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Genetic Variability for Mesocotyl Length in Maize †

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Abstract: The possibility of developing deep-sowing tolerant (DST) maize to absorb moisture from subsoil zones is crucial to maize adaptation to water-stress environments. The function of the mesocotyl in field emergence of seedlings is established in grasses. However, information is scarce on the extent of genetic variability for mesocotyl length (ML) in maize. Sixty-eight maize genotypes were studied using Completely Randomized Design in a laboratory experiment to investigate the extent of genetic variability for ML, and the relationship of seed biochemical components with ML. Ten seeds of each genotype were germinated for 10 days in the dark. Mesocotyl length was determined by placing cut mesocotyl against a flexible measuring tape. Biochemical contents of seeds were determined at a standard diagnostic laboratory. Analysis of variance revealed highly-significant ($p \leq 0.01$) genotype mean square, indicating sufficient variability for genetic improvement. Broad-sense heritability and genetic advance were high and implied that ML was heritable. Mean ML for genotypes ranged from 0.58 to 9.02 cm; thus, planned crosses can be made for ML improvement. Dendrogram from cluster analysis based on Ward's minimum variance cluster analysis classified 65 of the genotypes into clusters I, II, and III with ML (mean ± standard deviation) of 0.49 ± 0.18, 4.25 ± 0.96, and 9.16 ± 0.93 cm respectively. Crosses can be planned involving genotypes from clusters 1 and III, to exploit heterosis for ML in a hybrid program. The results obtained from this study provide a basis for the development of DST maize for drought-prone environments.

Keywords: adaptation; cluster; dendrogram; drought; heritability; maize; mesocotyl; variability

1. Introduction

Different crops have developed characteristic adaptive features for their seedlings to push through the soil. This is particularly essential in the arid and semi-arid regions of the world which are characterized by dry top soil owing to low water table. Farmers in these areas find it difficult or impossible to obtain good field establishment when water table is low. In crops like cowpea and Arabidopsis, the ssedlings breaks out of the soil through the hypocotyls while the seedlings of monocots (without hypocotyl growth) like maize, wheat, and rice do so by means of the mesocotyl [1,2]. However, maize lines/varieties that are able to emerge from soil depths below the common 2-5 cm are scarce or unavailable to farmers particularly in Africa and other drought-prone areas of the world. Therefore, efforts at developing maize varieties with elongated mesocotyl are strategic to the improvement of the crop's performance in drought-prone areas. Because it is highly regulated by phytohormones, much of the research on maize mesocotyl elongation has focused on hormonal regulation such as auxin [2], gibberellic acid [3,4], and brassinosteroids [5]. Information

on the genetics; variability and/or mode of inheritance, of maize mesocotyl length is thus sparse in literature or altogether unavailable. According to Niu et al. [1], critical genes determining mesocotyl elongation in maize remain unknown. Discovery of candidate genes for maize mesocotyl elongation, and identification of the mode of inheritance of the genes, will enhance the improvement of deep-sowing tolerance in maize, and provide the possibility of developing maize varieties for sowing in areas with low water tables. Investigating the extent of genetic variability for ML in available maize germplasm, and the influence of seed biochemical quality attributes on ML will provide information for determining the gene action controlling ML, and secondary traits for selection of DST maize genotypes. The objectives of this study were to determine the: (i) extent of genetic variability in maize; and (ii) influence of seed biochemical quality attributes on mesocotyl elongation in maize.

2. Experiments

2.1. Genetic Materials

Sixty-eight maize genotypes were used in this study, comprising of 66 genotypes obtained from the International Institute of Tropical Agriculture and two cultivars obtained from a local seed market.

2.2. Experimental Setup

Ten seeds of each genotype were grown in the dark for 10 days using locally-available materials. Jute sack was cut into A4 paper-size and soaked in water. Soaked cut jute materials were laid flat on the laboratory workbench and seeds of each genotype were arranged in two rows of five each. The setup was rolled and held in place at the tips using rubber bands. Completely Randomized Design (CRD) was used with two replicates. Both replicates were placed in a cupboard at the Agronomy Laboratory of the Department of Crop Production and Horticulture, Lagos State Polytechnic, Ikorodu, Nigeria. Seedlings were retrieved to determine mesocotyl lengths at 10 days after setup.

2.3. Data Collection

- Mesocotyl length

Elongated mesocotyl was cut from 10 seedlings per replicate, and placed against a measuring tape to record the length in cm. The arithmetic mean of the ML was then recorded for each maize genotype.

- Seed biochemical attributes

Approximately 10-g seed samples of the genotypes were taken to Tetra "A" Analytical and Diagnostic Laboratory, Abeokuta, Ogun State, to determine biochemical composition with respect to iron (Fe), zinc (Zn), Selenium (Se), crude protein (CP), free fatty acid (FFA), oil, linoleic acid, and amylase contents. Seeds of genotypes †PVAEH 33 and †PVAEH 40 were not sufficient and the genotypes were excluded from the biochemical analysis.

2.4. Data Analysis

Means of the ML were subjected to analysis of variance using the 'glm' procedure of SAS (SAS Institute, Cary, NC, USA). Variance components were computed based on expected mean squares obtained from 'proc varcomp' of SAS (SAS Institute, Cary, NC, USA). Estimates of genetic components were obtained in microsoft excel (Microsoft Corporation, Washington, DC, USA) using the formulae of Singh and Chaudhary [6]. Values of ML were converted to Euclidean distance estimates which were subjected to Ward's minimum variance cluster analysis, in SAS (SAS Institute, Cary, NC, USA). Relationship among ML and seed biochemical contents was studied via correlation analysis performed using 'proc corr' in SAS (SAS Institute, Cary, NC, USA).

3. Results and Discussion

As shown in Table 1, analysis of variance of revealed significant ($p \leq 0.01$) genotype mean square for mesocotyl length. Also, genotypic variance (8.79) was close to phenotypic variance (13.19) leading to high broad-sense heritability (0.67) while genotypic (GCV) and phenotypic coefficients of variation (PCV) were high at 0.57 and 0.70 respectively. In addition, genetic advance (as a % of mean) was very high (almost unity). There was also, difference between the computed genotypic and phenotypic variances indicating the role of environmental factors in the elongation of mesocotyl in maize. The significant genotype mean square, as well as the close correspondence between the genotypic and phenotypic variances indicated the existence of sufficient variability for mesocotyl length among the maize genotypes. Thus, there is possibility of genetic improvement of the trait for future breeding programs. The estimate of environmental variance suggested the need to test the maize genotypes in more environments to investigate the effect of genotype × environment interaction in the elongation of mesocotyl. Similar observations have been reported for agronomic traits in maize. Sivasubramanian and Menon [7] ranked GCVs and PCVs as low, moderate and high when the values are <0.10, 0.10–0.20, and >0.20 respectively. The high GCV (0.57) observed indicated potential for improvement of ML within the germplasm. The higher value of PCV than GCV is an indication of the role of the environment in the phenotypic expression of ML, and further suggested the need to test in more environments. According to Johnson et al. [8], heritability values < 0.30, between 0.30 and 0.60, and above 0.60 are classified as low, moderate and high respectively. Thus, the observed heritability estimate of ML was high and implied that the trait was majorly controlled by the genotype and is therefore heritable. Olayiwola and Soremi [9] have suggested that broad-sense heritability should not be solely used in determining the genetic potentials of a trait since it (broad-sense heritability) is composed of both additive and non-additive genetic variances and as a result, high heritability is not always associated with high genetic advance [10]. Earlier, Johnson et al. [8] recommended that estimates of heritability and genetic advance should be jointly considered in predicting the value of selection. The high genetic advance (as a % of mean) of mesocotyl length is an indication that the trait will respond favourably to selection.

Table 1. Mean squares and genetic components of maize genotypes for mesocotyl length after 10 days in the dark.

Source of Variation	DF	Mean Square
Genotype	67	13.19 **
Error	68	4.40
R^2		0.75
Genotypic variance		8.79
Environmental variance		4.40
Phenotypic variance		13.19
Broad-sense heritability		0.67
Genotypic coefficient of variation		0.57
Phenotypic coefficient of variation		0.70
Genetic Advance (% of Mean)		0.96

**, significant at 99% level of confidence; DF, degrees of freedom; R^2, R-squared.

Grouping genotypes into clusters has been found effective to minimize the genotype pool and ease the process of selection **[11]**. Sixty-five of the maize genotypes were clearly delineated into three clusters based on mesocotyl length while three genotypes; PVAQEH-4, LY1919-14, and A1804-66, were ungrouped (Table 2, Figure 1). Cluster I was composed of 10 genotypes with no or short mesocotyl growth. Cluster II was composed of 20 genotypes with moderate mesocotyl growth ranging from 2.45 cm for †PVAEH 36 to 5.56 cm for PVAEH 29 while cluster 3 had 36 genotypes characterized by long observed mesocotyls ranging from 5.95 cm for †PVAEH 32 to 9.02 cm for LY1901-18. The observed clustering pattern implied the existence of considerable genetic diversity for ML among the maize genotypes. Several past works have classified maize genotypes into clusters

based on a single trait. For instance, Oyetunde et al. [12] and Badu-Apraku et al. [13] classified maize genotypes into heterotic groups based on specific combining ability for grain yield. Crosses can be planned involving genotypes from clusters 1 and III (the most divergent groups) to exploit heterosis for ML.

Sixty-two of the maize genotypes tested developed measurable mesocotyl. The mean mesocotyl length ranged from 0.58 cm for A1312-12 to 9.02 cm for LY1901-18 (Table 2). The differences in ML lengths of the maize genotypes further indicated the existence of variability of the trait, and genotypes with high ML values, such as LY191-01, †PVAEH 34, and †PVAEH 31 have potentials to draw moisture from the subsoil and could be adaptable to drought-prone areas. The standard deviation of a set of numbers is an indication of the spread of the numbers from the mean. A low standard deviation value implies that the majority of the numbers are close to the average while a high value implies that most numbers are far from the mean.

Table 2. Mean ± standard deviation (Std) of mesocotyl length (ML) and classification into clusters based on ML of maize genotypes germinated in the dark for 10 days.

ID	Genotype	Mean	Std	Cluster	ID	Genotype	Mean	Std	Cluster
G25	A1804-14	0	0	1	G2	PVAEH 30	5.99	1.57	3
G56	LY1001-18	0	0	1	G37	LY1901-11	6.08	3.11	3
G57	LY1409-14	0	0	1	G20	Oba super 2	6.12	1.27	3
G58	LY1312-11	0	0	1	G9	†PVAEH 41	6.2	0.83	3
G59	LY1901-15	0	0	1	G21	LY1312-23	6.2	0.99	3
G61	A1804-67	0	0	1	G8	†PVAEH 43	6.28	1.22	3
G23	A1312-12	0.58	0.19	1	G34	LY1901-23	6.3	2.12	3
G24	LY1501-7	1.18	0.22	1	G31	LY1501-8	6.31	0.86	3
G27	LY1901-14	1.36	0.55	1	G38	†PVAEH 40	6.45	2.81	3
G28	LY1913-3	1.74	1.02	1	G4	PVAEH 28	6.47	1.54	3
G60	A1804-66 *	2.02	2.86	-	G63	A1736-6	6.57	1.51	3
G19	†PVAEH 36	2.45	3.46	2	G66	LY1001-23	6.77	2.73	3
G53	Local check	2.65	3.75	2	G67	Mkt-cultivar A	6.88	1.27	3
G51	M1124-31	3.06	3.62	2	G35	LY1901-20	6.93	3.15	3
G54	LY1501-6	3.23	1.1	2	G68	Mkt-cultivar B	6.98	0.76	3
G6	PVAQEH-4 *	3.28	1.64	-	G14	PVAQEH-6	7.02	2.88	3
G44	LY1901-12	3.57	3.01	2	G64	LY1901-24	7.02	0.68	3
G49	A1802-4	3.67	0.61	2	G45	A1706-2	7.04	2.88	3
G26	A1802-12	3.7	0.95	2	G22	LY1901-22	7.05	3.1	3
G36	LY1901-17	4.1	0.11	2	G1	PVAEH 26	7.06	2.43	3
G41	LY1409-21	4.11	2.42	2	G65	LY1501-9	7.19	0.24	3
G11	PVAQEH-3	4.4	0.62	2	G46	Ife hybrid-4	7.29	4.26	3
G18	†PVAEH 37	4.65	6.58	2	G48	LY1914-14*	7.67	3.95	-
G47	LY1901-19	4.78	4.1	2	G29	LY1501-5	7.68	0.99	3
G62	LY1501-1	4.89	1.99	2	G39	†PVAEH 33	7.87	0.52	3
G43	LY1901-25	4.94	1.92	2	G17	†PVAEH 44	7.89	0.78	3
G55	LY1901-21	5.05	1.53	2	G33	A1736-13	8.18	0.85	3
G42	LY1901 -16	5.11	1.2	2	G50	A1804-15	8.22	2.8	3
G40	Ife hybrid-3	5.24	0.62	2	G30	LY1913-16	8.38	2.38	3
G5	Check (RE)	5.39	0.69	2	G15	PVAEH 27	8.42	0.28	3
G52	LY1901-13	5.39	0.87	2	G12	Local check	8.52	0.51	3
G7	PVAEH 29	5.56	0.81	2	G13	†PVAEH 31	8.73	1.6	3
G3	†PVAEH 32	5.95	2.5	3	G16	†PVAEH 34	8.94	0.03	3
G10	PVAQEH-5	5.98	0.72	3	G32	LY1901-18	9.02	0.42	3

*, not classified.

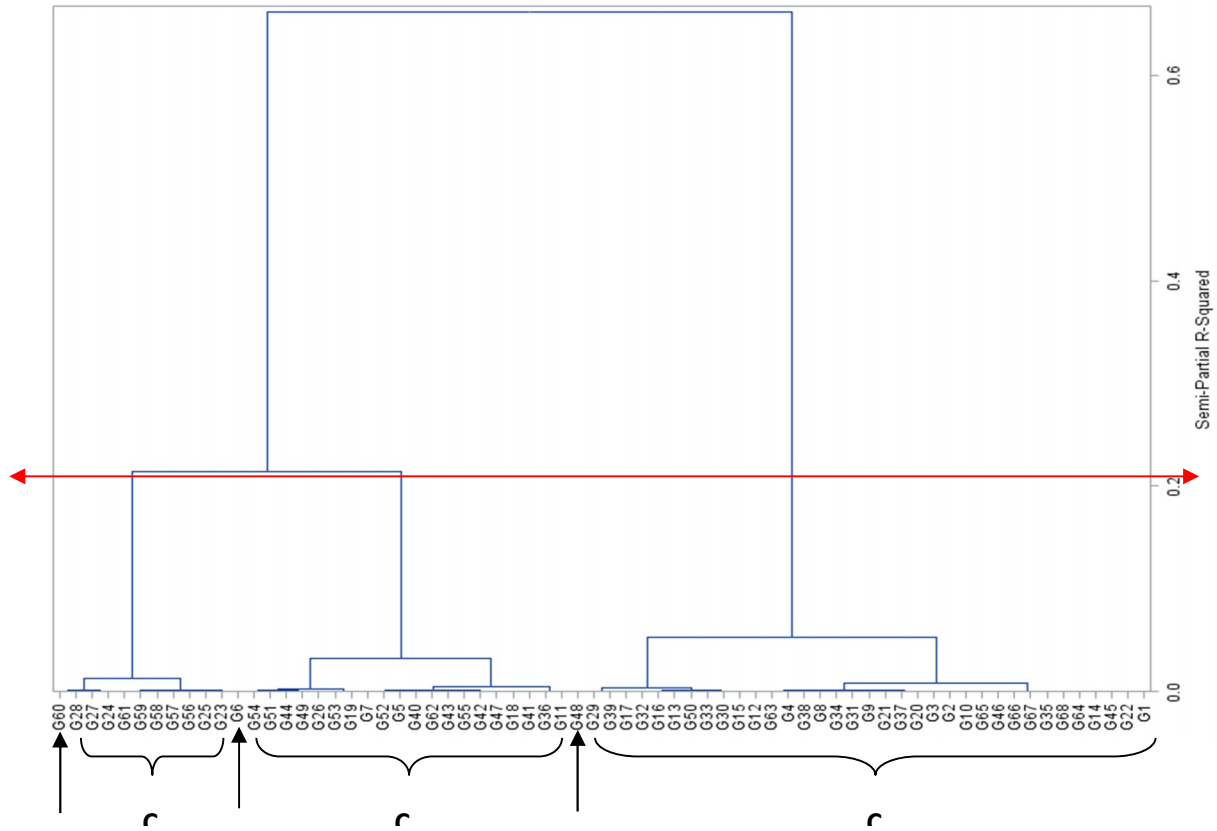

Figure 1. Dendrogram of relatedness among maize genotypes (*X*-axis), based on genetic similarity (*Y*-axis) from Ward's minimum variance cluster analysis. The red double-arrowed line delineates the genotypes into clusters at approximately 65% level of similarity; C1, C2, C3 are clusters 1, 2, and 3, respectively; single-arrowed line signifies ungrouped genotypes.

Genotypes with low standard deviation estimates should, therefore, be more reliable for prediction. Hence, genotypes that combined high ML with low standard deviation values; such as LY1901-18 and †PVAEH 34 would be more reliable in predicting ML of maize. In addition, planned crosses can be made involving the genotypes A1312-12, LY1501-7, and LY1901-14 to develop maize hybrids with potential for adaptation to drought conditions. Also, genotypes with high standard deviation values could be useful for development of inbred lines useful in future hybrid programs with focus in developing deep-sowing tolerant maize

Information on seed micronutrients and biochemical composition which affects the seedling vigor and organ development may provide some insight to the understanding of the mechanism underlying mesocotyl elongation. In this study, significant ($p \leq 0.05/0.01$) associations were observed in the relationship of ML with all the measured biochemical parameters except selenium (Table 3). Furthermore, all the significant associations were positive except for the negative association between ML and amylase content. The significant levels of association observed implied the possibility of simultaneous improvement of each of iron, zinc, crude protein, free fatty acid, oil content, linoleic acid, and amylase content with mesocotyl length of maize. The quality attributes could serve as selection criteria for ML. Muhammad [14] employed nutrient priming to reveal the impact of seed reserves on seedling development and root biomass in maize. According to Martinez-Ballesta et al. [15], seed biofortification enhances seed vigour, and can play a role in abiotic stress tolerance.

Table 3. Pearson correlation coefficients of seed biochemical parameters with mesocotyl length (ML).

Biochemical Content	ML
Iron	0.31 *
Zinc	0.29 *
Selenium	0.01ns
Crude protein	0.33 **
Free fatty acid	0.26 *
Oil	0.30 *
Linoleic acid	0.28 *
Amylase	−0.31 *

* and **, significant at 5 and 1%; ns, not significant.

4. Conclusions

There was substantial genetic variability among the 68 maize genotypes, allowing the grouping into different clusters. Genotypes †PVAEH 31, †PVAEH 34, and LY1901-18 have potentials for improvement as DST maize. Biofortification has the potential to enhance the elongation of mesocotyl in maize during germination. The results obtained from this study provide basis for the development of DST maize for drought-prone environments.

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Conflicts of Interest: The authors declare no conflict of interest.

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