Screening for resistance to the banana weevil (*Cosmopolites sordidus*)

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Introduction

Banana weevils are a very important pest, besides nematodes, that contribute to decline in production and disappearance of bananas in some regions of Uganda (Gold *et al.*, 1993). This pest can cause yield loss ranging from 5% in the first cycle to 40% in the fourth ratoon cycle (Rukazambuga *et al.*, 1998). However, loss of up to 100% can also be obtain starting from the fourth ratoon cycle (Sengooba, 1986; Gold *et al.*, 2004). This is because banana weevil populations build up slowly but exponentially over the years (Rukazambuga, 1996), therefore the damage is often greatest as the crop ratoon increases (Mitchell, 1980). The damage caused by weevils is a result of the larvae feeding on the corm, hence weakening the plant, creating wounds from which secondary pests and fungi access the inner parts thus destructing and decomposing the rhizome tissues (Rukazambuga *et al.*, 1998). This can later result into premature death of the plant, snapping of the corm, reduced bunch weights and reduced banana standing life (Gold *et al.*, 2001).

The measures used to control banana weevil's damage vary widely depending upon the type of banana production systems practiced (Padmanaban and Sathiamoorthy, 2001). One of the control measures is the use of chemicals (Masanza, 2003), mainly under commercial production (Gold and Messiaen, 2000).Cultural control strategies are also being applied and they are of greater significance to resource-limited farmers cultivating banana for subsistence production (Padmanaban and Sathiamoorthy, 2001) and these practices include planting of clean planting material coupled with improved agronomic practices so as to increase the plant vigor and proper field sanitation through proper management of crop residues (Gold *et al.*, 2001). Some biological control measures have been applied and these involve the use of exotic natural enemies, endemic natural enemies, secondary host associations, and microbial control for example entomo-pathogens, endophytes and entomo-phagous nematodes (Gold *et al.*, 2001). However, biological control is difficult due to the weevil's boring habit (Gold *et al.*, 2001), and methods like use of entomo-pathogenic fungi as biopesticides may not be affordable by most farmers (Tinzaara *et al.*, 2009).

The available biological control measures of banana weevils using natural enemies has so far been unsuccessful (Gold *et al*., 2001). Germplasm improvement to develop resistant cultivars is the most sustainable solution towards control of banana weevils (Tinzaara *et al*., 2009), especially in developing countries where farmers lack the resources for other control measures (Frison, 1999). However, the genotypes used in the banana improvement for weevil resistance need to be evaluated for resistance against banana weevils. Banana being a long cycle crop, field screening for weevil resistance takes long (Kiggundu *et al*., 2000). It is also labour intensive and require large space as each banana plant occupies 4m^2 to 9 m^2 depending on the planting density (Sadik *et al*., 2010). Therefore, this standard operating procedure is developed to provide guidance when evaluating for weevil resistance among the genotypes to be used in the banana breeding program in a reliable and time saving manner.

Materials and methods

Plant materials

1) TC generated plant material including:
   a) Test genotypes (Parental genotypes and newly developed hybrids)
   b) Resistant checks:
      i. Calcutta 4
   c) Land race controls:
      i. Mchare
      ii. Mbwazirume
      iii. TM-28 OBINO LEWAI

Other materials

1. Sterile forest soil
2. Sterile sand
3. Weevil proof nets
4. 13-liter plastic pots
5. Watering cans
6. Weevils

Multiplication, weaning, and transplanting of tissue culture plantlets

1. Genotypes from the tissue culture laboratory are left in the nursery under humid chamber for 4 weeks and later harden under shade in the nursery for four more weeks
2. After hardening off, genotypes are planted in 13-litre volume plastic buckets that are filled with a mixture of sterilized topsoil, farm manure and saw dust in the ratio of 3:1:1 respectively.

3. Buckets are then sealed off with weevil poof nets to prevent weevils from the nearby fields from entering them, the bucket are then organized according to the design in open space under shade (Fig. 1).
4. Genotypes are allowed to establish themselves for three months in order to attain a suitable corm size before the introduction of weevils.
5. Watering is done regularly to enable the establishment of the suckers.

Figure 1. Established potted trial to evaluate genotypes for weevil resistance

Experimental design

Parental genotypes

Partially replicated experimental design (P-Rep) with three blocks will be adopted with each parental genotype occurring in duplicate and the checks in triplicate for the entire experimental set up. Each plot will constitute four plants per genotype. P-rep designs are useful when plant materials and space are limiting factors. In fact, P-rep designs allow for repeated trial evaluations at different locations. The experimental design for the available 31 parental genotypes including checks is shown in Table 1 below. The average efficiency 0.98 indicating that the design is optimal.

Table 1. P-rep lay out for banana weevil screening for the available 51 parental lines including checks

	1	2	3	4	5	6	7	8	9	10	11	12
1	29364S-2	SH 3362	TMPX 32(PITA 6	BORNEO	C V ROSE	5610S-1	MSHALE	365K-1	TMB2X 7197-2	29275S-4	246K-1	CALCUTTA 4
2	29364S-2	SH 3362	TMPX 32(PITA 6	BORNEO	C V ROSE	5610S-1	MSHALE	365K-1	TMB2X 7197-2	29275S-4	246K-1	CALCUTTA 4
3	29364S-2	SH 3362	TMPX 32(PITA 6	BORNEO	C V ROSE	5610S-1	MSHALE	365K-1	TMB2X 7197-2	29275S-4	246K-1	CALCUTTA 4
4	29364S-2	SH 3362	TMPX 32(PITA 6	BORNEO	C V ROSE	5610S-1	MSHALE	365K-1	TMB2X 7197-2	29275S-4	246K-1	CALCUTTA 4
5	10969S-1	25447S-57	29275S-1	716	1201K-1	SH 3142	PITA 7(1658-4	TMP-28 OBINO L	9128-3	20108K-4	PITA 4(582-4)	MBWAZIRUME
6	10969S-1	25447S-57	29275S-1	716	1201K-1	SH 3142	PITA 7(1658-4	TMP-28 OBINO L	9128-3	20108K-4	PITA 4(582-4)	MBWAZIRUME
7	10969S-1	25447S-57	29275S-1	716	1201K-1	SH 3142	PITA 7(1658-4	TMP-28 OBINO L	9128-3	20108K-4	PITA 4(582-4)	MBWAZIRUME
8	10969S-1	25447S-57	29275S-1	716	1201K-1	SH 3142	PITA 7(1658-4	TMP-28 OBINO L	9128-3	20108K-4	PITA 4(582-4)	MBWAZIRUME

	1	2	3	4	5	6	7	8	9	10	11	12
1	TMP-28 O	20108K-4	SH 3217	246K-1	ZEBRINA GF	MSHALE	201071K	TMPX 32(PITA 6	GALEO(TMB-13)	29275S-1	CALCUTTA 4	29364S-2
2	TMP-28 O	20108K-4	SH 3217	246K-1	ZEBRINA GF	MSHALE	201071K	TMPX 32(PITA 6	GALEO(TMB-13)	29275S-1	CALCUTTA 4	29364S-2
3	TMP-28 O	20108K-4	SH 3217	246K-1	ZEBRINA GF	MSHALE	201071K	TMPX 32(PITA 6	GALEO(TMB-13)	29275S-1	CALCUTTA 4	29364S-2
4	TMP-28 O	20108K-4	SH 3217	246K-1	ZEBRINA GF	MSHALE	201071K	TMPX 32(PITA 6	GALEO(TMB-13)	29275S-1	CALCUTTA 4	29364S-2
5	9128-3	660K-1	KOKOPO	8075-7	TMB2X6142	716	C V ROSE	365K-1	2829-62	MBWAZIRUME	222K-1	1201K-1
6	9128-3	660K-1	KOKOPO	8075-7	TMB2X6142	716	C V ROSE	365K-1	2829-62	MBWAZIRUME	222K-1	1201K-1
7	9128-3	660K-1	KOKOPO	8075-7	TMB2X6142	716	C V ROSE	365K-1	2829-62	MBWAZIRUME	222K-1	1201K-1
8	9128-3	660K-1	KOKOPO	8075-7	TMB2X6142	716	C V ROSE	365K-1	2829-62	MBWAZIRUME	222K-1	1201K-1

	1	2	3	4	5	6	7	8	9	10	11	12
1	BORNEO	GALEO(TMB-1	PITA 7(1658-4)	25447S-57	SH 3142	TMP-28 OBIN	SH 3362	MBWAZIRUME	660K-1	ZEBRINA GF	1154K-1	2829-62
2	BORNEO	GALEO(TMB-1	PITA 7(1658-4)	25447S-57	SH 3142	TMP-28 OBIN	SH 3362	MBWAZIRUME	660K-1	ZEBRINA GF	1154K-1	2829-62
3	BORNEO	GALEO(TMB-1	PITA 7(1658-4)	25447S-57	SH 3142	TMP-28 OBIN	SH 3362	MBWAZIRUME	660K-1	ZEBRINA GF	1154K-1	2829-62
4	BORNEO	GALEO(TMB-1	PITA 7(1658-4)	25447S-57	SH 3142	TMP-28 OBIN	SH 3362	MBWAZIRUME	660K-1	ZEBRINA GF	1154K-1	2829-62
5	TMB2X 71	MSHALE	8075-7	SH 3217	29275S-4	TMB2X6142	201071K	CALCUTTA 4	222K-1	PITA 4(582-4)	10969S-1	5610S-1
6	TMB2X 71	MSHALE	8075-7	SH 3217	29275S-4	TMB2X6142	201071K	CALCUTTA 4	222K-1	PITA 4(582-4)	10969S-1	5610S-1
7	TMB2X 71	MSHALE	8075-7	SH 3217	29275S-4	TMB2X6142	201071K	CALCUTTA 4	222K-1	PITA 4(582-4)	10969S-1	5610S-1
8	TMB2X 71	MSHALE	8075-7	SH 3217	29275S-4	TMB2X6142	201071K	CALCUTTA 4	222K-1	PITA 4(582-4)	10969S-1	5610S-1

Note: *Names in black colour used in the blocks are the parental genotypes to be screened, and those in red colour are for the checks and controls in each block.*

Hybrid genotypes

The augmented design resulting into an incomplete block design where standard checks are replicated in each block and test genotypes will not be adopted for the hybrids. Such augmented designs are very useful where very large numbers of hybrids are produced with limited planting materials and reduced space.

Collection and rearing of banana weevils

1. Banana weevils are collected from the old, infested banana plantations using pseudo stem traps.
2. The pseudo stems are cut into pieces of about 30cm long and then split into two halves.
3. The split pseudo stems are then placed near the banana mats and left for three days as described by (Viljoen *et al.*, 2017).
4. The trapped weevils are collected into 30-litre ventilated buckets and kept in a cool conducive place.
5. The banana weevils are cultured and maintained by feeding them with fresh pared rhizomes of highly susceptible variety (Nakyetengu) and these corms are changed every week.

Determination of the sex of the banana weevils

1. Because infestation of the genotypes with weevils is done by adding 3 female and 3 male weevils to each pot/bucket, therefore it is important to determine their sex.
2. Once the weevil colony is established, the sex of each weevil is determined by viewing the weevil through a stereo microscope.
3. The male has fully punctuated rostrum and the female has less than half punctuated rostrum (Viljoen *et al.*, 2017).
4. The sex of the banana weevil is later confirmed by considering the shape of the last abdominal segment.
5. In the male when the last abdomen segment is viewed laterally, it curves more sharply downwards than that of the female which is flatter (Roth and Willis, 1963).

Inoculation of the experiment

1. Three months after the establishment of the experiment, three female and three male weevils are placed at the base of each plant in the bucket.
2. Then each bucket is sealed off again using a weevil proof net to prevent the introduced banana weevils from escaping.
3. After 60 days from the time of banana weevil introduction into the buckets, the plants are uprooted and the damage caused by the banana weevils is estimated basing on cross-section method as described by (Gold *et al.*, 1994).

Data collection

1. Banana weevil damage is assessed 60days after inoculation.
2. The following traits are evaluated:
    a. Upper cross-section outer damage (%)
    b. Upper cross-section inner damage (%)
    c. Lower cross-section outer damage (%)
    d. Lower cross-section inner damage (%)
    e. Total cross-section damage (%).
3. The cross-section damages are assessed by cutting a transverse cross-section both at the collar (upper cross-section) and 2cm below the collar (lower cross-section).
4. Weevil damage is scored as percentage damage on the upper cross-section and lower cross-section for both the inner corm (central cylinder) and the outer corm (cortex).
5. For each cross-section, weevil damage is assessed independently for the central cylinder and the cortex by estimating the percentage of corm tissue damaged by the weevil in each area.
6. The mean of the four scores (upper cross-section inner, upper cross-section outer, lower cross-section inner and lower cross-section outer) is calculated to generate a total cross-section damage estimate.

Figure 2. Cross-sectional damage on the banana corm

Data analysis

1. To determine the variation among genotypes, analysis of variance is carried out using the following linear model: *Genotype response = μ+ genotype effect + block effect + block/rep effect + error*
2. Both Dunnett's test and fishers protected least significate different test using GenStat or any other powerful statistical package are used to separate the resistant genotypes from susceptible genotypes with comparison to the positive and negative checks.

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