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Research Article

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Variation in Phenotypic and Genotypic Characteristics of Yam Zygotic Seeds Raised In Drip System Hydroponics

Ossai, C.O[1*]., Shonde, T.E[2]., Edemodu, A[3]., Udofia, F.A[4]., Olusola, T[5]., Balogun, M.O[6]

[1-6]*International Institute of Tropical Agriculture, Ibadan, Nigeria.*

***Corresponding author**
Ossai, C.O, International Institute of Tropical Agriculture, Ibadan, Nigeria.

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Abstract

Yam is traditionally propagated using the tuber part (seed yam) as the use of yam seed is constrained by the non-synchronization of flowering and the rare occurrence of zygotic seed production will generate segregation in the offsprings (not true-to-type) theoretically. However, there is dearth of information on the validation of this theory in practical terms. This study, thus evaluates individual zygotic seeds of 2 improved open pollinated improved varieties (TDr 98/00917 and TDr 95/00893 x TDr 95/00903) and one landrace (TDr 3010). Ten zygotic seeds of the three accessions were planted in hydroponics trough containing buffered and moistened cocopeat substrate. The individual plants were phenotyped following standard operating procedures. DNAs were extracted from young fresh leaves at 6 weeks old and were used for Polymerase Chain Reaction (PCR) with 10 codominant SSR markers. The PCR bands were scored based on presence and absence. The phenotypic and genotypic scores were used to generate a dendogram and principal clusters. Result obtained revealed varied clustering arrangements among the individual plants, irrespectively of the accessions in both phenotypic and genotypic dendograms. This reveals the differences that exists between individual plants of same accession.

Keywords: Yam, Zygotic Seeds, SSR Markers, Phenotype, Genotype, Hydroponics

Introduction

Yam (*Dioscorea* species) is an important staple in Africa where about 90% of world's output is produced and it makes up an integral part of the socio-cultural traditions of the people in the region [1]. In addition to food, it contributes immensely to the Gross Domestic Product (GDP) of the member countries of Africa, especially in the yam zone of West Africa comprising of the Republic of Cote d'Ivoire, Ghana, Togo, Benin, Nigeria and Cameroon (Scarcelli *et al.*, 2019). In other parts of the world like Japan, reports of the medicinal application of yam exists [2]. The genus Dioscorea comprises of about 600 species, of which species like D. rotundata Poir. (white yam), *D. cayenensis* Lam. (yellow yam), *D. esculenta* Burk (lesser yam), *D. alata* L. (water yam), *D. dumentorum* (Kunth) Pax., and *D. bulbifera* L. are the most popular [3]. While the white Guinea yam (*Dioscorea rotundata*) among the species is the most important in West Africa where it originated from (Sugihara *et al*., 2020) in terms of food and market demand [4].

Despite the economic importance of yam in this region of the world, constraints such as unavailability of disease free planting materials, tuber dormancy coupled with its low propagation ratio has hindered the attainment of its potential yield of 22 tonnes per ha as against the 9.4 tonnes per ha currently obtained [5, 6, 7]. Success in yam improvement has been made in fast tracking the multiplication ratio through advanced technology like the aeroponics and hydroponics systems [8, 9]. However, yam improvement through breeding programs takes long time period as it is majorly constrained by the rare occurrence and non-synchronization of flowering in yam, thus making its hybridization difficult [10].

Traditionally yam seedlings for breeding purposes are raised from botanical seeds using seed trays and nursery bags and appropriate media. The seed are initially sown into seed trays from where they are transplanted to nursery bags individually, and these steps takes time to accomplish with risk of losing some of the seedlings due to transplanting. With the successful adoption of the hydroponics system in fast propagating yam seedlings, to fully understand the half sib progenies (zygotic seeds) generated through the pollination of two parent lines with unique components of breeding interests, it is important to exploit the use of hydroponics system to

propagate the zygotic seeds. This study thus evaluates the performance of yam botanic seeds using the hydroponics system relative to their phenotypic and genotypic characteristics.

Materials and Methods

Crop husbandry

Dissolved and buffered cocopeat substrate was poured into a 4 kg capacity hydroponic troughs (10 cm by 10 cm dimension). Ten (10) zygotic seeds each of two accessions of open pollinated improved varieties (TDr 98/00917-Female plant and TDr 95/00893-Female plant x TDr 95/00903-Male plant) and one landrace (TDr 3010-Female) of *Dioscorea rotundata* genotypes were planted in the hydroponics system and watered every other day. At 4 weeks after planting, 250 g poultry manure was added to each trough to provide nutrient for the crop growth and development. At 6 weeks after planting, each planting unit was twined to ensure proper access to photoperiodism.

Data collection

The percentage survival, growth and yield parameters were collected. Also, the plants were phenotyped using information collected on the leaf waxiness, presence of wings, hairs, spines, vigor, thickness, leaf color, leaf shape etc. following the standard operating protocol for yam variety performance evaluation as recommended by Asrat (2016).

DNA Extraction

Total genomic DNA was isolated from young, fresh and healthy leaves of six weeks old plants from each of the sampling unit using CTAB method with modification by [11]. Twenty milligram (20 mg) of tender leaves of the sampled leaves of each accessions was weighed into 2 ml eppendorf tube, ground to fine powder in liquid nitrogen using the geno grinder. To each tube, 1ml of HEPES buffer (1.94g L-Ascorbic acid, 180 ml autoclave distilled water 20 ml HEPES buffer solution 2 g of PVP) was added to each sample to remove polyphenols and polysaccharides. It was homogenised and centrifuged at 10,000 rpm for 5 minutes and repeated thrice before adding 800 µl of lysis buffer containing 2 g CTAB, 2 g PVP, 28 ml NaCl, 4 ml EDTA (pH 8.0), 10 ml Tris–HCl (pH 8.0), and 1 ml Beta–mercaptoethanol was added under lamina flow and shaken several times until a homogenous mixture was obtained to lyse the nuclear membranes.

The mixture was incubated at 65 ºC for 30 min with intermittent vortexing by hand three times at 10 minutes interval to ensure uniform temperature within the tube. The samples were cooled at room temperature. The protein contaminants from the cell lysate were then removed by adding equal volume (500 µl) of chloroform : isoamyl-alcohol (24:1) and mixed gently by inversion of the tube. The samples were centrifuged at 10000 rpm for 10 min using centrifuge and the upper phase containing aqueous phase transferred into clean 1.5 ml tubes. The same volume (500 µl) of chloroform isoamyl-alcohol (24:1) was added again to ensure total removal of the protein contaminants as possible. The nucleic acids were precipitated by adding two-thirds volume of ice-cold isopropanol (500 µl) and the tube gently inverted five times. The precipitation was enhanced by storing the samples at -20 ºC in a refrigerator for one hour. The samples were centrifuged again at 10000 rpm for 10 min to pelletize the nucleic acid and the isopropanol (supernatant) was decanted and discarded. The DNA pellet was washed with 500 µl of 70% ethanol then centrifuged at 10000 rpm for 5 minutes. The ethanol was decanted and the DNA pellets were air-dried at room temperature (25 oC) on the laboratory bench for 10 min to remove the ethanol smell. The DNA pellets were then suspended in 95 µl of 1X Low salt TE buffer (Tris-ethylenediaminetetracetic acid) and 5 µl of RNase.

DNA Quality and Quantity Estimation

The DNA quality and quantity were determined using the NanoDrop spectrophotometer (ND-1000) (Thermo Fisher Scientific, USA) and visualized by Agarose gel electrophoresis (Sunrise 96, Biometra, Germany). The diluted DNA samples were loaded to the cuvette of the spectrophotometer for estimation of the absorbance. The DNA quality was assessed using the absorbance ratio of 260 nm to 280 nm wavelengths (A260/A280). Each well contained a mixture of 2 µl of loading dye and 3 µl of the genomic DNA sample. The gel was run with 1X TBE buffer from the cathode to the anode with a constant voltage of 120 V for 45 min. They were visualized after electrophoresis with a UV Trans illuminator camera.

Polymerase Chain Reaction Process

The PCR comprises of 25 ul of the reaction mixture (3 ul (25 ng/ul), I ul of 50 mM, 2 ul of 2.5 mM DNTPs, 2.5 ul 10x Reaction Buffer, 1 ul DMSO, I ul of Forward and Reverse primer (Table 1), 0.1 ul Taq Polymerase and 13.4 ul H2O) per reaction. The PCR reaction mixture was loaded in a Veriti 96 Well (Applied Biosystems, USA) thermal cycler according to the following thermal profile: a program denaturing at 94 oC for 5 min, annealing at 94 oC, 55 oC for 90 sec and 72 oC for 1 minute and final extension at 72 oC for 10 minutes.

Table 1: Primer sequences used in the polymerase chain reaction processes

Name of primer	Forward Sequence	Reverse Sequence
YM 25	GAT GGA GAT GAG GCC G	TTC GAA GCC AGA GCA AGT G
YM 43	GCC TTG TTT TGT TGA TGC TTC G	CCA GCC CAC TAA TCC CTC C
YM 50	TTG CCC TTG GGA TGT AGG G	CAT CCC CGT TGT ATC CTG C
YM 54	CAC TTG CTC TCT CAT CGG C	TTG ACA ACC TCT ATT TTG CCC
YM 16	TGA AGA GAA TGT TGA CGT ACC	TAT CCG GCC CTCTCA TTG G
YM 44	CGC AAC CAG CAA AGG ATT TA	ATT CTG TCT CTC AAA ACC CCT
YM 30	GTG GTA GGG TGT GTA GCT TCT T	CCA CAA CTA AAA ACA CAT GGA C
YM 28	CCA TTC CTA TTT AAG TTC CCC T	GAT GAA GAA GAA GGT GAT GAT G
YM 27	TCC AGC TCT TTA GCA CAG G	AGG AGC ATA GGC AAC AAG C
YM 61	AGT GGT GCT GTA GTA ACT GGA A	CAT GAC TAC CTT TCC TCA ATC A
YM 69	CTC TCT ACC CAA CAA AAA C	AAT CTT GCA CCA CCT TTT CTA C

Results and Discussion

Results obtained showed that 2 individual seeds of the accession TDr-893x903 took the shortest number of days to sprout, while 4 individual seeds of TDr-3010 took the longest number of days to sprout (Fig 1). However, out of the 10 seeds planted per accession, 40%, 70% and 70% of TDr-893x903, TDr-3010 and TDr-98/917, respectively sprouted. This shows a higher seed viability rate in TDr-3010 and TDr-98/917 accessions, but they required longer time to break dormancy relative to TDr-893x903. There are dearth of information on the cultivation of yam using the zygotic seeds as propagule, except few reports on its usage in vitro in the generation of callus for propagation through somatic embryogenesis techniques [12]. Since, there is lack of information on the viability of the zygotic seeds, the planting substrate becomes inconsequential in terms of its effect on the seeds viability. However, in this research, soil constraints are being circumvented in using sterile cocopeat substrate in hydroponics system [13].

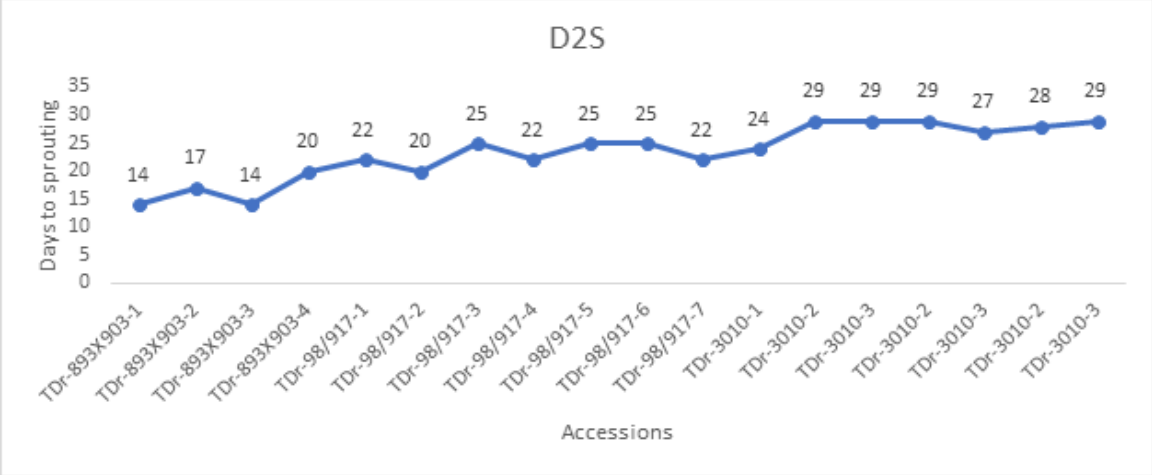


Figure 1: Number of days taken by individual zygotic seeds of three accessions of white yam

The plants grew steadily as they grow older. At 8 weeks after planting, the plant height ranged from 15.1 cm (TDr-98/917-7) to 22.0 cm (TDr-3010-1) (Fig. 2). Among the individual seeds in the three accessions, TDr-3010-1 grew taller than the rest individual plants. As the plants grew older, the number of leaves produced increased with a range of 5 (TDr-3010-3) to 20 (TDr-893x903-1) at 8 weeks old (Fig. 3). Expectedly, production of leaves within the vegetative stage of plants development is essential for their photosynthetic activities and food production. However, in this case, the tallest plants did not produce the highest number of leaves, which shows longer internode in the individual plant. Also, when compared to planting yam with the tuber part as seed, higher growth rate and leave production has been reported within 8 weeks interval than what was achieved using the zygotic seed, despite sprouting approximately same time interval (2 – 4 weeks) [14].

There were varied sizes and number of tubers produced by the individual zygotic seeds of the three accessions. Only TDr-893x903-1, TDr-98/917-1 and 2, and TDr-3010-1 produced 2 tubers each, while the rest individual plants produced 1 tuber each. The tuber sizes ranged from 2.3 (TDr-3010-2) to 24 (TDr-98/917-3) (Fig. 4). However, despite TDr-98/917-3 producing 1 tuber, the tuber weighed more than the individuals with more than 1 tuber each. This still reaffirms the low propagation ratio of yam which several research has been devoted into finding a lasting solution to improve [5].

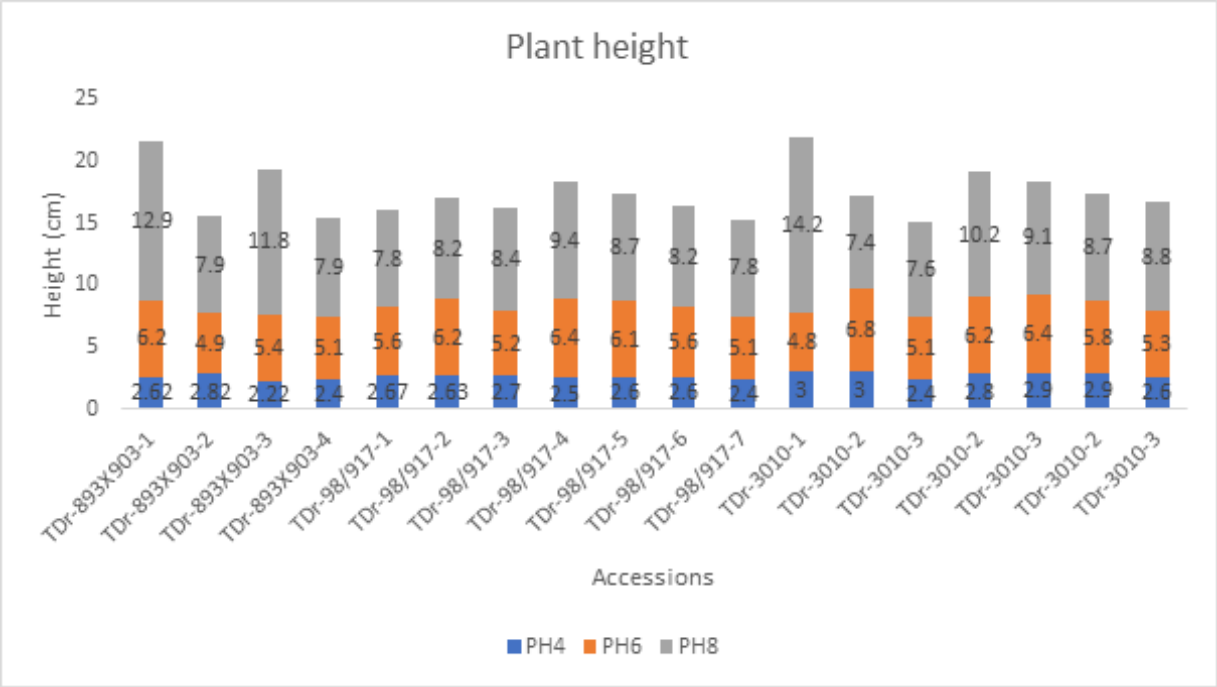


Figure 2: Plant height of individual zygotic seeds of three accessions of white yam at 4, 6 and 8 weeks after planting. PH4, PH6 and PH8 are plant height at 4, 6 and 8 weeks old respectively.

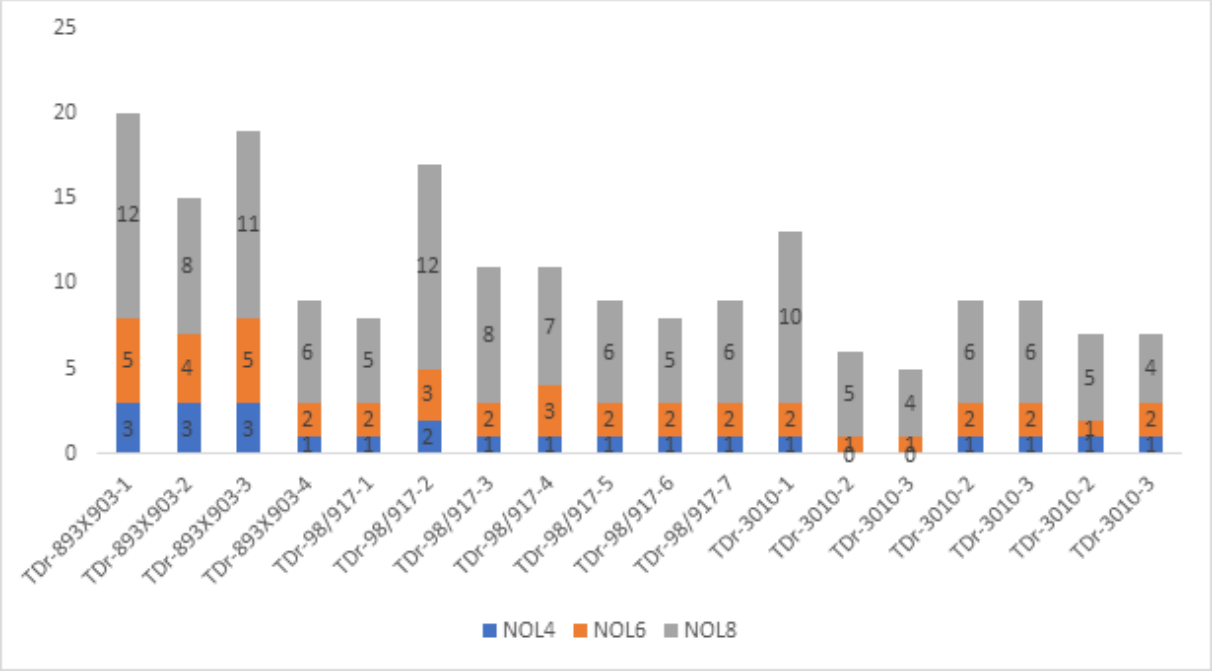


Figure 3: Number of leaves of individual zygotic seeds of three accessions of white yam at 4, 6 and 8 weeks after planting. NOL4, NO6 and NO8 are number of leaves at 4, 6 and 8 weeks old repectively.

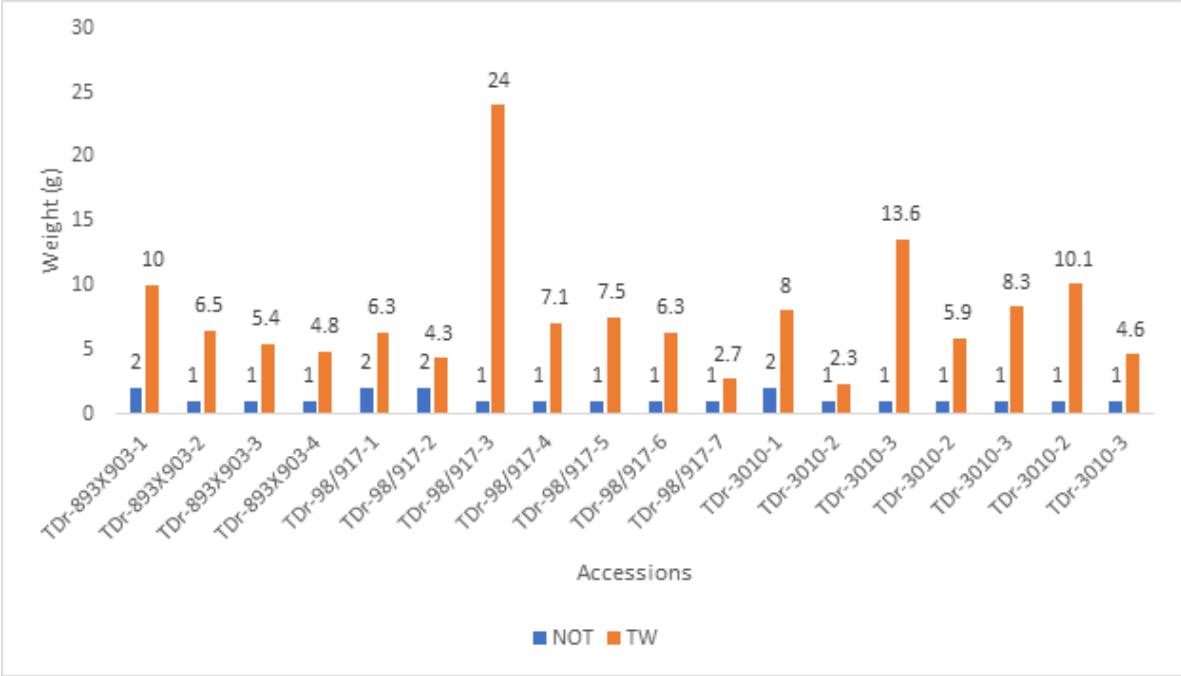
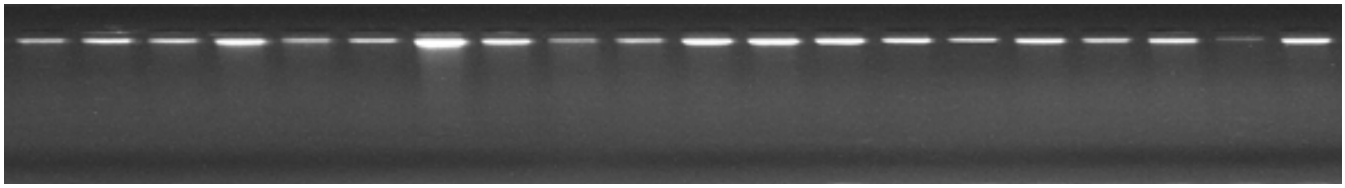


Figure 4: Number of tubers and tuber weight produced by individual zygotic seeds of three accessions at harvest in hydroponics system. NOT and TW are numbers and weight of tubers respectively.

Following the protocol of in extracting the DNA of the individual plants in the three accessions, good quality DNAs were extracted (Plate 1) [11]. The genotypic profiling of the individual plants in the three accessions with 10 codominant SSR markers revealed a varied clustering arrangement not limited to accession groups. Six clusters were observed, with cluster 1 having 2 TDr-3010 and 1 TDr-893x903 individuals, cluster 2 and 5 had TDr-3010 (2) and TDr-893x903 (1) and TDr 98/917 (1) individuals, cluster 3 had one individual each of TDr-3010, TDr-893x903 and TDr 98/917. Cluster 4 had TDr 98/917 (3) only, while cluster 6 had only one individual of TDr 98/917 (Fig 5a). Just like the cluster result, the Principal Component groupings presented a scattered arrangement of the individual plants of the three accessions (Fig 5b).

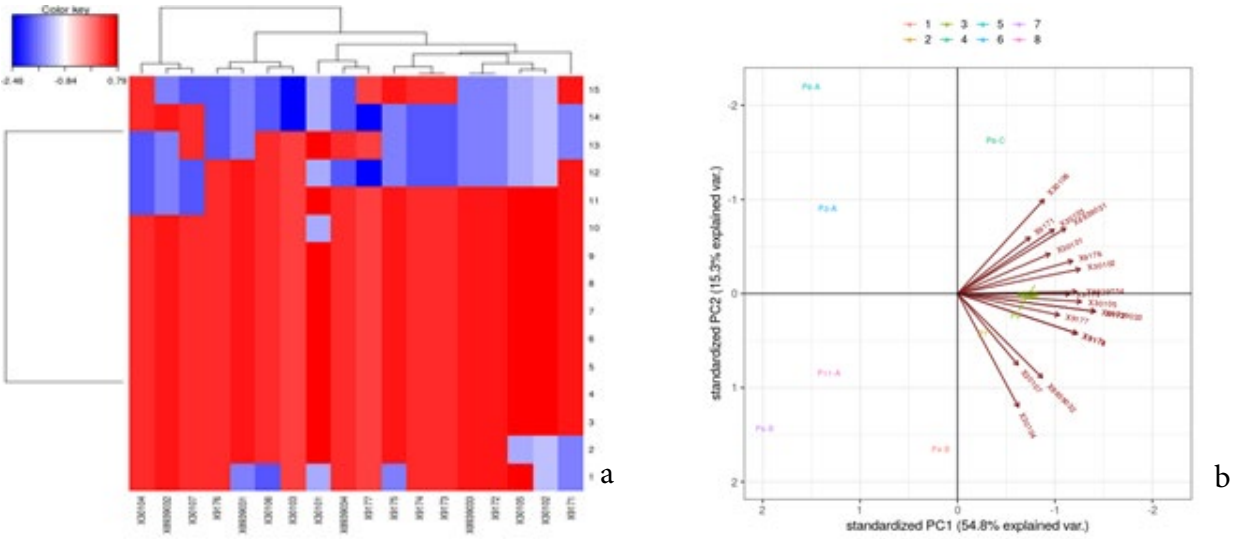
Based on the phenotypic information, the individuals were grouped into 3 clusters, with cluster 1 having 4 individuals each from TDr-3010 and TDr/98-917, cluster 2 had 3 individuals each from TDr-3010 and TDr/98-917 and 1 from TDr-893x903 accession, while cluster 3 had 3 individuals from TDr-893x903 accession (Fig 6a). Also, based on the Principal Component Analysis, the individual plants were grouped into 2, with all individuals from TDr-893x903 accession being grouped together, while the TDr 98/917 and TDr-3010 individuals were grouped together (Fig. 6b). Yam is traditionally propagated using the tuber part [15]. This method help maintains the genetic purity of the plant from one generation to another as the offspring originates from the somatic part, thus they are true to type and conventionally termed 'seed yam' [16]. However, yam sometimes produces flowers and bears fruit, which can be harvested and used for breeding programs [17]. Through this means, 'yam seed' is being produced. But the seeds are not true to type, which is not in the interest of commercial seed companies but the breeders that requires such in generating improved varieties for release.

On the down side, there is non-synchronization of flowering in yam, thereby elongating yam breeding period [17]. The yam seed originating from the flowering part developed through pollination and cross fertilization explains why progenies of same accession tends to differ in the genotypic and phenotypic characteristics as half of the genetic components of the parent lines are being contributed to form the offspring. Then the issue of contribution proportionality, dominant and if the system of inheritance obeys mendellian laws or a deviant of it coupled with the environmental conditions controls the genotypic constituent of the progeny. On the phenotypic characters exhibited, progenies of same accession tends to cluster more closely as the phenotype is a multiplicative result of genotype and environment. Thus, they exhibited epigenetic control, as though there are slight genetic changes in the offsprings as revealed by the genetic analysis using the codominant markers, they tend to exhibit similar phenotypic features.



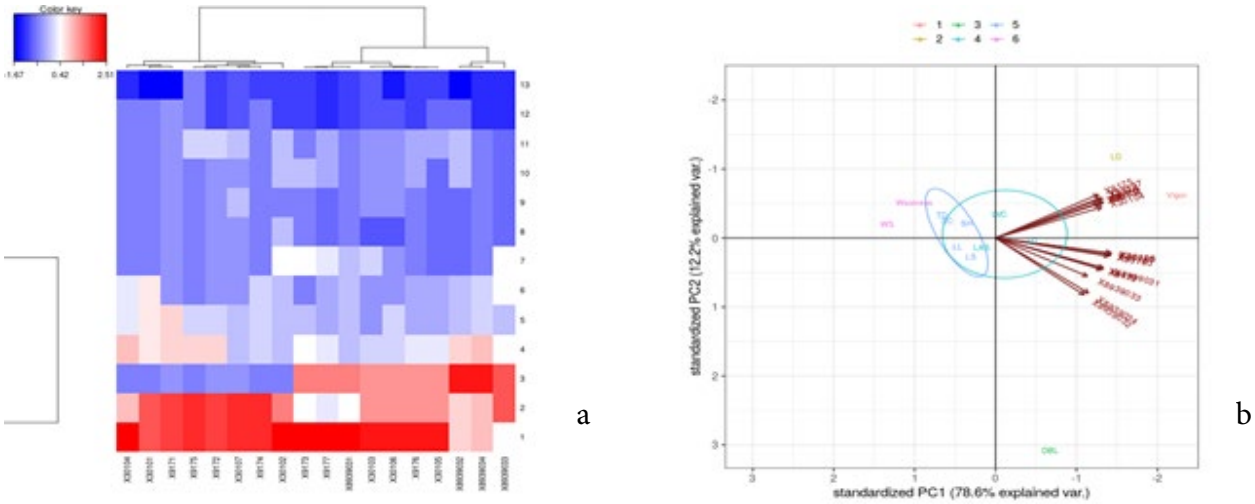
1ulof 50ng lambda
917 (R1)
917 (R2)
917 (R3)
917 (R4)
917 (R5)
917 (R6)
917 (R7)
3010 (R1)
3011 (R2)
3012 (R3)
3013 (R4)
3014 (R5)
3015 (R6)
3016 (R7)
893x932 (R1)
893x932 (R2)
893x932 (R3)
893x932 (R4) 2ul of 50ng lambda

Plate 1: Integrity check of extracted RNAs from of yam using agarose gel electrophoresis.



a b

Figure 5: (A): Genotypic dendogram and (B): clusters of individual zygotic seeds of three yam accessions



a b

Figure 6: (A): Phenotypic dendogram and (B) clusters of individual zygotic seeds of three yam accessions

Conclusion

Yam seeds, unlike the seed yam, originate from the zygotes and are very important in breeding programs. The findings of this research validates the variations in the progenies of same accession/parents in same environment, the hydroponics system. However, the extent of diversity is less revealed phenotypically relative to the genotypic make up. It is therefore recommended that the variation and resemblance of these seed progenies be compared to their parent lines to fully understand the pattern of inheritance [18, 19].

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