Inheritance of Low Phytate in Africa Biofortified Sorghum

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ABSTRACT

Sorghum is an important food crop in Northern Nigeria. Although, sorghum is highly valued for its hardiness and drought tolerance, but as with most staples, phytic acid an antinutrient inhibits the bioavailability of micronutrients such as iron and zinc leading to micronutrient malnutrition on populations who depend on it as a dietary source. The present study was conducted to study the inheritance of the phytate content in Africa Biofortified Sorghum and to assess the nature of gene action involved in its inheritance. This was done by crossing a transgenic sorghum (ABS188) and a non- transgenic sorghum (SAMSORG 17) to generate F_1, F_2, BC_1P_1 and BC_1P_2. Field evaluation was conducted at the confined field trial (CFT), Samaru in a Randomized Complete Block Design (RCBD) with two replications. Observations were recorded on ten characters and analysis of variance revealed significant differences among the six generations. Estimates of gene effect showed that additive, additive x additive and additive x dominance gene effect are important in the inheritance of phytate level in Africa Biofortified Sorghum. Both additive and non-additive gene effects are essential for the yield improvement in sorghum and breeding strategies like reciprocal recurrent selection would satisfy the breeding objectives.

Keywords: Sorghum, Phytate, Heritability, Genetic-Advance, Variability

INTRODUCTION

Sorghum *(Sorghum bicolor L)* belongs to the order of *Poales* and to the family of *Poacae.* In Africa, the major staple foods are cassava, maize, yam, sorghum plantains, rice (23 million tons), wheat, millet, sweet potato, and bananas FAOSTAT, 2008). Among these staples, sorghum occupies a unique position due to its hardiness as a crop. Sorghum is particularly unique in that it grows in both temperate and arid climates. It is photosynthetically efficient because it is a C4 plant (plants that use the C4 carbon fixation pathway). Sorghum is drought-tolerant and resistant to water-logging (Doggett, 1988), and grows in various soil conditions (Dillon, *et al*. 2007). These characteristics contribute toward it being the staple crop of Africa's most food-insecure people, who live in the desert- margin, semiarid tropics—about 300 million people.

Sorghum nutritional quality is dictated mainly by its chemical composition and the presence of anti-nutritional factors, such as phytate. Kumar *et al.* (2010) also noted that, phytic acid is one of prime concern for human nutrition and health management. The effects of phytate in human and animal nutrition are related to the interaction of phytic acid with proteins, vitamins and several minerals, and thereby restrict their bioavailability (Ali *et al.* 2010). Phytate is the principle means of phosphorus storage in a number of crop plants (Raboy, 2002), including cereal grains, particularly in the bran, and legumes (Bender, 1999). In fact, marginal zinc deficiency has been found to be widespread in people who maintain diets rich in legumes (Cichy, *et al.* 2005). Phytic acid has a strong binding affinity to important

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minerals, such as calcium, magnesium, iron, and zinc. When a mineral binds to phytic acid, it becomes insoluble, precipitates and will be nonabsorbable in the intestines. This process can therefore contribute to mineral deficiencies in people whose diets rely on these foods for their mineral intake.

In view of the anti-nutritional effects of phytate, many attempts were carried to reduce it. Various food processing and preparation techniques, such as decortications, soaking, cooking, germination and fermentation, are the major efforts made to reduce the amounts of phytate in foods (Elmaki *et al.* 2007; Sangronis and Machado, 2007; Liang *et al.* 2008; Khattab and Arntfield, 2009; Wang *et al.* 2010; Kumar *et al.* 2010). The most effective treatments are fermentation (Marfo *et al.* 1990) and germination (Honke *et al.* 1998) but their application remains limited because of the additional workload they imply or the particular organoleptic properties they induce. Keeping in view the importance of phytic acid as a potent inhibitor for the bioavailability of micronutrients viz. Fe, Ca, Mn, Zn, Mg, Cu, etc. a study was conducted.

This study was initiated by crossing two contrasting groups of sorghum genotypes, SAMSORG17 and ABS188 (with high and low phytate content respectively), with the objective of enhancing the nutritional profile of SAMSORG17 through decreasing the inhibitory effect of phytic acid and enhancing the bioavailability of micronutrients and macronutrients to humans. The specific objectives of this research were to determine the phytic acid profile and other agronomic traits and to estimate of their heritability.

MATERIALS AND METHODS

The research was conducted at the Confined Field Trial site of the Institute for Agricultural Research (IAR), Samaru, Ahmadu Bello University Zaria, Kaduna State, Nigeria. Samaru is located on latitude $11^{0}11^{1}$N and longitude $07^{0}38^{1}$E in the Northern Guinea savannah ecological zone at an altitude of 686m above sea level. (Kowal and Knabe, 1972).

The F_1 population was developed by making a biparental cross between the ABS 188 and SAMSORG 17. The Nigerian local variety SAMSORG 17 designated as P_2 was used as female. It was emasculated, by covering with cellophane bags in order to abort the pollens. The parentals, P_1 and P_2, F_1s, F_2s, and the backcross populations (BC_1P_1 and BC_1P_2) were evaluated under field conditions at the CFT site of the Africa Biofortified Sorghum, IAR farm Samaru. The experiment was laid out in a Randomized Complete Block Design (RCBD) with two replications. Plot size was 3mx2m for each of the nonsegregating generations and 3mx4m for each of the segregating generations. Inter and intra row spacing was 75cm and 30cm with two plants per hill. All the agronomic practices such as land preparations, ridging, fertilizer application, weeding were done based on recommended practices.

Data was collected for the following: Number of leaves, Heading date, Plant height (cm), Panicle length (cm), Panicle length Panicle weight (g), Grain weight (g), 1000 seed weight (g), Number of grain per panicle, Determination of phytate content (mg/100g): Determination of phytate content was carried out by the procedure described by Reddy *et al. (*1982).4.0g of ground sample was soaked in 100ml of 2% Hydrochloric acid (HCL) for 3hours and filtered. 25ml of the filtrate was taken into a conical flask and 5 ml of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of iron (iii) chloride until a brownish yellow colour persists for 5 minutes. The titre value was noted and the amount of phytate content in mg/100g is derived by the formula; $Y = X \times 0.00195 \times 1.19 \times 100$; where X= titre value.

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Both genotypic and phenotypic coefficients of variability were computed as per the method suggested by Burton and Devane (1953).

GCV and PCV values were categorized as low, moderate and high as suggested by Sivasubramanian and Menon (1973). To estimate mode of gene interaction, the generation mean analysis (GMA) was used to measure genetic parameters following the procedure described by Gamble's (1962).

The F_2 variances were used to compute the broad sense heritability according to Mahmud and Kramer (1951). The narrow sense heritability estimates were computed using the formulae described by Warner (1952). The heritability percentage was categorized as low, moderate and high as given by Robinson *et al.* (1949).

RESULTS

Analysis of variance (ANOVA) was carried out for the different characters evaluated during the 2013 cropping season by using the replicated data on the same characters from parents (ABS 188 and SAMSORG 17), F_1, F_2, BC_1P_1, BC_1P_2. Mean squares for the different traits are presented in Table 1. It was observed from the table that variances among the generations were highly significant for all the characters evaluated.

Estimates of Gene Effects

Scaling tests was done to test the adequacy of simple additive dominance model in the genetic control of the traits. The result on scaling tests (A, B and C) is presented in Table 2. Scaling test A was significant for phytate content and seedling vigor indicating the presence of epistasis. Scaling test B was significant for panicle length and number of grain per panicle indicating the presence of epistasis. No scaling test was found significant for number of leaves and plant height which reveals the absence of epistasis. For heading date, scaling test A and C were significant which reveals the presence of epistasis. The significance of scaling test B and C for panicle weight and scaling test C for grain weight reveals the presence of epistasis in these traits.

The results obtained for the estimates of gene effect are presented in table 3.The mean effect (m) was highly significant for all the traits evaluated in this study. For phytate level, additive and additive x dominance were highly significant, while additive x additive was significant. The opposite signs observed for dominance and dominance x dominance indicates the role of duplicate epistasis. Among the gene effect, additive and additive x dominance were significant for seedling vigor. The opposite signs observed in dominance and dominance x dominance indicates the role of duplicate epistasis for the expression of this trait.

For heading date, Additive gene effect was found to be highly significant (-21.08) but with a negative sign and played a major role in the control of the trait. Among the epistasis gene effect, dominance x dominance (-96.89) was significant but also with a negative sign. For number of leaves, except additive gene effect with negative sign and highly significant, which played a major role, none of the other gene effects were significant for the trait. The opposite signs observed in dominance and dominance x dominance indicates the role of duplicate epistasis for the expression of the trait. For panicle length, Additive gene effect was found to be highly significant. Among the epistasis gene effects, additive x dominance gene effect was found to be highly significant for the trait. The opposite signs observed in dominance and dominance x dominance indicates the role of duplicate epistasis for the expression of the trait.

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For plant height, only additive gene effect with a negative sign was found to be significant for this trait. The opposite sign observed for dominance and dominance x dominance gene effect indicates the presence of duplicate epistasis for this trait.

All the epistatic gene effects were found significant for panicle weight with additive x additive gene effect having negative sign. Dominance x dominance gene effect played a prominent role in the control of this trait (236.22). The opposite signs observed in dominance and dominance x dominance indicates the role of duplicate epistasis for the expression of the trait.

For grain weight, both the dominance and additive × additive types of gene interaction effects were found to be highly significant and negative. Additive x dominance gene effect was also significant for the trait. Further, the observation on opposite signs of dominance and dominance x dominance gene effects indicated the expression of the trait under the control of duplicate epistasis. Except additive gene effect with a negative sign, which played a prominent role none of the gene effects were found significant for 1000 grain weight. The opposite signs noticed for dominance and dominance x dominance gene effects indicated evidence of duplicate epistasis for this trait. With respect to number of grain per panicle, additive x dominance gene effect was found to be significant which played a prominent role in the control of the trait; no other gene effect was found to be significant.

Estimates of Heritability and Genetic Advance

The estimates of heritability and genetic advance obtained are presented in Table 4.The broad sense heritability estimates were high for all the traits apart from plant height (0.46), 1000-grain weight (0.37) and grain number per panicle (0.42) which were moderately high. Phytate content (0.70), panicle length (0.63), and panicle weight (0.67) recorded high estimate for narrow sense heritability while, number of leaves (0.39), panicle length (0.47) and grain weight per panicle (0.59) had moderately high values for narrow sense heritability. Negative narrow sense heritability estimates were recorded for, seedling vigour, plant height, 1000-grain weight and grain number per panicle. The lowest estimate for narrow sense heritability was recorded for heading date (0.02).

High estimates of genetic advance as percent of means were recorded for all the traits except, seedling vigor, heading date and 1000-grain weight which recorded low estimates for genetic advance of mean.

DISCUSSION

The highly significant mean squares obtained from the analysis of variance for genotypes indicate that considerable amount of genetic variation exist among the genotypes. This wide range of variation observed for all the characters would offer scope for selection for development of desirable genotypes.

Gene Effects

Inheritance of phytate content was governed by additive, additive x additive and additive x dominance gene effect. Preponderance of additive gene effect implies that favourable genes in both parents contribute to the expression of phytate content. The Additive, additive x additive gene effect is indicative of a good potential for the improvement of this trait and thus selection for low phytate content can be done in the early segregating generations. Ijaz *et al.* (2013) in their findings reported the importance of non-additive gene effect in the inheritance of phytic acid in bread wheat.

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Additivity of gene effects observed for heading date, number of leaves, plant height and 1000- grain weight indicates that additive gene action played a greater role in the inheritance of these traits. This further indicates that favourable genes in both parents contributed to the expression of these traits and that there is a good potential for the improvement of these traits as selection can be done in the early segregating generation. The presence of additive and dominance x dominance appears to have promoted earliness in days to flowering.

Inheritance of grain weight was governed by dominance gene effect. This implies that this trait can be utilized successfully in the formation of hybrids. The prevalence of epitasis in the inheritance of seedling vigor, panicle length, panicle weight and number of grain per panicle is indicative of greater genetic diversity in the parental lines. The opposite sign observed for dominance and dominance x dominance for all the traits indicates the duplicate type of epistasis in the inheritance of the traits. Additive and epistasis is more important than dominance to the breeder of self-pollinating species, because dominance will be broken by segregation following hybridization. Also epistasis does not depend on heterozygosity and can therefore permit more gene combinations than dominance.

Genetic Variability, Heritability and Genetic Advance

The close resemblance between the corresponding estimates of PCV and GCV in almost all the characters except, 1000 grain weight indicated the least role of environmental influences for expression of the characters studied. The high estimates of PCV and GCV for phytate elucidate the agreement between the genotype and phenotype for the trait, i.e. environment had least effect on the character. Similar result was reported by Reddy *et al.* (2005).

The high genotypic coefficient of variation observed for majority of the traits, revealed the preponderance of broad base genetic background as well as a good potential that they would respond positively to selection. This is in corroboration of the report of Ahmed *et al.* (2012). Gururaj Rao and Patill (1996) also reported moderate estimate for PCV and GCV. Prabhakar (2001) and Rizwan and Haris (2001) reported moderate GCV and PCV values for heading date, while Prabhakaran (2001) reported high estimate for variability for this trait in sorghum.

The low values for the estimate of narrow sense heritability indicate that non additive gene action is important in the control of the trait. Whereas, moderate narrow sense heritability suggests that the trait is controlled by additive and non- additive gene actions, thus some appreciable amount of variation could exist for selection. On the other hand, high values of narrow-sense heritability imply the superiority in the control of the trait concerned. All the negative narrow sense heritability estimates were considered low.

In the present study, phytate level and panicle weight showed high estimates for both broad and narrow sense heritability implying that the traits would respond positively to selection. The low narrow sense heritability estimate obtained for seedling vigour, heading date, plant height, 1000-grain weight, and number of grain per panicle may be due to the presence of epistasis.

Moderate estimates for narrow sense heritability were observed for number of leaves and grain weight per panicle and this suggests that, these traits can be directly selected for, and it implies that an appreciable amount of additive gene action controls the expression of these traits. This corroborates the reports of Bello *et al.* (2007), and Biwas, *et al.* (2001).

Plant height showed moderate estimate for broad sense heritability which is in contrast with the results obtained by Tiwari *et al.*(2003) who recorded high heritability estimates for plant height. The narrow differences between PCV and GCV for phytate content, which are amply reflected in their high heritability, suggest that selection for low levels of phytate content

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would be highly effective. The high heritability estimates also implies that, the trait can easily be transferred to the offspring.

The high narrow sense heritability for phytate confirms the presence of additive genetic variability for phytate, as was observed in the generation mean analysis. In the present investigation, high heritability coupled with high genetic advance as per cent of mean was observed for phytate content, number of leaves, panicle length and panicle weight, grain. Thus these traits are predominantly under the control of additive gene action and hence selection in early segregating generation would be highly effective for their improvement. Similar observations were recorded by While, Khanure (1993), Biradar (1996) observed similar result for inheritance of panicle weight in sorghum.

Moderate heritability with low genetic advance was recorded for plant height, 1000 grain weight and number of grain per panicle. These traits appear to be under the control of both additive and non-additive gene actions as observed in the generation mean analysis.

CONCLUSIONS AND RECOMMENDATIONS

Analysis of variance revealed highly significant differences among the six generations of a cross (Samsorg17 X ABS188) and estimates of gene effect showed that, phytate level and most of agronomic parameter studied were under the control of additive gene action. Other parameters including, seedling vigor, panicle length, seed size and grain number per panicle were controlled by additive x dominance gene action. Panicle and grain weight were under the control of additive x additive gene effect. Panicle and grain weight were under the control of dominance x dominance and dominance gene actions respectively. Panicle weight was under the control of dominance x dominance gene action. So both additive and non-additive gene actions are essential for the yield improvement in sorghum and breeding strategies like reciprocal recurrent selection would satisfy the breeding objectives.

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Table 1. Mean Squares from the analysis of variance for different characters evaluated CFT, Samaru, 2013

Sov	*df*	*Phytate (mg/100g)*	*SV*	*HD*	*NL*	*PaL(cm)*	*PH(cm)*	*PaW(g)*	*GrW(g)*	**1000-GrW(g)**	**GNP**
rep	1	0.0002	0.0005	7.316	1.584	0.219	184.318	0.128	0.612	6.900	57413.867
Gen	5	0.084**	0.0234**	1261.413*	42.584**	23.265*	6429.002*	1717.856**	2766.880**	156.776**	556987.892*
error	5	0.0012	0.0008	3.984	0.832	6.053	1296.596	13.673	49.897	5.629	64824.401

*significant at 5% level of probability, **significant at 1% level of probability

SV= Seedling vigor, HD = heading date, NL = Number of leaves, PaL= panicle length (cm), PH = Plant height (cm), PaW = panicle weight(g), GrW = Grain weight per panicle (g), 1000-GrW = 1000 grain weight per panicle (g), GNP = number of grain per panicle (g)

Table 2. Estimates of mean of agronomic traits in six generations of the cross SAMSORG 17 X ABS188 evaluated at CFT

Generation	*Phytate level (mg/100g)*	*SV*	*HD*	*NL*	*PaL (cm)*	*PH(cm)*	*PaW(g)*	*GrW/pa*	*1000 GrW/plt*	*GNP*
P_1	0.4640^{de}	1.93^{c}	55.00^{f}	9.85^{d}	26.97^{bc}	119.63^{d}	36.76^{f}	27.72^{e}	37.18^{de}	766.63^{d}
P_2	0.8169^{a}	2.21^{a}	125.00^{a}	23.40^{a}	35.10^{a}	250.30^{a}	119.17^{a}	129.20^{a}	58.55^{a}	2227.79^{a}
F_1	0.5577^{b}	2.14^{ab}	80.00^{e}	15.20^{c}	29.31^{bc}	167.05^{dc}	81.92^{b}	54.05^{c}	38.95^{de}	1384.41^{bc}
F_2	0.4730^{ef}	2.12^{ab}	99.89^{c}	15.28^{c}	30.64^{ab}	202.30^{bc}	84.81^{b}	73.82^{b}	45.68^{c}	1566.10^{b}
BC_1P_1	0.4630^{de}	2.20^{a}	94.02^{d}	14.35^{c}	31.84^{bc}	141.75^{bcd}	51.33^{d}	38.18^{de}	34.76^{e}	1198.45^{bcd}
BC_1P_2	0.4680^{f}	2.08^{b}	115.04^{b}	19.20^{b}	25.23^{b}	241.2^{ab}	54.42^{d}	40.24^{de}	48.36^{b}	902.19^{cd}

Means with the same alphabet in a column are not significantly different from each other.

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Table 3. Estimates of scaling test in a Cross SAMSORG 17 X ABS188 Evaluated at CFT Samaru, 2013

Scaling test	*Phytate(mg/100g)*	*SV*	*HD*	*NL*	*PaL (cm)*	*PH(cm)*	*PaW (g)*	*GrW (g)*	*1000-GrW (g)*	*GNP*
A	2.823*	2.741*	7.780*	0.656	0.539	-0.162	0.032	0.043	0.473	0.322
B	0.666	1.78	0.340	1.369	2.975*	-1.460	3.034*	-0.02	0.127	13.847*
C	0.687	0.06	4.392*	0.050	0.861	0.0089	2.502*	5.607*	-0.325	0.451

*significant at 5% probability level

Table 4. Estimates of Gene effect of a Cross SAMSORG 17 X ABS188 Evaluated at CFT Samaru

Gene effect	*Phytate(mg/100g)*	*SV*	*HD*	*NL*	*PaL (cm)*	*PH(cm)*	*PaW (g)*	*GrW (g)*	*1000-GrW (g)*	**GNP**
m	0.47**	2.15**	99.89**	15.28**	30.65**	202.30**	84.81**	73.82**	45.68**	1566.1**
	±0.04	±0.02	±3.0	±0.67	±1.07	±5.46	±7.00	±5.36	±1.15	±121.58
d	0.38**	0.12*	-21.08**	-4.83**	6.70**	-100.1**	-2.90	-1.67	-13.87**	307.16
	±0.08	±0.05	±7.31	±1.58	±1.96	±1.14	±0.75	±2.17	±3.28	±3.83
h	0.66	0.06	8.67	4.65	-10.27	-75.92	-123.9	-162.9**	-25.05	-2165
	±0.41	±0.30	±0.92	±1.05	±3.95	±4.03	±3.99	±6.88	±4.95	±542.5
i	0.74*	-0.02	18.67	6.08	-8.54	-43.00	-127.8*	-138.5**	-16.16	-2052
	±0.33	±0.18	±2.92	±5.83	±0.20	±0.24	±4.58	±5.78	±1.14	±74.0
j	0.56**	0.26*	13.92	1.94	10.77**	-19.78	38.30*	49.07*	-3.19	1037.7*
	±0.12	±0.13	±2.31	±2.01	±2.80	±6.27	±2.47	±17.92	±4.56	±449.44
l	-0.98	-0.15	-96.89*	-9.63	15.20	10.83	236.22*	246.81	23.24	3602.4
	±0.66	±0.53	±1.54	±1.43	±1.61	±1.10	±19.99	±2.32	±1.31	±98.7

*significant at 5% probability level, **significant at 1% level of probability.

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Table 5. Estimates of heritability genetic advance and genetic advance as percent of mean for the character evaluated in a cross SAMSORG 17 x ABS188 at Samaru, 2013

Traits	*Broad Sense Heritability*	*Narrow Sense Heritability*	*Genetic Advance 10%*	*GA as% of Mean*
Phytate	0.89	0.70	0.4	66.77
SV	-1.28	-0.36	-0.1	4.72
HD	1.00	0.02	1.0	1.54
NL	0.82	0.39	3.2	20.74
PaL(cm)	0.63	0.47	15.7	53.20
PH(cm)	0.46	-2.46	-160	-80.72
PaW(g)	0.70	0.67	58.2	80.65
GrW(g)	0.72	0.59	40.2	66.42
1000-GrW(g)	0.37	-0.17	-2.5	-5.69
GNP	0.42	-0.62	-955	-71.00

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