

Abstract

Genetic analysis of sweetpotato resistance to the African sweetpotato weevils (*Cylas spp.*)

Sweetpotato weevils (*Cylas spp.*) are the major pests of sweetpotatoes in Uganda and sub-Saharan Africa. They cause losses ranging from 60-100% particularly during dry periods and in drought prone areas. Host plant resistance is currently considered as one of the most viable control options for these pests given their obscure feeding habits. However, breeding for resistance to *Cylas spp.* requires a clear understanding of the genetics underlying this trait. The genetic basis for sweetpotato weevil (SPW) resistance though, has never been fully elucidated despite years of research on this trait. Genetic analyses in this crop are typically challenging due to the crop's polyploid, highly heterozygous, and outcrossing nature. This study was aimed at improving our understanding about the genetics underlying resistance to the African sweetpotato weevils, *Cylas puncticollis* and *Cylas brunneus*, using a population developed from an 8x8 partial diallel cross of sweetpotato, known as the Mwanga Diversity Panel (MDP).

The test population was initially phenotyped in field trials with the aim of quantifying the heterotic gains, transgressive segregation and fitness costs associated with sweetpotato weevil resistance expression in sweetpotato. A total of 1,896 genotypes were tested together with their parental genotypes at two field sites located in Lira and Arua districts in Uganda for two seasons each, using an augmented design. Data on weevil severity (WED), weevil incidence (WI), storage root yield (SRY) and dry matter content (DM) were obtained and thereafter analyzed using R statistics software. Best linear unbiased predictors (BLUPs) for each genotype across environments were used to estimate heterotic gains and for regression analyses to establish fitness costs related to sweetpotato weevil resistance expression in sweetpotato.

In general, relatively low mid-parent heterotic gains were detected, with the highest favorable levels recorded for SRY (14.7%) and WED (-7.9%). About 25% of the crosses exhibited desirable and significant mid-parent heterosis for weevil resistance. Over 16% of the genotypes displayed superior transgressive segregation, with the highest percentages recorded for SRY (21%) and WED (18%). The best performing genotypes across all traits were MDP449, MDP1514 and MDP920. Transgressive segregation was observed in all traits although crosses 'Wagabolige' x NASPOT 10 O and "New Kawogo" x "Ejumula", particularly produced the highest number of superior segregants with regards to weevil resistance. Out of the 64 crosses, only NASPOT 11 x NASPOT 7 demonstrated desirable heterosis and had superior transgressive segregants for all the traits examined. A yield penalty of 10% was observed to be associated with SPW resistance whereas no decline in DM was detected in relation to the same. The yield penalty detected due to SPW resistance suggests that a trade-off may be necessary between maximizing yields and developing weevil-resistant cultivars if the current needs for this crop are to be met in weevil-prone areas.

In order to study the inheritance of SPW resistance and other test traits, general combining ability (GCA), specific combining ability (SCA) and heritability analyses were conducted in the MDP population. To achieve this, 1,360 genotypes that survived in the field were further evaluated in three rounds of a no-choice feeding laboratory bioassay using a randomized complete block design. This was done at the sweetpotato entomology laboratory at NaCRRI.

In the no-choice bioassay, three clean, healthy storage roots of each genotype were placed in separate plastic jars and artificially inoculated with 2-week-old gravid female adult weevils (*C. puncticollis*). The weevils were allowed to feed and oviposit for 24 hours, after which they

were removed from each jar and the number of feeding holes on the roots counted and recorded. The eggs that were laid were left to incubate until emergence at room temperature, and thereafter, the number of adults that emerged from the roots was counted on a weekly basis. Data from this experiment was augmented with field data to perform subsequent analyses.

Results revealed significant GCA effects for parents and SCA effects for families for most traits. All variance components were highly significant ($P \leq 0.001$). Narrow-sense heritability estimates for WED, SRY and DM were 0.35, 0.36 and 0.45, respectively. Parental genotypes with superior GCA for weevil resistance included 'Mugande', NASPOT 5, 'Dimbuka-bukulula', and 'Wagabolige'. On the other hand, families that displayed the highest levels of resistance to weevils included 'Wagabolige' \times NASPOT 10 O, NASPOT 5 \times 'Dimbuka-bukulula', 'Mugande' \times 'Dimbuka-bukulula', and NASPOT 11 \times NASPOT 7. Superior parents and families identified through this study could be deployed in further research involving the genetic improvement of these traits. The moderate levels of narrow-sense heritability observed for the traits, coupled with the significant GCA and SCA effects, suggest that there is potential for their improvement through conventional breeding via hybridization and progeny selection and advancement.

Finally, genome-wide association (GWAS) studies were conducted with the aim of identifying genomic regions (quantitative trait loci (QTL)) associated with SPW resistance, storage root yield and dry matter content in sweetpotato. A subset of the MDP population consisting of 822 genotypes was sequenced via a high throughput sequencing platform, OmeSeq. Quality filtering and variant calling were done using bioinformatic pipelines, ngsComposer and GBSapp. The quality SNPs together with BLUPS from the phenotypic data were used to test for significant marker-trait associations via GWASpoly, while modeling different types of polyploid gene action including additive and all hexaploid dosage-based dominance SNP effects. GWASpoly identified 18 significant SNPs associated with sweetpotato weevil resistance, located on chromosomes 4, 7, 10 and 12. The additive model and the simplex dominant gene action model (1-dom-alt) were found to be most important for SPW resistance signifying the importance of both additive and non-additive gene action in the control of SPW resistance. This is the first GWAS study to report significant SNPs associated with sweetpotato weevil resistance. The QTL identified will be quite resourceful in next-generation genomics and/or marker-assisted breeding in the genetically complex hexaploid sweetpotato and will expedite the development of high yielding, weevil resistant varieties.

From this study, the following are recommended: 1) Further tests and selection of the best performing genotypes for potential variety release or deployment in research as parental genotypes; 2) Further tests to confirm the yield penalty due to sweetpotato weevil resistance followed by strategic decisions such as developing targeted weevil resistant genotypes for the drier weevil-prone areas; 3) Validation of the genomic regions detected to be controlling sweetpotato weevil resistance in another sweetpotato population to confirm their usefulness before they are deployed in genomic and/or marker assisted selection, and 4) Identification of specific genes controlling sweetpotato weevil resistance using a higher marker density, to facilitate future molecular or genomics-related research.