Journal of Underutilized Legumes
Vol. 6 (1): 17 - 30 (March 2024)

Society for Underutilized Legumes
https://sulegumes.org/
e-ISSN: 2705-3776

Expression of abscisic acid using the candidate Gene NCED1 on drought stress bambara groundnut (*Vigna subterranea* (L.) Verdc) accessions.

[1,2*]Odesola, K. A., [1]Akamihe, C. A., [1]Ogunsola, K. E., [2]Shonde T

[1] Department of Biological Sciences, Bells University of Technology, Sango Ota, Ogun State Nigeria
[2] International Institute of Tropical Agriculture, Idi-Ose, Oyo Road, Oyo State, Nigeria

*E-mail: kaodesola@bellsuniversity.edu.ng

Received September 13, 2023
Accepted for publication October 18, 2023
Published March 31, 2024

Abstract

Abscisic acid (ABA) functions as one of the numerous plant hormones governing a range of physiological processes in plants, including responses to water stress. In most plants, abscisic acid levels are supposedly increased under conditions of water deficit, leading to stomatal closure and reduced transpiration and ultimately a decrease in relative water content. There is little to no information related to the expressions of abscisic acid linkage to the drought tolerance usually attributed to Bambara groundnut. The study investigated the expression of Abscisic Acid using the candidate gene *9-cis-epoxycarotenoid dioxygenase (NCED1)* in seven (7) *Vigna subterranea* [L.] Verdc accessions under both well-watered and water stress conditions. Three healthy seeds were planted per bag, with 500 mL water administered to the plant once a day on both treatments until two weeks after flowering when the water-stressed pots received no further watering. The leaf samples were collected for RNA extraction and then differing bands intensity from amplified cDNA bands were captured for densitometric analysis using the Image J imaging software. On the other hand, data was collected on relative water content (RWC) on the individual samples. The RWC values among the accessions ranged from 82.12% to 89.24% under the well-watered condition and 62.00% to 79.43% under the water-stressed condition. The observed ABA hormone gene expression profile suggests that all the seven Bambara groundnut accessions express ABA adequately under the two water conditions identified. This indicates tolerance to water stress and the effectiveness of identifying NCED1 as a marker in drought tolerance expression studies.

Keywords: NCED1; cDNA; ABA; *Vigna subterranea*, drought

Introduction

Numerous significant weather variations brought on by climate change have been found to pose a danger to food security. As a result of these effects, developing nation currently experiences hunger, famine, and population displacement [1] while the developed nations suffer significant economic loss and increased health risks [2]. Drought is one of the key abiotic barriers to rain-fed agricultural productivity [3]. It is a recognized

below-average occurrence that impacts the amount of water in streams, rivers, lakes, and groundwater as well as the soil moisture levels [4]. For most crops, this water scarcity is known to result in a greater than usual yield loss of roughly 50%. [5, 6]. Relative water content (RWC) is one useful metric for determining how much water is absorbed by plant and how much is lost [7]. The mechanism of osmoregulation, which is the process by which plants maintain proper concentration of water if well understood can ultimately improve knowledge of turgor pressure and its preservation for plant use [8]. Datta et al. [9] observed better genotypes performance under environment which had optimum root length, shoot length and RWC for wheat genotypes which were tagged drought tolerant when subjected to normal and water-deficient situations. Over time, several plants have evolved ways of mitigating mechanisms to adapt to these extremes, enabling them to sense stressors and respond to them in specific constructive ways [10].

Abscisic acid (ABA) plays a crucial role as a hormone during times of stress aiding in the reduction of adverse stress reactions [11, 12]. It serves as a vital messenger by causing a variety of metabolic events in numerous plant tissues in response to lack of water [13]. It is particularly important in its ability to trigger immediate plants responses to any environmental stresses, inclusive of drought, cold tolerance, heavy metal ion tolerance, soil salinity, freezing tolerance, and heat stress [14]. ABA is present in various parts of plants and has the ability to quickly change the osmotic potential in most stomatal guard cells, resulting in their rapid shrinking and subsequent closure [15] particularly in a bid to salvage the whole plant from dyeing at the onset of drought. ABA does not directly affect abscission; instead, it encourages senescence, which eventually results in abscission as this also results in other physiological processes.

The importance of cultivating climate smart crop types cannot be overstated, especially considering the recurring climate change prediction that the world's temperature would climb by 4°C in 2100 [16]. Bambara groundnut (BG) is a native African legume with known potential to be a climate-smart crop because it can grow in marginal soils and tropical climes [17]. In comparison to other cereal legumes, it is thought to be the most drought resistant (18, 19]. Morphological, physiological, biochemical, and its genomics has been extensively studied [19, 20, 21, 22, 23]. The work of Ma, [24] identified the fact that plants generally release solutes such as jasmonic acids, proline, polyols, abscisic acids, etc. and the over expression or suppression response of these solutes can be adopted for drought stress studies in plants. Thus, it is possible to use this sizable collection of over 1900 wild types [25] kept in the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA) to enhance the plant's nutritional value and adaptability [26].

NCED literally means 9-cis-epoxycarotenoid dioxygenase gene, which is linked to a group of genes responsible for the production process of abscisic acid [27]. In the majority of plants, the concentration of ABA is primarily controlled by the activity of the NCED gene, which encodes enzymes at the transcriptional level. This gene plays a significant role in this context for its responsiveness to certain environmental (salt and drought) signals which directly or indirectly affect the biosynthesis of ABA [14]. Furthermore, it had been linked to other plant traits like fruiting and ripening in crops like tomatoes [28], strawberry [29], kiwi [30] and for legumes. Research reports emphasized that plants demonstrate enhanced performance under water scarcity conditions when the genes responsible for overexpressing NCED3 in the ABA biosynthetic pathway are activated [30, 31]. Additionally, it was found that Arabidopsis overexpressing AtNCED3 specifically targeted the transcription of genes induced by dehydration and ABA resulting in lowered transpiration rates and increased resilience to drought in the crop. As these differing RWC physiological mechanisms and ABA responses in Bambara groundnut remain poorly understood, thus the study aimed at investigating the possible expression of Abscisic acid (ABA) through the candidate gene NCED1 in varying drought and water availability scenarios in selected Bambara Groundnut (Vigna subterranea) accessions using PCR technique and establish the relationship between the RWC and the ABA synthesis.

Materials and Methods

Source of plant materials

Seeds of seven Bambara groundnut accessions (Table 1) were collected from the seed bank of the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan Nigeria. The seven accessions were selected based on previous study at IITA from which the accessions were observed to have shown drought tolerance traits [33]. The research was conducted at the Department of Biological Sciences, Bells University of Technology, Ota, Ogun State, Nigeria.

Table 1: Accession number of selected accessions

S/N	Accession name
1	TVSu – 203
2	TVSu – 2
3	TVSu – 22
4	TVSu – 421
5	TVSu – 423
6	TVSu – 989
7	TVSu – 266

TVSu – **T**ropical ***Vigna subterranea*** *Source: Genetic Resources Centre IITA*

Drought treatment and relative water content analysis

For each accession of the watered and stressed treatment, two seedlings were planted in a 5kg pot containing sterilized sandy loam soil in the screen house. For a strong plant establishment, seedlings were reduced to one plant per pot following germination while every plant received a daily 500 ml watering. Watering stopped for the stressed treatment at the onset of 50% flowering which coincides between 43 and 50 days after planting till the end of the experiment. Fresh leaf samples were weighed, immersed in distilled water, and then subjected to drying at 70°C for a duration of 48 hours. before being weighed to calculate the RWC.

$$\text{RWC} = \frac{FW - DW}{TW - DW} X\ 100$$

Dhopte and Manuel (2002)

DW stands for dry weight, TW for turgor weight, and FW stands for fresh weight of foliage samples.

RNA Extraction and PCR profiling

The sampling of leaf for DNA was done exactly four weeks after planting for the well-watered treatment while leaf from the stressed plant was sampled for extraction at the termination of the stress experiment. Each central leaflet which was placed into a well labelled Eppendorf tubes for the total nucleic acid (tNA) extraction startup step (all reagents prepared with 1% DEPC water) using a modified method [35]. To get quality RNA from the tNA, the RNA clean and concentrator kit (Cat No: R1016 and Lot No: ZRC 202397) was used. The concentration of the RNA was normalized to 100ng/ul using nuclease free water. For further analysis RNA was converted to cDNA using the cDNA synthesis kit (NEB cat and samples stored at 20^0C). The Purity and quantity of RNA were then assessed via gel electrophoresis and Nanodrop spectrophotometer, respectively. Prior to converting RNA into cDNA, the RNA samples were thinned out to a concentration of 100 ng using water that was free of nucleases. Following this, the MMLV Reverse Transcriptase 1st-strand cDNA synthesis Kit (NEB) was utilized according to the instructions provided by the manufacturer to convert RNA into cDNA. The resulting cDNA was stored at a temperature of -20^0C.

PCR amplification was performed to determine the candidate gene, using the primers sequence according to the report of Ajayi [36]. The gene sequence, alongside the actin gene primer used as internal control was submitted for synthesis to Inqaba biotech (Table 2). In a total volume of 10 L, the following ingredients were used to make the PCR mixture: 2 ng of genomic DNA, 5 pmo1 of each forward and reverse primer, 1X Taq buffer, 200 mmol/L dNTPs, 2 mmol/L MgCl2, and 1 U Taq DNA polymerase. Amplification conditions were denaturation at 94^0 C for 5 min, 35 cycles of the annealing stage at 94^0 C for 30 sec, 55^0 C for 30 sec, and 72^0 C for 30 sec then final elongation for 5 min at 72^0 C.

Table 2: Primer sequence of the P-actin gene and the candidate genes used for the study.

S/N	Target primers	Forward primers	Reverse Primer
1.	P-Actin	5'TGCCAAGAACAGCTCCTCAG3'	3'GAAGCACTTCCTGTGGACGA 5'
2.	NCED1	5' GATAAGGCTGAACTTAAGGA 3'	3' TACAGTAAACCGTAACACAT 5'

Assessment of Reverse Transcriptase

The amplicons from the PCR were electrophoresed in 1.5% agarose gel using 0.5X TBE buffer with 0.5 µl ethidium bromide. The Gel documentation method was used to record the in-gel expression bands under the UV-transilluminator. (ENDUROTM Apelegen). Bands were subsequently analyzed using the Image J imaging software.

Results

Relative Water Content

The RWC for all accessions was significantly ($p<0.01$) higher under the two water regimes. This high RWC of the accessions under the differing water conditions could adjoined all accessions to be regarded as tolerant. The mean values for the relative water content for the seven accessions varied significantly from 62.00% to 79.43% under water-stressed to its non-significant values of 82.12% to 89.24% under the well-watered conditions (Table 3). The RWC of water stressed plants decreased by 10.12%, 11.39%, 8.86%, 21.51%, 16.45%, 2.53% for TVSu-266 to maintain the highest value at 79.43% while 6.74%, 3.37%, 5.62%, 10.11%, 2.25%, 2.25% percentage differences were observed under the well-watered condition as TVSu-22 was highest at 89. 21%.

Table3: Relative water content (RWC) mean values of the Bambara groundnut accessions under the water stressed and well-watered conditions. Results are presented as Mean±StdDev

Accession	Water-stressed	Well-watered
TVSu_203	0.71±0.06	0.83±0.11
TVSu_2	0.70±0.05	0.86±0.03
TVSu_22	0.72±0.03	0.89±0.03
TVSu_421	0.62±0.15	0.84±0.04
TVSu_423	0.66±0.21	0.80±0.13
TVSu_989	0.77±0.04	0.87±0.02
TVSu_266	0.79±0.04	0.87±0.07
	A 85.143 WW	
	B 70.857 WS	

Gene Expression Profiling

The quality check on the extracted RNA from each single representative plant of the seven Bambara groundnut accessions from the two treatments is shown on the gel electrophoresis (Fig 1) while the quality range was between 1.80-2.00 (Table 4). A very clear amplification band with no trace of contaminants indicated its efficiency for conversion to complimentary RNA and then further upstream application.

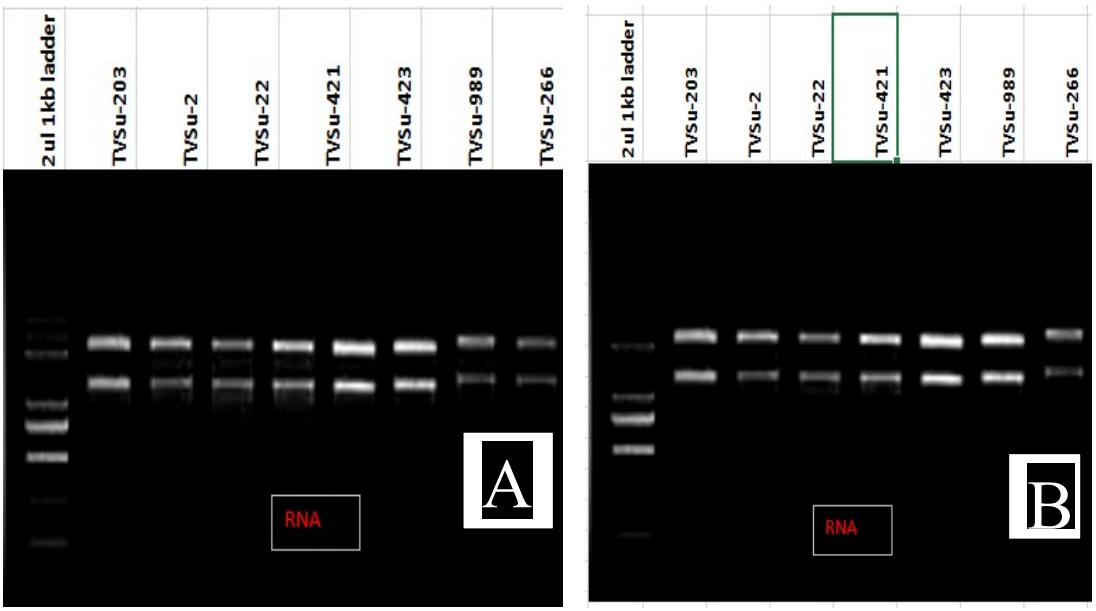

Fig 1: Gel image of the extracted DNA from the leaf tissues sampled from the well-watered (A) and water stressed (B) conditions.

Table 4: Quantity of RNA evaluated with Nanodrop spectrophotometer.

S/N	Accession Name	RNA quantity	A260(Abs)	A280(Abs)	260/280
Water stress treatment.					
1.	TVSu-203	223.0	5.575	2.479	1.90
2.	TVSu-2	204.8	5.120	2.562	2.00
3.	TVSu-22	167.2	4.179	2.239	1.87
4.	TVSu-421	224.2	5.604	2.556	2.00
5.	TVSu-423	181.6	4.539	2.280	1.99
6.	TVSu-989	138.1	3.452	1.678	1.89
7.	TVSu-266	200.5	5.013	2.516	1.99
Well-watered treatment					
8.	TVSu-203	148.7	3.718	1.847	1.98
9.	TVSu-2	921.8	23.04	11.00	2.00
10.	TVSu-22	476.8	11.92	5.775	1.90
11.	TVSu-421	563.8	14.09	6.850	1.80
12.	TVSu-423	91.10	2.278	1.045	1.87
13.	TVSu-989	370.4	9.259	4.523	1.85
14.	TVSu-266	641.0	16.025	7.594	1.88

RNA–Ribonucleic Acid**, Abs–**Absorbance factor

Complimentary DNA Conversion and P-Actin Validation of cDNA

The MMLV reverse transcriptase 1st strand cDNA synthesis kit for the conversion also resulted in a clear gel band production (Fig 2). P Actin amplification as an internal control gene showed full expression of the gene as amplified bands intensity was highly identical in all the seven accessions from leaf tissues sampled under the different water regimes (Fig 3).

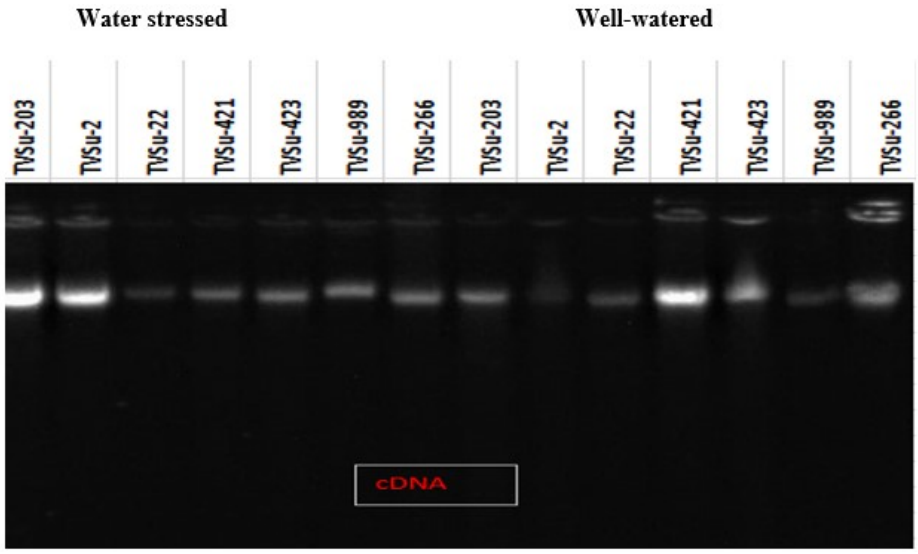

Fig 2: Gel image of the complimentary DNA after conversion from RNA

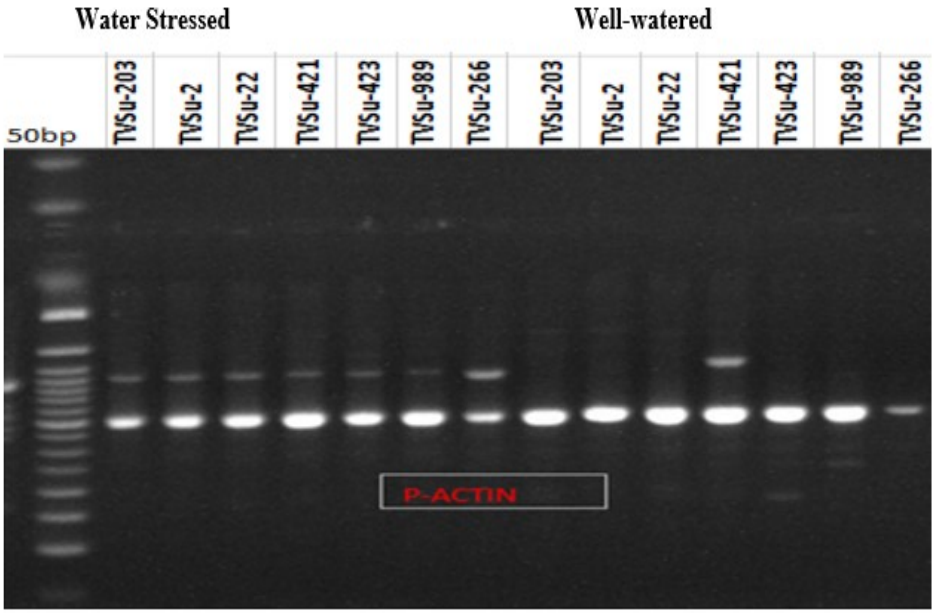

Fig 3: Gel picture of the internal control, P-Actin

NCED1 Transcript Expression

The NCED1 transcript expression gene was observed among all the accessions for both treatments (Fig 4). This was observed in varied amounts with band intensities been higher under the well-watered conditions than for the water stressed for some accessions. The differing NCED1 expression intensity was observed to be higher for TVSu-266, TVSu-989, and TVSu-421 for the water stressed condition while TVSu-203, TVSu-2, TVSu-22, and TVSu-423 showed increased intensity with water availability. Converting the gel picture intensity for a clearer expression with Image J produced numerical values (Table 4) for easy comparison and further depicted by the bar chart (Fig 4). The densitometrical presentation of band intensity also shows an expressive image intensity of the NCED1 expression as viewed under the UV transilluminator (Fig 5).

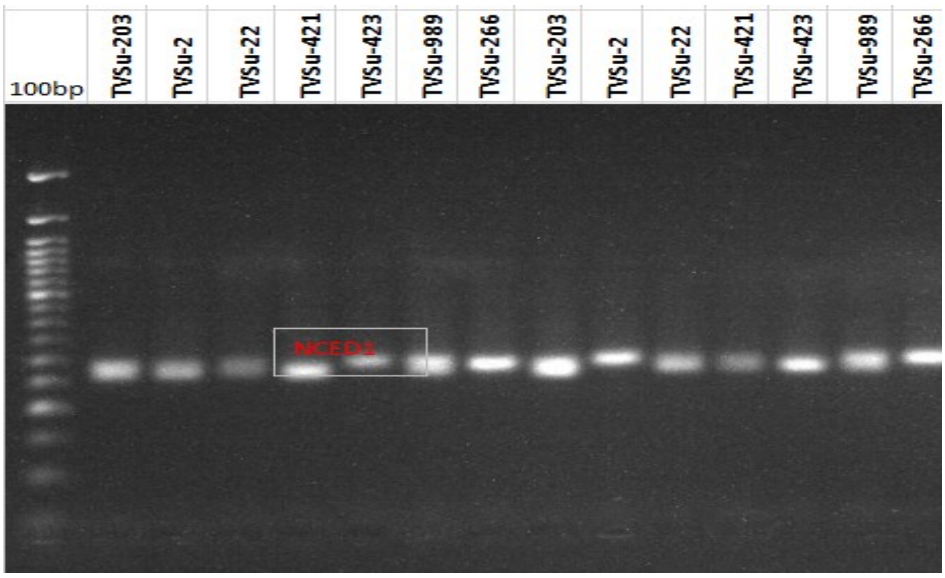

Fig 4: Gel picture of the NCED1 primer amplification

Discussion

Crop production is expected to be impacted by climate change which is already forecasted to make water shortages worse in many regions of the world in the near future [37]. In light of the fact that they have little to no study to support them, Bambara groundnut is one of many crops currently recognized as climate smart crops and if given enough study attention, especially in the areas of nutrition and its drought resistance, then it can be found competing with major crops of the world. When it comes to identifying the key internal water status of plants during drought conditions, one effective approach is to use a measure called RWC, which provides a comprehensive measure compared to other methods [38]. The high RWC expressed by the seven accessions their high level of drought tolerance. The high RWC can be attributed to a number of mechanisms that help plants withstand drought stress, such as stomata that regulate gas exchange, reduced leaf area, maintenance of a comparatively high leaf water status, and high levels of photosynthesis [39]. The overall high RWC identified in this research was similarly observed by Nazran et al. [40] who identified cultivars of mung bean that were resistant to drought having more RWC despite the extensive decreasing soil moisture conditions. There was a reduction in RWC of the well-watered treatment in comparison to the stressed, a finding consistent with what was documented in snap bean studies.

Due to its crucial function in influencing the cytoskeleton and plant physiology, actin is being adjudged a potential reference gene for expression analysis. It's utilized to standardize gene expression studies when comparing different target genes because its expression remains stable in various circumstances. Peng et al. [41] affirmed the use of actin as an internal control, contributing to gene expression as he observed it to be found in nearly all parts of the plant, including seeds, actively growing tissues, stems, roots, flowers, and the leaves, on his study on chickpea where it was isolated and designated as CarACT1. Its counterpart actin alpha (Act α) and actin beta (Act-β) were validated suitable internal control for most abiotic (cold, drought, heat, and salt) stresses [42].

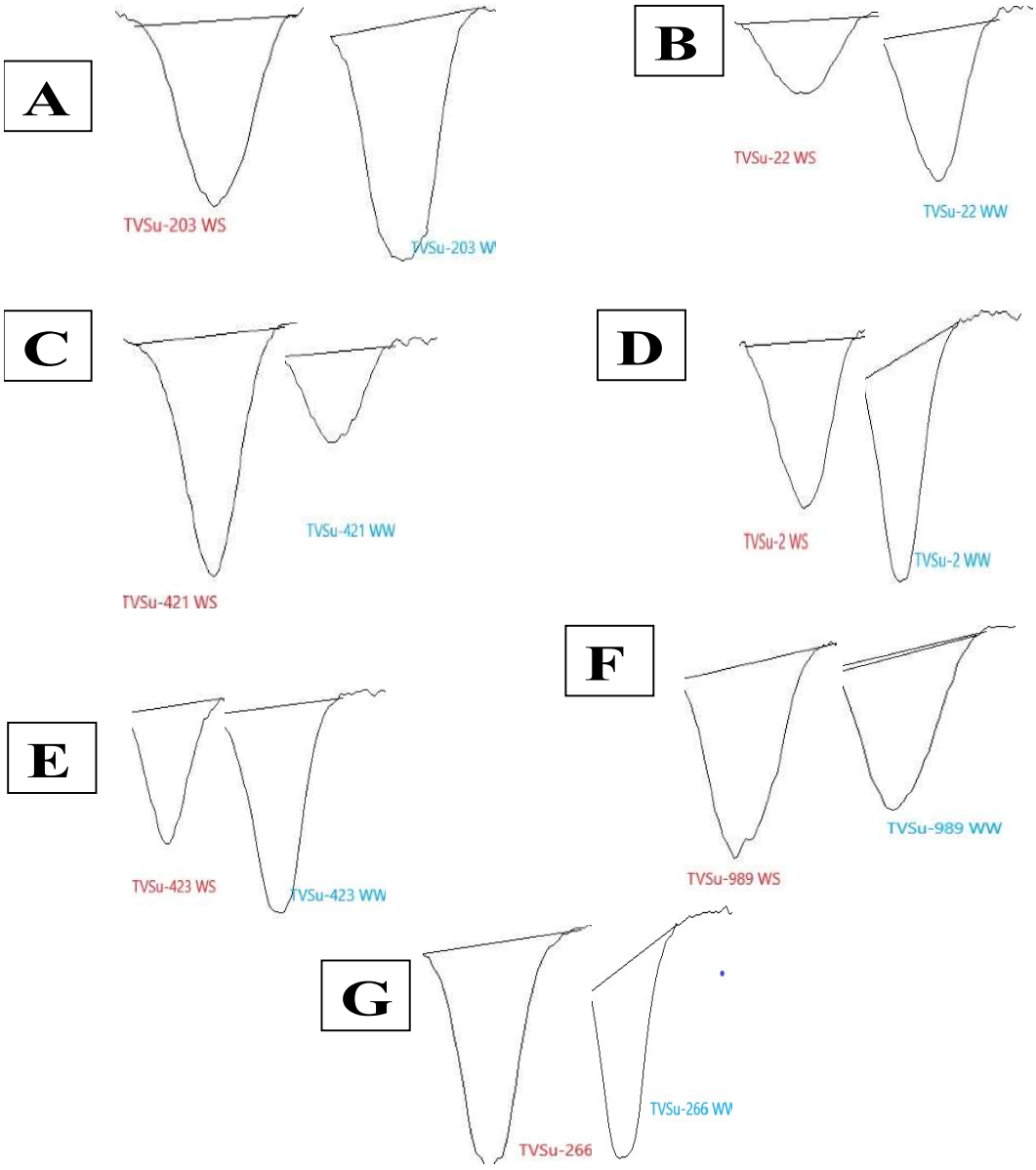

Fig 5: Density graphs as illustrated by the Image J software; A-F.
WS- water stressed WW-well watered.

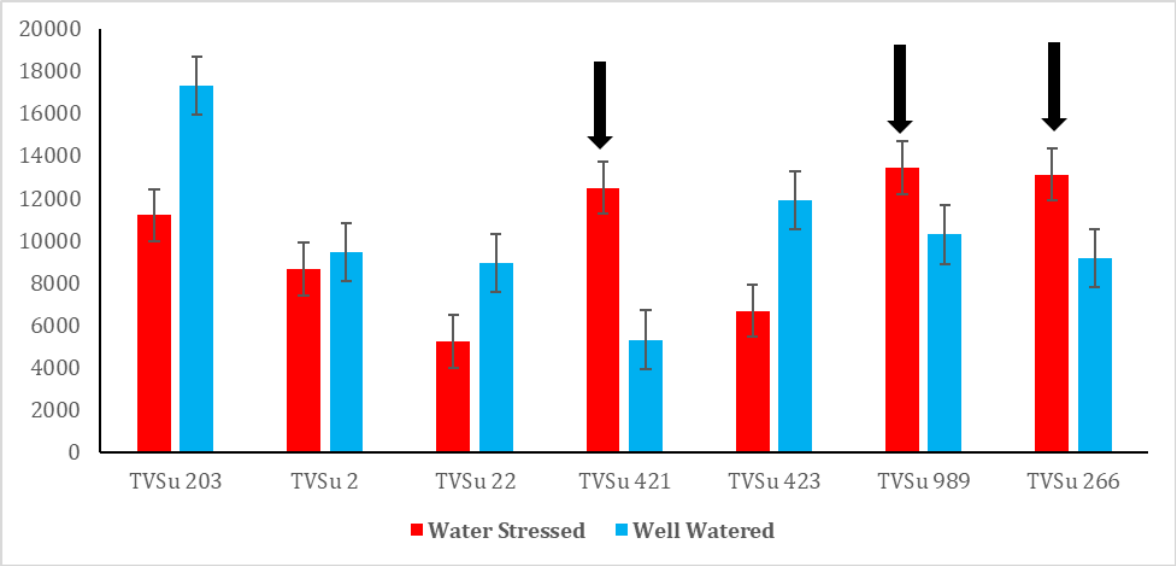

Fig 6: Comparison of NCED1 expression between the water stressed and well-watered Bambara Groundnut Accessions

The accession studied showed differing level of NCED1 expression particularly for a supposed drought tolerant accession. Previous studies by Ajayi et al. [43] showed that drought greatly stimulated NCED1 expression in cowpea observed to be drought-tolerant, and that the expression degree correlated with the length of drought stress. For the accessions showing expression of NCED1 progressively under well-watered conditions, Changan et al. [44] findings buttressed similar observation in rice under the watered conditions than in stressed conditions, where roots were observed to have an increased relative level of expression of NCED1 than leaves, to express the inhibition of ABA biosynthesis through feedback regulation. Perhaps a study of the root system of the studied accessions could portray such a higher expression which thus could translate to the possibility of NCED1 being expressed more in other parts of the plant. Bambara Groundnut is a very beneficial legume, and the results of this study offered a better understanding of these seven accessions. The accessions were observed with different degrees of variance in the expression of the candidate gene NCED1 of selected accessions for this study. Due to the prospects of Bambara groundnut as an environmentally resilient crop, greater number of accessions should be investigated to obtain a more detailed knowledge of the drought tolerance of the crop.

Statement and Declaration

The authors declare no conflict of interest.

Acknowledgement

The Genetic Resources Centre at the International Institute of Tropical Agriculture (IITA) in Ibadan, Oyo State, Nigeria, supplied the seeds for this study.

Author Contributions

KAO, CAA and TS conceived and designed the experiments; KAO and KEO performed the statistical analysis; KAO wrote the manuscript and prepared the references; KAO, KEO, CAA, and TS revised the manuscript.
All authors have read and agreed to the published version of the manuscript.

Funding

No funding was provided

References

1. Choudhary A., Pandey P., and Senthil-Kumar M. Tailored responses to simultaneous drought stress and pathogen infection in plants. Drought Stress tolerance in plants. Physiology and biochemistry. 2016 Sept 23; 1:427-438. Available from: http://223.31.159.10:8080/jspui/handle/123456789/655

2. United Nations Framework Convention on Climate Change (UNFCCC). Adaptation to & mitigation of climate change in agriculture [Internet]. 2017 [cited 2023 sept 23]; Available from: www.unfccc.int.

3. Stephan R., Terzi S., Erfurt M., Cocuccioni, S., Stahl, K., and Zebisch, M. Assessing agriculture's vulnerability to drought in European pre-Alpine regions. EGUsphere. 2022 Sept 23; 1-28. Available from: https://doi.org/10.5194/nhess-23-45-2023

4. Van Loon A.F., Stahl, K., Di Baldassarre, G., Clark, J., Rangecroft, S., Wanders, N., Gleeson, T., Van Dijk, AI. J. M., Tallaksen, L.M., Hannaford, J., Uijlenhoet, R., Teuling, A.J. Hannah, D.M., Sheffield, J., Svoboda, M., Verbeiren, B., Wagener, T., and Van Lanen, H.A.J. Drought in a human-modified world: reframing drought definitions, understanding, and analysis approaches. Hydrology and Earth System Sciences. 2016 sept 23; 20(9):3631-3650. Available from: https://doi.org/10.5194/hess-20-3631-2016

5. Mafakheri A., Siosemardeh A.F., Bahramnejad B., Struik P.C., & Sohrabi, Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian journal of crop science. 2010 sept 23; 4(8):580-585. Available from: https://search.informit.org/doi/abs/10.3316/informit.857341254680658

6. Caparas M., Zobel Z., Castanho A.D., and Schwalm C.R. Increasing risks of crop failure and water scarcity in global breadbaskets by 2030. Environmental Research Letters. 2021 sept 23; 16(10): 104013. Available from: https://iopscience.iop.org/article/10.1088/1748-9326/ac22c1/meta

7. Bilal M., Rashid R., Rehman S., Iqbal F.; Ahmed J., Abid M., Ahmed Z., and Hayat A. Evaluation of wheat genotypes for drought tolerance. Journal of Green Physiology Genetics Genomics. 2015 sept 23; 1:11–21. Available from: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Bilal%2C+M.%2C+Rashid%2C+R.%2C+Rehman%2C+S.%2C+Iqbal%2C+F.%3B+Ahmed%2C+J.%2C+Abid%2C+M.%2C+Ahmed%2C+Z.%2C+and+Hayat%2C+A.+%282015%29+Evaluation+of+wheat+genotypes+for+drought+tolerance.+Journal+of+Green+Physiology+Genetics+Genomics&btnG=

8. Soltys-Kalina D, Plich J, Strzelczyk-Żyta D, Śliwka J, and Marczewski W. The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-derived potato cultivars. Breeding Science. 2016 sept 23; 66(2):328-31. Available from: https://www.jstage.jst.go.jp/article/jsbbs/66/2/66_328/_article/-char/ja/

9. Datta J., Mondal T., Banerjee, A., and Mondal N. Assessment of drought tolerance of selected wheat cultivars under laboratory condition. Journal of Agricultural Technology. 2011 sept 23; 7:383–393. Available from: https://www.thaiscience.info/journals/Article/IJAT/10842519.pdf

10. Macheroux P. More important than ever: understanding how plants cope with stress. The FEBS Journal. 2022 sept 23; 289(7):1720-1722. Available from: https://febs.onlinelibrary.wiley.com/doi/full/10.1111/febs.16434

11. Wani S. H. and Kumar V. Plant stress tolerance: engineering ABA: a potent phytohormone. Transcriptomics. 2015 sept 23; 3(2):1000113. Available from: https://febs.onlinelibrary.wiley.com/doi/full/10.1111/febs.16434

12. Sah S.K., Kaur G. and Wani S.H. Metabolic Engineering of Compatible Solute Trehalose for Abiotic Stress Tolerance in Plants. Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies. 2016 sept 23; pp.83–96. Available from: https://link.springer.com/chapter/10.1007/978-81-322-2616-1_6

13. Kuromori T., Seo M. and Shinozaki K. ABA Transport and Plant Water Stress Responses. Trends in Plant Science. 2018 sept 23; 23(6):513–522. Available from: https://www.cell.com/trends/plant-science/abstract/S1360-1385(18)30085-2?dgcid=raven_jbs_etoc_email

14. Finkelstein R. Abscisic Acid Synthesis and Response. The Arabidopsis Book / American Society of Plant Biologists. 2013 sept 23; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3833200/

15. Rosales M. A., Maurel C. and Nacry P. Abscisic Acid Coordinates Dose-Dependent Developmental and Hydraulic Responses of Roots to Water Deficit. Plant Physiology. 2019 sept 23; 180(4):2198–2211. Available from: https://academic.oup.com/plphys/article/180/4/2198/6117611

16. Thuiller W. Climate change and the ecologist. Nature. 2007 sept 23; 448(7153):550-552. Available from: https://www.nature.com/articles/448550a

17. Mayes S., Ho W.K., Chai H.H., Song B., Chang Y., and Massawe F. Bambara Groundnut (*Vigna subterranea* (L) Verdc)—A climate smart crop for food and nutrition security. In Genomic Designing of Climate-Smart Pulse Crops. 2019 sept 23; pp. 397-424. Available from: https://link.springer.com/chapter/10.1007/978-3-319-96932-9_8

18. Jørgensen S.T., Liu F., Ouédraogo M., Ntundu W.H., Sarrazin J., and Christiansen J. L. Drought responses of two bambara groundnut (*Vigna subterranea* L. Verdc.) landraces collected from a dry and a humid area of Africa. Journal of Agronomy and Crop Science. 2010 sept 23; 196(6):412-422. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1439-037X.2010.00435.x

19. Olanrewaju O.S., Oyatomi O., Babalola O.O., & Abberton M. Breeding potentials of Bambara groundnut for food and nutrition security in the face of climate change. Frontiers in Plant Science, 2022 sept 23; 12:3186. Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2021.798993/full

20. Olukolu B.A., Mayes S., Stadler F., Quat Ng N., Fawole I., Dominique D., Azam-Ali S.N., Abbott A.G. and Kole C. Genetic diversity in Bambara groundnut (*Vigna subterranea* (L.) Verdc.) as revealed by phenotypic descriptors and DArT marker analysis. Genetic Resources and Crop Evolution 2012 sept 23; 59(3):347-358. Available from: https://link.springer.com/article/10.1007/s10722-011-9686-5

21. Fatimah S., and Ardiarini N.R. (2018). Genetic diversity of Madurese Bambara groundnut (*Vigna subterranea* L. Verdc.) lines based on morphological and RAPD markers. SABRAO Journal of Breeding & Genetics. 2018sept 23; 50(2). Available from: https://openurl.ebsco.com/EPDB%3Agcd%3A11%3A14026747/detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A130162149&crl=c

22. Konate M., Nandkangré H., Ouoba A., Zida S., Ouedraogo M., Sawadogo N.S., Genetic diversity of bambara groundnut accessions from Burkina Faso using random amplified polymorphic DNA markers. Agricultural and Food Science Journal of Ghana. 2019 sept 23; 12:1050-1059. Available from: https://www.ajol.info/index.php/afsjg/article/view/193104

23. Osundare O.T., Akinyele B.O., Odiyi A.C., Paliwal R., Oyatomi O.A., and Abberton M.T. Genetic diversity and population structure of some Nigerian accessions of Bambara groundnut (*Vigna subterranea* (L.) Verdc.,) using DArT SNP markers. Genetic Resources and Crop Evolution. 2022 sept 23; 1-15. Available from: https://link.springer.com/article/10.1007/s10722-022-01472-w

24. Ma Y., Dias M.C., and Freitas H. (2020). Drought and salinity stress responses and microbe-induced tolerance in plants. Frontiers in Plant Science. 2020 sept 23; 11591911. Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2020.591911/full

25. Paliwal R., Abberton M., Faloye B., and Olaniyi O. Developing the role of legumes in West Africa under climate change. Current Opinion in Plant Biology. 2020 sept 23; 56: 242-258. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1369526620300546

26. Abberton M., Paliwal R., Faloye B., Marimagne T., Moriam A., and Oyatomi O. Indigenous African Orphan Legumes: Potential for Food and Nutrition Security in SSA. *Frontiers in Sustainable Food Systems.* 2020 sept 23; 6 (83). Available from: https://www.frontiersin.org/articles/10.3389/fsufs.2022.708124/full

27. Li Q., Yu X., Chen L., Zhao G., Li S., Zhou H., Dai Y., Sun Na., Xie Y., Gao J., Li D., Sun X., and Guo N. Genome-wide identification, and expression analysis of the NCED family in cotton (Gossypium hirsutum L.). Plos one. 2021 sept 23; 16(2): e0246021. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246021

28. Ji K., Kai W., Zhao B., Sun Y., Yuan B., Dai S., Li Q., Chen P., Wang Y., Pei Y., Wang H, Guo Y., and Leng P. SlNCED1 and SlCYP707A2: key genes involved in ABA metabolism during tomato fruit ripening. Journal of Experimental Botany, 2014. Sept 23; 65(18):5243-5255. Available from: https://academic.oup.com/jxb/article/65/18/5243/2884963

29. Liao X., Li M., Liu B., Yan M., Yu X., Zi H., Liu R., and Yamamuro C. Interlinked regulatory loops of ABA catabolism and biosynthesis coordinate fruit growth and ripening in woodland strawberry. Proceedings of the National Academy of Sciences, 2018 sept 23; 115(49): E11542-E11550. Available from: https://www.pnas.org/doi/abs/10.1073/pnas.1812575115

30. Gan Z., Shan N., Fei L., Wan C., and Chen J. Isolation of the 9-cis-epoxycarotenoid dioxygenase (NCED) gene from kiwifruit and its effects on postharvest softening and ripening. Scientia Horticulturae. 2020 sept 23; 261:109020. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0304423819309069

31. Bhaskara G.B., Thao Thi N., and Verslues P.E. Unique drought resistance functions of the highly ABA-induced clade A protein phosphatase 2Cs. Plant Physiol 160:379–395. doi:10.1104/pp.112.202408. 2012 sept 23; 160:379–395. Available from: https://academic.oup.com/plphys/article/160/1/379/6109775

32. Behnam B.K., Iuchi, S., Fujita, M., Fujita, Y.I., Takasaki H., Osakabe, Y., Yamaguchi-Shinozaki K., Kobayashi M., and Shinozaki, K.O. Characterization of the promoter region of an Arabidopsis gene for 9-cis-epoxycarotenoid dioxygenase involved in dehydration-inducible transcription. DNA research. 2013 sept 23; 20(4):315-324. Available from: https://academic.oup.com/dnaresearch/article/20/4/315/353210

33. Odesola K.A., Olawuyi O.J., Paliwal R., Oyatomi O.A., and Abberton M.T. Genome-Wide association analysis of phenotypic traits in Bambara groundnut under drought-stressed and non-stressed conditions based on

DArTseq SNP. Frontiers in Plant Science. 2023 sept 23; 14:1104417 Available from: https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2023.1104417/full

34. Dhopte A. M. and Manuel L. M. Principles and techniques for plant scientist 2002 [cited 2023 sept 23]; Available from: https://www.abebooks.co.uk/9788177541168/Principles-Techniques-Plant-Scientists-A.M.Dhopte-8177541161/plp

35. Dellaporta S. L., Wood, J., and Hicks, J.B. (1983). A plant DNA minipreparation: version II. Plant molecular biology reporter. 1983 sept 23; 1:19-21. Available from: https://doi.org/10.1007/BF02712670

36. Ajayi O.B., Oyetayo F.L. Potentials of Kerstingiella geocarpa as a health food. Journal of Medicinal Food. 2009 sept 23; 12(1):184-187. Available from: https://www.liebertpub.com/doi/abs/10.1089/jmf.2008.0100

37. González-Villagra J., Rodrigues-Salvador A., Nunes-Nesi A., Cohen J.D. and Reyes-Díaz M. M. Age-related mechanism and its relationship with secondary metabolism and abscisic acid in Aristotelia chilensis plants subjected to drought stress. Plant Physiology and Biochemistry. 2018 sept 23; 124:136–145. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0981942818300160

38. Mishra B.K., Srivastava J.P., and Lal J.P. Drought resistance in lentil (Lens culinaris Medik.) in relation to morphological, physiological parameters and phenological developments. International Journal of Current Microbiology of Applied Science. 2018 sept 23; 7(1):2288-2304. Available from: https://www.researchgate.net/profile/Brajesh-Mishra-3/publication/322601389_Drought_Resistance_in_Lentil_Lens_culinaris_Medik_in_Relation_to_Morphological_Physiological_Parameters_and_Phenological_Developments/links/5a621d414585158bca4b01c7/Drought-Resistance-in-Lentil-Lens-culinaris-Medik-in-Relation-to-Morphological-Physiological-Parameters-and-Phenological-Developments.pdf

39. Chai H.H., Massawe F., and Mayes S. Effects of mild drought stress on the morpho-physiological characteristics of a bambara groundnut segregating population. *Euphytica.* 2016. sept 23: 208:225-236. Available from: https://link.springer.com/article/10.1007/s10681-015-1581-2

40. Nazran A., Ahmed J.U., Karim A. J. M. S., and Ghosh T.K. Physiological responses of mungbean (Vigna radiata) varieties to drought stress. Bangladesh Journal of Agricultural Research 2019 sept 23; 44(1):1-11. Available from: https://www.researchgate.net/profile/Totan-Ghosh-4/publication/332322406_Physiological_responses_of_mungbean_Vigna_radiata_varieties_to_drought_stress/links/61547e4814d6fd7c0fc09150/Physiological-responses-of-mungbean-Vigna-radiata-varieties-to-drought-stress.pdf

41. Peng H.H. Cheng X. Yu Q. Shi H. Zhang J. Li and Ma H. Molecular analysis of an actin gene, CarACT1, from chickpea (Cicer arietinum L.). Molecular Biology Report. 2010 sept 23]; 37(2):1081-1088. Available from: https://link.springer.com/article/10.1007/s11033-009-9844-4

42. Mufti F.U., Aman S., Banaras S., Shinwari Z.K. and Shakeel S. Actin Gene Identification from Selected Medicinal Plants for their use as Internal control for Gene Expression Studies. Pakistan Journal of Botany. 2015 sept 23]; 47(2):629-635. Available from: https://pakbs.org/pjbot/PDFs/47(2)/30.pdf

43. Ajayi A.T., Gbadamosi A.E., Olumekun V.O., and Omotuyi I.O. Variable Expression of the Candidate Gene NCED1 Among Cowpea Accessions under Different Drought Stress Conditions. Journal of Genetic Resources.

2021 sept 23; 7(1):133–143 Available from: https://sc.journals.umz.ac.ir/article_3128_ff5413c92d3502b39aeee21f1833b82a.pdf

44. Changan S.S., Ali K., Kumar V., Garg N.K. and Tyagi A. Abscisic acid biosynthesis under water-stress: anomalous behavior of the 9-cis-epoxycarotenoid dioxygenase1 (NCED1) gene in rice. *Biologia Plantarum*. 2018 sept 23; 62(4):663–670. Available from: https://link.springer.com/article/10.1007/s10535-018-0807-2