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Response of provitamin-A maize germplasm to storage weevil *Sitophilus zeamais* (Motschulsky)

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Abstract

Development and dissemination of biofortified maize is essential for communities at risk of malnutrition. This further requires that the improved maize varieties retain all their nutritional attributes up to the end users. Weevils (*Sitophilus zeamais*) are among the most economically important storage pests of maize in the tropics and present a permanent threat to the availability of maize nutrients for human consumption. This study aimed at evaluating resistance to maize weevils in 24 provitamin-A maize inbred lines and their single cross hybrids. Evaluation was done by artificial infestation and the Dobie Index of Susceptibility was used to group the genotypes. Results showed that Provitamin-A maize genotypes evaluated were in general susceptible to *S. zeamais*. Two inbred lines CLHP0014, and CLHP0005; and five single crosses; CLHP00434/CLHP0020, CLHP00306/CLHP0005, CLHP00434/CLHP0003, CML486/CLHP0005, CLHP0331/CLHP0020 were moderately resistant to *S. zeamais*. Grain damage (GD) was strongly positively correlated with number of F1 weevils which emerged (R^2= 0.74, P<0.001). The index of susceptibility (IS) was positively and strongly correlated with number of weevils which emerged (R^2= 0.86, P<0.001) and grain damage (R^2= 0.69, P<0.01). Susceptibility of the maize genotypes was not correlated with differences in kernel colours. Lines with moderate resistance to weevil were identified and could be improved for the use in developing nutritional qualities maize varieties to minimize loss due to storage pests.

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Introduction

Maize (*Zea mays* L.) is an important source of daily calories, especially in areas of severe malnutrition and food insecurity (Nelson *et al.*, 2010; WHES, 2015). It is a dominant food crop in much of sub-Saharan African and the American countries, where 17 to 30 % of children under 5 years age are vitamin A deficient (Harjes *et al.*, 2008). Although maize is a staple cereal crop, the white kernels maize varieties which are very popular among the African population, have low nutritional value, mainly with respect to protein and essential micronutrients such as carotenoids (FAO, 1992; Sands *et al.*, 2009; Chandler *et al.*, 2013). Carotenoids molecules in maize are precursors of vitamin A (Provitamin-A) in human diet, and represent an important source of vitamin A particularly for populations of developing countries, whose poor economic conditions do not allow an easy diet balance of plants and animal foods (Sivaranjani *et al.*, 2013).

Food biofortification seek thus to enhance the concentration of various micronutrients in maize, especially the provitamin-A carotenoids, so as to address the problem of micronutrient deficiencies in developing countries (Hotz and McClafferty, 2007; Stein, 2010; Bouis and Welch, 2010). Consequently, provitamin-A maize cultivars are introduced in regions of severe malnutrition. However, success in biofortification of maize for areas at risk of malnutrition requires that the improved maize varieties retain all their nutritional attributes up to the end users. Even if significant progress have recently been made to increase provitamin-A content in maize, there is a danger that if the losses caused by storage pests are not well managed, the improved provitamin-A maize will not be available in sufficient quantity and quality to meet the overwhelming need of provitamin-A maize varieties in areas at risk of vitamin A deficiency (VAD).

Maize weevils (*Sitophilus zeamais*) represent a threat to the sustainable storage of maize grains in tropics. They are economically important field-to-store pests of maize that start to infest the ripening maize crop in the field as early as when the grain moisture content is still 50-55% (Ojo and Omoloye, 2012).

In many tropical countries, *S. zeamais* can cause 30 to 80% seed weight loss in storage (Ajayi and Soyelu, 2013). In the absence of proper post-harvest insect control measures, seed loss can be high as 90% (Giga *et al.*, 1998; Derera *et al.*, 2014). This results in loss, reduction in nutritional value of maize; reduction in market value of the crop as well as loss of seed vigor (Abebe *et al.*, 2009, Tongjura *et al.*, 2010). Hence, damage due to storage weevils must be controlled to minimize the losses incurred by maize producers, since cares and expenditures for pest control in field crops would be of no use, if the product was attacked and destroyed when stored (Alleoni and Ferreira, 2006). Development of resistant maize varieties offers an effective way and long term solution to the damage due to *S. zeamais* (Gudrups *et al.*, 2001; Mwololo *et al.*, 2012b). A number of studies have been conducted to screen maize varieties against *S. zeamais* and have identified genotypes having resistance genes that can therefore be used to introgressing genes of resistance into available germplasm (Kim and Kossou, 2003; Abebe *et al.*, 2009; Mwololo *et al.*, 2012a). However, the genotypes used in most of these studies were not known as provitamin-A maize and to date there is a need to identify genotypes with good performance for provitamin A trait and resistant to storage weevils. Study was thus conducted to assess the response of provitamin-A inbred lines and their single crosses to storage weevils under controlled storage conditions.

Materials and methods

Experimental materials

A sub set of 24 elite provitamin-A maize inbred lines (Table 1) were selected from introduction from CYMMYT and used to develop 72 single crosses hybrids by crossing four of the inbred lines (CML300, CLHP0020, CLHP0005, CLHP0003) used as males to the other 20 inbred lines (females) at the National Crop Resources Research Institute (NaCRRI) of Uganda in 2014 (September to December). Concurrently, seeds of the inbred lines were multiplied during that season and used for a first screening of the parental lines.

At the onset of first rainy season in 2015 (April to August), seeds of the provitamin-A inbred lines and their single crosses were self-pollinated at NaCRRI in Namulonge and the National Semi-Agricultural Research Institute (NaSAARI) in Serere. The inbred lines were selfed to produce enough seeds for screening while F1s seeds were advanced to produce F2 grains. F2 grains are the generation of grains normally stored by farmers and therefore the most vulnerable to weevils damage (Siwale *et al.*, 2009). Seeds of yellow conversions of popular single cross testers CML202/CML395, CML312/CML442 and Longe10H a popular three ways maize variety in Uganda were multiplied at the same time. Longe 5, an open pollinated maize variety highly susceptible to weevils was included as a susceptible check.

Experimental sites

Seeds multiplication was done at the National Crop Resources Research Institute (NaCRRI), Namulonge and the National Semi-Agricultural Research Institute (NaSAARI), Serere in Uganda. NaCRRI, is located in Wakiso District, central Uganda at an elevation 1200 masl. It is within the bimodal rainfall region and has a tropical wet and mild dry climate with slightly humid conditions(NARO, 2005). NaSSARI is located in Eastern Uganda at an elevation of 1080 masl; between the bimodal rainfall regions and the unimodal rainfall regions (Mubiru *et al.*, 2012). The trials for on grains resistance test were conducted in the entomology laboratory unit at NaCRRI.

Mass rearing of weevils for the kernel screening

The weevils used in this study were mass reared in entomology laboratory mass rearing unit at NaCRRI. A total of 200 unsexed adult insects were sampled and used to infest 1000 grams of Longe 5 (highly susceptible to weevils). The grains were put in 3000 cm^3 plastic jars and covered with lids which were first perforated and stuck with wire mesh to allow maximum aeration without the insects escaping from the jars. The unsexed adult weevils were given 10 days to oviposit after which they were removed and the samples were left for 35 days of incubation to ensure that enough F1 insects were produced.

The F1 insects at 1 to 10 days old were sieved out with mesh sieve (Endecotts Ltd, UK) and used in the kernel screening.

Hybrids kernel colour scoring

Kernel colour of the hybrids was visually scored prior to the grain resistance test as described by Chandler *et al.*(2013). This involves grouping a total of 100 randomly selected seeds of each genotype into colour classes using an ordinal standardized colour scale ranging from 1 (lightest yellow) to 12 (darkest orange). The score of a specific genotype was computed as an average of the different colour scores observed in the seeds bulk.

Preparation and infestation of grains samples with weevils

After a proper cleaning operation, four subsamples (replicates) each of 50 grams seeds were weighed for each of the 100 genotypes (24 inbred lines and 76 hybrids) using a compact balance electronic weighing scale brand type. The weighed samples were thereafter labelled and wrapped in polythene bags and then frozen at -20°C for 14 days to kill any insect/egg that could have attacked the grains in the field (Siwale *et al.*, 2009). After freezing, the seed samples were transferred to 250 cm^3 evaluation jars and left to achieve uniform temperature and moisture content before the infestation. A total of 32 unsexed insects was used to infest the maize kernels in each glass jar (Derera, Pixley, & Giga, 2010). The jars were sealed as described earlier. The inbred lines were laid in 4 x 6 alpha Lattice Design while single crosses were set in 4 x 19 Alpha Lattice design within the laboratory (Dhliwayo *et al.*, 2005). A Thermo-Hygrometer was used to monitor relative humidity (70±5%) and temperature (28±2°C) status during the experimentation period (Mwololo *et al.*, 2012a)

Data collection

Samples were given 10-days oviposition period after which all adult insects, dead and living were removed and counted. The samples were then incubated for 25 days to allow a development period of 35 days which is the average period for the weevils to complete one cycle, under optimum conditions (Gwinner *et al.*, 1996; Derera *et al.*, 2001a).

F1 progeny insects were then counted and removed from the jars at 2 days interval until no more insect emerged from the jars. This interval of counts prevents any chance of F1 progeny laying eggs in the maize samples to produce F2 generation weevils. This is based on the fact that individuals of *S. zeamais* do not mate before they are three days old. Five parameters were assessed for each genotype, including adult insect mortality, number of F1 insects emerged during the storage period, median development period, Dobie's index of susceptibility and grain damage as described below.

Adult mortality

Adult mortality was estimated as percentage of the number of dead adult insects after ten 10 days of oviposition period.

Number of F1 insects emerged (N° F1 insects)

It represents the cumulative sum of F1 progeny insects counted and removed from the jars at 2 days interval until no more insects emerged from the jars.

Median development period (MDP)

The median development period was computed as the number of days from the middle period of oviposition (5 days) to the middle emergence of progeny (50% emergence of F1 insects), (Dobie, 1974; Siwale *et al.*, 2009).

Index of susceptibility (IS)

The index of susceptibility was calculated using the method proposed by Dobie (1977). This involves the number of F1 progeny and the median development period.

$$(IS) = \frac{\log_e(\text{total of F1 progeny emerged})}{\text{Median development period}} \times 100$$

Grain damage (GD)

Once all adult insects which emerged were removed from the infested jars, 100 grains were randomly taken from each jar and the number of damaged grains (holed grains) by weevil feeding was determined as a proportion of the total number of grain sampled (Caneppele *et al.*, 2003; Abebe *et al.*, 2009).

Statistical analysis

Data were subjected to an analysis of variance across locations using the Restricted Maximum Likelihood (ReML) in GenStat (12th Editon). The genotypes were considered as a fixed effect while environment and replications were random. The linear model for the across environments was as follows:

$$y_{ijk} = u + l_i + r/l_{ij} + g_k + gl_{ik} + e_{ijk}$$

Where y_{ijk} = observed value from each experimental unit, u = grand mean, l_i= effect of the i^{th} environment,r/l_{ij}= effect of the j^{th} replication nested to the i^{th} environment,g_k= effect of k^{th} genotype, gl_{ik} = interaction effect of k^{th} genotype by the i^{th} environment and e_{ijk} is the experimental error.

Means were separated using Least Significant Differences (LSD) at a 5% probability level. The index of susceptibility scale, ranging from 0 to 11, was used to classify the response of the maize genotypes into resistance groups. The resistance classes were 0 - 3 = resistant, 4 - 7 = moderately resistant, 8 - 10 = susceptible and ≥11 = highly susceptible (Issa *et al.*, 2011). kernel colour scale of 1 to 12 (Chandler *et al.*, 2013) was used to categorize the hybrids maize genotypes into four groups (1-4= yellow, 4.1-6 = orange pale, 6.1-8=orange and 8.1 -12= deep orange). Correlation analyses were performed between measured traits. The kernel colour scores of the hybrids were also regressed against their index of susceptibility to check if there is a relationship between these two traits.

Results

Response of provitamin-A inbred lines to maize weevil infestation

There were significant differences among the inbred lines (Table 2) for adult mortality (P < 0.01), Number of F1 insects emerged (P <0.001), Median development period (P < 0.01), Index of susceptibility (P < 0.001), and grain damage (P < 0.001). Genotypes by Environment Interaction was also significant for all the five grain susceptibility parameters (Table 2).

Adult mortality in the inbred lines was generally low with an average value of 17.52% (Table 3). Line CML451 had the lowest percent of adult mortality while the line CLHP0014 exhibited the highest mean of adult mortality.

Number of F1 insects emerged varied with the mean value of 83 insects (Table 3). Lines CLHP00328 and the CLHP0301 had the highest number of weevils emergency while the line CLHP0014 had the lowest number of F1 insects emerged.

Table 1. Provitamin-A inbred lines evaluated for resistance to storage weevils.

Genotype	Pedigree
CML304	CML304-B-B-B-B
CML486	CML486-B-B-B
CML451	CML451-B-B-B
CLHP00306	(KUIcarotenoidsyn-FS17-3-1-B-B-B-B//(KUIcarotenoidsyn-FS17-3-1-B-B-B/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/KUI3×SC55)))-plant19(HH)-3-1-B-B-B-B-B-B
CLHP00478	(CLQRCWQ97-B///((KUIcarotenoidsyn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)//CarotenoidSyn3-FS11-4-3-B-B-B))-B-25-6-B-B-B-B-B-B
CLHP00476	(CLQRCWQ97-B///((KUIcarotenoidsyn-FS17-3-2-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)//CarotenoidSyn3-FS11-4-3-B-B-B))-B-25-2-B-B-B-B-B-B
CLHP0310	((CML506//CML506/CML305)//((CML506//CML506/CML305)/KUIcarotenoidsyn-FS11-1-1-B-B-B-B)-B)-6-3-B-B-B-B-B
CLHP0290	([DTPYC9-F65-2-3-1-1-B-BxDTPYC9-F65-2-2-1-1-B-B]-3-4-2-B-B-B//([DTPYC9-F65-2-3-1-1-B-BxDTPYC9-F65-2-2-1-1-B-B]-3-4-2-B-B/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/KUI3×B77)))-plant3(H)-1-3-B-B-B-B-B-B
CLHP00308	((CML506//CML506/CML305)//((CML506//CML506/CML305)/(KU1409/DE3/KU1409)S2-18-2-B-B-B)-B)-11-1-B-B-B-B-B
CLHP0302	(CML486/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/KUI3×B77))-B-11-1-B-B-B-B-B-B
CLHP0352	([DTPYC9-F65-2-3-1-1-B-BxDTPYC9-F65-2-2-1-1-B-B]-3-4-2-B-B/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/KUI3×B77))-B-21-1-B-B-B-B-B-B-B
CLHP00294	(KUIcarotenoidsyn-FS17-3-1-B-B-B-B//(KUIcarotenoidsyn-FS17-3-1-B-B-B/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/KUI3×SC55)))-plant19(HH)-4-2-B-B-B-B-B-B
CLHP00328	([[[NAW5867/P30SR]-43-2/[NAW5867/P30SR]-114-1]-9-3-3-B-1-B/CML395-1]-B-13-1-B-4-#/[BETASYN]BC1-8-1-1-1-B-B/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/KUI3×B77))-B-1-1-B-B-B-B-B-B-B
CLHP0301	(CML486/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/KUI3×B77))-B-11-1-B-B-B-B-B-B
CLHP0331	(CML491-B///((KUIcarotenoidsyn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)//[[[NAW5867/P30SR]-40-1/[NAW5867/P30SR]-114-2]-16-2-2-B-2-B/CML395-6]-B-20-1-B-3-#/[BETASYN]BC1-3-1-1-#-B-B-B))-B-3-1-B-B-B-B-B
CLHP0289	(CML496//(CML496/(CML297-B×KUICarotenoidsyn-FS17-3-2-B/A619×SC55)))-plant11(HF)-1-3-B-B-B-B-B-B
CLHP00434	((([CLRQ00502xB109]-F2)-04-05-K1/KUIcarotenoidsyn-FS17-3-2-B-B-B-B)//(([CLRQ00502xB109]-F2)-04-04-K1/CarotenoidSyn3-FS11-4-3-B-B-B-B)//(KUIcarotenoidsyn-FS11-1-1-B-B-B/(KU1409/DE3/KU1409)S2-18-2-B)-B-2)-12-1-B-B-B-B-B
CLHP0014	CarotenoidSyn3-FS8-4-3-B-B-B-B-B-B-B-B-B-B-B
CLHP0002	CML489/[BETASYN]BC1-2-#-B-B-B-B-B-B-B-B-B-B-B
CLHP0006	CML488/[BETASYN]BC1-15-7-1-1-1-B-B-B-B-B-B-B
CML300	CML300-B-B-B
CLHP0005	MAS[206/312]-23-2-1-1-B-B-B/[BETASYN]BC1-11-3-1-#-B-B-B-B-B-B-B-B-B-B-B-B
CLHP0003	CML537/[BETASYN]BC1-10-3-#-B-B-B-B-B-B-B-B-B-B-B-B
CLHP0020	KUIcarotenoidsyn-FS17-3-2-B-B-B-B-B-B-B-B-B-B-B

The median development period of weevils on the inbred lines maize varied from 43 days to 54 days for the lines CLHP0014 and CLHP0005, respectively (Table 3). Percentage of damaged grains due to weevil infestation was high for the lines CML304, CLHP0006, CLHP00328 and CLHP0301 while the line CLHP0014 had the lowest percent of damaged grain (Table 3).

A classification of the 24 provitamin-A inbred maize lines into four resistance classes based on their index of susceptibility, revealed lines CLHP0014 and CLHP0005 as moderately resistant lines while the rest of the inbred lines were susceptible or highly susceptible genotypes. The most susceptible genotypes were the lines CLHP0352 and CLHP00328 (Table 3).

Table 2. Mean squares for five grain susceptibility parameters of the 24 provitamin-A maize lines across environments.

Source	Df	Mean squares				
		Adult Mortality	N° F1 Insects	MDP	IS	GD
Environments (E)	2	6622.24***	2281.20	6622.24***	49.43**	2250.12*
Rep/E	9	1070.1***	5022.3***	82.57***	12***	1125.6***
Genotypes (G)	23	1450.84**	6503***	1450.84**	25.92***	2403.36***
G x E	46	592.00***	1521*	592 ***	6.32***	723.20***
Pooled error	157 -181	116.36	783.22	15.57	2.37	251.76
Grand Mean		17.52	82.53	46.53	9.12	39.54
CV (%)		61.57	33.91	8.48	16.88	40.13

Rep/E= Replications nested to Environments, Adult Mortality= percentage of dead insects after 10 days of oviposition, MDP=Median development Period, IS= Index of susceptibility, GD= Grain damage. ***Significant at P<0.001, **significant at P<0.01, *significant at P<0.05.

Table 3. Mean response of the 24 lines and testers for five grain susceptibility parameters across environments.

ENTRY	Adult[b] mortality	N° F1 Insects	MDP	GD	IS	Category
CML304	18.58	121.49	46.86	62.85	10.3	S
CML486	12.82	102.54	44.36	47.46	10.4	S
CML451	6.97	87.03	46.94	48.54	9.5	S
CLHP00306	25.44	74.05	45.91	31.88	9.3	S
CLHP00478	23.83	57.77	48.25	29.78	8.3	S
CLHP00476	22.75	74.52	45.78	34.11	9.3	S
CLHP0310	7.73	70.17	46.15	39.11	9.1	S
CLHP0290	12.81	62.66	45.52	31.25	9.0	S
CLHP00308	10.77	79.16	46.86	41.51	9.3	S
CLHP0302	17.85	62.94	45.36	34.75	9.2	S
CLHP0352	11.11	127.64	43.48	51.25	11.2	HS
CLHP00294	39.80	61.98	47.00	27.99	8.4	S
CLHP00328	9.90	157.73	45.30	58.59	10.8	HS
CLHP0301	9.02	155.62	44.44	57.94	11.2	HS
CLHP0331	9.45	73.45	46.96	34.50	8.9	S
CLHP0289	16.18	72.67	45.36	39.50	9.4	S
CLHP00434	13.74	81.14	49.02	47.99	9.1	S
CLHP0014	53.10	18.22	43.41	4.75	4.5	MR
CLHP0002	7.64	72.55	44.61	43.52	9.7	S
CLHP0006	12.03	104.27	49.23	60.56	9.9	S
CML300	15.46	102.76	45.80	50.15	10.0	S
CLHP0005	25.42	54.45	53.75	20.25	6.5	MR
CLHP0003	27.40	54.35	48.48	22.60	7.7	S
CLHP0020	10.76	51.49	47.79	28.04	7.9	S
Grand Mean	17.52	82.53	46.53	39.54	9.12	
LSD	8.69	22.54	3.18	12.79	1.24	

HS= highly susceptible, S= Susceptible, MR= Moderately Resistant.

Responses of single cross hybrids provitamin A maize to S. zeamais

Significant differences among the single crosses of provitamin-A maize genotypes were obtained for all susceptibility parameters evaluated except the Median development period (Table 4). Genotype by environment interaction had also a significant effect on the performance of the genotypes for all parameters across environments (Table 4).

Mean values of the provitamin-A maize hybrids for the five grain susceptibility parameters evaluated (Table 5), showed that hybrid CLHP00478/CLHP0005 had the lowest adult mortality while hybrid CLHP00434/CLHP0020 exhibited the highest adult mortality . The number of F1 insects emerged varied from 22 insects for the hybrid CLHP0331/CLHP0020 to 107 insects for CML486/CML300. The median development period was the lowest in the susceptible check (Longe 5, MDP = 41) as compared to the other genotypes. On the other hand, grain damage was the highest for hybrid CML486/CML300 and the lowest for CLHP0331/CLHP0020 (14.28%).

Table 1. Mean squares for five grain susceptibility parameters for the 76 genotypes evaluated for resistance to S. zeamais across environments in Uganda.

Source	Df	Mean squares				
		Adult Mortality	N° F1 Insects	MDP	GD	IS
Environments (E)	1	2597.12	36941.56*	13620.9***	26275.56*	838.73**
Rep/E	6	2335.07***	4900.4***	141.67***	2799.6***	25.30***
Genotypes (G)	75	343.84**	2395.88***	26.68	980.9***	6.80**
G x E	75	185.08***	986.2***	23.70***	489.2***	3.43***
Pooled error	412-444	88.25	387.42	11.53	153.77	11.48
Grand Mean		13.96	54.34	46.78	32.55	8.41
SEM		3.83	8.03	1.39	5.06	0.50
CV (%)		67.28	36.22	7.26	38.10	14.45

Adult Mortality= percentage of dead insects after 10 days of oviposition. MDP=Median development Period, IS= Index of susceptibility, GD= Grain damage. ***Significant at $P<0.001$, **significant at $P<0.01$, *significant at $P<0.05$.

Table 2. Mean response of the 15[a]hybrids provitamin-A maize genotypes for five grain susceptibility parameters assessed across environments in 2015.

Genotypes	Adult Mortality	N° F1 Insects	MDP	GD	IS	Category
CLHP00434/CLHP0020	34.72	23.71	46.56	15.63	6.28	MR
CLHP00306/CLHP0005	20.84	29.21	49.41	17.35	6.50	MR
CLHP00434/CLHP0003	13.06	25.34	47.37	16.19	6.74	MR
CML486/CLHP0005	18.15	28.34	48.69	19.15	6.76	MR
CLHP0331/CLHP0020	15.59	21.59	43.98	14.28	6.91	MR
CLHP00478/CLHP0005	2.50	36.81	48.90	25.69	7.28	S
CML486/CLHP0020	13.06	38.59	49.93	23.88	7.28	S
CLHP0002/CLHP0020	18.01	36.96	46.93	22.18	7.34	S
CLHP0289/CLHP0005	4.53	36.59	49.40	21.79	7.34	S
CLHP0006/CLHP0005	15.89	44.59	49.19	31.27	7.39	S
CML486/CLHP0003	12.93	83.59	45.08	52.48	9.91	HS
CLHP0352/CML300	18.83	92.34	45.78	46.49	10.08	HS
CLHP0352/CLHP0003	20.81	89.34	44.67	44.89	10.15	HS
CLHP0002/CML300	7.09	81.59	42.83	55.22	10.41	HS
CML486/CML300	8.98	106.84	44.91	61.47	10.44	HS
Susceptible check						
Longer 5	6.45	74.84	41.00	43.05	10.52	HS
Grand Mean	13.96	54.34	46.78	32.55	8.41	-
LSD	9.23	19.34	-	12.19	1.19	-

HS= highly susceptible, S= Susceptible, MR= Moderately Resistant. 15[a]= 5 highly susceptible genotypes, 10 susceptible genotypes and 5 Moderately Resistant genotypes.

Index of susceptibility ranged from 6.28 in CLHP00434/CLHP0020 to 10.52 in the susceptible check (Longe 5). Genotypes Longe 10H, CML312y/CML444y and CML202y/CML395y had an index of susceptibility of 10.2, 9.08 and 8.27, respectively. A classification of the 72 provitamin-A maize hybrids in different category of resistance to weevils showed that genotypes had a distribution skewed toward susceptibility (Fig. 1). The provitamin-A hybrids maize CLHP00434/CLHP0020, CLHP00306/CLHP0005, CLHP00434/CLHP0003, CML486/CLHP0005 and CLHP0331/CLHP0020 were moderately resistant to the maize weevils attack.

Table 6. Mean squares for kernel colour of the hybrids provitamin-A maize genotypes.

Source	Df	Mean squares
		Kernel colour[d]
Environments (E)	1	1.26
Rep/E	2	2.85**
Genotypes (G)	74	7.15***
G x E	74	0.52
Pooled error	80	0.81
Grand Mean		6.75
SEM		0.21
CV (%)		13.38

[d]=Kernel colour scored on a scale of 1(lightest yellow) to 12 (darkest orange).

Table 7. Kernel colour scores of the first 5 deepest orange and last 5 lightest yellow hybrids provitamin-A maize genotypes across environments in Uganda in 2015.

Hybrids	Kernel colour scores
CLHP00306/CLHP0020	9.25
CLHP0310/CLHP0020	9.00
CLHP0352/CLHP0020	9.00
CLHP00294/CLHP0020	9.00
CLHP0302/CLHP0020	8.75
CLHP0301/CLHP0020	8.65
CML486/CLHP0020	8.50
CLHP0289 /CLHP0005	8.50
CLHP0290/CLHP0020	8.25
CLHP00434 /CLHP0020	8.25
CLHP00328/CLHP0005	5.75
CLHP00328/CLHP0003	5.75
CLHP0002/CLHP0003	5.75
CLHP00476/CLHP0005	5.63
CLHP00308/CLHP0005	5.38
CLHP00328/CML300	5.25
CLHP0331 /CLHP0005	5.25
CML451/CML300	5.08
CML451/CLHP0003	4.57
CML451/CLHP0005	3.75
Grand Mean	6.25
LSD	0.59

Thirty-height genotypes were classified in the susceptible group while the rest of the genotypes were highly susceptible (Fig.1). The high susceptible hybrids harboured three to four times more F1 weevils and have considerably higher percentage of grain damage than moderately resistant ones (Table 5).

There were significant differences in kernel colour ($P<0.001$) between provitamin-A hybrids screened (Table 6). Hybrid CML451/CLHP0005 was the lightest with the kernel colour score of 3.75 whilst the deepest orange kernel (9.25) was obtained from the cross between the lines CLHP00306 and CLHP0020 (Table 7).

The frequency distribution of the hybrids into four kernel colour classes showed that genotypes were normally distributed (Fig. 2). On average the genotypes were orange in colour with the kernel colour score mean of 6.75.

Relationship between grain susceptibility parameters

Correlations analysis between grain susceptibility parameters (Table 8) showed strong positive relationship between the number of F1 insects emerged and index of susceptibility (R^2= 0.86, $P<0.001$). The index of susceptibility was also positively correlated with Grain damage (R^2= 0.69, $P<0.001$). Grain damage was strongly correlated with number of F1 insects emerged (R^2= 0.74, $P<0.001$). Negative correlation was obtained between the Median development period and the index of susceptibility (r= -0.6, $P<0.001$) while no significant linear relationship was found between IS and adult mortality on the grains of the provitamin-A hybrids maize.

There was no consistent relationship between kernel colour of the genotypes and their index of susceptibility ($r=0.004^{ns}$).

Table 8. Relationship between grain susceptibility parameters.

Parameters	Coefficient of correlation (r)	Coefficient of determination (R^2)
Adult Mortality vs. IS	-0.21	0.04^{ns}
F1 insects emerged vs. IS	0.93	0.86^{***}
MDP vs. IS	-0.6	0.4^{***}
GD vs. IS	0.83	0.69^{***}
GD vs. F1 insects emerged	0.86	0.74^{***}
Kernel colour vs. IS	0.004	$1.60E\text{-}05^{ns}$

***Significant at $P<0.001$, **significant at $P<0.01$, *significant at $P<0.05$, ^{ns} = non-significant.

Discussion

Results from the laboratory screening of the inbred lines and resultant crosses for resistance to storage weevils showed considerable variation among the genotypes for the grain susceptibility parameters evaluated. These differences indicate the inherent ability of a particular genotype to resist weevil infestation (Abebe *et al.*, 2009, Ajayi and Soyelu, 2013). There was a significant genotype by environment interaction effect, indicating that the environment plays a role in the resistance of the maize varieties to weevils.

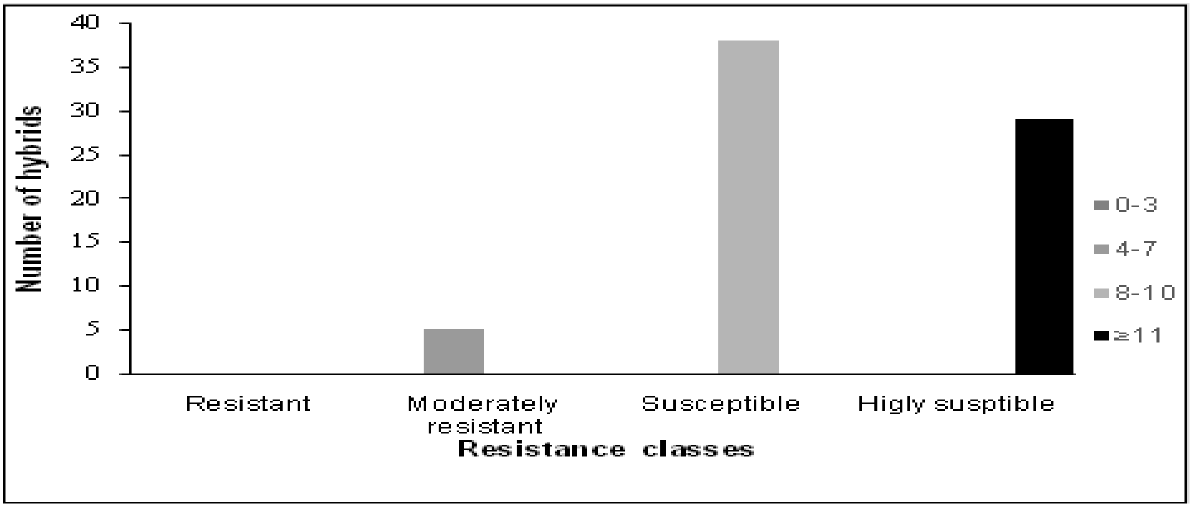

Fig. 1. Frequency distribution of the 72 hybrids provitamin-A maize into different susceptibility classes.

This finding is supported by Kim and Kossou (2003). Whereas the screened lines were in general susceptible to the weevils; two genotypes (CLHP0014 and CLHP005) out of the 24 inbred lines had a moderate resistance to weevil's infestation. This response among inbred lines may explain the high rate of susceptibility observed in their resulting crosses. These hybrids crosses are yellow to orange maize genotypes and their index of susceptibility range from 6.24 to 10.44; which was not correlated to the variation in the hybrids kernel colour scores(Table 8). Differential responses of yellow/orange maize genotypes have been reported by previous studies where the index of susceptibility varied from 7.8 to 15.2 in the yellow/orange kernel coloured maize varieties(Dobie, 1974, Santos *et al.*, 2006). Although most single crosses based on the Dobie's index of suceptibility were classified as susceptble/higly susceptible to weevils in this study, the cross combinations CLHP00434/CLHP0020, CLHP00306/CLHP0005, CLHP00434/CLHP0003, CML486/CLHP0005 and CLHP0331/CLHP0020 had moderate resistance to weevils may be improved for reducing the threat due to the maize weevils.

This response developed against the maize weevils in the moderate resistant genotypes could have been triggered by intrinsic resistance mechanisms in their grains. Studies have identified antibiosis and non-preference as basis of grain resistance to S. zeamais (Hall, 1975; Derera *et al.*, 2001a, 2001b), and this suggests further experiments in order to elucidate the type of mechanism present in these moderately resistant genotypes. The genotypes assessed in the test for resistance to weevils generally showed a relatively long median development period. The inbred line CLHP0005 had the longest Median development period and was moderately resistant. The inbred lines differed in their median development period but did not induce substantial genetic variation in their progenies for the same parameter. The median development period of the hybrids was negatively correlated with the index of susceptibility. Similar result was reported by Abebe *et al*(2009) who pointed out that the weevils on varieties having a high index of susceptibility displayed reduced periods of completion and that a prolongation of development periods will also result in reduction of number of generations of the weevils in the season.

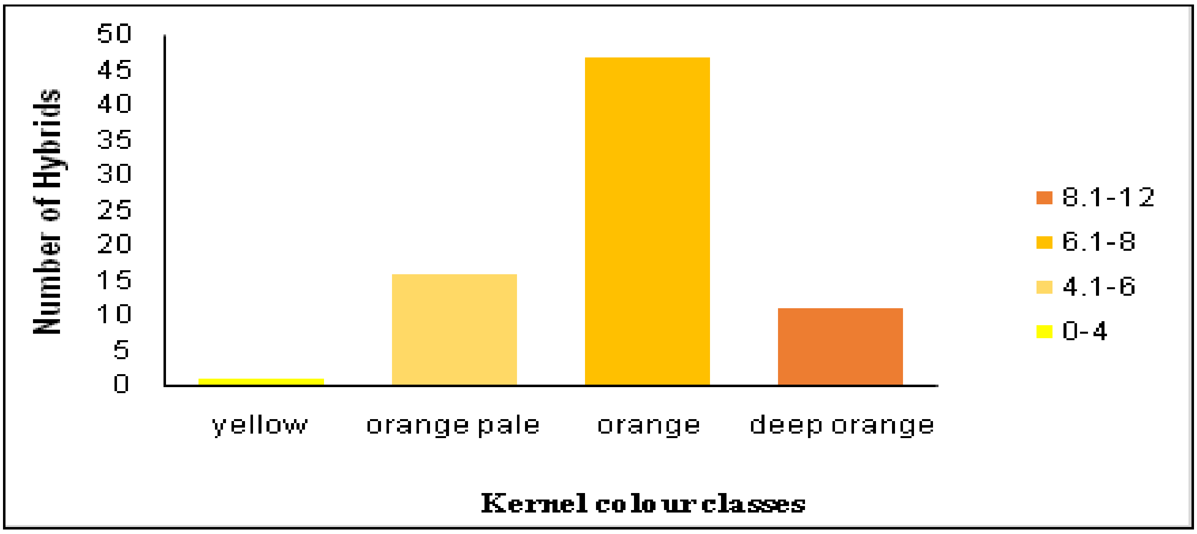

Fig. 2. Frequency distribution of the 72 hybrids provitamin-A maize into four colour classes.

In the screening of the inbred lines, CLHP0014 had the lowest number of F1 emergency and low percentage of grain damage (GD) with the lowest index of susceptibility. A strong positive correlation was also found between these three parameters (F1 insects emerged, GD, IS) in the response of hybrids to the maize weevils attack. Thus, highly susceptible hybrids had more F1 weevils and have considerably higher percentages of grain damage than the moderately resistant ones.

In maize grains, weevils grow from larvae to adult eating the maize grains from inside to out, creating holes on the grain, thus leading to the susceptibility status of the genotypes. Many studies found that the number of F1 insects emerged is high in susceptible genotypes and is positively correlated to grain damage (Abebe *et al.*, 2009; Siwale *et al.*, 2009; Keba and Sori, 2013). In the present study adult mortality was in average low among the hybrids maize and was not significantly correlated with susceptibility. Thus, this parameter may not be an adequate factor in discriminating the genotypes in their response to weevils' attack (Dobie, 1974), indicating the usefulness of considering multiple traits in assessing resistance to *S. zeamais* in maize germplasm.

Conclusion

Overall, this study showed inbred lines and hybrids that are moderately resistant to the maize weevils and the need for their improvement for use in the developing areas would reduce the high incidence of weevil's damage. This would help in preserving the nutritional quality of the stored maize grains up to the consumers and contribute to fight-off micronutrients deficiency. Most of the prtovitamin A genotypes screened exhibited a susceptible response toward the maize weevils' attack, suggesting that breeding activity for developing provitamin-A maize varieties should be conducted concurrently with effort of introgressing weevils' resistance genes into the maize varieties.

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