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GENETIC ANALYSIS OF WEEVIL (*COSMOPOLITES SORDIDUS*) RESISTANCE IN AN F_2 DIPLOID BANANA POPULATION

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A B S T R A C T

The East African highland bananas (*Musa spp*. AAA), an important staple food in Uganda, are highly susceptible to the banana weevil (*Cosmopolites sordidus* Germar). Sources of host plant resistance to the banana weevil exist in wild diploid bananas. The use of wild diploid bananas to improve East African highland bananas can be facilitated by studying genetics of host plant resistance of inter-diploid crosses. The objectives of this study were a) to identify segregating weevil resistance and agronomic traits in an F_2 diploid population, and, b) to determine the inheritance of banana weevil resistance and agronomic traits based on an F_2 banana diploid population. An F_1 population developed from *Musa acuminata* subsp. *banksii* acc. Kasaska (ITC0591) and *M. acuminata* subsp. *microcarpa* acc. Borneo (ITC0253) was selfed to generate an F_2 diploid population. The F_2 population was screened against weevil resistance and agronomic traits in the laboratory, pot and field experiments. There were significant differences ($P < 0.05$) among the different genotypes for banana weevil resistance traits such as head capsule width, body length, body weight, larval mortality, total damage, peripheral damage, dead weevils and larvae retrieved. There were also significant differences ($P < 0.05$) for agronomic traits such as inner corm hardness and total corm hardness. The histograms for the banana weevil resistance traits such as head capsule width, body length, body weight and larval mortality, total damage, peripheral damage, cross sectional inner and outer damage, larvae retrieved and dead weevils showed continuous distribution. Similarly, histograms for agronomic parameters such as height of plant at flowering and girth at 1 meter at flowering showed continuous distribution. The Chi-square test of goodness of fit indicated that weevil growth and damage parameters had significant modifications of the expected 9:3:3:1 ratio for two independent loci, thus suggesting epistasis affects their inheritance.

Keywords: F_2 banana diploid population, host plant resistance, weevil damage traits, weevil growth traits, weevil resistance.

INTRODUCTION

Bananas represent a major staple food for 400 million people in the tropics and subtropics (INIBAP, 2000). In Africa, banana and plantain provide more than 25% of food energy requirements for around 70 million people (Vander Stichele *et al*., 2005; Evans and Fredy, 2013). The banana industry is important for generation of export earnings and employment of hundreds of thousands of people in distribution networks and supermarkets worldwide (Evans and Fredy, 2013). Banana is a staple to about 10 million Ugandans; and 66% of the country's urban population depend on this crop (FAO, 2011; PIBID-Uganda, 2012). Uganda is the second largest producer of banana (9.2 million MT) in the world with the highest per capita consumption, which ranges from 230 to 450 kg person^{-1} year^{-1} (FAO, 2012). The East African highland cooking banana, used for a dish known as matooke in Uganda, is mainly grown for consumption and as source of rural revenue that offers the highest returns to family labour (Bagamba *et al.,* 1994; Embrechts *et al.,* 1996).

Banana production is limited by a number of factors including biotic stresses such as banana nematodes and weevils (Gold *et al*., 2004; Ocan *et al*., 2008), black

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Sigatoka (Barekye, *et al*., 2009), banana bacterial wilt, Fusarium wilt (Biruma *et al*., 2007) and abiotic stresses such as low soil fertility and drought (Lescot and Ganry, 2008; Wachira *et al*., 2013). Banana weevil (*Cosmopolites sordidus* Germar) is an important pest of banana, plantain and ensete. Most triploid (2*n* = 3*x* = 33 chromosomes) East African highland bananas (EAHB) are highly susceptible to this insect pest (Gold and Messiaen, 2000; Kiggundu *et al*., 2007) and it can cause up to 50% yield loss after the 3rd ratoon cycle in new plantations (Gold *et al*., 2004; Ocan *et al*., 2008). The loss due to banana weevil damage can go up to 100% in severe infestations (Sengooba, 1986).

Attempts to control the weevil using cultural, chemical and biological methods have not been effective because they are labour intensive, expensive and environmentally unfriendly (Gold *et al*., 1993). Breeding for resistant cultivars is a more sustainable solution to the banana weevil problem (INIBAP, 1998). Sources of resistance to banana weevil have been reported among wild diploid (2*n* = 2*x* = 22) bananas (Kiggundu, 2000). The host plant resistance mechanisms reported include antibiosis, antixenoxis and corm hardness (Gertrude, 2010). These mechanisms affect the banana weevil directly by deterring the weevil, and or making it hard for the weevil to feed or indirectly by retarding the growth of the weevil larvae (Ortiz et al., 1995; Getrude, 2010). Other banana weevil resistance mechanisms reported include: increased larval mortality (Abera, 2000), increased larval development time (Mesquita *et al*., 1984; Gertrude, 2010), and damage parameters such as cross sectional inner and outer damages (Kiggundu, 2000).

To exploit the potential of wild diploids in improvement of EAHB, the National Agricultural Research Organization (NARO) and the International Institute of Tropical Agriculture (IITA) adopted a strategy whereby female fertile East African highland bananas (triploids) are crossed with the wild diploid banana to generate hybrid tetraploids and diploids. The tetraploid banana hybrids that are generated are then crossed with the secondary (improved) diploids to generate secondary triploid banana cultivars with superior hybrids in terms of bunch yield, resistance to pests and diseases, and fruit quality traits as stated by Tushemereirwe *et al*. (2014) in the NARITA report. This strategy utilizes the diploids to introgress resistance into the edible EAHB while retaining the farmer-preferred traits in EAHB. Inter-diploid crosses are carried out to improve the diploids before crossing with tetraploids to reduce the undesirable traits from the wild diploids.

The genetics of host plant resistance to banana weevil, as measured by its heritability and defined by its gene action, as well as linkage to other characters within the secondary banana diploids is yet to be determined. This information is important for further improvement of bananas for weevil resistance. An F_2 population offers adequate segregation in banana where resistance mechanisms can be easily investigated (Brown and Caligari, 2008; Muhammad *et al.,* 2014). Little research has been, however undertaken to screen F_2 banana diploid offspring to determine their levels of host plant resistance to banana weevil. The purpose of this study was therefore to identify segregating weevil resistance traits and determine the inheritance of weevil resistance in an F_2 diploid population derived from crossing *Musa acuminata* subsp. *banksii* acc. Kasaska (ITC0591) and *M. acuminata* subsp. *microcarpa* acc. Borneo (ITC0253).

MATERIALS AND METHOD

Generation of segregating population: The diploids Kasaska (ITC. 0591) and Borneo (ITC. 0253) were crossed to generate an F_1 population. Kasaska is susceptible to weevil damage and was used as female parent, while *Borneo* is resistant to weevil damage and was used as the male. The F_1 generation was moderately resistant to weevil. One random F_1 plant was selected and selfed to generate an F_2 segregating population for this study.

Field experiment

Planting field: Two hundred forty two F_2 plants were planted without replication together with five replications of the susceptible parent, five replications of the resistant parent, and five replications of the F_1 genotype in a weevil free field at the National Agricultural Research Laboratories (NARL) in Kawanda. NARL - Kawanda is located 13 km North of Kampala at 0°25°N, 32°32°E and 1,195 m above sea level.

Holes of size 45 × 45 × 45 cm were prepared at a spacing of 2 × 2 m. The holes were filled half way with equal quantities of top soil and well decomposed farm yard manure before planting, which was done on 14th September 2012. The individual plants were randomly planted in 25 columns and 11 rows. Hand weeding and mulching were used to manage weeds. Mulch was applied two months after planting and again after 1 year. The

plants were allowed to grow and produce as many suckers as possible without desuckering. Fourteen F_2 plants failed to grow, thus only 228 plants were assessed.

Raising weevils and hatching weevil eggs: The inoculation of the plants with weevil was done artificially in the laboratory. The weevils were raised on corms of the susceptible EAHB cultivar Mbwazirume that were changed after 3 days. The weevils were fed on fresh peeled pseudostem of Mbwazirume where eggs were laid. The eggs were sterilised in 25 ml of distilled water containing 5 ml of ethanol (20%) and 2 or 3 drops of sodium hypochlorite in a petri dish. The eggs were then spread on a moistened kitchen towel tissue in the petri dish using painter's brush. The eggs were stored at room temperature on the petri dishes and wetted daily to avoid desiccation. After 6 or 7 days the larvae heads emerged from the eggs and were reddish in colour at first instar. On the fifth day from the time the eggs were inoculated, one sucker was detached from each of the 228 F_2 plants, their parents and F_1 plants using a de-suckering spear and the corms dug out of the soil. The corms were cleaned and weighed to determine the weight of the whole corm. The suckers that were detached from each plant were estimated to have an average number of 7 leaves, average diameter at base of 29 cm and an average height of 137 cm.

Determination of dry matter and dry mass: A cube of about 3 × 3 × 3 cm was obtained from the inner ring of the corm and kept overnight. From the remainder of the corm, 150g were weighed from a representative sample, chopped into small pieces and used to determine the moisture content in the laboratory. The samples were placed in an oven at 80 ^{o}C until they were completely dry (feeling crunchy when felt with hands). The samples were then removed and weighed again as subsamples or whole samples. The dry matter content of subsample, dry mass of the whole sample and moisture content of the subsample were determined by the below formulae (Timothy *et al*., 2005; Mickan, 2005);

$$\text{Dry mass} = \frac{\text{Subsample dry mass} \times \text{Fresh mass of the whole sample}}{\text{Subsample fresh mass}}$$

$$\%\ \text{Dry matter content of subsample} = \frac{\text{Final Dry Weight (g)}}{Initial\ Wet\ Weight\ (g)} \times 100$$

$$\%\ \text{Total Moisture} = 100 - \%\ \text{Total DM}$$

Inoculation of fresh corms with weevil larvae: The next day when the eggs had hatched, the larvae were inoculated into the corm section. The corm section was bored with four holes on one side. A larva was placed in each hole, covered with corm tissue and placed in a petri dish. The petri dish was covered, sealed well with cling film and labelled. The petri dishes were then placed in plastic boxes and kept at room temperature for eight days after which the larvae were removed for measurements. This procedure was repeated three times every after thirty days and these were taken as replications over time. After eight days, the larvae were retrieved from the corm section and head capsule width, body length, body weight and larval mortality were recorded.

Determination of corm hardness: At harvest, a corm was taken to the laboratory and corm hardness determined using digital gauge penetrometer (General FHT803 Fruit Hardness Tester for Large, Hard fruits) from Tequipment NET (USA), as described by Ortiz *et al*. (1995); Kiggundu, (2000). The machine measured corm hardness in Newtons (N = 105 dynes). Four transversal and four longitudinal random measurements were taken on each corm fragment by punching the machine in corm using tip size of 7.9 mm. However, this tip had a blunt end and could not penetrate the corm so another tip was fabricated that had a sharper end and a mark was made at 12 mm from where the hardness would be read when reached.

Pot experiment: Field screening for weevil resistance generally takes several years, and are labour-intensive and require large space as each banana plant occupies 4 to 9 m2 depending on the planting density. For breeding purposes, a quick, reliable and effective screening method for resistance to the banana weevil facilitates selection and/or development of resistant banana genotypes (Sadik *et al*., 2010).

The two hundred forty two F_2 plants, their parents and F_1's that were planted and allowed to grow for seven months after which two hundred plants were selected for re-initiation in the tissue culture lab. A sucker from each clone was detached and initiated on proliferation medium, multiplied to obtain 4 to 8 copies, and then rooted on rooting medium in vitro. One hundred fifty genotypes made it successfully through tissue culture with 4 or more clones and were weaned in the nursery. The plantlets were left in the nursery under humid

chamber for 3 weeks, after which they were left to harden under shade in the nursery for 4 weeks and these were transferred to big pots (black polythene bags of 101.6 × 127 cm) for pot experiment. The potting mix for the pot experiment was prepared by mixing black soil and farmyard manure in ratio of 4:1, sterilised and left to cool before use.

The pot experiment was laid out in a randomised complete block design (RCBD) with 2 replications and each replicate consisted of 2 to 4 plants. The plants in the potting bags were left to grow for 3 months to attain a reasonable size before inoculation. The pots were dressed with net bags tightened on the pseudostem at the point where it touches the soil to avoid escape or entrance of foreign weevil and to make sure weevils do not eat the pseudostem. Thereafter, 6 weevils in male to female ratio of 1:1 were introduced. The number of weevils was adjusted from 5 males and 5 females (Sadik *et al*., 2010) because in this experiment tissue culture plants with small corms were used. The inoculated plants were left to grow for 90 days, the corms were uprooted and damage parameters scored for each line.

Data collection

Data collected is presented in table 1.

Table 1. Data collected from different experiments at different growth stages.

Parameter	Experiment	Unit	Stage of growth
Head capsule width	Field(Artificial innoculation)	60×	18 months
Body length	Field(Artificial innoculation)	40×	18 months
Body weight	Field(Artificial innoculation)	mg	18 months
Larval mortality	Field(Artificial innoculation)	%	18 months
Total damage	Pot	%	6 months from planting
Peripheral damage	Pot	%	6 months from planting
Total cross sectional damage	Pot	%	6 months from planting
Total cross sectional inner damage	Pot	%	6 months from planting
Total cross sectional outer damage	Pot	%	6 months from planting
Number of larvae retrieved	Pot		6 months from planting
Number of Dead weevils	Pot		6 months from planting
Girth at 1 m	Field	cm	At flowering
Height of a plant	Field	cm	At flowering
Inner corm hardness	Field	cm	At harvest
Outer corm hardness	Field	cm	At harvest
Total corm hardness	Field	cm	At harvest

Data analysis: The field and pot experiment data were analysed by GenStat 14th edition (ANOVA) using the following linear model Response = General mean + Genotype + Replication + error?

Broad sense heritability (H) was determined using the variance component formula:

Heritability = Genetic variance/(Genetic variance + Error variance)

The mode of inheritance was investigated using frequency histograms for the traits recorded in the study, and Chi-square test of goodness of fit was used to define putative gene segregation.

RESULTS

Field response in an F_2 banana diploid population for banana weevil and agronomic traits: There were significant differences ($P < 0.05$) among the different genotypes for inner corm hardness and total corm hardness. However, there was no significant difference ($P < 0.05$) for agronomic traits such as girth at 1 m, height of plant at flowering and outer corm hardness leaves at flowering (Table 2).

Response of F_2 banana diploid genotypes, their parental lines and F_1 to weevil growth parameters in the laboratory: There were significant differences ($P < 0.001$) among the parental lines and $F_1$for head capsule width (HCW), body length and body weight. Larval mortality was significant at ($P > 0.05$) (Table 3a). There were significant differences ($P < 0.05$) among the different genotypes for head capsule width (HCW), body length, body weight and larval mortality. Dry matter content and dry mass were not significant ($P > 0.05$) (Table 3b).

Table 2. Mean squares of agronomic traits in an F_2 banana diploid population.

Source of variance	Degrees of freedom [z]	Girth at 1 m	Height of a plant	Inner corm hard-ness	Outer corm hard-ness	Total corm hard-ness
Replication	1	5528.9	20543	76.9	523.2	250.3
Genotype	187-191	17.7 [NS]	692.8 [NS]	3.4**	9.5[NS]	4.1**
Error	41-199	18.3	690.3	1.2	8.9	2.6
Total	230-391	38. 9	1476.7	2.5	10.5	4.0
CV (%) [y]		12.62	10.47	18.3	38.4	23.5

[z] = Given as range because degrees of freedom vary according to number of genotypes and plants within genotypes for each trait.

[y] = Coefficient of variation.

[NS], * and ** indicate non-significant ($P > 0.05$), significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$).

Table 3a. Mean squares of weevil growth parameters in the parental lines and F_1 of banana diploid population.

Source of Variation	Degrees of freedom	HCW	Body Length	Body Weight	Larval Mortality
Replication	4	30.6	51.5	1.4	770.8
Genotype	2	371.6**	567.7**	44.5**	2000.0*
Error	8	24.2	57.2	0.9	333.3
Total	14	75.7	128.5	7.2	696.4
CV (%)[y]		6.9	4.5	7.4	40.1

Table 3b. Mean squares of weevil growth parameters in an F_2 banana diploid population.

Source of variance	Degrees of freedom [z]	HCW	Body length	Body weight	Larval mortality	Dry matter	Dry mass
Replication	2	1693.6	58409.8	200.3	20991	3368.9	1375891
Genotype	227	609.7**	564.8**	65.2**	1056.5*	84.0 [NS]	64738 [NS]
Error	378-408	360.7	400.4	26.1	840.4	98.1	65361
Total	607-638	457.9	653	41.3	980.8	103.8	69585
CV (%) [y]		34.9	33.9	57	50.2	42.9	36.7

[z] = Given as range because degrees of freedom vary according to number of genotypes and plants within genotypes for each trait.

[y] = Coefficient of variation.

[NS], * and ** indicate non-significant ($P > 0.05$), significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$).

Response of F_2 diploid banana genotypes, their parental lines and F_1 to weevil damage parameters under glasshouse screening: There were significant differences ($P < 0.05$) among the parental lines and F_1different genotypes for total damage, peripheral damage, dead weevils and larvae retrieved. Weevil damage parameters such as total cross sectional inner and outer damages and total cross sectional damage were non-significant ($P < 0.05$) (Table 4a).

Table 4a. Mean squares of weevil damage parameters in the parental lines of banana diploid population.

Source of Variation	Degrees of freedom	Peripheral damage	Total damage	cross sectional damage	cross sectional inner damage	cross sectional outer damage	Dead Weevils	Larvae retrieved
Replication	1	118.8	270.6	484.0	325.8	673.4	0.4	0.1
Genotype	2	1786.7*	1521.9*	1288.6[NS]	2041.1[NS]	716.7[NS]	1.6*	3.8*
Error	2	54.1	130.7	279.5	157.8	442.2	0.1	0.1
Total	5	760	715.2	724.1	854.7	598.2	0.7	1.6
CV(%)[y]		21.5	37.1	58.2	45.9	71.3	3.8	18.2

There were significant differences ($P < 0.05$) among the different genotypes for total damage, peripheral damage, dead weevils and larvae retrieved. These parameters showed that the screened population was segregating for weevil resistance. Weevil damage parameters such as total cross sectional inner and outer damages and total cross sectional damage were non-significant in this population but these parameters add up to total damage which was significant (Table 4b).

Table 4b. Mean squares of weevil damage parameters in an F_2 banana diploid population.

Source of Variation	Degrees of freedom	Peripheral damage	Total damage	cross sectional damage	cross sectional inner damage	cross sectional outer damage	Dead Weevils	Larvae retrieved
Replication	1	2060.7	7120	36.4	48	26.5	87.7	0.3
Genotype	118	141.9**	421.2**	48.5^{NS}	46.7^{NS}	55.6^{NS}	2.4**	0.4*
Error	606	87.1	216	50.8	48.8	59.3	1.6	0.3
Total	725	98.7	259	50.4	48.4	58.6	1.9	0.3
CV(%)^{y}		40	47	29.1	34.9	24.9	10.5	19.4

^{y} Coefficient of variation

^{NS}, * and ** indicate non-significant ($P > 0.05$), significant ($P \leq 0.05$) or highly significant ($P \leq 0.01$)

Nature of inheritance for weevil resistance traits among an F_2 diploid banana population: Histograms for head capsule width (Figure 1) and girth of a plant at 1 m (Figure 3) are not skewed, while those for body weight (Figure 1), total damage, peripheral damage, cross sectional inner and outer damage and larvae retrieved (Figure 2), were skewed towards the resistant parent. The histogram for plant height at flowering also had a normal distribution (Figure 3). Histograms for body length (Figure 1), and dead weevils (Figure 2) were skewed towards the susceptible parent, whereas histogram for larval mortality (Figure 1) showed a binomial distribution. In general histograms for weevil growth traits showed more continuous distribution as opposed to weevil damage trait histograms.

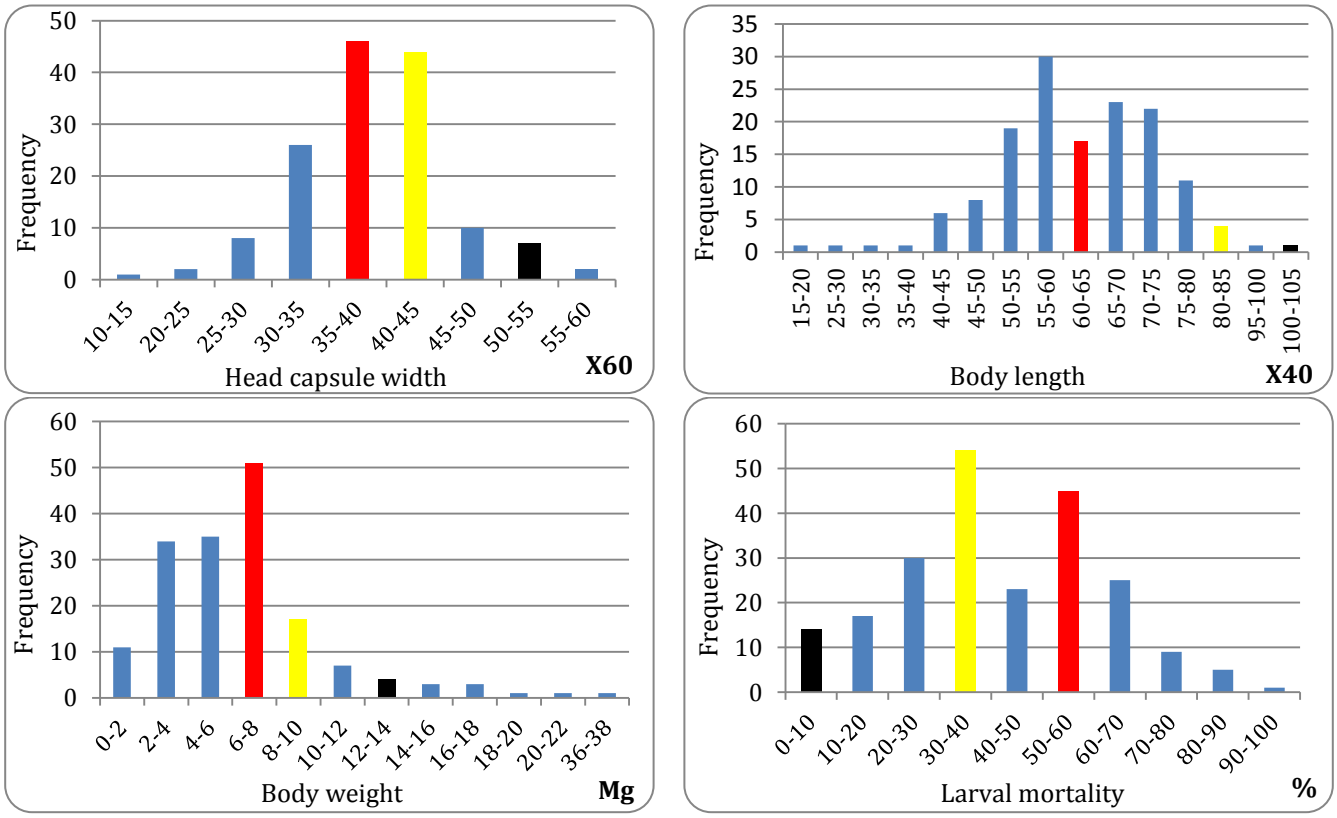

Figure 1. Frequency of larval traits expressed among an F2 banana diploid population.

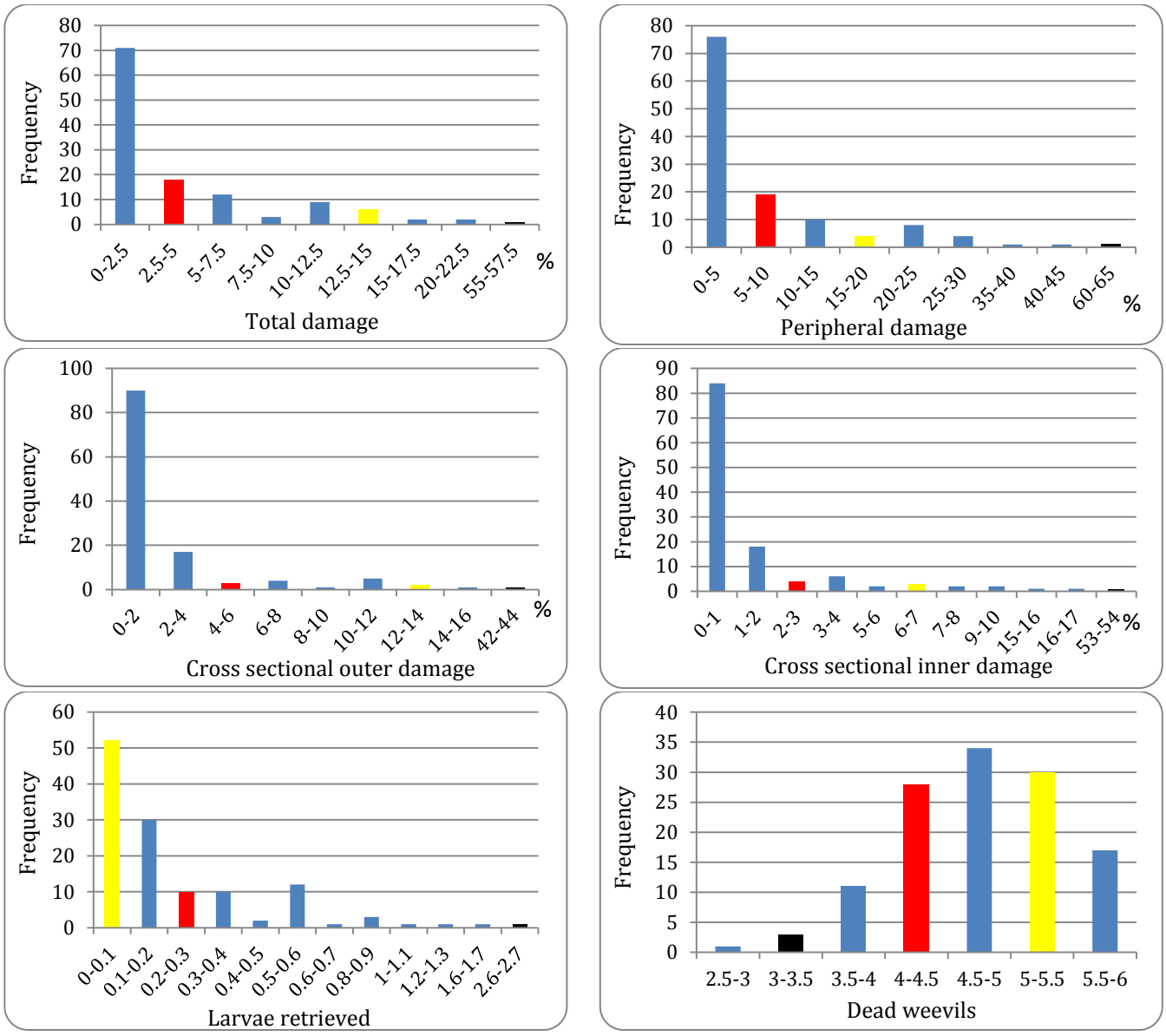

Figure 2. Frequency of weevil damage parameters among an F2 banana diploid population

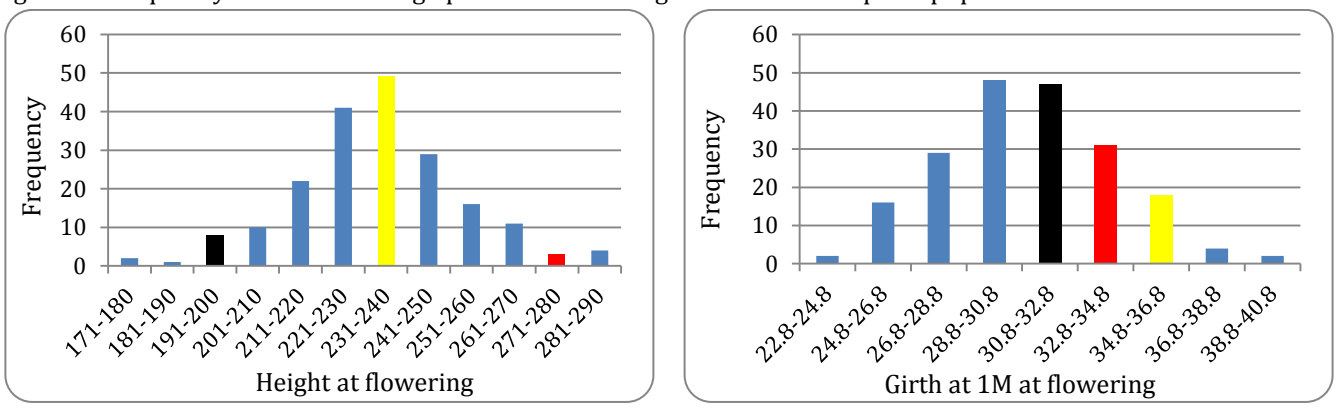

Figure 3. Frequency of agronomy parameters expressed among an F2 banana diploid population

Key for Figure 3

Parent 1/ susceptible parent

Parent 2/ resistant parent

F1/ off spring

Genetic ratios among an F_2 diploid banana population: Chi-square for body length showed a ratio of 15:1 when performed using the resistant parent as a check whereas larval mortality and body weight showed ratios of 15:1 when using the susceptible parent as a check, and dead weevils showed a ratio of 1:15 when using the susceptible parent as a check. Chi-square for total damage and larvae retrieved showed 3:1 ratios when using the resistant parent as a check, whereas larval mortality and dead weevils showed 1:3 ratios when using the resistant parent as check. Chi-square for peripheral damage showed a 9:7 ratio when using a resistant parent as a check, while body weight showed a 7:9 ratio using the resistant parent as a check (Table 5).

Table 5. Genetic ratios involved in selected weevil growth traits and weevil damage parameters in an F2 banana diploid popula tion.

Trait	Resistant/ Susceptible parent	Type of check used	Observed number of genotypes	Total	Ratio tested	Expected forward (e.g. 1:3)	Expected reverse (e.g. 3:1)	Calculated Chi- square	Chi-square distribution (*P*)	Significance level
Body length	Resistant parent	Resistant	135	146	1:3	36.5	109.5	354.4	4.6E-79	***
		Susceptible	11		3:1	109.5	36.5	23.8	1.1E-06	***
					9:7	82.125	63.875	77.8	1.1E-18	***
					7:9	63.875	82.125	140.8	1.8E-32	***
					15:1	136.875	9.125	0.4	0.6	ns
					1:15	9.125	136.875	1852.1	0	
	Susceptible parent	Resistant	145	146	1:3	36.5	109.5	430.0	1.6E-95	***
		Susceptible	1		3:1	109.5	36.5	46.0	1.2E-11	***
					9:7	82.125	63.875	110.0	9.7E-26	***
					7:9	63.875	82.125	183.2	9.9E-42	***
					15:1	136.875	9.125	7.7	0.01	*
					1:15	9.125	136.875	2158.1	0	
	Resistant parent	Resistant	76	171	1:3	42.75	128.25	34.5	4.3E-09	***
		Susceptible	95		3:1	128.25	42.75	85.1	2.8E-20	***
					9:7	96.1875	74.8125	9.7	0.1E-03	***
					7:9	74.8125	96.1875	0.0	0.8	ns
					15:1	160.3125	10.6875	709.5	2.6E-156	***
					1:15	10.6875	160.3125	425.7	1.4E-94	***
	Susceptible parent	Resistant	158	171	1:3	42.75	128.25	414.3	4.3E-92	***
		Susceptible	13		3:1	128.25	42.75	27.6	1.4E-07	***
					9:7	96.1875	74.8125	90.8	1.6E-21	***
					7:9	74.8125	96.1875	164.4	1.2E-37	***
					15:1	160.3125	10.6875	0.5	0.5	ns
					1:15	10.6875	160.3125	2165.9	0	

Larval mortality	Resistant parent	Resistant	62	226	1:3	56.5	169.5	0.7	0.4	ns
		Susceptible	164		3:1	169.5	56.5	272.7	2.9E-61	***
					9:7	127.125	98.875	76.3	2.5E-18	***
					7:9	98.875	127.125	24.4	7.6E-07	***
					15:1	211.875	14.125	1696.3	0	
					1:15	14.125	211.875	173.1	1.6E-39	***
	Susceptible parent	Resistant	211	226	1:3	56.5	169.5	563.3	1.6E-124	***
		Susceptible	15		3:1	169.5	56.5	40.6	1.8E-10	***
					9:7	127.125	98.875	126.5	2.4E-29	***
					7:9	98.875	127.125	226.0	4.3E-51	***
					15:1	211.875	14.125	0.1	0.8	ns
					1:15	14.125	211.875	2927.0	0	
Total damage	Resistant parent	Resistant	95	124	1:3	31	93	176.2	3.3E-40	***
		Susceptible	29		3:1	93	31	0.2	0.7	ns
					9:7	69.75	54.25	20.9	4.9E-06	***
					7:9	54.25	69.75	54.4	1.6E-13	***
					15:1	116.25	7.75	62.2	3.2E-15	***
					1:15	7.75	116.25	1047.8	7.5E-230	***
	Susceptible parent	Resistant	123	124	1:3	31	93	364.0	3.7E-81	***
		Susceptible	1		3:1	93	31	38.7	4.9E-10	***
					9:7	69.75	54.25	92.9	5.4E-22	***
					7:9	54.25	69.75	154.9	1.5E-35	***
					15:1	116.25	7.75	6.3	0.01	*
					1:15	7.75	116.25	1828.1	0	
Periheral damage	Resistant parent	Resistant	81	124	1:3	31	93	107.5	3.4E-25	***
		Susceptible	43		3:1	93	31	6.2	0.01	*
					9:7	69.75	54.25	4.1	0.06	ns
					7:9	54.25	69.75	23.4	1.3E-06	***
					15:1	116.25	7.75	171.0	4.4E-39	***
					1:15	7.75	116.25	738.5	1.2E-162	***
	Susceptible parent	Resistant	123	124	1:3	31	93	364.0	3.7E-81	***
		Susceptible	1		3:1	93	31	38.7	4.9E-10	***
					9:7	69.75	54.25	92.9	5.4E-22	***
					7:9	54.25	69.75	154.9	1.5E-35	***

					15:1	116.25	7.75	6.3	0.1E-01	*
					1:15	7.75	116.25	1828.1	0	
Larvae retrieved	Resistant parent	Resistant	102	124	1:3	31	93	216.8	4.5E-49	***
		Susceptible	22		3:1	93	31	3.5	0.06	ns
					9:7	69.75	54.25	34.1	5.3E-09	***
					7:9	54.25	69.75	74.7	5.4E-18	***
					15:1	116.25	7.75	27.9	1.2E-07	***
					1:15	7.75	116.25	1222.6	7.4E-268	***
	Susceptible parent	Resistant	123	124	1:3	31	93	364.0	3.7E-81	***
		Susceptible	1		3:1	93	31	38.7	4.9E-10	***
					9:7	69.75	54.25	92.9	5.4E-22	***
					7:9	54.25	69.75	154.9	1.5E-35	***
					15:1	116.25	7.75	6.3	0.1E-01	*
					1:15	7.75	116.25	1828.1	0	
Dead weevils	Resistant parent	Resistant	31	124	1:03	31	93	0.0	1	ns
		Susceptible	93		3:1	93	31	165.3	7.7E-38	***
					9:7	69.75	54.25	49.2	2.3E-12	***
					7:9	54.25	69.75	17.7	2.6E-05	***
					15:1	116.25	7.75	1000.3	1.6E-219	***
					1:15	7.75	116.25	74.4	6.4E-18	***
	Susceptible parent	Resistant	8	124	1:3	31	93	22.8	1.8E-06	***
		Susceptible	116		3:1	93	31	310.8	1.5E-69	***
					9:7	69.75	54.25	125.0	5.2E-29	***
					7:9	54.25	69.75	70.1	5.7E-17	***
					15:1	116.25	7.75	1612.8	0	
					1:15	7.75	116.25	0.0	0.9	ns

Broad-sense heritability: The heritability for inner corm hardness was 48% (Table 6); i.e., intermediate. Body weight had a heritability of 33.4% and higher than other weevil growth traits such as head capsule width, body length, and larval mortality (12%, 10% and 7.9 %, respectively; Table 7). For pot experiment, peripheral damage and total damage in pot experiments had broad-sense heritability of 32% and 24%, respectively. Other traits assessed in pot experiments like dead weevils, larvae retrieved, total cross sectional damage and total inner and outer damages had low heritability; i.e., below 21% (Table 7).

Table 6. Growth trait heritability (H) in an F_2 diploid banana population.

Trait	H (%)
Inner corm hardness	48.0
Total corm hardness	22.5
Outer corm hardness	3.4
Girth at 1 m	1.6
Plant height	0.2

Table 7. Heritability (H) of weevil growth and weevil damage traits among an F2 diploid banana population.

Trait	H (%)
Body weight	33.4
Body length	12.0
Head capsule width	10.0
Larval mortality	7.9
Larvae retrieved	14.0
Dead weevils	20.0
Peripheral damage	32.0
Total damage	24.0
Total cross sectional damage	2.3
Total cross sectional outer damage	3.2
Total cross sectional inner damage	2.2

Phenotypic correlations in an F_2 banana diploid population: Plant height at flowering was positively correlated with girth of the plant at flowering ($P < 0.001$;). The number of larvae retrieved was positively correlated ($P < 0.001$) with peripheral damage, total damage, total cross sectional damage, total cross sectional inner and outer damages. All the weevil damage traits were also directly and positively correlated with each other, peripheral damage was positively correlated ($P < 0.05$) with total damage, total cross sectional damage and outer damages. Furthermore, total damage was positively correlated ($P < 0.001$) with total cross sectional damage, total cross sectional inner and outer damages. Total cross sectional damage was positively correlated ($P < 0.001$) with total cross sectional inner and outer damages. Moreover, total cross sectional inner damage was positively correlated ($P < 0.001$) with total cross sectional outer damage.

Table 8. Correlations of weevil damage parameters and corm hardness in an F_2 diploid banana population.

Trait		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Body length	1	-																	
Body weight	2	0.7**	-																
Dry matter	3	0.1	-0.1	-															
Girth	4	-0.1	-0.1	-0.1	-														
HCW	5	0.9**	0.8**	0.1	-0.1	-													
Plant height	6	-0.1	-0.1	0.1	0.4**	-0.1	-												
ID	7	-0.1	0.1	-0.1	0.1	0.1	-0.1	-											
ICH	8	0.1	-0.1	0.1	-0.1	0.1	-0.1	0.3**	-										
LR	9	-0.1	0.1	0.1	-0.1	-0.1	-0.1	0.1	0.1	-									
LM	10	-0.4**	-0.3**	-0.1	0.1	-0.4**	0.1	-0.2	0.1	-0.1	-								
LW	11	-0.1	-0.1	-0.1	0.1	-0.1	0.1	0.1**	0.1	-0.1	0.2*	-							
OD	12	-0.1	-0.1	-0.1	0.1	0.1	0.1	0.8	-0.2	-0.1	-0.1	0.1	-						
OCH	13	0.1	0.1	-0.1	0.1	0.1	-0.1	0.1	0.1*	-0.1	-0.1	0.1	-0.1	-					
PD	14	0.1	0.1	-0.1	-0.1	0.1	-0.1	0.1	0.1	0.7**	-0.1	-0.1	-0.1	0.1	-				
TD	15	0.1	0.1	-0.1	-0.1	0.1	-0.1	-0.1	0.1	0.7**	-0.1	-0.1	-0.1	0.1	0.9**	-			
TXD	16	-0.1	0.1	-0.1	-0.1	-0.1	-0.1	-0.3	0.1	0.3**	0.1	0.1	-0.3**	0.1	0.3**	0.5**	-		
TXI	17	-0.1	0.1	-0.1	-0.1	-0.1	-0.1	-0.3	0.1	0.2*	0.1	0.1	-0.3**	0.1	0.2	0.4**	0.9**	-	
TXO	18	-0.1	0.1	-0.2	-0.1	-0.1	-0.2	-0.2	0.1	0.3**	0.1	-0.1	_0.2*	0.1	0.4*	0.6**	0.9**	0.8**	-
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

HCW = head capsule width, ID = inner damage, ICH = inner corm hardiness, LM = larval mortality, LR = larvae retrieved, LW = live weevil , OD = outer damage, OCH = outer corm hardiness, PD = peripheral damage, TD = total damage, TXD = total cross sectional damage , TXI = total cross sectional inner damage, TXO = total cross sectional outer damage

DISCUSSION

Segregation of weevil resistance and agronomic traits in an F_2 diploid banana population: Morphological traits like corm hardness, corm size, physiological or other traits like chemical compounds of the host plant may affect the insect population and growth by negatively affecting its biology such as larval growth in weight and length or reducing the severity of attack (Smith, 1989) quoted in Ortiz *et al*. (1995). This study found significant differences in inner and total corm hardness showing that these parameters could be used to characterise the F_2 diploid banana population. Ortiz *et al*. (1995) reported significant differences in corm hardness, both inner and outer corm hardness among euploid hybrids whereby resistant cultivars had increased corm hardness.

Host plant resistance in the form of antibiosis can be expressed as increased mortality, delayed development, reduced body size and reduced fecundity of the weevils and weevil larvae (Gertrude, 2010). When the weevil growth traits were used to screen the F_2 diploid population, head capsule width, body length, body weight and larval mortality were significant among the genotypes implying that the population was segregating for them. The above traits therefore can be used to screen populations for weevil resistance. This method is an indirect way of measuring host plant resistance because it depends on the ability or inability of the larvae to feed on a corm tissue. The strategy is that larvae that feed on a susceptible corm tissue will feed a lot and consequently will grow faster as measured by body weight, body length and head capsule width while the larvae that feed on resistant corm tissue have reduced body size, weight and consequently will die faster. So we expect more larval mortality in resistant corm tissue.

Weevil damage traits such as total damage, peripheral damage and traits like dead weevils and larvae retrieved from the pot experiment were significantly different among the genotypes indicating that the population was segregating for them, and could be used to screen populations for weevil resistance within a short period in a glasshouse (Sadik *et al*., 2010) other than waiting for long duration field trials.

Peripheral damage, the damage on the outer part of the corm and total damage, the overall damage on a corm arrived at after summing all damage indices were significant and could be used for weevil assessment in diploid bananas. The results indicated that most of the damage on diploid corms was not big inside the corm, thus implying that the larvae attempted to eat the corms but never penetrated deep. The inner and outer cross sectional damages in the segregating population were non-significant indicating that they are not good traits for assessing weevil damage but they are necessary since they contribute to the total damage, which was significant. Dead weevil and larvae retrieved were significant, thereby implying that they are indicators of weevil resistance mechanisms that lead to weevil mortality or reduction in number of larvae retrieved.

Nature of inheritance for weevil resistance and agronomic traits in an F_2 diploid banana population: Histograms for distribution of weevil damage, weevil growth and agronomic traits in an F_2 banana population for head capsule width, girth of a plant at 1 m plant at flowering and plant height at flowering showed normal distribution but their goodness of fit was that of a modification from the 9:3:3:1 Mendelian ratio. Histograms for body weight, total damage, peripheral damage, cross sectional inner and outer damage and larvae retrieved were skewed to the resistant parent. Most of the F_2 offspring were on the side of the resistant parent with a few on the side of the susceptible parent. Ortiz *et al*. (1995) reported diploid hybrids to be more resistant compared to polyploids in a euploid population derived from crossing the wild diploid banana Calcuta 4 and West African French plantains.

Histograms for body length and dead weevils were skewed towards the susceptible parent, thus indicating that, most of the offspring were on the side of susceptible parent, which indicates that the offspring response was in an opposite to other traits. All the above histograms suggest that two or more genes may be involved in trait segregation except for larval mortality which had a binomial distribution. The histograms for weevil growth as measured by body weight, body length and head capsule width showed continuous distribution vis-á-vis weevil damage traits. This result could have resulted from inoculating larvae in the corm pieces, which obliges the larvae to feed on the corm for survival, whereas the in the pot experiment the weevils had freedom to avoid feeding on resistant offspring and could move around in the soil.

Segregation ratios for weevil resistance traits among F_2 diploid banana population: The segregation ratios for weevil resistance show that body length and larval

mortality (when tested using resistant parent as a check), and number of dead weevils and body weight (when using a susceptible parent as check) suggest duplicate dominant epistasis, whose ratio is 15:1; whereas segregation for peripheral damage and body weight (when tested using resistant parent as a check) fit the 9:7 ratio of duplicate recessive epistasis. Epistasis causes such phenotypic ratios, which deviate from Mendelian segregation, because an allele at one locus masks the effect of an allele in another locus. The ratios for total damage and larvae retrieved (when tested using resistant parent as a check), larval mortality and dead weevils (when using a susceptible parent as check) suggest that these traits might be controlled by a single gene because they fit the expected 3:1 Mendelian ratio.

Heritability for weevil resistance and agronomic traits in an F_2 diploid banana population: Kiggundu (2000) estimated heritability in triploid bananas for various weevil resistance traits. He found that total inner damage had the highest heritability (87%). This trait recording is, however, best done in a destructive experiment and often at harvest, which takes a long time. Furthermore, his research estimated heritability of other weevil resistance traits such as upper inner cross section damage (35%), lower inner cross section damage (34%), upper outer cross section damage (29%) and lower outer cross section damage (29%). Due to its intermediate heritability (48%), as estimated in our research, inner corm hardness can be used to select offspring with corm, body weight had the highest heritability (33.4%) among weevil growth traits, while peripheral damage and total damage had the highest heritability (32.4% and 24%, respectively) among weevil damage traits, thus suggesting that they can be also used for selection of weevil resistant offspring in diploid banana breeding populations.

Phenotypic correlations for weevil resistance and agronomic traits in an F_2 diploid banana population: The more larvae found in the corm, the more severe the damage on the corm. When weevils lay eggs at the peripheral of the corm and in the pseudostem sheaths and when they hatch, the larvae burrow into the corm making tunnels while feeding causing damage to the corm. The weevil damage parameters were directly and positively correlated with each other, thus suggesting that any of these damage traits may suffice to assess weevil resistance in diploid banana offspring. Weevil growth traits were also positively correlated with each other, which indicate that any of them can be used to determine weevil growth. There were low and non-significant correlations between corm hardness and weevil damage implying that corm hardness does not affect weevil damage.

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REFERENCES

Abera , A.K., Gold, C.S., Kyamanya , S. and Karamura , E.B., 2000. Banana weevil (*Cosmopolites sordidus* Germar) ovipositional preference, timing of attack and larval survival, survivorship in a mixed cultivar trial in Uganda. Acta Horticulturae, 540:487-496.

Abera, A., 1997. Oviposition prefrences and timing of attack by Banana weevil *(Cosmopolites Sordidus Germar)* in East African Highland bananas*(Musa spp).* Msc.Thesis Makerere University- Kampala.

Bagamba, F., 1994. Resource allocation efficiency in a banana based cropping system in Uganda. M.Sc. Thesis Makerere University, Kampala. 116.

Barekye, A., Tongoona, P., Derera, J. and Tushemereirwe, W.K., 2009. Variation in diploid (AA) and tetraploid (AAAA) banana populations for black Sigatoka resistance and agronomic traits. African Crop Science Journal, 9: 509-16.

Biruma , M., Pillay, M., Tripathi , L. and Blomme, G., 2007. Banana Xanthomonas wilt: A Review of the Disease, Management Strategies And Future Research Directions. African Journal of Biotechnology, 6: 953-62.

Brown, J. and Caligari, P., 2008. An introduction to plant breeding. Wiley-Blackwell Publishers.

Embrechts, J., Bananuka , J.A., Couveur, Y. and Bagamba, F., 1996. Banana sub-sector review, Republic of Uganda. (STABEX), Service Contract No. SCl26196.

Edward, E. and Fredy, B., 2013. Banana Market. Florida: University of IFAS extension.

FAO, 2011. Examination of technical feasibility of analyzing and monitoring the economic significance of banana varieties other than Cavendish. Yaoundé, Cameroon, 2011. Bioversity Intenational, CIRAD and IITA.

FAOSTAT, 2012. The Agricultural production. FAO.

Gertrude, N., Gold, S.C. and Alison, G., 2010. Survivorship and development rates of banana weevils reared

on excised plant material of different banana cultivars. International Journal of Tropical Insect Science , 30(2): 77-83.

Gold, C.S., Kagezi, G.H. and Night, G., 2004. The effects of banana weevil, Cosmopolites sordidus (Germar) damage on highland banana growth, yield and stand duration in Uganda. Applied Biology, 145: 263-69.

Gold, C.S., Latigo, M.O. and Tushemereirwe, W., 1993. Farmer perceptions of banana pest constraints in Uganda: In: Results from a rapid rural appraisal. Ibadan, Nigeria: Biological and Integrated Control of Highland Banana and Plantain.

Gold, C.S. and Messiaen, S., 2000. The banana weevil Cosmopolites sordidus. Montpellier, France: INIBAP.

INIBAP, 1998. Mobilising IPM for sustainable banana production in Africa. In Frison, A.E., Gold , C.S., Karamura, E.B. and Sikora, R.A., eds. Workshop on banana IPM. Nelspruit, South Africa, 1998.

Kiggundu, A., 2000. Host plant reactions and resistance mechanisms to banana weevil, *Cosmopolites sordidus (Germar)* in Ugandan Musa germplasm. University of the Orange Free Stae, Bloemfontain, South Africa: MSc.Thesis.

Kiggundu, A., Gold, C., Labuschagne, M.T. and Vuylsteke, D., 2007. Components of resistance to banana weevil (Cosmopolites sordidus) in Musa germplasm in Uganda. *Europe PubMed Central*, 122(1): 27-35

Lescot, T. and Ganry, J., 2008. Plantain cultivation in Africa: Production and research in the past two decades. Montpellier - France: CIRAD.

Mesquita, A.M., Alvers, E.J. and Caldas, R.C., 1984. Resistance of banana cultivars to Cosmopolites sordidus (GERMAR, 1824). Fruits, 39(4), 254-257.

Mickan, F., 2005. Dry Matter Content of Conserved Forages: Measurement of DM Content (reviewed December 2008). Melbourne, Victoria: Department of Environment and Primary Industries.

Mickan, F.E., 2005. Dry Matter Content of Conserved Forages: Measurement of DM Content. (Frank Mickan, Ellinbank 2005 and reviewed December 2008).

Muhammad , J.B., Naila, G., Wajid, A.J. and Raja, Z.B., 2014. Genetic Constitution of Multigenic Traits in F2 Populations of Intrahirsutum Crosses. Advances in Agricultural Sciences, 2(2): 35-47.

Ocan, D., Mukasa, H. and Rubaihayo, R.P., 2008. Effects of banana weevil damage on plant growth and yield of East African Musa genotypes. Applied Biosciences, 9(2): 407-415.

Ortiz, R., Vuylsteke, D., Dumpe, B. and Ferris, R.B., 1995. Banana weevil resistance and corm hardness in Musa germplasm. *Euphytica*, 86: 95-102

Pavis, C. and Minost, C., 1993. Banana resistance to the banana weevil borer Cosmopolites sordidus Germar (Coleoptera: Curculionidiae); role of pseudostem attractivity and physical properties of the rhizome. In J. Ganry, ed. Breeding banana and plantains for resistance to diseases and pests. Proceedings of international symposium on genetic improvement of banana for resistance to diseases and pests. Montpellier France: CIRAD-FLHOR. pp.129-142 CIRAD.

PIBID-Uganda, 2012. Banana Industrial Development-Presidential Initiative on Banana Industrial Development (PIBID). Kampala, Uganda: PIBID.

Pillay, M., Ssebuliba, R., Hartmann, J. and Vuylsteke, D., 2004. Conventional breeding strategies to enhance the sustainability of Musa biodiversity conservation for endemic cultivars. African Crop Science Journal, 12: 63-69.

Sadik, K., Moses, N. and Michael, P., 2010. A screening method for banana weevil (Cosmopolites sordidus Germar) resistance using reference genotypes. African Journal of Biotechnology, 9(30), 4725-30.

Sebastião, S.O., Manoel , S.T., Élio, A.J. and Marcelo, B.L., 2001. Banana breeding program at Embrapa. Crop Breeding and Applied Biotechnology, 1(4): 399-436.

Sengooba, T., 1986. Survey of banana pest problem complex in Rakai and Masaka Districts in Uganda. Namulonge, Uganda: Namulonge Research Station.

Smith, C.M., 1989. Plant resistance to insects. New York: John Wiley and Sons Inc.

Timothy, P., Walker, S. and Sandra, B., 2005. Sourcebook for Land Use, Land-Use Change and Forestry Projects.

Tushemereirwe, W., Batte , M., Barekye , A. and Swennen , R., 2014. Performance of narita banana hybrids in the preliminary yield trial, uganda. NARITA Report. Kampala: http://www.banana.go.ug/ National Agricultural Research Organization (NARO), International Institute of Tropical Agriculture (IITA) and Bill and Melinda Gates Foundation.

Vander Stichele, M. and van der Wal, S., 2005. Critical Issues in the Fresh Fruit and Vegetable *chain*. Study. Amsterdam: Stichting Onderzoek Multinationale Ondernemingen(SOMO), Centre for Research on Multinational Corporations SOMO.

Wachira, M.P., Kimenju, W.J., Kiarie, W.J. and Kihurani, 2013. Incidence of pests and diseases affecting banana in a commercial banana production setting in Kenya. International Journal of Research In Agriculture and Food Sciences, 1(1): 1-7.