

Analyzing genetic variation for important yield-contributing attributes in genotypes of orange-fleshed sweet potatoes

Abstract

An experiment was conducted to estimate genetic variability of 47 orange-fleshed sweetpotato (OFSP) genotypes. It was established in two locations (Umudike and Igbariam) in a RCBD fashion with three replications. Data collected were subjected to ANOVA using the software; AGD-R. The results showed that all the characters studied were highly significant ($p < 0.01$) for genotypes. All the traits were significant for location except the vine length, days to 50% flowering and root yield. Most of the characters were also significant for genotypes-location interaction. Significant differences observed among genotypes for most traits indicated the presence of genetic variation among the materials. The genotypic coefficient of variation (GCV) was very high for beta carotene (73.8%) and vitamin A (70.0%). Phenotypic coefficient of variation (PCV) was very high for beta carotene (183.5%), root yield per hectare (151.6%), total root weight per plant (150.6%) and total number of roots per plant (113.5%). The PCV was higher than their corresponding GCV for all the characters studied, indicating that the expression of these characters was influenced by environment. The GCV was consistently higher than PCV in all the traits. The genotypic variance was high for days to 50% flowering (373.16), dry matter (220.30), root girth (140.73), root length (68.10) and root yield per hectare (349.66). The magnitude of V_A was consistently larger than that of V_D for all the traits suggesting that the additive genetic variance was more important than the dominance genetic variance in the inheritance of most studied traits. All the characters studied had high heritability (>60%). High heritability estimates for those traits indicated a high response to selection.

Keywords: orange-fleshed sweetpotato genotypes, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), genetic advance (GA), heritability

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Abbreviations: GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; GA, genetic advance

Introduction

Sweetpotato (*Ipomoea batatas* (L.) Lam) is a member of *Convolvulaceae* family.¹ Sweetpotato ranks third among tuber and root crops worldwide.² In Nigeria however, sweetpotato has been identified to be the fourth most vital root crop after Cassava, Yam, and Cocoyam.³ Orange-fleshed sweetpotato (OFSP) is now becoming an important member of the tropical root crops having huge potentials as a dependable source of vitamin A.⁴ The young leaves and shoots are sometimes eaten green for its anthocyanin pigments which have anti-inflammatory and anti-carcinogenic properties.⁵ Its starchy root contain vitamin A and some other minerals that are comparable to those of many fruits. The majority of sweet potatoes cultivated globally are processed into potato food diets and food components for human, pig, cow, chicken feed, and starch for industries. Only around 50% of sweet potatoes are eaten fresh. Polystyrene and other polymers can be replaced by its starch since it is 100 percent biodegradable.⁶ In spite of being the cheap source of energy, the roots are high in vitamin A in the form of β -carotene. Because yield is used by African farmers to accept or reject varieties, the most often prioritized qualities for orange-fleshed sweetpotato development programs globally are yield and yield contributing characters.

Heritability, the transfer of favourable traits into new varieties, and the type of accessible genetic variation all play a significant role in crop improvement. High variation within the available germplasm

can increase the performance of breeding programs by enabling the plant breeder to create new varieties or enhance the qualities of existing crop variety more quickly.^{7,8} An effective biometric method for assessing genetic variability is the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and genetic advance (GA).^{9,10} Because of this, the development of new genotypes requires knowledge of the genetic variability that is heritable in the crop's germplasm in order to establish an effective breeding program. Knowledge of genetic variability may also be used to determine if certain variations are heritable or not.^{11,12} Effective genetic improvement is enhanced by knowledge of the type and degree of genetic variation controlling the inheritance of traits.¹³ Therefore, before the commencement of any breeding program, it is important to characterize the genetic background of orange-fleshed sweetpotatoes and establish breeding values.¹⁴ As a result of numerous interactions between several traits that contribute to yield, yield is a complex polygenic trait. Taking into account the aforementioned information, the current research study was conducted to evaluate genetic variability, heritability, and genetic advancements among yield and yield-contributing characters for orange-fleshed sweetpotato genotypes. This study offers knowledge that might help future breeding programs develop desirable orange-fleshed sweetpotato genotypes for high root yield.

Materials and methods

This research was carried out in two locations, namely; the "NRCRI" known as National Root Crops Research Institute (NRCRI), Umudike. This institute is located at Latitude 05°29' N, Longitude

07°33'E and at an altitude of 122m asl and at Igbariam experimental farm which is located at Latitude 06°15' N, Longitude 06°52'E and at an altitude of 81m above sea level from 2015 – 2017. Umudike experiences yearly precipitation of between 2000 and 2500mm and average temperatures of approximately 26°C. Igbariam temperature ranges between 21 to 32°C during the year. Vines of 47 accessions of orange fleshed sweetpotato (OFSP) obtained from the NRCRI were used. The research was established at the on-set of rainy season (April - May) in the two locations; Umudike and Igbariam. Three replicates of a Randomized Complete Block Design (RCBD) were employed. Three-month old vine cuttings of OFSP measuring between 25 – 30cm long, with 3 – 4 nodes were planted in the field with 1.0 m between rows and 0.3 m within rows. The plant density was 33,333 plants per hectare, while the plot size was 1 × 3m (3m²). A single seedling was planted per stand. Weeding was done manually using hoe as the need arises. Vacant stands were supplied after 2 weeks from the planting date. Compound fertilizer, NPK 15:15:15 was used at 1 month after planting at the rate of 400kg/ha. The field was sprayed regularly, especially against sweetpotato weevils, leaf scab, and caterpillars that feed inside the flowers. Other crop management practices such as weeding once per month was applied following research recommendations for the site. The plants were harvested when the leaves began to senesce.

Statistical analysis: The collected data for the various parameters were carefully gathered and arranged for statistical analysis. Each trait's estimated genetic contributions and heritability were computed based on Feher.¹⁵

$$\text{Broad sense heritability}(bs) = \delta^2g / \delta^2p$$

$$\text{Whereas Genotypic variance } (\delta^2g) = \text{MSG} - \text{MSE}/r$$

$$\text{Phenotypic variance } (\delta^2p) = \text{MSE}/r$$

MSG = Genotypic mean squares from the analysis of variance (ANOVA)

$$\text{MSE} = \text{Error mean squares of ANOVA}$$

$$r = \text{number of replications}$$

The broad sense heritability estimates were divided into three categories: low, moderate, and high.

Low is defined as 0 to 30%, Moderate as 31 to 60%, and High as > 60%.¹⁶

$$\text{Broad sense heritability} = \frac{Vg}{Vp} = \frac{Va + Vd + Vi}{Va + Vd + Vi + Ve}$$

Where Va = additive variance, Vd = dominance genetic variance, Vi = epistasis, Ve = environmental variance, Vp = phenotypic variance. Similarly, the formula used by Johnson *et al.* (1955) was used to evaluate the coefficients of variation at the phenotypic and genotypic levels. as: GCV (%) = $\{(\sqrt{\delta^2g}/X) \times 100/1\}$,

PCV = $\{(\sqrt{\delta^2p}/X) \times 100/1\}$. The range of variation was classified according to Sivasubramanian and Madhavamenon's¹⁷ classification system as: 10% = low, 10-20% = moderate, >20% = high; Where, δ^2g = genotypic standard deviation, δp = phenotypic standard deviation, and X = Grand mean for the characteristic x; PCV and GCV are phenotypic and genotypic coefficient of variation, respectively. This was analysed using the software; Analysis of Genetic Designs (AGD-R) written by Francisco *et al.*¹⁸ Expected Genetic Advance (GA) was computed as follows for each trait: $GA = K\sqrt{Vp} H^2$; Where, K = 2.06 at 5.0% selection intensity; H² = Broad Sense Heritability and Vp = Phenotypic variance for trait.

Results

Analysis of variance: The results of the analysis of variance revealed (Table 1) that the genotype differences for all the investigated traits were significant. Similarly, all the traits were significant for location except the vine length, days to 50% flowering and root yield that were non-significantly different. Most of the characters were also significant for genotypes-location interaction except traits like vine length, root length, total number of roots per plant, marketable roots per plant and root yield.

Estimate of genetic parameters: Evaluation of environmental (δ^2e), additive (δ^2_A), dominant (δ^2_D) and genotypic variances (δ^2g), phenotypic coefficient (PCV) and genotypic coefficient of variance (GCV), heritability (%), and genetic advance (GA) are shown in Table 2.

Table 1 Analysis of variance for different morphological plant traits of 47 orange-fleshed sweetpotato genotypes

TRAITS	df	Vine Length (cm)	Days to 50% Flowering	Root Girth (cm)	Root Length (cm)	No. of marketable Roots Per plant	Weight of marketable Roots Per plant(kg)	Total no. of roots Per plant	Total root Weight per Plant (kg)	Root Yield (t/ha)	B-carotene Content (Mg/100g FW)	Dry Matter (%)
Replication	2	8159.0	1.17	7.50	5.69	1.99	2.07	1.10	1.26	193.70	0.01	0.067
Genotypes (A)	46	13083.0 **	2204.69**	165.44**	84.13**	18.97 **	3.77 **	37.91**	7.54 **	362.19**	80.25**	200.10**
Location (B)	1	3212.0ns	203.58 ns	205.96**	111.60**	7.05 *	129.11 **	12.97 *	338.47 **	46.31ns	14.75**	1600.1**
A X B	46	619.0ns	162.36 **	18.24*	5.34 ns	0.67 ns	2.59 **	2.48 ns	6.37 **	14.68ns	0.2643**	128.0**
Residual	186	1549.0	77.89	9.38	5.19	0.89	0.44	2.23	1.16	20.55	0.0026	0.089

** Indicates significant at 0.01 probability level and * indicates significant at 0.05 probability level.

Table 2 Estimate of mean, genetic components of variance, heritability and genetic advance of orange -fleshed sweetpotato combined across locations

Character	Mean	GCV (%)	PCV (%)	δ^2g	δ^2e	δ^2_A	δ^2_D	$h^2(bs)$ (%)	GA
Vine length(cm)	164.70	34.7	85.5	12162.79	38.00	9084.09	3040.70	98.9	1533.1
Days to 50% flowering	64.62	14.3	38.13	373.16	0.02	295.12	78.04	98.9	268.84
Root girth (cm)	21.95	25.2	68.5	140.73	0.01	111.97	28.76	99.2	165.49
Root length (cm)	17.87	21.0	58.4	68.10	0.02	54.48	13.62	99.5	144.87
Total number of roots per plant	6.94	41.0	113.5	38.57	0.00	30.75	7.82	99.6	87.08

Table Continued...

Character	Mean	GCV (%)	PCV (%)	δ^2_g	δ^2_e	δ^2_A	δ^2_D	$h^2(bs)$ (%)	GA
Total root weight per plant (kg)	2.27	56.2	150.6	7.00	0.01	5.44	1.56	99.4	36.82
Root yield per hectare (t/ha)	15.93	56.4	151.6	349.66	0.02	271.75	77.91	99.5	260.98
Beta carotene content	5.24	73.7	183.5	57.85	8.83	34.56	14.46	99.5	106.09
Dry matter (%)	42.26	17.8	44.6	220.30	29.50	135.72	55.08	99.6	207.29
Starch content (%)	24.32	31.3	75.9	196.09	0.00	147.84	48.25	97.2	192.93
Vitamin A	407.90	70.0	174.7	316313.49	45794.72	191440.40	79078.37	99.5	7854.10

GCV (%), Genotypic coefficient of variation; PCV (%), phenotypic coefficient of variation; δ^2_g genotype variance; δ^2_e environmental variance; δ^2_A additive variance; δ^2_D dominance variance; $h^2(bs)$, broad sense heritability(%); GA, genetic advance.

Estimates of phenotypic and genotypic coefficients of variation:

The range of genotypic coefficient of variation was high for most of the traits considered and ranged from 14.3% (days to 50% flowering) to 73.8% (beta carotene). The genotypic coefficient of variation was very high for beta carotene (73.8%) and vitamin A (70.0%). However, root yield per hectare (56.4%), total root weight per plant (56.2%), total number of roots per plant (41.0%) and vine length (34.7%) had moderate genotypic coefficient of variation. The genotypic coefficient of variation for days to 50% flowering (14.3%), root girth (25.2%), root length (21.0%) and dry matter (17.8%) were low. The phenotypic coefficient of variation was also high for the majority of the characteristics examined, but it was very high for beta carotene (183.5%), vitamin A (174.7%), root yield per hectare (151.6%), total root weight per plant (150.6%), total number of roots per plant (113.5%), vine length (85.5%). However, root girth (68.8%) was high, while root length (58.4%), dry matter (44.6%) and days to 50% flowering (38.13%), were moderate.

Estimates of genetic variance, environmental variance, additive and dominance genetic variances:

The overall mean of genotypic variance was high for some traits and moderate for others. However, it was high for vine length (12162.79), days to 50% flowering (373.16), dry matter (220.30), root girth (140.73), root length (68.10), root yield per hectare (349.66) and vitamin A (316313.49), while root length (68.10) and Beta carotene content (57.85), were moderate. The overall mean of additive genetic variation was high for vitamin A (191440.40), days to 50% flowering (295.12), root girth (111.97), root yield per hectare (271.75), dry matter content (135.72), while root length (54.48), total number of root per plant (30.75) and beta carotene content (34.56) were moderate. However, total root weight per plant (5.44) was low. The overall mean of dominance genetic variance was high for vitamin A (79078.37), while vine length (3040.70), days to 50% flowering (78.04), dry matter (55.08) and root yield per hectare (77.91) were moderate. However, root girth (28.76), beta carotene content (14.46), total number of root per plant (7.82), root length (13.62), and total root weight per plant (1.56) had low dominance genetic variance (Table 2).

Broad sense heritability estimates: In general the values of heritability in broad sense considered for all the attributes were in the experiment with total number of roots and dry matter content having the highest with the value of 99.6%, each.

Estimates of genetic advance: From this study, high value of GA was recorded for such traits as vine length (1533.1), days to 50% flowering (268.84), root girth (165.49), root length (114.87), root yield per hectare (260.98), beta carotene content (106.09), dry matter (207.29), total number of roots per plant (87.08) and vitamin A (7854.1). While low GA was obtained for total root weight per plant (36.82).

Discussion

The significant difference from ANOVA results showed that the genotypes had genetic variations between them that affect how they

behave in terms of the characters examined. Bekele et al.,¹⁹ reported a similar result. Significant disparities between genotypes for the majority of the examined characters observed by Dagne et al.,²⁰ revealed the presence of genetic variety among the materials, which allows for the improvement of the traits. According to the current study, all of the studied parameters had higher phenotypic coefficient variances (PCV) than their corresponding genotypic coefficient variances (GCV), showing that the environment had an impact on how these traits were expressed. Singh et al.,²¹ and Tuhina-Khatun et al.,²² observed similar outcomes in rice. The characters that exhibited a higher difference between PCV and GCV, such as total root weight per plant, root yield per hectare, beta carotene content, and vitamin A content, suggested that the environment had a greater impact on the expression of those traits. For traits like vine length, total number of roots per plant, total weight of roots per plant, root yield per hectare, beta carotene, and vitamin A, the largest genotypic and phenotypic coefficients of variation were found, indicating that selection may be used on the traits to identify more productive genotypes. Different scientists also reported similar observations in upland cotton.^{23,24} On the basis of dry matter, root girth and root length, moderate PCV and GCV were noted. The moderate phenotypic and genotypic coefficients of variation for several characters were also observed by Abbas et al.,²⁴ and Amare et al.,²⁵ who hypothesized that some traits may improve through active selection. Days to 50% flowering, for example, showed low PCV and GCV, indicating that breeders should look for sources of high variability to enhance these parameters. Additionally, for every trait, the magnitude of VA was consistently greater than the VD. This result demonstrates that in the inheritance of the majority of the examined characters, additive genetic variation was more important than dominant genetic variance, demonstrating the efficacy of selection in the early segregating generations for increasing such traits. The prevalence of additive gene action for plant height, the number of productive tillers, and the days to 50% flowering in rice was similarly observed by Sobita Devi et al.,²⁶ and Verma et al.²⁷ They emphasized that homozygosity was associated with predominant additive genetic variance, which means that it may be fixed in nature and that selection for these traits will be extremely successful. Other important selection criteria are heritability and genetic progress. When taken into account as a whole, they are more effective as selection tools. Understanding the sort of gene action underlying distinct polygenic traits can be aided by estimates of genetic advance. According to Johnson et al.,²⁸ a combination of strong genetic advance and high heritability estimates is more useful in predicting gain under selection than just high heritability estimates alone. Thus, if there is significant genetic advance, the heritability estimates will be accurate. This finding suggests that these characters could easily be improved through selection. According to Al-Tabbal et al.,²⁹ the heritability's predictive role in demonstrating the validity of the phenotypic value as a guide to breeding value is its most significant function in the genetic research of quantitative characters. The heritability of every character under the study was high (>60%). High heritability estimates for these characters suggested that these

characters had a strong response to selection. Dutta et al.,³⁰ and Raffi et al.,³¹ provided findings that are consistent with the current findings. According to Wolie et al.,³² genetic advance (GA) under selection is the improvement of traits' genotypic values for the new population as compared to the base population during a single cycle of selection at a given selection intensity. The highest GA value was found in vitamin A (7854.10), while the lowest value was found in total root weight per plant (36.82). The result from GA estimates 36.82 kg for total root weight per plant. Suggesting that the average total root yield of offspring could be increased by 36.82 kg per plant whenever we choose the best, 1% high yielding genotypes as parents.

However, it was found that the PCV, δ^2g , δ^2e , δ^2_A , δ^2_D and **GA** for some traits were more than 100. In OFSP, the high estimates may be due to competition among the genotypes.³³

Conclusion

The genetic coefficient of variation, heritability, and genetic advance (GA) are important parameters to consider when establishing an effective breeding program for orange-fleshed sweetpotato. The result from the current study suggested that these parameters are potential for improving root yield of orange-fleshed sweetpotato through selection. This is because breeders can utilize additive gene effects, transgressive segregation, and heterosis to increase yield when there is enough genetic variation.

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None.

Conflicts of interest

There are no conflicting interests declared by the authors.

References

- Austin DF. Hybrid polyploids in *Ipomoea* section *Batatas*. *J Hered*. 1977;68:259–260.
- Food and Agricultural organization of the United Nations, FAOSTAT data: <http://faostat.fao.org/faostat/form collection production. Crops Primary and Domain. 2005. 32 p.
- Okonkwo JC, Amadi CO, Nwosu KI. Potato production, storage, processing and utilization in Nigeria. National Root Crops Research Institute, Umudike, Nigeria. 2009;36:1–14.
- Horton DE. World patterns and trends in sweetpotato production. *Tropical Agricultural African Crop Science Journal*. 1988;16:147–160.
- Anthony R. Purple Sweet Potato Pigments Could Be Used To Color Foods; Replacing Synthesized Dyes And Bugs. *Medical Daily*, USA. 2013;1(2):136–145.
- Kapinga R. Present status of sweetpotato breeding for Eastern and Southern Africa. In: Ree D, editor. Sweetpotato post-harvest assessment: Experiences from East Africa, NRI, CPHP, DFID, CIP and Ministry of Agriculture Tanzania, Chatman, UK; 2003. 88 p.
- Ranganatha HM, Patil SS, Manjula SM, et al. Genetic variability studies in segregating generation of upland cotton (*Gossypium hirsutum* L.), *Molecular Plant Breeding*. 2013;4:84–88.
- Yared S, Misteru T. Variability, heritability and genetic advance analysis for some morphological characters in oilseed brassica breeding lines. *Molecular Plant Breeding*. 2016;7(20):1–8.
- Vimala B, Hariprakash B. Variability of morphological characters and dry matter content in the hybrid progenies of sweetpotato (*Ipomoea batatas* (L.) Lam). *Gene Conserve*. 2011;10:65–86.
- Falconer DS, TFC Mackay. Introduction to quantitative genetics. 4th ed. Longman, Essex, England; 1996;2(3):20–22.
- Konate AK, Zongo A, Kam H, et al. Genetic variability and correlation analysis of rice (*Oryza sativa* L.) inbred lines based on agromorphological traits. 2016;11(35):3340–3346.
- Perera UIP, Disna R, Senanayake SGJN, et al. Genetic parameters and correlations of yield attributing characteristics of sweetpotato in Sri Lanka. *JNSF, Sri Lanka*. 2014;42(4):299–306.
- Yadav SK, Suresh BG, Pandey P, et al. Assessment of genetic variability, correlation and path association in sweetpotato. *J Bio-sci*. 2010;18:1–8.
- Agong SG, Schittenhelm S, Fried W. Genotypic variation of Kenyan tomato (*Lycopersicon esculentum* Mill.) germplasm. *Journal of Food Technology in Africa*. 2001;6(1):13–17.
- Fehr WR. Principles of cultivar development. 1987;1:351–353.
- Lu GQ, Sheng JL. Application of near infrared reflectance spectroscopy (NIRS) in sweet potato quality breeding. *Scientiagricultura Sinica*. 1990;23:76–81.
- Sivasubramanian S, Madhavamenon P. Combining ability in rice. *Madras Agricultural Journal*. 1973;60:419–421.
- Francisco R, Gregorio A, Angela P, et al. Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT); *Biometric and Statistical Unit*. Mexico. 2015. 12 p.
- Bekele BD, Naveen GK, Rakhi S, et al. Genetic evaluation of recombinant inbred lines of rice for grain zinc concentrations, yield related traits and identification of associated SSR markers. *Pakistan Journal of Biological Sciences*. 2013;16(23):1714–1721.
- Dagn W, Habtamu Z, Labuschagne MT, et al. Heterosis and combining ability for grain yield and its component in selected maize inbred lines. *S Afr J Plant Soil*. 2007;24(3).
- Singh A, Singh AK, Singh V, et al. Genetic variability among traits associated with grain yield of rice (*Oryza sativa* L.) exposed to drought at flowering stage. *Afr J Res*. 2014;9(16):1252.
- Tuhina-Khatun M, Hanafi MM, Yusop MR, et al. Genetic variation, heritability, and diversity analysis of upland rice (*Oryza sativa* L.) genotypes based on quantitative traits. *BioMed Res Intr*. 2015;290861.
- Amir S, Farooq J, Bibi A, et al. Genetic studies of earliness in *Gossypium hirsutum* L. *IJAVMS*. 2012;6(3):189–207.
- Abbas HG, Mahmood A, Ali Q. Genetic variability, heritability, genetic advance and correlation studies in cotton (*Gossypium hirsutum* L.). *International Research Journal. Microbiol*. 2013;4(6):156–161.
- Amare B, F Abay, Y Tsehay. Evaluation of sweet potato (*Ipomea batata* L.) varieties for total storage root yield in south and south east zones of Tigray, Ethiopia. *American Journal Trade Policy*. 2014;1:74–78.
- Sobita Devi L, Raina FH, Manish K, et al. Genetic parameters of variation for yield and its components in rice (*Oryza sativa* L.) *Crop Research*. 2006;32:69–71.
- Verma RS, Yadav RDS, Giri SP. Genetics of yield and its important components in rice (*Oryza sativa* L.). *Crop Research*. 2006;31:142–146.
- Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybeans. *Agronomy Journal*. 1955;47:314–318.
- Al-Tabbal JA, Al-Fraihat AH. Genetic variation, heritability, phenotypic and genotypic correlation studies for yield and yield components in promising barley genotypes. *J Agric Sci*. 2012;4:3.
- Dutta P, Dutta PN, Borua PK. Morphological Traits as Selection Indices in Rice: A Statistical View. *Univ J Agric Res*. 2013;1(3):85–96.

31. Raffi MY, Zakiah MZ, Asfaliza R, et al. Grain quality performance and heritability estimation in selected rice genotypes. *Sains Malaysiana*. 2014;43(1):1–7.
32. Wolie A, Dessalegn T, Belete K. Heritability, variance components and genetic advance of some yield and yield related traits in Ethiopian collections of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes. *Afr J Biotechnol*. 2013;12(36):5529–5534.
33. Searle SR. Topics in variance component estimation. *Biometrics*. 1971;27:1–74.