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GRAIN YIELD AND WEEVIL (SITOPHILUS ZEAMAIS MOTSCH.) RESISTANT POTENTIAL OF 10 Maize (*Zea mays* L.) VARIETIES AND THEIR INTER-VARIETAL HYBRIDS.

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ABSTRACT

Availability of high yielding and weevil resistant maize varieties will contribute significantly to yield stability and also lower the cost of keeping maize grains viable during storage. This study was conducted to assess grain yield and weevil-resistant potential of 10 local maize varieties and 27 inter-varietal crosses produced from by intermating the 10 local varieties in a pair-wise manner. The parents and crosses were evaluated for grain yield and agronomic traits using a two-replicate randomized complete block design at the Teaching and Research farm of the University of Ilorin, Nigeria (Latitude of 8° 30'N and Longitude 4° 33'E). Physical and biochemical properties of the seeds were examined in the laboratory followed by screening the seeds for susceptibility to the maize weevil (*Sitophilus zeamais* Motsch.) using parental survival, F_1 weevil progeny emergence, median development period, seed weight loss, total phenolics and Dobie index of susceptibility. Data collected on morphological attributes, grain yield and susceptibility parameters were subjected to analyses of variance and significant means were separated using the least significant differences at 5% probability level. The results showed that both parents and crosses were similar for grain yield and agronomic parameters. However, parents differed significantly ($\rho < 0.05$) for almost all seed physical attributes, the crosses differed significantly ($\rho < 0.05$) only for 100 seed weight. The results of Dobie's classification showed that all the parents were either susceptible or highly susceptible to *S. zeamais* infestation while 16 of the 27 crosses were either moderately resistant or resistant to the pest. Seed physical characteristics (size, texture, colour and 100 seed weight) were more important in conferring resistance in the parents while total phenolic content was more important in conferring resistance in the crosses. Resistant genotypes among the crosses had lower number of total emerged F_1 progeny and low seed weight loss compared to the susceptible genotypes. It was concluded from the study that the five crosses (Eruwa 1, Abeokuta 3, Ago-Iwoye 2, Ago-Iwoye 4, and Idi-Ayunre 3) which combined resistance to *S. zeamais* with acceptable grain yield should be used as source populations from which high yielding and weevil resistant maize varieties can be developed.

Key words: Maize varieties, *Sitophilus zeamais*, Susceptibility index, resistance, yield

INTRODUCTION

The maize weevil (*Sitophilus zeamais* Motsch.) is one of the most destructive pests of maize grains in storage in tropical and sub-tropical regions of the world. It is a field-to-store pest which attack maize grains by creating hole on the whole grains in the process of breeding (Murata and Imanura, 2008) thereby causing grain weight loss of between 20 – 90 percent depending on the variety, storage condition and level of infestation (Boxall *et al.*, 2002; Chaubey, 2008; Abebe *et al.*, 2009; Suleiman *et al.*, 2014) as well as loss of seed viability. The insect's feeding habit also reduces the grain lot to powder, make it unfit for human consumption such that the product is rejected at the local and international markets (Luke *et al.*, 2015).

The main approach adopted in the control of the pest is the use of chemicals and synthetic insecticides. However, apart from the prohibitive cost for purchase, majority of the resource-poor farmers do not have the necessary skills to safely apply the chemicals which often cause health hazards. The FAO (2003) reported evidence of improper use of chemicals in different African countries resulting in loss of life and loss of efficacy. Furthermore, the global concern for use of pesticide due to associated environmental hazards, adverse effect on non-targeted organisms and eco-toxicity (Lorini *et al.*, 2007; Umoetok *et al.*, 2009), development of resistance to chemicals by insect pest and presence of residues in food (Nwosu and Nwosu, 2012) has evoked regulatory restrictions on the use of chemicals for insect pest control. The increased public awareness and concern for environmental safety have further necessitated the need for

reorientation of research efforts to the development of cheaper, effective and safer alternative management strategies in the control of *S. zeamais*. In order to overcome the challenge posed by the use of chemicals, there is the need to exploit the plant's inherent ability to resist or tolerate insect infestation. This can be achieved through the development of varieties that possess genes for resistance to the pest. However, such varieties must also be able to combine resistance with high yield potential as well as general adaptation to the target ecologies. In other words, identification and utilization of weevil resistant germplasm will be an important step in yield stability, the safety of the consumers and a reduction in environmental pollution.

The development and release of weevil resistant maize genotypes has several advantages which include yield stability even in the presence of upsurge of weevil biotypes (Mwololo *et al.*, 2012), enhancement of improvement in both quantity and quality of stored grain for planting and human consumption (Issa *et al.*, 2011) and increase in farming efficiency through reduction in the amount expended on purchase of insecticides and the risk of yield losses from insect damages. Studies conducted by several authors have revealed the occurrence of weevil resistance in maize germplasm (Derera *et al.*, 2001; García-Lara *et al.*, 2009; Mwololo *et al.*, 2010; Kasozi *et al.*, 2016) and that there exists, high genetic variability to the pest in a number of maize germplasm investigated (Siwale *et al.*, 2009; Dari *et al.*, 2010).

In the search for sources of resistance to *S. zeamais*, several studies have provided information on maize seed attributes that confer resistance to the pest. These include seed physical attributes (grain colour, shape, size, hardness), protein and moisture content (Osipitan and Odebiyi, 2007; Tongjura *et al*, 2010), sugar and phenolic acid as well as structural protein and diferulates of grain hull (Munjoma, 2004; Garcia-Lara 2004; Luke *et al.*, 2015). However, independent studies conducted by Shafique and Chaudry (2007) as well as Astuti *et al.*, (2013) have also shown that the basis of maize grain resistance to stored products insects is still not clear but vary from insect to insect. The preliminary step in the development of weevil resistant maize varieties is to screen available germplasm for their resistance (or susceptibility) to the pest followed by composing varieties from genotypes that combine the desired agronomic traits (for example high yield) with weevil resistance. The approach is believed to likely assist in identifying genetic materials that can be utilized in the development of high yielding and weevil resistant maize varieties.

The objectives were to determine grain yield potential and agronomic attributes, assess the relationship among resistance parameters and classify the maize genotypes for either resistance or susceptibility using Dobie's susceptibility index.

MATERIALS AND METHODS

Description of Experimental Materials

The genetic materials used for the study comprised 10 local maize varieties (as parents) and 27 inter-varietal hybrids. The varieties which were initially collected from different parts of the south western states of Nigeria (Table 1) were sibmated by maize scientists at the Institute of Agricultural Research and Training, (IAR&T), Moor Plantation, Ibadan, Oyo State, Nigeria in order to attain uniformity with respect to morphological traits prior to their release for evaluation. The inter-varietal crosses were derived by intercrossing the 10 varieties in all possible combinations and were evaluated for grain yield and agronomic potentials during the 2015 cropping season out of which 27 pair-wise crosses with sufficient seeds for evaluation were utilized for evaluation during the 2016 cropping season (Table 1).

Field Experimentation

The field trials were conducted at the Teaching and Research (T & R) Farm, University of Ilorin, Ilorin, Kwara State, Nigeria. The location is in the southern guinea savanna agro-ecology of Nigeria (Latitude of 8° 30'N and Longitude 4° 33'E). The annual rainfall is 1186 mm with a bimodal distribution and peaks in June and September. The mean annual temperature is 29°C with average annual relative humidity of 85%. The trial for both parents and crosses were established in two (2) row plots, 5 m long with inter and intra row spacing 0.75 m x 0.5 m during the 2015 growing season (parents) and 2016 growing season (crosses). For each trial, two (2) seeds were planted per hole to attain a plant population of 53,333 plants/ha. Pre-emergence herbicide (Primextra @ 2.5 kg ai/ha) was applied at planting followed by supplementary hand weeding as required. The first dose of fertilizer (NPK 15:15:15) was applied at three (3) weeks after planting while the second dose of fertilizer

comprising only urea was applied at tasseling. There was serious armyworm infestation in 2016 cropping season which adversely affected many of the plants both at vegetative and flowering stages (Plate 1). The pest was controlled with Spirotetramate 75 g/L + Flubendiamide 100 g/L.

Data collection from field experiment

Data were collected on growth and yield parameters. Days to anthesis and silking were determined as the number of days from planting to the time when 50% of the plants in a plot had tassels which had shed pollen and extruded silk. Anthesis-silking interval was determined by the difference between days to 50% pollen shed and days to 50% silking. Plant and ear heights were obtained as the distance from the base of the plant to the flag leaf and the node subtending the upper ear, respectively. The measurement was taken from five (5) plants selected at random in a plot. Ear aspect, root and stem lodging were scored on a scale of 1-5 where 1 = clean, uniform, large and well filled ears and 5 = rotten, variable, small and partially filled ears (ear aspect) and 1= not lodged and 5= heavily lodged (stem and root lodging). Grain weight was obtained by weighing all the grains on the ears harvested in a plot on a scale and recorded in kilogramme/plot and was later converted to tones/hectare after adjusting for moisture at harvest.

Laboratory Experiment

The laboratory study involved detailed measurement of the seed physical attributes and the analysis of total phenolic compounds in the maize grains of both parents and the crosses, as well as weevil bioassay of the maize grains for relative resistance to *S. zeamais*.

Analysis of phenolic compounds

The quantitative analysis of total phenolic compounds was carried out at the Central Research Laboratory and Diagnostic Center, Ilorin, Nigeria. This was determined by using the Folin – Ciocalteu method (Singleton *et al.*, 1999) with the aid of the UV- Spectrophotometer. Gallic acid equivalent (GAE) was used as a standard and each sample was done in three replicates.

Seed physical attributes

The physical attributes of the seeds included seed size, seed texture, seed colour and seed weight. Seed size was determined by the use of micrometer screw gauge which measured the length, width and thickness of 10 seeds of each maize sample while seed texture was determined by the proportion of soft and hard endosperm and the level of indentation of 10 seed kernels of each maize sample. Maize seed kernels with high proportion of soft endosperm and high level of indentation belonged to seed textural class dent and were coded as 1 while maize seed kernels with high proportion of hard endosperm and low level of indentation belonged to seed textural class flint and were coded as 0. Seed colour was determined by visually observing 10 seeds of each maize sample in three replications and assigning colours to them. Four classes of colours (cream, yellow, deep yellow and orange) were established. The weight of 100 seeds was determined by measuring 100 seeds of each sample on a digital weighing balance and this was done in three replications.

Weevil bioassay for relative resistance to S. zeamais.

The treatments for weevil bioaasay were arranged in a Completely Randomized Design with three (3) replicates at a room temperature of 27°C and a relative humidity of 43 – 60%. Maize weevils were collected from bags of weevil infested maize harvested from field trials at the T & R Farm (University of Ilorin) during the previous growing season. Unsexed weevils of mixed age, in a no choice experiment were used (Serratos *et al.*, 1997). The weevil bioassay was done using the modified Dobie's method (Dobie, 1977). The dried seeds of each maize sample were stored in a cold storage to kill any previous infestation by insects which include the adults, larvae or eggs (Kossou *et al.*, 1993). Thereafter, the seeds were air dried to get acclimatized to the new environment. One hundred (100) seeds of each maize sample were placed in plastic containers. Muslin cloths allowing ventilation and preventing the escape of insects were used to cover the plastic containers with the aid of rubber bands to secure and to put them in place. Ten (10) unsexed weevils of mixed age, in a no choice experiment (Serratos *et al.*, 1997) were introduced into the plastic containers containing the maize seeds. Weevils were kept for ten (10) days to oviposit as described by Derera *et al.*, (2001). Live and dead adult weevils were counted, removed and discarded after 10 days. Counts were made on progeny emergence at every other day and this began 20 days after the

removal of the parent insects and ended when all F_1 progeny had emerged. Emerged progenies were removed from the jars at each count (Siwale *et al.*, 2007).

The experiment lasted for 90 days after which the genotypes were rated for resistance or susceptibility using Dobie's susceptibility index where 0.0 to 4.0 = Resistant; 4.1–7.0 = Moderately Resistant, 7.1–10.0 = Susceptible and ≥ 10.1 = Highly Susceptible (Dobie, 1974).

This was calculated using the formula:

$IS = (\text{Log}_e X / MDP)\ 100$

where:

IS = Dobie's susceptibility index

LogeX = Natural logarithm of the total number of the F_1 progeny emerged

MDP = Median development period. This was determined by calculating the time (days) from the middle of the oviposition period to the emergence of 50% of the F_1 progeny (Dobie, 1977).

Data Analyses

All data collected from the field and laboratory studies were subjected to Analyses of Variance (ANOVA). Correlation analysis was used to assess relationships between the grain resistance parameters for maize weevil.

The separation of significant means was done using the Least Significant Difference (LSD) at 5% level of probability according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Seed physical attributes among parents and crosses

The results of the seed physical attributes for parents and crosses showed that the parents differed significantly ($\rho < 0.05$) for all the seed physical attributes except seed texture while the crosses differed significantly ($\rho < 0.05$) only for seed width and 100 seed weight (Table 2). Majority of the crosses had higher mean values than their respective parents for these attributes and the overall mean for these traits were higher in the crosses than their respective parents. The ranges in the means among the crosses for seed attributes especially length, width and thickness were also larger than in the parents indicating that they were more variable for these attributes than their respective parents. One of the parents (Idi-Ayunre) had the largest 100 seed weight which was superior to those of other parents except NACGRAB. This variety also transferred this trait to the progenies either when used as male or female parent. A similar observation was recorded in the crosses where NACGRAB 2 and Abeokuta 1 with larger seed width exhibited resistance to the maize weevil. Thus, it can be inferred that large seed size in maize has the potential to confer resistance against weevil infestation than small seed size, which is similar to reports from earlier studies (Gudrups *et al.*, 2001; Luke *et. al.*, 2015; Makanjuola *et al.*, 2009). Although study conducted by Suleiman *et al.*, (2014) revealed that dent maize is known for resistance to *S. zeamais*, none of the dent varieties among the parents was resistant to the weevil while only few of the crosses exhibited certain level of resistance to the weevil. Hence, susceptibility in the dent genotypes in this study may be due to other factors other than seed physical attributes.

The distribution of the parents and crosses with respect to colour were cream, yellow, deep yellow and orange, however, majority of the genotypes produced seeds with deep yellow colour. None of the four (4) colour classes could be directly linked to resistance or susceptibility to *S. zeamais* which contradicts the report of Nwosu *et al.*, (2015) who noted that 83.33% of white coloured genotypes screened exhibited various degree of resistance to the maize weevil and therefore hypothesized that white colour on a maize grain is likely to be a positive indicator of good resistance to *S. zeamais*. However, parental lines and the crosses with deeper colour showed some level of resistance compared to those with lighter colours in the present study which tend to agree with the study of Luke *et al.*, (2015) that colour combinations appeared to favour resistance to *S. zeamais*.

Susceptibility parameters in parents and crosses

The parents and their respective crosses were similar in their ratings for all the susceptibility parameters except that the parents differed significantly ($\rho < 0.05$) for seed weight loss (Tables 3 and 4). However, the crosses had lower values for almost all the susceptibility parameters compared to their respective parents except for parental survival for which they were similar and total phenolics in which the crosses had higher values. The parents and crosses also had high weevil parental survival ranging between 90 and 100 percent (%) for the parental maize and 80-100 % for the crosses. The high parental survival

recorded for the parents and their respective crosses in this study indicate that mortality of adult *S. zeamais* was not affected by the genotypes of the individual parents and crosses. This observation corroborates earlier report (Dobie, 1974) that parental survival is not a good indicator of susceptibility in a genotype especially since the study of Abraham (1991) has shown that adult weevils can survive without food for more than 10 days under laboratory condition.

One parent (NACGRAB) and two crosses (Ago-Iwoye 4 and Eruwa 2) which were among the genotypes with maximum (100%) parental survival also had the lowest number of emerged F_1 progenies in their respective groups. Among the parents, the difference in number of emerged F_1 progenies between NACGRAB and Idi-Ayunre which had the highest weevil emergence was 51. Two other parents (Ago-Iwoye and Itawure-Ekiti) in that order also had low F_1 progeny emergence. Among the crosses, the differences between the two crosses with the lowest emerged F_1 progenies and NACGRAB 1 with the highest number of emerged F_1 progenies were 51 and 48 respectively. Keba and Sori (2013) hypothesized a positive correlation between parental survival and number of eggs laid with the probability of higher number of emerged F_1 progenies. While data from two (2) parents (Idi-Ayunre, Ago-Iwoye) and two (2) crosses (Kila 2 and Kila 4) supported this hypothesis, our results also showed that one (1) parent (NACGRAB) and three (3) crosses (NACGRAB 2, Ago-Iwoye 4 and Eruwa 1) with 100 parent survival had very low total F_1 progeny emergence. This observation corroborates earlier reports (Shafique and Chaudry, 2007; Astuti *et al.*, 2013) that the basis of maize grain resistance to stored products insects is still not clearly understood.

There was no trend in the values of MDP and total phenolics in both parents and crosses that could be associated with susceptibility to *S. zeamais* infestation in this study. According to Abebe *et al.*, (2009), the combination of lower weevil emergence and longer MDP should confer resistance to maize genotypes. Although, the number of total F_1 progeny emergence recorded in this study supports this assertion, data in respect of MDP showed that only one (1) cross (Kila 2) had a longer MDP than the susceptible and moderately resistant genotypes. In other words, the results for MDP in respect of the genotypes assayed did not support this assertion. Rather, seed weight loss in combination with total F_1 progeny emergence (Adams, 1976; Serratos *et al.*, 1997; Derera *et al.*, 2001; Siwale *et al.*, 2007) and total phenolics (Dobie, 1974; Classen *et al.*, 1990; Arnason *et al.*, 1994; Sen *et al.*, 1994; Arnason *et al.*, 1997; Derera *et al.*, 2001) were more predictive of susceptibility in the maize genotypes screened.

All the parents had high susceptibility index ranging from 9.30 in Kila to 12.25 in Idi-Ayunre and were classified as either susceptible or highly susceptible. The crosses on the other hand had a range of 1.33 (Ago-Iwoye 4) to 8.67 (Kila 4) and were classified as resistant, moderately resistant and susceptible respectively. The highly susceptible parents were characterized by high parental weevil survival (93.30 –100.00), high emerged F_1 progenies (51– 80) high seed weight loss (7.02–11.30) and high susceptibility index (10.22–12.25) while the resistant genotypes among the crosses were characterized by low weevil progeny emergence (4.70–21.30), low seed weight loss (0.83–2.70) higher total phenolics (183.81–191.70) and low susceptibility index (1.33–3.94). Seven (7) of the crosses (Ago-Iwoye 4, Kila 2, Idi-Ayunre 1, Ijebu-Igbo 1, Idi-Ayunre 2, Itawure-Ekiti 2 and Eruwa 3) were classified as resistant to weevil infestation, 12 (Eruwa 1, Kila 5, Kila 6, Abeokuta 1, Ago-Iwoye 2, Aramoko 1, Isara 2, Ago-Iwoye 5, Abeokuta 2, Kila 1 and NACGRAB 2) were classified as moderately resistant while the remaining eight (8) were classified as susceptible based on Dobie's (1974) classification.

Grain yield and agronomic traits in the parents and crosses

Within group differences for the agronomic traits as well as grain yield was not significant (Table 5). However, the crosses were more variable for grain yield than their parents judging by the large range in means for this trait. There was no appreciable heterosis for grain yield in the crosses which could be due to the fact that the parents were collected from the same agro-ecology and are therefore mere ecotypes. In other words, they probably belong to the same heterotic block with the likelihood of lack of genetic diversity among them for grain yield. Alternatively, the seeming lack of heterosis in the crosses for grain yield may also be due to the

outbreak of armyworm ***(Spodoptera sp.)*** **in 2016 cropping season which did not occur in 2015 when the parents were evaluated on the field.**

Association of seed physical attributes with susceptibility parameters in varietal crosses

Correlation analysis revealed significant relationship among different resistance parameters. There were positive and significant relationships between susceptibility index and total F_1 progeny, seed length and seed colour (Table 6). Positive and significant correlations were also observed between susceptibility index and median developmental period, susceptibility index and seed weight loss, seed weight loss and total F_1 progeny, seed width and 100 seed weight as well as seed width and seed thickness. The positive relationship between susceptibility index and total F_1 progeny displayed by the crosses indicated that genotypes with higher population of F_1 progeny emergence were more susceptible to the infestation of *S. zeamais* and vice versa. This significant relationship probably could be an indication that the indicators of susceptibility have a high chance of identifying resistant varieties among the crosses. This observation corroborates earlier report of Classen *et al.* (1990) in which the Index of Susceptibility (IS) has been described in detail and found to be correlated with important genetic resistant factors like grain weight loss number of F_1 progeny grain hardness and rate of insect increase.

Seed weight loss was found to be an index of susceptibility as the F_1 hybrids with higher seed weight loss percentage tended to show higher level of susceptibility compared to those with lower seed weight loss. The susceptibility in hybrids with higher seed weight loss percentage could be attributed to a high number of total progeny emergences recalling that susceptibility index is a function of median development period and total progeny emergence. Similar result was documented by **Abebe *et al.* (2009)** where he found seed weight loss to be positively related with susceptibility index.

Negative but significant relationship was observed between total phenolic compounds total F_1 progeny, susceptibility index and seed weight loss. The F_1 hybrids with high total phenolic compounds had lower susceptibility index indicating resistance while those with low total phenolic compounds had higher susceptibility index which indicates susceptibility. Also, more weevil F_1 progeny emerged on the F_1 maize hybrids with low total phenolic compounds which in turn led to increase in seed weight loss (Classen *et al.*, 1990; Demissie *et al.*, 2015).

The inverse relationship between seed length and parental weevil survival displayed by the F_1 maize hybrids indicated that more parents survived with decreased seed length. Some researchers have reported a relationship between grain size and the susceptibility parameters (Gudrups *et al.*, 2001). Smaller grains were more susceptible to weevil attack than larger grain in selected and shelled maize varieties. This could be attributed to the fact that the edible grain embryo is further away from the crown of large kernels where the emerging F_1 adult weevils would search before finding the emergence site thereby leading to more time before emergence (Kossou *et. al.*, 1992).

CONCLUSION

The genetic mechanism governing resistance to the maize weevil appears not to be very clear based on the results obtained from this study. For example, all the parents except Aramoko when used as pollen parent produced crosses that were either resistant or moderately resistant to *S. zeamais* infestation (Table 4) while five (5) parents (Isara, Kila, Abeokuta, Ago-Iwoye and Itawure-Ekiti produced seven (7) resistant crosses (Idi-Ayunre 1 and 2, Kila 2, Ago-Iwoye 4, Eruwa 2 & 3 and Itawure-Ekiti 2). One plausible explanation is that resistance to *S. zeamais* is governed by recessive genes and that the parents which produced resistant crosses actually carried the genes in the heterozygote form. Thus, it may be necessary to really investigate this hypothesis. However, five (5) crosses (Eruwa 1, Abeokuta 3, Ago-Iwoye 2, Ago-Iwoye 4 and Idi-Ayunre 3) combined acceptable grain yield with resistance to *S. zeamais*. These populations could therefore serve as sources of genes for development of maize varieties that combine weevil resistance with acceptable grain yield for further testing in farmers' fields.

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