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GENETIC DISSECTION OF LOW-N TRAITS USING TRIPLE TEST CROSS ANALYSIS IN MAIZE (*ZEA MAYS* L.)

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A B S T R A C T

Low-N maize is bred for its ability to tolerate low soil nitrogen (N) by growing and producing grain that compares appreciably to conventional varieties. This experiment was conducted to study the genetic effects of grain yield and other agronomic traits in Low-N maize using triple test cross analysis. Twelve low-N open pollinated maize varieties were converted to the inbred line after six generations of selfing and used for the experiment. Two inbred lines along with their F_1 were used as testers for ten inbred lines in a triple test cross pattern to generate 30 crosses and along with their parents and testers to make a total of 43 entries which were evaluated at the Teaching and Research Farms of Ekiti State University, Ado-Ekiti during in 2017. The design was a Randomized Complete Block Design (RCBD). Data was collected on plant height, ear height, days to 50% anthesis, days to 50% silking, the incidence of Curvularia leaf spot, blight, plant aspect, ear aspect, ear rot, stay green, cob per plant, ear weight, grain moisture content and grain yield. All data was subjected to analysis of variance and complete genetic estimates made. Additive and dominants were significant ($P<0.05$) for all traits, however, epitasis estimates were not significant for all the traits. The degree of dominance component indicated partial dominance for all the traits. Correlation coefficients for days to 50% anthesis and 50% silking, plant height, ear height, number of cob per plant and grain yield were positive and significant ($P<0.05$). Since both additive and dominance gene actions were important for low-N traits, the use of reciprocal recurrent selection procedure can be adopted in incorporating the trait into elite maize varieties.

Keywords: Genetic, dissection, Low-N, triple test cross, maize.

INTRODUCTION

Maize is an important source of carbohydrates, proteins, vitamins and minerals comparing favourably with other starchy crops such as rice and potatoes (Amudalat, 2015). It is prepared into various products such as maize-meal pap, porridge, mixed with a wheat meal to make bread and popcorns. In addition, it is fed to livestock as whole grain in the farms or can be processed into variety. It is an important cereal crop, due to its high yielding, ease of processing readily digested and cost less than other cereals (Jaliya *et al.*, 2008). It is cultivated worldwide on more than 160 million hectares every year and production was put at 785 million tons (Umar *et al.*, 2014). It is widely grown throughout the tropics and temperate regions as well as any place man can be found in all the continents of the world. Maize is an important crop in industrial and livestock production in the country (Vacaro *et al.*, 2002). It was reported by Amudalat (2015) that maize is always preferred to other crops and it is fast becoming an industrial crop in sub-Sahara African countries. The industrial carbohydrate used in the making of feed for the livestock, production of beer, industrial starch, baby foods, cornflakes, textile and pharmaceutical industry is made from maize. Most Africans depend on maize as their staple food (Bänziger and Diallo, 2001) to feed both rural and urban dwellers. Despite the economic importance of maize to the teeming populace in sub-Sahara Africa, it has not been produced to meet food and industrial needs. This could be attributed to poor genetic, biotic factors (like weeds, pests, insects, diseases), the types of varieties grown,

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unpredictable weather conditions, high post-harvest lost and storage losses, poor agronomic practices, abiotic stress (like drought and low soil fertility), high post-harvest, socioeconomic factors like market price fluctuation, or that farmers have not adopted improved technologies for maize production. Nitrogen is the most widely deficient nutrient limiting maize growth and can significantly affect yield(Azevedo *et al.*, 2003; Fakorede *et al.*, 2003; Martins *et al.*, 2008; Moser *et al.*, 2006). Nitrogen deficiency is one of the most important stresses affecting maize production in tropical areas (Banziger *et al.*, 2000; Martins *et al.*, 2008). It was reported that nitrogen deficiency can reduce maize yield by as much as 40% (Milander *et al.*, 2016). Nearly all cultivated maize in developed countries receives some form of N fertilizer and N use is increasing in developing countries where their impacts on raising yield from nutrient-poor soils are greatest. However, as a result of high cost of N fertilizer, poor distribution system and low purchasing power (Fakorede *et al.*, 2003), the use of N fertilizer in developing countries is hindered. The extensive use of N fertilizer not only increase crop input cost but also can cause environmental pollution (Egli, 2011). For these reasons, reducing the amount of supplemental N used in maize production by developing low-N tolerance varieties will have significant positive economic and environmental benefits to the world of agriculture. A possible approach to reduce N deficiency in soil is to lower crop demand for N through selection for low-N tolerance (Kogbe and Adediran, 2003; Shapiro *et al.*, 2008). Reports from several studies have indicated that using genetic potential exists in maize genotypes for the improvement of nitrogen use efficiency (Fageria and Baligar, 2005; Muurinen, 2007; Ortiz-Monasterio *et al.*, 2001). Breeding programme strategy is aimed at achieving higher yield. The breeder must have sound information on the genetic behavior of yield and agronomic traits responsible for low-N in maize. Such knowledge is essential for in incorporating the trait into elite maize varieties to develop improved varieties. In this study, TTC was used to estimate additive, non-additive and epistasis of low N traits in maize.

MATERIALS AND METHODS

Development of inbred lines of the genetic materials used for the experiment were done in Teaching and Research Farms of Osun State University, Ejigbo Osun State and Landmark University, Omu Aran, Kwara State from 2012 to 2016 cropping seasons. Evaluation of test crosses was done at Teaching and Research Farms of Ekiti State University, Ado-Ekiti during the 2017 cropping season.

Ado-Ekiti (7.6124° N, 5.2371° E) is located in the derived savanna zone of Nigeria. The field has been previously grown to various vegetables, cereals, root and tuber crops. Last cropping season, it was cropped to yam. The soil of the site is sandy loam, low in nitrogen and other microelements.

Eight soil core samples were taken from each plot using soil auger before planting. Cores for each plot were combined and the composite sample was air dried. The soil was passed through a 2 mm, and 0.5 mm sieve for chemical and physical analysis. The 0.5 mm soil was used for total N an organic matter content of the soil while 2 mm soil was thoroughly mixed, and subsamples were taken by coning technique for the determination of organic matter, available P, pH in (H_2O, 1: 1), exchangeable Ca, K, Mn, Na and Mg. the remaining soil was analyzed for particle size distribution using Hydrometer technique. Total N and organic matter content of the soil were determined by Kjeldahl (Black *et al.*, 1965) and Walkley and Black procedures (Nelson and Sommers, 1982), respectively. Soil pH was measured using a 1:1(w/v) soil/water suspension ration. Available P was analyzed using Bray P-1 method (Bray and Kurtz, 1945), Potassium, Ca and Mg were first extracted using NH4OAC. Thereafter, K was determined by flame emission in the Perkin-Elmer 5000 spectrophotometer and Mg and Ca by atomic absorption.

The materials used for this study were twelve open pollinated low-N maize obtained from International Institute of Tropical Agriculture (IITA) Ibadan (Table 1). These materials were subjected to 6 cycles of selfing to obtain inbred lines starting from cropping season of 2013. During selfing, the materials were planted out in each row of 5 m length.

The 30 test crosses, testers (L1, L2 and F1) with 10 inbred parents P1 to P10 (43 entries) were evaluated. The evaluation was carried out at Teaching and Research Farm of Ekiti State University, Ado- Ekiti with two replicates. Entries were made in a row plot of 5m long; spacing was 75cm inter-rows and 50cm intra-rows. Three seeds were initially planted on a hill but were later thinned to two in three weeks after planting to give a planting density of 53,333 plants ha^{-1}.

Table 1. Description of Experimental Materials.

Name	Code
SINT MAR 20CA LARGA	L1
BR99 72L COMPI	L2
SINT MAR 20CA LARGA x BR99 72L COMPI	F1
LN TP YC7	P1
72PB PROL C4	P2
LA POSTA SEQUIA C6	P3
72L COMP IC6 LNCI	P4
DMR ESR W LN	P5
72PB PROL C3 SYN	P6
LN TP YC6 SYN	P7
DMR ESR Y LN	P8
TZPB PROL C_3 LNSYN	P9
M_{13}-1881	P10

Two inbred lines from the 6th generation of selfing were used as parents. Each parent SINT MAR 20CA LARGA (L1) and BR99 72L COMPI (L2) were crossed together to generate F_1. Each of the three testers (L 1, L 2 and F_1) was used to cross the remaining 10 inbred lines to generate 30 crosses (Table 1, Figure 1).

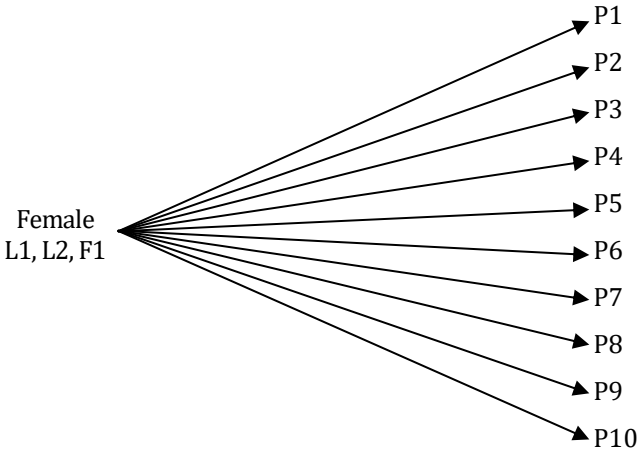

Figure 1. Conceptual model for crossing of the parents.

Seed were planted on the 6th May, 2017. One row plot of 5 meter length was used. Three seeds were planted per hill at an intra-row spacing of 0.5 m and inter-row spacing of 0.75 m. Thinning was done at 2 weeks after planting (WAP) to retain only 2 plants per hill, thus giving a plant population of about 53,333 plants ha^{-1}.

Weeds were controlled with a pre-emergence application of atrazine at the rate of 4kg ha^{-1} and dragon (paraquat) at the rate of 2 liters per ha^{-1}. Chemical weed control was supplemented by manual weeding at 6 WAP in Ado-Ekiti. At 11 WAP, the dragon was applied using a guarded sprayer in the two locations. Caterpillar force was applied at 5 WAP to check the attack of armyworm. Earthing-up was done to minimize lodging.

Data was collected for the following traits: Plant Height, Ear Height, Days to anthesis, Days to silking, Plant Aspect, Ear Aspect, stay green, Ear rot, Ear weight, Grain moisture, Grain yield: Grain yield (Kg ha-1) adjusted to 15% moisture and based on 80% shelling percentage (Dhillon *et al.*, 1976).

The analysis of variance was performed following the method described by Singh and Chaudhary (1999) to determine the significance of variations among the hybrids, parents, lines, testers, L1 + L2 vs F1, P1 vs P2, lines vs testers and hybrids vs parents for each trait using TTC technique. The detection of epistasis was performed according toSingh and Chaudhary (1999).

Based on the genetic model:

$$Lijk = m + Gij + R\,k + Eijk$$

Where,

Lijk = Phenotypic value of cross between tester Li and line j in Kth *replication*

m = Overall mean of all single and three way crosses.

Gij = Genotypic value of cross between tester Li and line j.

Rk = Effect of Kth replication.

Eijk = error.

Y_{ijk} is the kth observation of i x jth progeny

u is the general mean

m_i is the effect of ith male

f_i is the effect of jth female

$(m \times f)_{ij}$ is the interaction effect, and

e_{ijk} is the error associated with each observation.

RESULTS

Analysis of Variance for Growth, Flowering, Disease, Aspect Rating, Stay Green and Yield Traits: Growth and flowering traits among entries were significantly different ($P<0.05$) except for ASI (Table 2). Also, the differences among the hybrids showed significant differences for growth and flowering traits except for ASI. The differences among the lines were not significant except for days to 50% silking and plant height. The comparison between lines and tester showed significant differences only for plant height and ear height. The comparison between the parent and their F_1 as testers showed significant differences only for plant height and ear height.

Table 2. Means Squares Arising from Analysis of Variance (ANOVA) for Growth and Flowering Traits in Maize.

Source of variation	Df	Days to 50% anthesis	Days to 50% Silking	ASI	Plant height	Ear height
Entries	42	6.42**	6.26**	0.74	5202.91**	1621.74**
Hybrid	29	7.61**	7.61**	0.87	2101**	742.61**
Lines	9	3.89	3.75**	0.37	562.06**	159.74
Lines vs tester	1	0.59	0.72	0.01	11851.22**	8210.94**
P1 + P2 vs F1	1	6.75	5.33	0.08	13931.56**	12610.08**
P1 vs P2	1	4.00	0.25	2.25	2.07	36.00
Error	82	0.14	0.21	0.01	319.31	70.00

*, ** Significant at P<0.05 and P < 0.01 level of probability respectfully, ASI = Anthesis-silking intervals.

Comparing the parental lines used as tester showed no differences for all the growth and flowering traits. Anthesis-silking interval was consistently not significant for all the sources of variation.

There were no significant differences (P<0.05) among entries for disease and aspect rating except blight (Table 3). The differences among hybrid for disease and aspect rating were significant for the streak, plant aspect and ear aspect. However, the differences among hybrids for Curvularia, blight and ear rot were not significant. There were no significant differences among lines for disease and aspect rating. Similarly, the differences among lines vs tester and between the parental lines used as tester were not significant for disease and aspect rating. The differences for disease and aspect rating were only significant for ear aspect in the comparison between parents and their F_1.

Table 3. Means Squares Arising from ANOVA for Disease and Aspect Rating.

Source of variation	Df	Curvularia	Blight	Streak	Plant aspect	Ear rot	Ear aspect
Entries	42	0.22	0.18*	8.67	10.11	1.19	44.25
Hybrid	29	0.17	0.17	0.42*	0.57*	0.43	0.58**
Lines	9	9.18	0.18	37.09	42.66	3.71	182.10
Lines vs tester	1	0.05	0.05	8.06	8.48	0.97	34.77
P1 + P2 vs F1	1	0.18	0.42	0.26	0.08	0.02	1.88**
P1 vs P2	1	0.02	0.02	0.39	0.06	0.06	0.14
Error	82	0.01	0.02	0.20	0.03	0.01	0.07

*, ** Significant at P<0.05 and P < 0.01 level of probability respectfully.

Yield components and stay green traits among entries were not significantly different (P<0.05) except for yield (Table 4). Also, the differences among the hybrids showed significant differences for yield but not significantly different for stay green and number of cob per plant. The differences among lines were only significant for yield. Similarly, the comparison between the parents and their F_1 as tester showed significant difference only for yield. The differences in comparing the parental lines used as tester were not significant for all the yield components and stay green. The comparison between lines and tester showed no significant differences for stay green and number of cob per plant. The yield was consistently significant for all the sources of variation.

Table 4. Means Squares Arising from ANOVA for Yield Components and Stay Green in Maize.

Source of variation	Df	Cob per plant	Stay green	Yield
Entries	42	0.05	7.57	23148960.40**
Hybrid	29	0.03	0.31	8607357.50**
Lines	9	0.002	31.78	5266625.64**
Lines vs tester	1	0.16	2.59	59356791.47**
P1 + P2 vs F1	1	0.70**	3.00**	96631190.72**
P1 vs P2	1	0.01	0.06	19548377.00**
Error	82	0.01	0.02	333.42

*, ** Significant at P<0.05 and P < 0.01 level of probability respectfully.

Estimation of Epitasis for Growth, Flowering, Disease, Aspect Rating, Stay Green and Yield Components of Low-N Maize: The result of ANOVA for estimation of epitasis for growth, flowering, disease, aspect rating, stay green and yield components are shown in Table 5. Analysis of variances for i (additive x additive) epistasis for growth, flowering, disease, aspect rating, stay green and yield components are not significantly different (P<0.05). Similarly, the differences of j+l (additive x dominance) epistasis were not significant for all the traits. Interaction variances (i x rep, j+i x rep) was not significantly different for growth, flowering, disease, aspect rating, stay green and yield components. This is suggesting homogeneity of interaction variable.

Table 5. Means Squares Arising from ANOVA for Estimation of Epistasis.

	i	j + l	Total epistasis	i x rep	J + l x rep	Total epistasis x rep
Df	1	9	10	2	9	10
Days to 50% anthesis	10.30	16.21	17.47	87.33	8.31	16.24
Day to 50% silking	0.83	20.48	18.52	92.17	10.03	18.25
ASI	7.5	1.06	1.70	8.50	2.08	2.73
Plant height	33057.24	12200.39	14286.07	71430.35	8099.00	14432.32
Ear height	18963.10	3992.83	3595.44	27449.30	8664.73	10543.18
Curvularia	0.40	1.05	0.99	4.96	0.48	0.93
Blight	0.07	0.22	0.21	1.04	0.62	0.66
Streak	1.80	1.74	1.75	8.79	0.97	1.75
Plant aspect	0.03	1.87	1.68	8.41	0.82	1.58
Ear rot	0.53	0.50	0.50	2.50	0.70	0.88
Ear aspect	4.03	0.29	0.67	3.34	0.74	1.00
Cob/ plant	0.45	0.23	0.26	1.08	0.51	0.57
Stay green	3.67	0.32	0.66	3.29	0.94	1.17
Yield	9.20	6.47	5.909	2.95	1.20	1.37
Error	0.02	0.01	0.04	0.05	0.03	0.03

i= additive x additive epistasis, j + l = additive x dominance and dominance x dominance epistasis

Estimation of Degree of Dominance and Direction of Dominance for Growth, Flowering, Disease, Aspect Rating, Stay Green and Yield Components of Low-N Maize: The significant additive component (D) was consistently significant for all growth, flowering, disease, aspect rating, stay green and yield traits (Table 6). Also, dominant component (H) for days to 50% anthesis, days to 50% silking, plant height, ear height, number of cob per plant and yield were significant. However, the dominant genetic variance was not significant for ASI, Curvularia, blight, streak, plant aspect, ear aspect and stay green.

Table 6. Estimation of Degree of Dominance and Direction of Dominance for Growth, Flowering, Disease, Aspect Rating, Stay Green and Yield Components of Low-N Maize.

	D	H	$(H/D)^{1/2}$	R
Days to 50% anthesis	3.80**	0.53**	0.37	0.59**
Day to 50% silking	9.33**	4.68**	0.71	0.51**
ASI	4.68**	0.15	0.18	0.54
Plant height	349.64**	318.70**	0.95	0.79**
Ear height	470.64**	460.70**	0.99	0.30**
Curvularia	0.05**	0.03	0.77	0.19
Blight	0.12**	0.11	0.96	0.28
Streak	0.33**	0.07	0.46	0.41
Plant aspect	0.07**	0.05	0.84	0.58
Ear rot	0.33**	0.01	0.55	0.46
Ear aspect	1.73**	0.28	0.40	0.55
Cob/ plant	0.13**	0.04**	0.55	0.08**
Stay green	0.27**	0.15	0.75	0.04
Yield	11379572.00**	528004.97**	0.21	0.07**

*, ** Significant at P<0.05 and P < 0.01 level of probability respectfully, D= Additive, H = Dominance, (H/D)1/2 =Degree of Dominance, r= correlation coefficient.

The magnitude of additive variance (D) was consistently higher for all the traits since the presence of common alleles in testers increases the magnitude of the additive components. The degree of dominance $(H/D)^{1/2}$ was less than one for growth, flowering, disease, aspect rating, stay green and yield components. It was highest for ear height (0.99) and lowest for ASI (0.18). The correlation coefficient (r) for ASI, Curvularia, blight, streak, plant aspect, ear rot, ear aspect and stay green were not significantly different. Conversely, the correlation coefficient of days to 50% anthesis, days to 50% silking, plant height, ear height, number of cob per plant and yield were positive and significant.

DISCUSSION

Triple test cross provides good estimates for genetic components of variation for quantitative traits. Some maize breeders have used triple test analysis in maize that proposed by Kearsey and Jinks (1968). Information of the type of gene action involved in the inheritance of traits is helpful in deciding the breeding procedures to be followed for plant improvement and is necessary for efficient utilization of available germplasm in a plant breeding program. In this study, triple test cross has been helpful to provide unbiased estimates of additive and dominant components of low N traits in maize since epistasis is absent.

The highly significant differences among entries, lines and hybrids in some of the traits indicate considerable genetic variation that may exist in the lines, testers and hybrids. The significant differences reported in the yield and some traits of the two inbred parents SINT MARZOCAL LARGA and BR9972L COMP1 clearly disclose that SINT MARZOCAL LARGA, BR9972L COMP1 and SINT MARZOCAL LARGA x BR9972L COMP1 provided an estimate of additive and dominance variation with equal precision as reported by Kearsey and Jinks (1968); Khattak *et al.* (2004); Doerksen *et al.* (2003); Lamkey and Edwards (1999).

The additive x additive, additive x dominance and dominance x dominance epistasis were not significant for all the traits in this experiment although some earlier workers (Kulshreshtha *et al.*, 1993; Vijayakumar *et al.*, 1996) indicated evidence of epistasis for all the traits investigated. The absence of epistasis provides unbiased estimates of additive and dominance components (Koumber, 2011; Menshawy, 2008; Morad, 2012; Singh and Yunus, 1986). Genotype x environment interactions may have some influence on the epistatic effect. Such influences have been reported elsewhere in wheat and mungbean (Ketata *et al.*, 1976; Khattak *et al.*, 2004). The cause of the presence of absence of epistasis could be genetic and or environmental (Menshawy, 2008). Estimation of gene action and predicting new recombination lines in bread wheat cross using F2 triple test cross analysis (El-Massry, 2009) and thus, it may not always be related to the inherent capacity of a genotype. Similarly, Sunil and Singh (2003) reported that components of variance changed to a different degree over the environments. The environmental influences have also been reported in wheat (Pawar *et al.*, 1994).

The additive genetic variance was found to be much larger in magnitude than the Dominance variance for all traits studied. Consequently, it could be concluded that selection procedures based on the accumulation of additive effects would be successful in improving all these traits studied. However, to maximize selection advance, procedures which are known to be effective in shifting gene frequency when both additive and non-additive genetic variance are involved could be preferred. This result corroborates earlier result obtained by Esmail (2007); Koumber (2011); Morad (2012) and Dawwam *et al.* (2015). The degree of dominance was less than unity for traits studied suggesting the role of partial dominance in the inheritance of these traits and ascertain the fact that in cross-pollinating crops, most genes are heterozygous, and the over-dominance is rare (Dawwam *et al.*, 2015; El-Massry, 2009; Esmail, 2007; Koumber, 2011; Lamkey and Edwards, 1999; Morad, 2012). Furthermore, the correlation coefficients between the sums and differences were found to be positive and significant for days to 50% anthesis, days to 50% silking, plant height, ear height, number of cob per plant and yield. This indicates that dominance seemed to be acting in one direction. However, the correlation coefficient for the remaining traits was insignificant indicating the genes with positive and negative effects were equally distributed among the low N genotypes including in this study. Regarding epistasis genetic correlation the results indicated positive and significant correlation in days to 50% anthesis, days to 50% silking, plant height, ear height, number of cob per plant and yield. This indicates that most of the characters were not associated with each other and confirmed that the triple test cross mating system was useful in breaking up undesirable linkage groups to obtain new recombinant lines. In this

regard, Menshawy (2008); Morad (2012) and Dawwam *et al.* (2015) reported the efficiency of triple test cross for obtaining new recombinant lines. This investigation could help to design how to use triple test cross analysis to obtained additional information about the type of gene actions, genetic correlation and predicting the likely performance of new recombinants that could be derived after series of selfing generations. This also could help breeders for a rightful decision about an effective breeding method to be applied for improving yield and its contributing traits of low N maize.

CONCLUSION

The following conclusions can be drawn from this study:

1. The additive and dominant components were significant for all traits. This indicates that selection procedure based on accumulation of additive effects would be successful in improving these traits
2. Epistasis for growth, flowering, disease, aspect rating and yield components are not significant
3. The degree of dominance was than unity for all traits. This is suggesting partial dominance in the inheritance of these traits.

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