

**TRANSGENIC BREEDING OF SWEETPOTATO FOR RESISTANCE TO
AFRICAN WEEVIL (*Cylas puncticollis*)**

BY

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ABSTRACT

Sweetpotato weevil (*Cylas puncticollis*) Boheman is a serious pest throughout Sub-Saharan Africa. The pest causes up to 28% production losses annually in Uganda. Due to difficulties to implement pest management measures and the low level of resistance to weevils in existing sweetpotato varieties, *cry* genes were introduced, prior to this research, by genetic engineering to express three different Cry proteins known to be toxic to the African weevils. The main objective of this study was to assess the efficacy of sweetpotato expressing Cry proteins to the African sweetpotato weevil and identify candidate sweetpotato genotypes to be incorporated into sweetpotato breeding programme. This study was based on the following specific objectives: i) to evaluate the efficacy of sweetpotato events expressing Cry proteins against the African sweetpotato weevil, ii) to determine the inheritance and expression of *cry7Aa1* gene in F1 sweetpotato progenies developed through transgenic breeding, iii) to evaluate the agronomic performance of these F1 genotypes, iv) to identify key non-target organisms exposed to weevil resistant transgenic sweetpotato.

Ten transgenic sweetpotato events expressing Cry7Aa1, Cry3Ca1, and ET33-34 proteins from *Bacillus thuringiensis* (Bt) were evaluated for resistance against *C. puncticollis*. Four bioassays were used to evaluate the activity of the proteins against the weevil. (i) 1st instar larvae in an artificial diet using root powder of transgenic events, (ii) whole root of transgenic events infested with female adults, (iii) root chip, and (iv) small roots of transgenic events both infested with egg-plugs. One event known to have a single copy of the *cry7Aa1* gene was then crossed with three Ugandan sweetpotato cultivars, namely, New Kawogo, NASPOT 1 and Tanzania to assess the segregation pattern of the *cry* gene. In the CIP410008.7 × New Kawogo cross, F1 progenies were evaluated for Cry protein expression in the storage roots. In addition, these F1 progenies were accessed to confirm if genetic transformation process generated any unexpected alterations. Vine vigour, vine internode length, vine length, foliage weight, root skin and flesh colour, carotenoid content, number of storage roots and weevil resistance were evaluated in the F1 genotypes. Furthermore, key non target arthropods which may potentially be affected by cultivation and inclusion of Bt sweetpotato in breeding programmes were also identified.

DAS-ELISA showed variation (0.1 - 0.4 $\mu\text{g g}^{-1}$) in Cry protein concentration in fresh roots of the transgenic events. The highest protein quantity was observed on the event carrying the *ET33-34* transcript while those carrying *cry7Aa1* and *cry3Ca1* transcripts had low protein quantities. In the insect bioassays, although whole root assay had low weevil handling injuries, this method requires a comparatively large number of adult females for oviposition and roots per event to be tested. The root chip method was the least desirable because it was prone to fungal and bacterial contamination. The most appropriate method for testing Bt efficacy in sweetpotato is the small root egg-plug bioassay. Generally, none of the transgenic events tested provided weevil control because of low Cry protein expression in storage roots. The F1 seedlings were analysed for the presence of *cry7Aa1* transgene using PCR. Chi-square test showed that all the three families followed a 1:1 segregation pattern of the transgene. In the CIP410008.7 \times New Kawogo family, no significant differences ($p > 0.05$) were observed in the protein expression between transgenic progeny with mean values of 0.2 $\mu\text{g Cry protein g}^{-1}$ storage root tissue. The insect bioassay revealed no significant activity against *C. puncticollis* in the transgenic progenies. Despite New Kawogo having high concentrations of resistance compounds (hydroxycinnamic acid esters) synergy between transgenic and natural resistance was not observed in the progeny. Hence, Cry protein expression transmitted as a dominant and Mendelian trait, was not influenced by the genotype of the F1 progeny, and did not synergise with host plant 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 of transgenic breeding. Nevertheless, 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. There was also no significant difference in production of roots between the transgenic and non-transgenic F1 progeny. Hence, we did not observe any unexpected alterations due to the *in-vitro* regeneration and genetic transformation of the sweetpotato crop. In transgenic breeding, the ground, rove, and ladybird beetles were identified as the primary relevant non-target organisms of Bt sweetpotato. These insects are important predators in sweetpotato fields. Additionally, honey bee was also considered as relevant due to its ecological role as a pollinator.

In conclusion, the absence of effective transgenic events to control weevils in storage roots entails that, more research is needed on additional transgenic events with new gene constructs for

effective weevil control. The *cry7Aa1* gene inheritance and expression was stable in F1 progenies, this implies that the transgenic sweetpotato could be used as a valuable insect-resistant germplasm to be employed in transgenic breeding programmes. The existence of continuous variation of agronomic traits observed in the F1 progeny enables evaluation and selection of sweetpotato clones with farmer preferred traits to be incorporated in a breeding programme. In the likelihood of successful transgenic breeding, there is need to do risk assessment on the non-target organisms at laboratory and field level to assess the impact of Bt sweetpotato on the non target organisms.