INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 3, No. 3, p. 28-37, 2013

RESEARCH PAPER OPEN ACCESS

Evaluation of progenies from crosses between Bt and non-transgenic sweet potato (*Ipomoea batatas*)

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Article published on March 28, 2013

Key words: Transgene, phenotypic characters, weevil resistance.

Abstract

In this study, transgenic event, CIP410008.7 was used as donor parent of *cry7Aa1* gene and a commercial cultivar, New Kawogo, was used as the recipient parent. A total 53 progenies were obtained and evaluated. The characters evaluated were vine vigour, vine internode length, vine length, foliage weight, root skin and flesh colour, carotenoid content, number of storage roots and weevil resistance. Significant variation in vine vigour, vine length, number of roots per plant and carotenoid content ($p<0.05$) was observed in the F1 progeny. However, no significant differences were observed for foliage weight and vine internode length. The existence of continuous variation of the measured traits indicated the quantitative nature of most sweetpotato traits. The progenies were also analysed for the presence of *cry7Aa1* using PCR. Expected 608bp bands were amplified in progenies that contained *cry7Aa1*. Chi-square test showed a 1:1 segregation ratio. There was no significant difference in production of roots between the transgenic and non transgenic segregants, showing that the transgenic plants were not affected by the presence of *cry7Aa1*. In insect bioassays, there were significant differences in the mean development period of the weevil ($p=0.03$), total number emerged ($p<0.001$) and susceptibility index ($P<0.001$) between the transgenic progeny and control sweetpotato roots.

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Introduction

Sweetpotato weevil (*Cylas puncticollis* Boheman) is a very serious pest occurring mainly in Sub-Saharan Africa (SSA) and is a big threat to sweetpotato cultivation (Fuglie, 2007). The adult weevils attack leaves whilst the larvae feed on roots and stems, producing larval tunnels and later, pupal chambers. Severe infestations or slightly damaged roots render them unpalatable and inedible to humans due to terpenoid production in response to weevil feeding (Stathers *et al.,* 2003). There is no effective chemical control measure for the larvae, or other stages found within the plant tissue. Insecticides only kill adult weevils that migrate in search of feeding and oviposition sites. Use of chemical control, however, is too expensive and impractical for most resource poor farmers in SSA. Alternative control strategies, such as biological insecticides, are available but not used in SSA because they are also expensive. Efforts to develop elite insect-resistant sweetpotato genotypes; with high yield and desirable agronomic traits, through conventional breeding of germplasm exhibiting endogenous insect resistance are not yet available (Stevenson *et al.*, 2009). The prospect of weevil control by introducing *Bacillus thuringiensis* (Bt) resistance genes through genetic transformation is an attractive option (Kreuze *et al.*, 2009).

Genetic transformation is an important approach for the introduction of novel desirable traits into crops (Mishra and Slater, 2012). For many desirable traits from unrelated plants or other organisms, genetic transformation is clearly the only source of variation for breeding programmes. However, not all the cultivated or elite lines of a crop species are amenable to genetic transformation (Visarada *et al.*, 2009). In rice highly responsive genotypes for *in vitro* culture have been identified and used for introduction of foreign genes. Rice genotypes belonging to the *japonica* subgroup and a few *indica* genotypes like IR65, IR72, and Radon and aromatic varieties like 'Basmati 370' and 'Pusa Basmati-1' were identified as model genotypes for genetic transformation (Ashikari *et al.*, 2004). Similarly, in sweetpotato, an American cultivar Jewel is amenable to *in vitro* genetic manipulation as compared to African cultivars (Luo *et al.*, 2006). As a result several events expressing Bt genes for sweetpotato weevil control are available from this model genotype. Unfortunately these transgenic events are not well adapted to African growing conditions and do not have farmer preferred traits and hence a series of crossing and selection is required to put the new genes into best cultivars.

In exploitation of transgenic Bt sweetpotato in cross breeding with elite varieties for insect resistance, it is important to assess the morphological and agronomic characteristics for regulatory purposes and variety release. Variation among traits apparently not related to a foreign gene has been reported. In transgenic rice (R_2 generation), plants were significantly shorter, flowered later, and were partially sterile as compared to their non-transgenic controls (Lynch *et al.*, 1995). Blanche *et al.* (2006) compared the transgenic cotton cultivars and their non transgenic counterparts. Transgenic cultivars were taller, had greater internodes ratio, larger seed, and lower lint percentages, and in some cases yielded more than their conventional parents. On contrary, integration of the synthetic Bt fusion gene C*ry1B–Cry1Ab* in the cabbage nuclear genome did not alter photosynthetic activity (Paul, 2003). Both T0 and T1 Bt transgenic plants flowered normally and set seeds normally like their wild-type counterpart. In sweetpotato however, research focusing on phenotypic variations in the morphological and agronomic traits between progenies of transgenic and non transgenic parents is still scanty. Given that sweetpotato is a highly heterozygous an understanding of the nature and magnitude of variation among sweetpotato genotypes from a cross between model lines and elite lines for traits of economic importance is vital to plan effective transgenic breeding programmes.

The objective of the present work was to examine the performance of progeny derived from a cross between transgenic and non transgenic agronomically superior genotypes grown under screenhouse conditions. This study evaluates the effect of Bt gene modifications on performance of sweetpotato and accesses effect of

cry7Aa1 gene against the insect pest in the transgenic progeny.

Material and methods

Plant material

A transgenic parental sweetpotato event CIP410008.7 carrying *cry7Aa1* gene and Ugandan cultivar, New Kawogo were crossed under screenhouse (Table 1). New Kawogo is ranked among the five superior and farmer preferred cultivars grown in Uganda (Mwanga *et al.*, 2001). The transgenic event CIP410008.7 was developed by transforming an American cultivar Jewel as previously described by Luo *et al.* (2006) using an *Agrobacterium* mediated technique. The transgenic event has a β-amylase promoter known to cause higher protein expression levels in the roots. Botanical seed obtained from crosses between CIP410008.7 and New Kawogo were scarified in concentrated sulphuric acid, floated in water and germinated in sterile soil, in seed boxes, and spaced 15 cm x 3 cm in screenhouse. The plants were fertilised and irrigated according to screenhouse standard regimes. Each progeny was assigned an identification code starting with JNK (Jewel x New Kawogo) and a number. The F1 progeny were transplanted into pots in the same screenhouse. The pots were arranged in a randomised complete block design with three replications; nine clonal plants from each genotype. The experiments were maintained under natural light. Relative humidity ranged from 65 to 80% and temperature averaged 25±2°C during the day and 20±2°C at night. The trial was harvested when the plants were 7 months old.

Evaluation of F1 progeny

Characterisation was based on aerial and below ground parts of all progeny using standard descriptors; morphological and agronomical descriptors. Quantitative measurements were taken for: (a) plant type: determined by length of the main vines: erect (<75cm), semi-erect (75-150cm), spreading (151-250cm), extremely spreading (>250cm); (b) vine internode length: very short (< 3cm), short (3-5cm), long (6-12cm), very long (>12cm). (c) foliage weight (d) number of roots/plant; (e) vine vigour: based on visual appearance using a scale of 1 to 9 where: 1-2 represents very low vigour (very weak); 2-4, low vigour (weak); 4-6, intermediate vigour (good vigour); and > 7 (high vigour); (f) root and skin colour and (g) β- carotene content - expressed as mg 100 g^{-1} fresh weight (FW) of the root; β-carotene value was recorded as per the sweetpotato colour chart developed by Burgos *et al.* (2009) from CIP, Lima, Peru.

PCR analysis of cry7Aa1 gene

Genomic DNA was isolated from all sweetpotato F1 genotypes leaf samples using the CTAB method (Stacey *et al.*, 2000). PCR amplification of the 608 bp DNA fragment of the *cry7Aa1* gene was carried out in a Multigene Thermocycler (Labnet International, Inc. NJ, USA) using 5′-ACAACTCATCACCATACCAAAC-3′ and 5′-AAGAGCAAGATGCAAGTTTG-3′ as forward and reverse primers, respectively. PCR took place in a total volume of 25 µl containing 50ng of total plant DNA made as follows 12.5 µl of Ready mix Taq® DNA polymerase (Sigma-Aldrich, Poole, UK) and primer F (0.5 µl) and primer R (0.5 µl) and adjusted to 25 µl with nuclease free water. PCR conditions were as follows: initial denaturation of DNA at 93°C for 2 minutes and then amplified through 35 thermal cycles of 93°C for 15 seconds, 55°C for 30 seconds, 72°C for two minutes and ending by a final extension step at 72°C for 7 minutes. The PCR amplicons were separated by electrophoresis on 1% agrose gel in Tris-EDTA (TE) buffer stained with ethidium bromide and visualized under UV light. The presence or absence of the transgenes in the hybrids was used to confirm the transgenic and null (non-transgenic) segregants.

Sweet potato weevil rearing

A sweetpotato weevil colony was established from a field collected population (about 500 insects) and maintained in the laboratory on storage roots in Collapsible cages (1450C, BioQuip Products, Inc, CA) at 25± 2°C and 70 ± 10% RH. In preparing experimental insects, adult weevils were transferred to fresh roots with old roots being incubated until the new generation emerged. Emerging weevils were collected and held in cages with new roots until

required for bioassays. Sexing of the weevils was done by using the shape of the distal antennal segment, males have a filiform shape and females have club like shape (Smit, 1997).

Insect bioassay

A total of 15 transgenic F1 progeny and a susceptible check cv. Tanzania as a control were used in this study to determine the effects of the transgenic sweetpotato on *C. puncticollis*. Ten *C. puncticollis* female adult (3-4 weeks old) were placed in individual 1 litre polystyrene jars with paper towels at the base. Single uninfested experimental roots were introduced into each jar and each jar represented a replicate. After 24 hours, the adults were removed. The roots were then incubated until adult weevil emergence. Insect count commenced 23 days post-infestation when the F_1 weevil progenies started emerging. Thereafter, any newly emerged adults were recorded and removed daily. The emergent adults were collected and counted until weevil emergence stopped. This set-up was replicated three times, in a completely randomized design including the progeny and control. The experiment was conducted at 25± 2°C and 70 ± 10% RH. In this bioassay, the total number of progeny that emerged and their mean development period were derived for each replicate. An index of susceptibility (Equation 1) was calculated for each genotype according to Dobie (1977):

Equation 1:

$$\text{Index of susceptibility} = 100\left[\frac{\log_e(\text{Total number of adults emerged})}{\text{Median development period}}\right]$$

The median development period was calculated as the number of days from oviposition to the emergence of progeny. Genotypes with a high susceptibility index were considered susceptible and those with a lower susceptibility index as partially resistant.

Data analyses

The frequencies of all traits in the progeny were classified and data subjected to ANOVA using GenStat (VSN International Ltd, 2011). The transgene segregation data was analysed by $\chi 2$ test to determine if the observed segregation ratios of *cry7Aa1* fit the expected Mendelian 1:1 phenotypic ratio. T-test was used to compare the number of roots per plant of transgenic and null segregants. In the insect bioassay the number of progeny that emerged, mean development period and susceptibility index of the events were also subjected to ANOVA using GenStat.

Results and discussion

In this study vine length was an indication of the plant type or growth habit and there was significant variation observed between the F1 progeny (Table 2). Four growth habits were observed erect (13.7%), semi erect (64.2%), spreading (18.9%) and extremely spreading (3.8%). The frequency distributions showed that majority of the clones were semi erect type showing an affinity to the female parent. Only 18.9 % of the clones showed affinity to the male parent which has a spreading growth habit. The two extreme types; erect and extremely spreading were also found at 16.98% and 5.66% respectively (Fig. 1). The root skin colour in the hybrid progeny segregated into a variety of colours varying from cream, light red, red light orange and orange. The progenies were predominantly cream and very few were light orange and orange (Fig. 2a). The variability in skin colour increases potential to breed and select cultivars with skin colour preferred by farmers. Rees *et al.* (2003) reported that the colour characteristic of sweetpotato roots constitutes an important factor in the choice of sweetpotato by consumers, and could be used as genetic marker in predicting yield and cooking attributes of the roots.

Various flesh colours were exhibited by the progenies such as cream, pale yellow, pale yellow orange, pale orange, intermediate orange and orange. Orange colour was predominant in the progeny with pale yellow orange (7.3%), pale orange (12.2%), intermediate orange (26.8%) and orange (7.3%) colour respectively which constituted about 53.7% of the total progeny. Orange fleshed sweetpotato contains carotenoids yet most consumers prefer varieties with white or yellow flesh which contain very little carotene (Takahata *et al.*, 1993). Cream colour was noticed in 29.3% of the progeny while 17.1 % of

the genotypes exhibited a pale yellow colour (Fig. 2b). In Africa, consumers have been reported to prefer cultivars with high dry matter content (Kapinga and Carey, 2003), and varieties of sweetpotato with high carotenoid contents tend to have lower dry matter content. This variability allows breeding of genotypes with little or no carotenoids and with inherent transgenic insect resistance.

Table 1. Morphological and agronomic descriptors of the sweet potato parental genotypes.

Descriptor	Cultivar	
	Jewel	New Kawogo
Plant type	Semi-erect	Spreading
Root skin colour	Orange	Red
Root flesh colour	Orange	Whitish cream
Vine vigour	Good	Good
β-carotene content	11.03 mg 100 g^{-1}	0.0 mg 100 g^{-1}

The flesh colour observed in the progeny as per the sweetpotato colour chart, had β-carotene values ranging from 0.0 to 11.03 mg 100 g^{-1} fresh weight (FW). Of the 53 hybrids, 4.9 % of the hybrids possessed a high value of 10.0 – 12.0 mg 100 g^{-1} FW, 9.8% had 6.1 – 8.0 mg 100 g^{-1} FW, 14.6% had 6.1 – 8.0 mg 100 g^{-1} FW and the rest of the hybrids had less than 2.0 mg 100 g^{-1} FW (Fig. 2c). Hybrids JNK 48 had JNK 50 possessed highest β-carotene value of 11.03 mg 100 g^{-1} FW which is similar to the maternal parent CIP410008.7 (Table 2). A total of eight F1 progeny had no β-carotene, like the paternal parent, New Kawogo. Takahata *et al.* (1993) found a strong positive correlation between β-carotene content and flesh colour in sweetpotato cultivars. This shows that root flesh colour could be used in rapidly estimating the β-carotene content in breeding programmes. The carotenoid rich genotypes obtained in this study also indicates the possibility of significantly improving the Vitamin A nutrition in Africa.

Though not statistically different, vine weight and average internode length were observed to vary according to morphological classification (Table 2). Majority of the clones were found to possess vine weight ranging from 0.3 – 0.4kg (70%) with a few weighing higher between 0.4-0.5kg (Fig. 3a). The average internode length (cm) was obtained by measuring the mean length of the internode between the nodes of the mid-section of four randomly sampled vines from each of genotype. It varied across the progeny, majority of the internodes were short (81.1%) and a few very short (13, 2%) and long (5.7%) respectively (Fig. 3b). Most of the F1 genotypes displayed good vine vigour (Fig. 3c) probably because they were grown under controlled screenhouse environment. However, there was significant variation ($p=0.01$) observed between the progeny in vine vigour (Table 2).High vine vigour value of 7.0 was recorded in JNK 7 and majority of the progeny were above average vine vigour with JNK 43 having the lowest vine vigour value (3.3) (Table 2). Similarly, there was significant variation in the number of roots/ plant in all 53 genotypes. The high ploidy level in sweetpotato could be responsible for variation in these traits due to increased mutations associated with polyploidy (Osborn *et al.*, 2003).

The PCR, confirmed that the transgene cassette was stably inherited in the F1 progeny (Fig. 4) and showed segregation at 1:1 ratio ($\chi^2 = 0.17$, $p = 0.68$). There were 25 transgenic progeny and 28 null segregants (non-transgenic). A T-test done on number of roots/plant of transgenic progeny plants (group 1, n = 25) compared with the null segregants (group 2, n = 28) gave a P-value of 0.44. The mean of group 1 is 1.64 (SD = 0.93; SEM = 0.19) while that of group 2 is 1.43 (SD = 1.05; SEM = 0.20). The t-test demonstrated that there were no significant differences in the number of roots/plant between the transgenic and non-transgenic segregants. These results suggest that introduction of the *cry7Aa1* construct had no effect on the number of roots/plant which is an important agronomic trait in sweet potato. Some of the progenies however, failed to produce roots seven months post planting. These

genotypes are most likely not to be considered in the next level of evaluation in a breeding programme.

Table 2. Mean morphological and agronomic trait values of the 53 F1 genotypes.

Genotypes	Vine vigour	Vine length (cm)	Internode length (cm)	Foliage weight (Kg)	Number of roots	β- carotene content (mg/100^{-1} FW)
JNK 1	4.3	105.7	3.8	0.45	2.3	0.15
JNK 2	5.0	138.7	4.9	0.39	1.7	1.76
JNK 3	5.3	130.3	3.5	0.41	1.0	1.65
JNK 4	4.7	221.3	2.9	0.38	2.3	0
JNK 5	4.3	152.0	3.0	0.41	2.0	1.65
JNK 6	6.7	133.7	3.3	0.43	1.3	0.02
JNK 7	7.0	98.7	4.2	0.38	0.0	-
JNK 8	4.7	105.7	3.0	0.35	2.7	1.38
JNK 9	4.3	107.3	4.2	0.38	2.0	1.76
JNK 10	4.0	215.7	3.5	0.39	0.0	-
JNK 11	4.7	79.0	2.2	0.41	2.3	4.92
JNK 12	4.7	143.7	4.5	0.43	2.3	0
JNK 13	5.7	115.7	4.0	0.39	0.0	-
JNK 14	5.0	124.0	4.7	0.37	3.0	0.12
JNK 15	5.0	90.3	4.2	0.39	1.7	4.92
JNK 16	5.3	167.0	3.5	0.42	0.0	-
JNK 17	5.0	76.7	3.3	0.37	3.0	0
JNK 18	6.0	123.7	3.3	0.38	0.0	-
JNK 19	5.7	252.7	5.2	0.43	2.3	0.02
JNK 20	4.0	127.0	3.7	0.37	0.0	-
JNK 21	4.3	121.7	4.4	0.37	1.7	0.02
JNK 22	4.7	121.0	3.0	0.41	1.7	4.41
JNK 23	4.7	58.3	3.2	0.36	1.3	0.12
JNK 24	3.7	99.7	3.6	0.41	1.3	0.15
JNK 25	5.7	197.0	5.2	0.39	2.7	0.12
JNK 26	5.7	120.7	5.0	0.46	0.0	-
JNK 27	4.0	110.7	3.8	0.37	0.0	-
JNK 28	4.0	103.0	3.0	0.36	1.3	4.92
JNK 29	4.7	107.3	3.0	0.43	0.0	-
JNK 30	5.3	104.0	3.1	0.37	0.0	-
JNK 31	4.7	92.7	2.2	0.41	2.0	0
JNK 32	4.3	101.0	2.8	0.39	2.3	0
JNK 33	4.0	75.0	4.0	0.39	2.0	3.03
JNK 34	4.0	96.3	3.7	0.40	1.3	3.03
JNK 35	5.0	74.0	4.1	0.41	1.7	0.02
JNK 36	6.0	72.7	3.8	0.37	2.0	0.12
JNK 37	4.0	64.3	4.2	0.39	0.0	-
JNK 38	5.3	131.7	4.3	0.39	1.3	0
JNK 39	5.0	120.0	4.7	0.39	3.0	0.12
JNK 40	6.0	102.3	3.4	0.37	2.3	0.12
JNK 41	5.3	93.7	3.2	0.45	2.3	6.12
JNK 42	4.7	101.7	4.0	0.43	3.0	0.12
JNK 43	3.3	73.7	3.9	0.38	0.0	-
JNK 44	4.3	125.0	3.0	0.34	3.0	7.23
JNK 45	4.3	102.7	4.0	0.40	1.7	0
JNK 46	4.0	186.3	3.9	0.39	2.7	0
JNK 47	5.7	190.0	2.8	0.38	2.0	1.38
JNK 48	3.7	103.3	3.5	0.43	2.7	11.03
JNK 49	4.0	121.3	2.9	0.37	1.0	3.03
JNK 50	4.0	59.0	2.9	0.39	1.3	11.03
JNK 51	4.0	81.0	4.1	0.39	1.7	5.46
JNK 52	4.0	106.0	3.8	0.41	1.0	7.23
JNK 53	3.7	266.7	3.3	0.40	1.0	4.61
Mean	**4.74**	**120.6**	**3.67**	**0.39**	**1.60**	**2.22**
LSD (0.05)	**1.74**	**80.42**	**1.96**	**0.07**	**1.98**	**0.04**
p-value	**0.01**	**<0.001**	**0.49**	**0.56**	**0.001**	**<0.001**

Table 3. Comparison of sweet potato genotypes for resistance to *C. puncticollis*.

Genotype	Number of weevil adults emerged (±SE)	Median development period (d) (±SE)	Dobie's susceptibility Index (DSI) (±SE)
JNK 3	56.0 (±1.00)	27.7 (±0.67)	14.6 (±0.38)
JNK 9	60.7 (± 3.48)	28.3 (±0.88)	14.5 (±0.56)
JNK 14	65.3 (±2.91)	27.7 (±0.33)	15.1 (±0.33)
JNK 15	63.3 (±3.18)	28.3 (±0.33)	14.6 (±0.19)
JNK 21	59.7 (±0.88)	28.0 (±1.00)	14.6 (±0.56)
JNK 25	60.3 (±5.17)	27.0 (±0.58)	15.2 (±0.63)
JNK 31	60.7 (±5.61)	28.0 (±0.58)	14.6 (±0.53)
JNK 33	62.7 (±3.76)	28.3 (±0.88)	14.6 (±0.25)
JNK 34	65.0 (±3.21)	27.7 (±0.33)	15.1 (±0.28)
JNK 36	60.3 (±4.81)	27.7 (±0.33)	14.8 (±0.13)
JNK 40	58.3 (±2.33)	28.3 (±0.33)	14.3 (±0.03)
JNK 41	56.3 (±4.37)	28.0 (±0.00)	14.4 (±0.27)
JNK 44	58.3 (±5.61)	27.0 (±1.00)	15.0 (±0.37)
JNK 46	64.0 (±3.21)	28.7 (±0.88)	14.5 (±0.58)
JNK 47	62.7 (±2.33)	28.3 (±0.67)	14.6 (±0.48)
Cv. Tanzania*	75.7 (±1.20)	22.7 (±0.33)	19.1 (±0.27)

*Susceptible check

In the insect bioassay analysis, visual observation revealed that the adult weevils fed the same way on transgenic roots as on non-transgenic control. When the roots were incubated after oviposition, the new adult weevils began to emerge 23 days post infestation in the susceptible check and between 26-29 days post infestation in the F1 progeny. There was significant difference ($p = 0.03$) between the transgenic progeny and the susceptible check in the total number of weevils that emerged with more weevils emerging from cv. Tanzania and the least from JNK 3 (Table 3). The median development time of *C. puncticollis* was significantly different ($p < 0.001$) between the transgenic F1s and the susceptible cv. Tanzania. Similarly, there was also significant difference ($p < 0.001$) in the susceptibility index between the progeny and the control.

The adults which fed and oviposited on transgenic roots provided no evidence that the adults are sensitive to the Cry protein expressed in sweetpotato. Even though no indication of a feeding deterrent behaviour was observed, this aspect should be given attention, as reduced feeding and decreased food utilisation could also impose a higher mortality, which has been reported in other insects (Hussein *et al.*, 2005). The fresh root bioassays provides some indication that *C. puncticollis* is affected by ingestion of transgenic sweetpotato plants expressing Cry7Aa1 protein. The mean development period of weevils in the F1 progenies was almost the same, indicating that transgenic events had inherent insecticidal properties which delayed development as compared to cv. Tanzania. This data also shows that transgenic clones have an effect on weevil populations by reducing the emergence of adult weevils. These factors could result in population suppression that would significantly reduce weevil damage in sweetpotato. This is based on the assumption that a few insect progenies would emerge out of a resistant genotype and insect progeny development would take a longer time in a resistant than in a susceptible genotype.

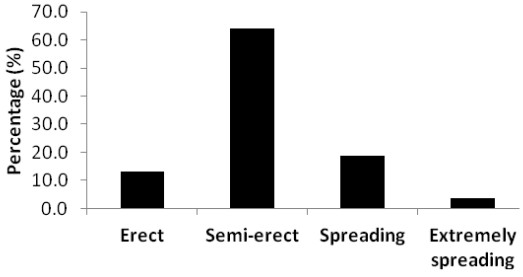

Fig. 1. Growth habit of the 53 F1 genotypes.

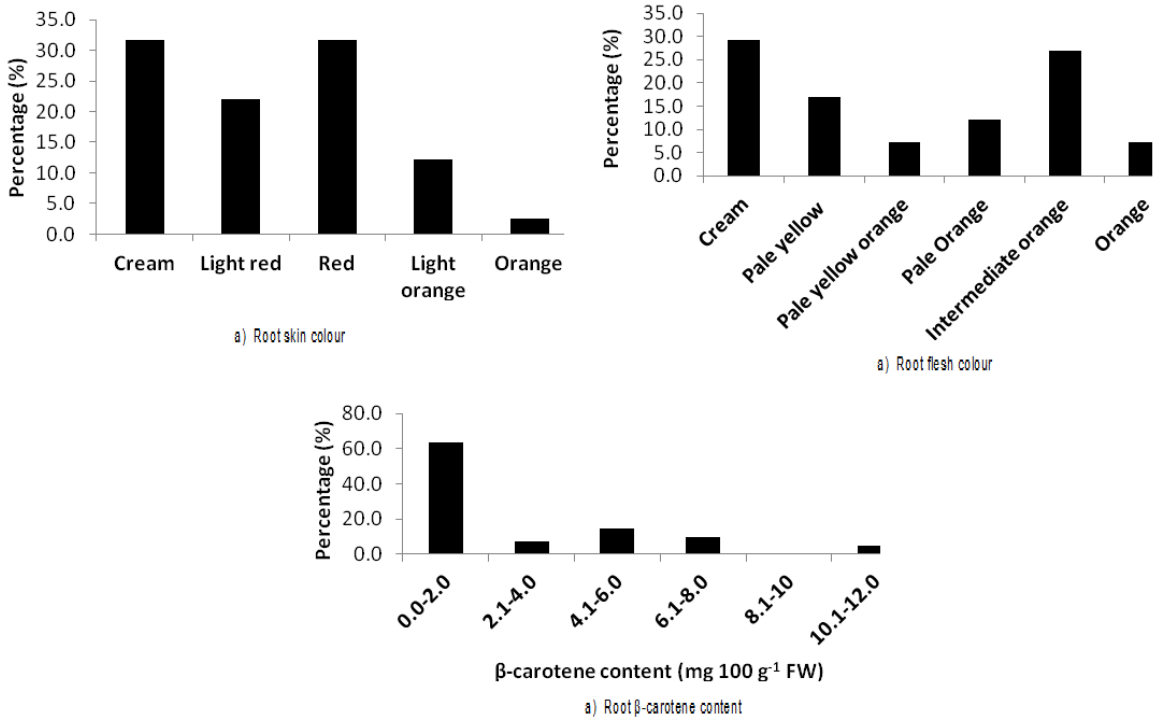

Fig. 2. Root characteristics of the 53 F1 genotypes (a) root skin colour; (b) root flesh colour and (c) root β-carotene content.

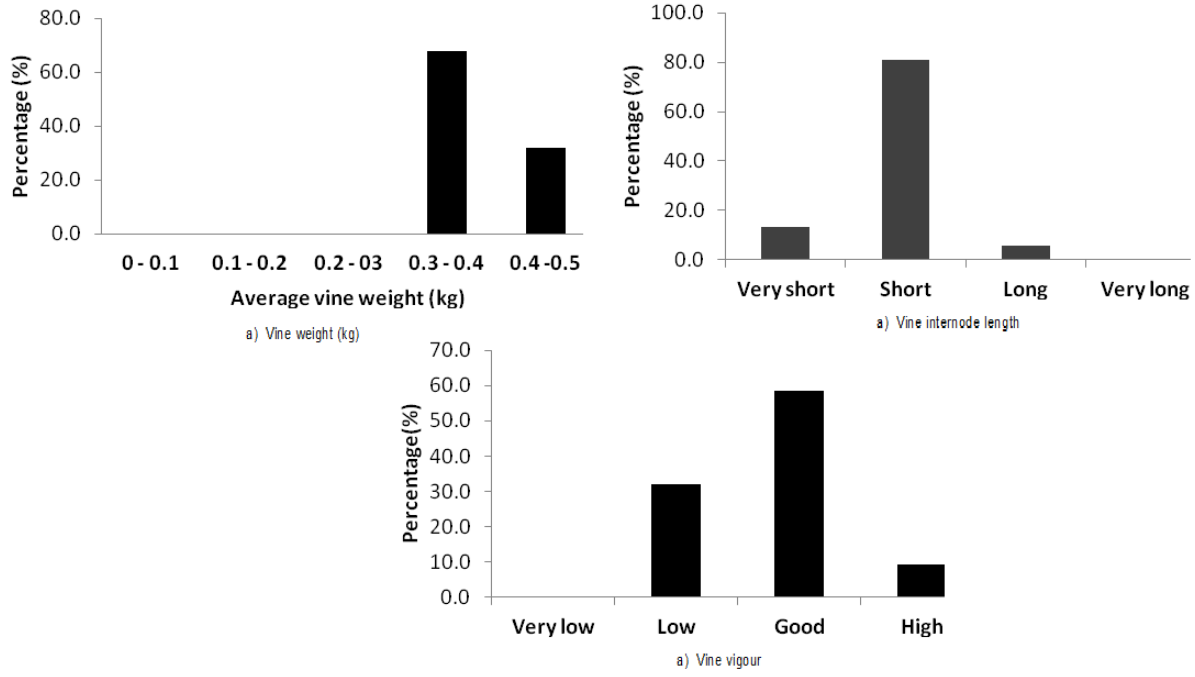

Fig. 3. Vine characteristics of the 53 F1 genotypes (a) average vine weight; (b) vine internode length and (c) vine vigour.

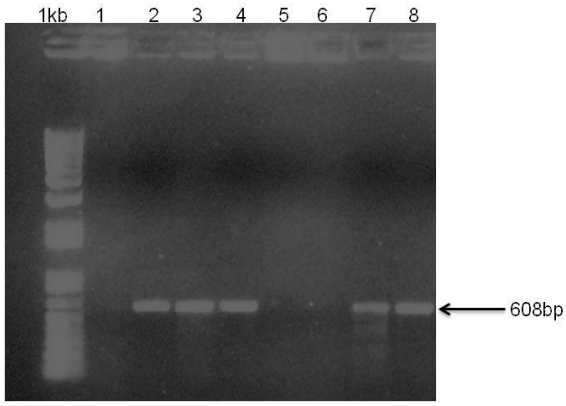

Fig. 4. PCR analysis for the F1 plants resulting from crosses between CIP410008.7 and New Kawogo. The pair of primers used amplified the *cry7Aa1* gene in plants yielding a 608-bp fragment. 1kb ladder, 1-7 plant numbers, positive control (8).

Conclusion

In this preliminary screenhouse and laboratory evaluation, the sweetpotato F1 genotypes showed variation in plant type, internode length, foliage weight, number of roots, vine vigour, root and skin colour, β- carotene content and resistance to *C. puncticollis*. The results presented here show that the transgene does not necessarily have an effect on morphological and agronomic traits. Since sweetpotato is a hexaploid, significant variation in all traits studied indicated segregation of many genes for a given trait. Evaluation and characterisation of sweetpotato clones could be used to identify and select genotypes from crosses involving transgenic and non-transgenic parents with farmer preferred traits for incorporation into breeding programmes. Our experiments were done under controlled environments because of the regulatory requirements; further field trials along with additional transgenic events would provide additional information on the effect of the transgene on agronomic performance.

Acknowledgements

We acknowledge Mr Moses Mwonda for taking care of transgenic plants in the screenhouse, Miss Justine Nanteza for assistance in rearing of the weevils. We also acknowledge International Potato Center and Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for funding this work.

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