suffering desiccation stress (Iuchi *et al*., 2000). Other crops in which expression of *NCED1* in conjunction with or without ABA accumulation have been characterized under drought stress include tomato mutants (Muñoz-Espinoza, *et al*., 2015) with no correlated relationship with ABA synthesis, soybean (Zhang *et al*., 2017), rice (Changan *et al*., 2018) with a negative relationship with ABA and drought stress, *Aristotelia chilensis* (González-Villagra *et al*., 2018), grapevine (He *et al*., 2018) with a positive relationship with ABA and tobacco (Chen *et al*., 2019). Regulation of stomata closure under pathogen invasion has also been linked to the expression of the *NCED1* gene and ABA accumulation in tomatoes (Du *et al*., 2014).

Although few reports exist on the expression of the *NCED1* gene in cowpea genotypes, none exists based on its differential expression under unstressed and drought stress conditions in Nigeria. Therefore, the objective of the present study was to assess the variations for differential expression of the candidate gene *NCED1* under drought and unstressed conditions in cowpea accessions. A part of the data involving the grouping of the accessions into different classes of drought tolerance based on stress tolerance indices (STI) of plant height and dry root weight and abscisic accumulation has been reported in Ajayi (2019). Further characterization of the accessions based on the relationships among drought tolerance indices (DTIs) of seed yield viz.; stress tolerance index (STI), yield index (YI), geometric mean productivity (GMP), mean productivity (MP), yield stability index (YSI) and drought resistance index (DRI) and seed yield have also been reported (Ajayi, 2020).

Materials and Methods

Experiments were conducted to evaluate the variations among 25 accessions of cowpea (Table 1) for expression of the candidate gene *NCED1* at the screen house of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The whole setup was conducted between January and March 2017.

Drought treatment

The seeds of 25 accessions were planted in plastic pots filled with 7 kg of sieved sandy loam soil without fertilizer in the screen house. After emergence, plants were thinned to three fairly uniform plants per pot with three pots per treatment (well-watered and drought-stressed) in three replications for each accession in a Completely Randomized Design (CRD). Each pot was watered with 500 ml of water per day for 3 weeks, after which watering was stopped for the drought-stressed condition for 10 days, while watering was maintained daily in the control condition until the end of the experiment.

Table 1. List of accessions of cowpea used for variable expression of *NCED1* under control (A) and drought stress (T) conditions.

Code	Accession name	Genebank ID	Biological status	Origin	DOI
AC01	TVu-7362	114096	Landrace	Ghana	10.18730/T6SG
AC02	TVu-185	108245	Landrace	Nigeria	10.18730/W9YK
AC03	TVu-199	108259	Breeding material	USA	10.18730/WAC
AC04	TVu-207	108267	Breeding material	USA	10.18730/WAM4
AC05	TVu-218	108278	Breeding material	USA	10.18730/WAZF
AC06	TVu-224	108284	Breeding material	USA	10.18730/WB5N
AC07	TVu-235	108295	Breeding material	Ghana	10.18730/WBG*
AC08	TVu-236	108296	Breeding material	Ghana	10.18730/WBH
AC09	TVu-239	108299	Breeding material	South Africa	10.18730/WBMU
AC10	TVu-241	108301	Breeding material	USA	10.18730/WBP1
AC11	IT98K-205-8	Unknown	Unknown	Nigeria	Unknown
AC12	IT98K-555-1	Unknown	Unknown	Nigeria	Unknown
AC13	TVu-4886	111947	Landrace	Niger	10.18730/ZXMN
AC14	TVu-4866	111927	Landrace	Niger	10.18730/ZX01
AC15	TVu-8660	115320	Landrace	Benin	10.18730/VD1K
AC16	TVu-9225	115884	Landrace	Tanzania	10.18730/VYNW
AC17	TVu-11986	118548	Landrace	Sudan	10.18730/PQXM
AC18	TVu-9256	115915	Landrace	Burkina Faso	10.18730/VZMP
AC19	TVu-9252	115911	Landrace	Burkina Faso	10.18730/VZGJ
AC20	TVu-11979	118541	Landrace	Sudan	10.18730/PQPD
AC21	IT97K-568-18	Unknown	Landrace	Nigeria	Unknown
AC22	IT89K-288	Unknown	Unknown	Nigeria	Unknown
AC23	IT96-610	Unknown	Unknown	Nigeria	Unknown
AC24	IT81-994	Unknown	Unknown	Nigeria	Unknown
AC25	IT89K-391	Unknown	Unknown	Nigeria	Unknown

On the 10th day of drought stress, leaves were collected in the drought stress and control conditions for RNA extraction.

Gene expression profiling

Fresh middle leaflets (of the terminal leaf) following the order of accession per treatment were collected into accession labeled Eppendorf tubes from the screen house. On the arrival at the laboratory, 50 µl of RNA Snap reagent was dispensed into the tubes across the board. Homogenization of samples was performed. Altogether, samples were incubated in the water bath at 65 ^{0}C and mixed by inversion every 10 min for 30 min. After incubation, all the samples were spun using a centrifuge at 16,000 rpm for 30 min. 50 µl of the supernatant was carefully aspirated into new, sterile, well-labeled, in the order of accession Eppendorf tubes.

Five microliters (5 µl) of 3 M sodium acetate (pH 5.3) were added and mixed gently by inversion. The amount of 700 µl of absolute chilled ethanol was added across the board, mixed by inversion, and was placed in the freezer for 1 hr. After the incubation, all samples were spun in the centrifuge at 16,000 rpm for 30 min to pellet the RNA. The supernatant was carefully decanted and the RNA pellet was washed by centrifugation at 16,000 rpm for 5 min in 70% ethanol (twice).

The supernatant was carefully decanted and the RNA pellet was air-dried across the board for 15 min at room temperature. All RNA pellets across the board were suspended in 50 µl nuclease-free, sterile water (VWR LIFE SCIENCE, Lot NO: 0596C320, Code: E476-500ML).

RNA yield and purity were then determined via spectrophotometry. Before cDNA conversion, RNA samples were diluted to 100 ng concentration using nuclease-free, sterile water. MMLV Reverse Transcriptase 1st-strand cDNA synthesis Kit (NEB) was used for RNA-cDNA

conversion according to the manufacturer's instruction.

Exponential amplification (PCR) for the determination of candidate genes whose primer (self-designed with the SnapGene software) are listed in Table 2 (Plant actin gene was used as control) was done using the following pipeline: PCR amplification was performed in a total mixture reaction volume of 10 µl for each cDNA accession. The reaction mixture containing 2 µl template (cDNA), 3 µl of nuclease-free, sterile water, 1 µl of primer pair (0.5 µl from the forward primer and 0.5 µl from the reverse primer), and 4 µl of ready Mix Taq® PCR Master Mix (2x) (inqaba biotech, Code: NEBM0482S). Amplification conditions were: Pre-denaturation at 94^0c for 5 min, Denaturation at 94^0C for 30 sec., Annealing at 55^0C for 30 sec., and Extension at 72^0C for 30 sec then 5 min at 72^0C by 35 cycles.

Table 2. Primer sequence of the candidate genes used for differential expression in cowpea under control (A) and drought stress (T) conditions.

S/N	Target genes	Forward 5' - 3'	Reverse 3' - 5'
1	*NCED1*	GATAAGGCTGAACTTAAGGA	TACAGTAAACCGTAACACAT
2	*P-Actin*	TGCCAAGAACAGCTCCTCAG	GAAGCACTTCCTGTGGACGA

Gel electrophoresis and image processing

Assessment of Real-Time Polymerase Chain Reaction (RT-PCR) products (amplicons) were electrophoresed in 0.5% agarose gel utilizing 0.5X TBE buffer (2.6 g Tris base, 5.0 g Tris boric acid plus 2 ml 0.5 M EDTA and adjusted to pH 8.3 with the sodium hydroxide pellet) with 0.5 µl ethidium bromide (as a fluorescent tag). The expression products were visualized as bands by UV-transilluminator. In-gel expression bands were captured using the iPhone-5c camera (Noir effect). Gel image post-processing was done on the Keynote platform on the MacBook Pro OS computer.

Determination of stress tolerance indices

Part of the data on stress tolerance indices based on plant height and dry root weight have been reported in Ajayi (2019). Plant height and dry root weight of one vigorous plant of each accession per pot in each replicate were used for determination of stress tolerance index (STI) as described by (Al-Rawi, 2016): (Ys)*(Yp)/(Grand mean of Yp)^2; where Ys = mean value under the stressed condition and Yp = mean value under the control condition and higher values of the index indicated tolerant to drought stress. Based on the indices, accessions were grouped into different classes of tolerance. The Meter rule was utilized in plant height measurement. Roots of the same set of plants were carefully removed from the soil, rinsed carefully in tap water, and were air-dried in the laboratory for two weeks.

The dry root weight was determined by a sensitive weighing balance. Further characterization of these accessions under drought stress was performed between March and June 2017, utilizing the relationships among leaf relative water content (RWC), seed yield and yield contributing traits under different drought conditions, and drought tolerance indices (DTIs) of seed yield as published in Ajayi (2020), available online.

Statistical analysis

The densitometric analysis was done using Image-J software (2.3.0 V, Mac version) and finally, the bar chart showing the gene expression of the target genes was done on the GraphPad Prism platform (version 7.04, for Mac). Means were analyzed in triplicates using a two-way analysis of variance (ANOVA), where treatment and accession were the factors utilizing GraphPad. SPSS program (Version 20) was used for analyzing the morphological data.

The biplot was constructed based on the relative expression of the gene under the stressed and unstressed conditions, morphological traits, and STIs with Paleontological Statistics software (Version 4.01).

Results

Expression of *NCED1* gene

High significant differences ($P < 0.0001$) were observed among the cowpea accessions for expression of *NCED1* gene; treatment and the interaction between drought treatment and accessions were also highly significant (Table 3). Expression patterns of the *NCED1* gene and the gel picture in response to drought stress amongst

cowpea accessions are presented in Figs. 1 and 2, respectively. *NCED1* was expressed in well-watered condition (control) for all accessions and was repressed by drought stress in most accessions excluding AC10, AC11, AC12, AC13, and AC14.

Table 3. Analysis of variance for *NCED1* expression profile under control (A) and drought stress (T) conditions.

Source of variation	DF	TSS	MS	P-value	P-value summary
Interaction	24	239576	9982	P<0.0001	****
Accession	24	308201	12842	P<0.0001	****
Treatment	1	207578	207578	P<0.0001	****
Residual	100	311.40	3.114		

****: Highly significant. DF: Degree of freedom; SS: Sum of square values; MS: Mean square values.

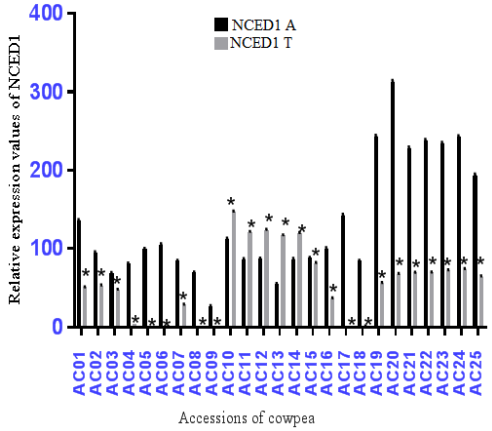

Fig. 1. Expression patterns of *NCED1* gene for accessions of cowpea under control (A) and drought-stressed (T) conditions. AC01- AC25 are accessions of cowpea. Each bar represents the mean value ± standard error (n = 3, where n represents the number of replicates). Means marked with '*' indicate significant differences between treatments at P < 0.0001 according to Tukey's multiple comparisons test.

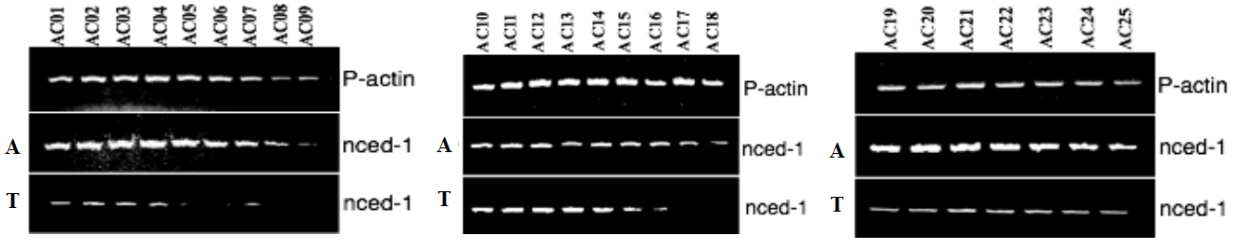

Fig. 2. Gel image of expression patterns of *NCED1* and *P-Actin* genes for accessions of cowpea under control (A) and drought-stressed (T) conditions. *P-Actin* gene was used as the internal control. AC01 – AC25 are accessions of cowpea.

All susceptible accessions experienced repressed expression of *NCED1* gene under drought stress except for AC12 which experienced higher expression of the gene under drought stress compared to control. All accessions with the highest level of drought tolerance experienced repression of *NCED1* gene under drought stress except for AC10, AC11, and AC13. All moderately tolerant accessions experienced repressed expression of *NCED1* under drought stress. Percentage repression ranged between 7.77% in AC15 to 99.90% in AC08, while percent increase ranged between 30.45% in AC10 and 111.91% in AC13.

Analysis of variance (ANOVA) for plant height and dry root weight indicated a significant effect ($P \leq 0.05$) of accession, treatment, and the trait interaction (Ajayi, 2019). Table 4 presents the mean performance of the accessions as grouped according to stress tolerance index (STI) of plant height and dry root weight (> 0.57 as drought-tolerant; < 0.57 as drought susceptible). Drought

stress reduced the plant height from -32.92 (AC24) to 4.61 percent (AC21), and the dry root weight from 82.47 (AC13) to 3.33 percent (AC13). The mean STIs of thirteen accessions were above 0.57 (the grand mean of STIs) with their plant height mostly less affected by drought stress. However, STIs were not determined for AC19 and AC05 for their inadequate number of plants (due to poor germination and growth) during the experiment from which collection of data on morphological traits could be made.

Table 4. Mean performance for plant height and root weight of accessions of cowpea under control (A) and drought stress (T) conditions.

ACC	PH-A	PH-T	RED (%)	RTW-A	RTW-T	RED (%)	PHSTI	RTWSTI	MEAN	Tolerant status
AC01	20.00^{cde}	16.67^{cde}	16.65	0.20^{a}	0.15^{a-d}	25.00	0.65	0.15	0.40	Susceptible
AC02	15.10^{ab}	14.07^{abc}	6.82	0.35^{a-d}	0.22^{c-f}	37.14	0.41	0.38	0.40	Susceptible
AC03	19.67^{b-e}	13.07^{ab}	33.55	0.43^{b-f}	0.18^{b-e}	58.14	0.50	0.38	0.44	Susceptible
AC04	25.33^{ghi}	14.00^{abc}	44.73	0.42^{b-f}	0.13^{abc}	69.05	0.69	0.27	0.48	Susceptible
AC05	-	-	-	-	-	-	-	-	-	-
AC06	21.67^{c-g}	17.00^{cde}	21.55	0.33^{abc}	0.24^{d-g}	27.27	0.72	0.39	0.56	Moderately tolerant
AC07	25.33^{ghi}	19.17^{efg}	24.32	0.49^{def}	0.26^{efg}	46.94	0.94	0.63	0.79	Tolerant
AC08	27.33^{ij}	18.00^{def}	34.14	0.69^{g}	0.14^{abc}	79.71	0.96	0.48	0.72	Tolerant
AC09	23.67^{d-i}	13.33^{ab}	43.68	0.31^{ab}	0.09^{ab}	70.97	0.61	0.14	0.38	Highly susceptible
AC10	24.33^{e-i}	20.60^{fg}	15.33	0.43^{b-f}	0.18^{b-e}	58.14	0.98	0.38	0.68	Tolerant
AC11	22.33^{c-h}	19.47^{efg}	12.81	0.68^{g}	0.15^{a-d}	77.94	0.85	0.50	0.68	Tolerant
AC12	19.00^{bcd}	15.33^{abc}	19.32	0.33^{abc}	0.22^{c-g}	33.33	0.57	0.36	0.47	Susceptible
AC13	20.33^{c-f}	15.50^{abc}	23.76	0.97^{h}	0.17^{a-e}	82.47	0.61	0.81	0.71	Tolerant
AC14	25.00^{f-i}	13.33^{ab}	46.68	0.40^{b-e}	0.13^{abc}	67.5	0.65	0.26	0.46	Susceptible
AC15	19.00^{bcd}	24.93^{h}	-31.21	0.38^{b-e}	0.30^{fg}	21.05	0.92	0.56	0.74	Tolerant
AC16	28.00^{ij}	14.63^{abc}	40.61	0.42^{b-f}	0.15^{a-d}	64.29	0.79	0.31	0.55	Moderately tolerant
AC17	26.67^{hi}	11.77^{a}	55.87	0.49^{def}	0.16^{a-e}	67.35	0.61	0.39	0.50	Moderately tolerant
AC18	26.67^{hi}	15.07^{abc}	43.49	0.32^{ab}	0.07^{a}	78.13	0.78	0.11	0.45	Susceptible
AC19	-	-	-	-	-	-	-	-	-	-
AC20	26.33^{ghi}	16.83^{cde}	36.08	0.56^{fg}	0.16^{a-e}	71.43	0.86	0.44	0.65	Tolerant
AC21	18.00^{abc}	17.17^{cde}	4.61	0.42^{b-f}	0.32^{g}	23.81	0.60	0.66	0.63	Tolerant
AC22	31.33^{j}	21.03^{fg}	32.88	0.38^{b-e}	0.16^{a-e}	57.89	1.28	0.30	0.79	Tolerant
AC23	24.00^{e-i}	21.33^{g}	11.13	0.49^{c-f}	0.21^{c-f}	57.14	0.99	0.51	0.75	Tolerant
AC24	13.67^{a}	18.17^{def}	- 32.92	0.30^{ab}	0.29^{fg}	3.33	0.48	0.43	0.46	Susceptible
AC25	18.67^{bc}	12.90^{ab}	30.91	0.54^{ef}	0.19^{b-e}	64.81	0.47	0.51	0.49	Susceptible
GM STI									0.57	

Means with the same superscript within a column are not significantly different from one another at $P \leq 0.05$ using Duncan Multiple Range Test (DMRT). ACC: Accessions of cowpea; RED: Percentage reduction. PH-A: Plant height under control; PH-T: Plant height under drought stress; RTW-A: Dry root weight under control; RTW-A: Dry root weight under drought stress; PH STI: Stress tolerance index of plant height; RTW STI: Stress tolerance index of dry root weight.

Bi-plot of principal component analysis of traits, STIs, and expression *NCED1* gene

The relationships between accessions, morphological traits, and STIs are shown in the biplot (Fig. 3) with PCs 1 and 2 contributing 59.19 percent of the total variation. PC 1 is majorly the *NCED1* expression and dry root weight under the stress axis, while PC 2 is more of plant height under the control axis. AC22, AC23, and AC15 are vertex accessions among the drought-tolerant group and contributed to by higher plant height under drought stress, higher PH STI, and higher expression of *NCED1* under drought stress. AC21 and AC24 were vertex accessions in the second group consisting of a mixture of tolerant and susceptible accessions, contributed to by higher expression of *NCED1* and dry root weight under drought stress.

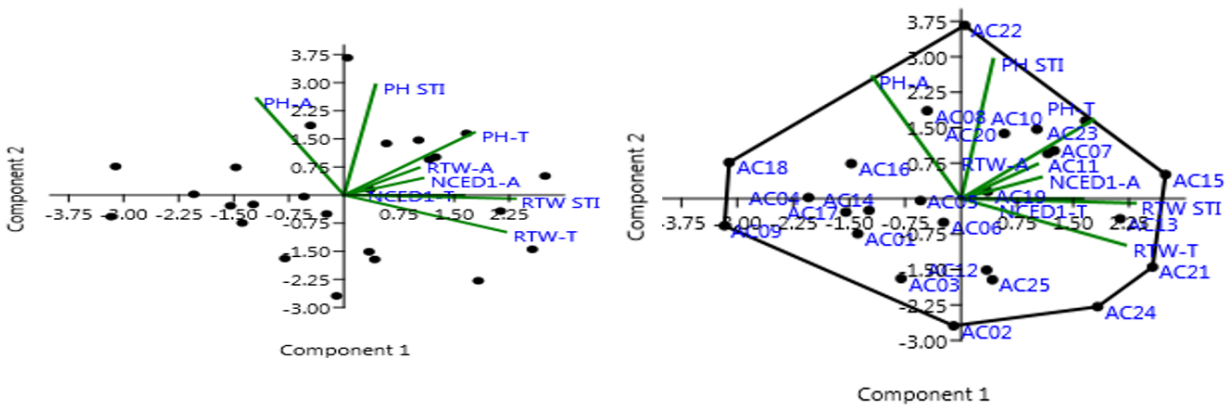

Fig. 3. Bi-plot (a) (showing relationships among traits) and polygon view (b) of Principal Component Analysis (PCA) based on morphological traits, STIs, and expression patterns of *NCED1* gene of accessions of cowpea under control (A) and drought stress (T) conditions. AC01-AC25 are accessions of cowpea. PH-A: Plant height under control; PH-T: Plant height under drought stress; RTW-A: Dry root weight under control; RTW-T: Dry root weight under drought stress; PH STI: Stress tolerance index for plant height; RTW STI: Stress tolerance index for dry root weight; *NCED1-A*: Expression of the gene under control; *NCED1-T*: Expression of the gene under drought stress.

Fig. 4. Cowpea plants growing in the screen house under control (A) and drought-stressed (T) conditions. The stressed plants showing signs of wilting with yellowish leaves at week five after planting.

AC18 was the only vertex accession in the plant height under the control sector. A high correlation existed among PH STI, plant height under drought stress, and dry root weight under control and *NCED1* under control, while RTW STI, *NCED1* under drought stress, and dry root weight under drought stress were positively correlated. Plant height under control was however negatively correlated with root weight under drought stress, RTW STI, and *NCED1* under drought stress. The biplot divided the accessions into four major groups if taken clock-wisely, group I consisted of eight accessions all of which were tolerant accessions with the most tolerant among them at the vertices (AC22, AC23, and AC15). Group II consisted of five accessions of both tolerant and susceptible statuses, while AC21 and AC13 were tolerant, others were susceptible. Group III consisted of a mixture of susceptible and moderately tolerant accessions (AC06 and AC17), with the highly susceptible accession (AC09) at the vertex. Group IV consisted of four accessions from tolerant (AC16 and AC08) and susceptible (AC18 and AC04). Fig. 4 presents the picture of the accessions of cowpea growing in the screen house; unstressed (A) and drought-stressed (T), at week five after planting (after the imposed stress).

Discussion

Insight into the knowledge of cowpea responses to dehydration stress at the molecular level and the variation in expression of genes involved under drought stress is the first step in breeding drought-tolerant cowpea plants. The drought reactive candidate gene, *NCED1,* and internal control, *P-Actin* were selected, and primers designed from them were used to amplify complementary DNA regions of the accessions of cowpea. This drought-responsive gene had been previously confirmed to be induced by dehydration in cowpea according to Iuchi *et al*. (2000) and Muchero *et al*. (2010). The strong significant differences among the cowpea accessions for expression of the candidate gene and the high significant accession effect for plant height and root weight under the control and drought stress indicated that the diversity among them is rich. This implies that selection for high drought tolerant individuals can be made from this collection for breeding purposes.

The results from the RT-PCR reactions disclosed that the primers designed for the study amplified DNA products from unstressed cowpea. They also amplified products from stressed cowpea; while expression was up-regulated in a few of the accessions, others had a down-regulated expression. Comparison between the drought-induced gene products and the unstressed products produced contradictory responses among highly drought tolerant, moderately tolerant, and susceptible accessions of cowpea. Differential expression of the gene in some of the accessions could be linked to their morphological traits. For instance, tolerant accessions such as AC22, AC15, AC23, AC10, AC11, AC13, and AC21 that combined higher plant height and dry root weight under drought stress with stress tolerance indices possessed higher gene expression under both control and drought stress conditions. Hence, the significant positive correlations between the gene expression under control condition with plant height under drought stress and STI of plant height, positive correlations between its expression in both conditions and positive correlation of its expression under drought stress with dry root weight and STI of root weight suggest that selection of cowpea based on a higher level of the gene expression under both conditions may be effective for breeding purpose. These relationships confirm that its expression might be involved in the drought tolerance of cowpea. However, selection based solely on plant height under control conditions will result in the selection of poor accessions because of its high negative correlation with the expression of the candidate gene, dry root weight under stress, STI of root weight, and uncorrelated response with plant height under drought stress. Higher expression of the gene under drought stress was reported among more drought-tolerant wild soybean genotypes compared to the less tolerant cultivated type (Zhang *et al*., 2017), it has also been linked to better growth and development under drought stress in grapevine (He *et al*., 2018).

Further characterization of these accessions under drought stress based on the relationships among relative water content (RWC), seed yield and yield contributing traits, and drought tolerance indices (DTIs) of seed yield confirmed AC21 (Gnt20), AC16 (Gnt15), AC20 (Gnt19), AC22 (Gnt21), AC06 (Gnt5), AC11 (Gnt10) and AC13 (Gnt12) as high drought-tolerant; while AC01 (Gnt1), AC03 (Gnt3), AC18 (Gnt17), AC14 (Gnt13) and AC04 (Gnt4) were confirmed as susceptible (Ajayi, 2020), available online. Among the tolerant accessions, AC21 and AC20 maintained above average seed yield per plant in both control (>18.94 g) and drought stress (>3.98 g) conditions (Ajayi, 2020). The fact that most of these tolerant accessions belonged to groups I and II corresponding to the higher level of the *NCED1* expression under both conditions suggests that selection involving these accessions would be effective for breeding drought-tolerant cowpea. This lies in the fact that the main target of breeding is to screen plant materials and identify genes with the capacity to withstand an environment with moisture deficit and maintain high yield (Chen *et al*., 2019).

The candidate gene was significantly down-regulated by dehydration in most accessions in contrast to Iuchi *et al*. (2000) but agrees with the findings of Changan *et al*. (2018) in rice. According to Otwe (2007), it is believed that if the expression of drought-responsive genes were constitutive, on the imposition of stress, the awareness of the stress will be communicated

through the signaling system for the plant to react to the stress by up-regulating or down-regulating certain genes to alleviate the effect imposed. The results from stressed and unstressed conditions suggest that this gene is constitutive (Ye *et al*., 2011), and in line with this, the up-regulation or down-regulation to combat the effect of an imposed stress may depend on many other factors, as many of the traits responsible for plant adaptation to droughts such as phenology, root weight and depth, hydraulic conductivity, and storage of reserves are associated with plant development and are constitutive rather than stressed induced (Chaves *et al*., 2002; Otwe, 2007).

According to Iuchi *et al*. (2000), expression of *NCED1* and *cprd86* were strongly induced by drought in a drought-tolerant cowpea plant, and the level of expression was shown to correspond to the duration of drought stress. Rehydration also repressed the expression of these genes. But the expression of other genes such as *cprd72* and *cprd76* involved was never induced by drought stress. He affirmed that cowpea plants stressed for 10 hours looked wilted, and the wilted plants recovered within 4 hours of relocation to well-hydrated soil. Furthermore, expression of *NCED1* was never detected before stress treatment and perhaps in the control treatment, making it seem to be non-functional under standard growing conditions but in drought or salt stress. The present findings did not go according to this, in contrast, all genotypes under normal growth conditions and stressed conditions expressed the *NCED1* gene, and drought however repressed the gene in most accessions, although there may be other factors responsible for this as suggested in tomato mutants by Muñoz-Espinoza *et al*. (2015). No relationships were also found between ABA in leaves, stress tolerance indices, plant height, and dry root weight among the present accessions (Ajayi, 2019). However, further studies are required in this regard.

As observed in the present study also, expression of the gene in control condition has been reported in many other crop species; the only difference in its expression in those instances compared to the present study lies in the fact that its expression was significantly higher under drought stress compared to unstressed condition (Zhang *et al*., 2017; González-Villagra *et al*., 2018; He *et al*., 2018; Chen *et al*., 2019). However, in support of the present study, the *NCED1* has been previously established as a housekeeping gene being significantly down-regulated by water stress (Ye *et al*., 2011); its repression under drought stress has been linked to feedback inhibition of its expression by the accumulation of ABA in leaves (Tian *et al*., 2004; Muñoz-Espinoza *et al*., 2015; Changan *et al*., 2018). Hence, further study of the gene expression concerning ABA accumulation under drought stress in cowpea will confirm their relationships as suggested by Changan *et al*. (2018).

Conclusively, it was observed that the gene *NCED1* was expressed under drought and in unstressed conditions employing the RT-PCR technique confirming its constitutive nature. The study also revealed the level of variations in its expression among the accessions of cowpea. Further analyses are therefore required for a better understanding of the role of the gene in drought tolerance among cowpea genotypes in Nigeria.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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