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Contribution of synthetic tetraploids (AAAA) and diploids (AA) to black Sigatoka resistance and bunch weight to their triploid progenies

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ABSTRACT

The generation of banana triploids from tetraploid–diploid crosses requires knowledge on the influence of the parents on black Sigatoka resistance and agronomic traits to the triploid progenies. The objective of this investigation was to determine the influence of tetraploid and diploid parents on black Sigatoka resistance and agronomic traits in the triploid progenies generated from tetraploid–diploid crosses. The mating scheme was designed as a 4 × 5 North Carolina II mating design. Due to problems in seed set and germination, progenies from 2 male parents with 4 female parents were evaluated at two sites in Uganda. The results showed that the male-parent triploid progeny heritability estimate for the number of leaves at harvest was greater than the female parent estimate. The diploid parents had higher correlation coefficients for the total leaves at harvest with the triploid progenies than tetraploid parents with triploid progenies. Disease development over time took more days in diploid parents than in the tetraploid parents with the triploid progenies as intermediates. These results suggested that diploids transferred black Sigatoka resistance to the triploid progenies as measured by the number of standing leaves and disease development overtime. There was a positive correlation ($P < 0.05$) between tetraploid female parents and triploid progenies for plant height and bunch weight. The triploid progeny–tetraploid female parent heritability estimates for plant height (0.92) and bunch weight (0.72) were highly significant. These results indicated that the female synthetic tetraploids influenced plant height and bunch weight in the triploid progenies. Therefore, it is important to select the tetraploids with heavy bunches to effectively improve yield in triploid progenies generated by tetraploid–diploid crosses. The tetraploid–diploid progenies had a significant ($P < 0.05$) family-by-site interaction for bunch weight indicating that new banana genotypes need to be tested across different environments to select stable genotypes to promote to end-users.

1. Introduction

The yield losses caused by black Sigatoka on bananas in Uganda have resulted in food insecurity problems especially in the subsistence sector. Black Sigatoka causes severe yield decline on bananas through reducing the photosynthetic area and thus causing poor bunch filling. All the East African highland banana cultivars are susceptible to black Sigatoka with a yield loss estimated at 37% (Tushemereirwe, 1996). Cultural and chemical management strategies for black Sigatoka have not proved effective as observed from available reports. Banana production in large commercial plantations depends greatly on fungicide sprays to control black Sigatoka. However, resistance of *Mycosphaerella fijiensis*, a pathogen that causes black Sigatoka, to benomyl in Honduras was reported (Stover, 1977; Romero and Sutton, 1997). Resource limited farmers who cannot afford chemicals use cultural control methods like deleafing to control black Sigatoka. Although, deleafing reduces disease inoculum (Carlier et al., 2000) reduction of leaves reduces the photosynthetic area available for fruit filling. In irrigated banana fields, efficient irrigation methods combined with optimum plant densities have been practised to reduce relative humidity (Wilemaker, 1990; Carlier et al., 2000) without stopping germination of ascospores. The use of host plant resistance appears to be the most economical and sustainable way of managing black Sigatoka on bananas and plantains.

Despite sterility in triploids, some cultivars among the East African highland bananas are fertile thus making it possible to improve the triploid bananas through hybridisation. The female fertile East African highland varieties were crossed with a diploid male parent Calcutta 4 to generate synthetic tetraploids (AAAA) (Ssebuliba et al., 2000). The synthetic tetraploids were resistant to black Sigatoka and conserved desirable traits from the original

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East African highland bananas. Other banana breeding programmes through this triploid × diploid breeding scheme have generated tetraploids that acquired resistance to black Sigatoka resistance (Vuylsteke et al., 1993a; Rowe and Rosales, 1996). However, with exposure to viable pollen, the synthetic tetraploids would form seeds. The seed formation would reduce the fruit quality hence making them unacceptable to farmers.

Pillay et al. (2004) and Tomekpe et al. (2004) proposed a scheme to produce sterile triploid bananas by crossing tetraploids with diploids to generate secondary triploids. The Ugandan National Banana Research Programme has adopted this scheme in the improvement of East African highland bananas to black Sigatoka and other biotic constraints.

The meiotic behaviour of autotetraploids complicates genetic studies between the tetraploid–diploid crosses. During meiosis, tetraploid chromosomes can form univalents, bivalents, trivalents, and quadrivalents in different proportions. For instance, Vuylsteke (2001) and Oselebe et al. (2006) observed more than 90% triploids progenies, 3% diploids and less than 1% tetraploids and pentaploids progenies from tetraploid plantain with diploid crosses. This implies that ploidy analysis has to be carried out to confirm progeny outcomes. The presence of dominant and recessive alleles at a locus is another factor that makes genetic studies in tetraploids difficult. The nulliplex, simplex, duplex, triplex and quadruplex genotypes will form different gamete frequencies depending on whether chromosomes form univalents, bivalents, trivalents and quadrivalents (Singh, 1993). The poor seed set in bananas, self-incompatibilities and low numbers generated from banana crosses makes it difficult to relate progeny outcomes with expected gamete ratios thus making it difficult to undertake genetic studies in autotetraploids.

Despite the challenges some inferences have been made on the outcomes of crosses involving tetraploid bananas. Tenkouano et al. (1998) reported that plantains female tetraploids parents were influential in determining yield ($\mathrm{t\,ha^{-1}\,yr^{-1}}$), leaf retention index and time to flowering in the secondary triploids generated through tetraploid–diploid crosses. After analysing the contribution of tetraploids to triploid progenies, Ortiz (1995) and Tenkouano et al. (1998) suggested that some traits like days to flowering and suckering behaviour can be improved in the tetraploid background. One approach can be by carrying out 4x by 4x crosses. This approach could help accumulate quality traits derived from the East African highland bananas in the current synthetic AAAA tetraploids, provided there would not be self-incompatibility and inbreeding problems within tetraploid progenies. The studies reported have been carried out on plantains. There is no information reported on the influence of synthetic tetraploid bananas on the triploid bananas generated from tetraploid–diploid crosses.

Despite reports that some traits could be improved in the tetraploid background, banana improvement has concentrated on improving traits in the diploid background. Apart from traits like disease resistance which has been confirmed to be inherited from the diploids (Vuylsteke et al., 1993a; Rowe and Rosales, 1996), the information on inheritance of other beneficial traits from diploids through tetraploid–diploid crosses to triploids is not available. This information will help breeders to identify which traits to improve in the parental background.

It is also important to understand the relationship between black Sigatoka resistance and other traits of importance if black Sigatoka resistant materials are to be acceptable to end-users. Ortiz (1995) reported a positive relationship between fruit size and bunch weight in diploids and a positive relationship between plant height and plant girth in plantain tetraploids. Later Ortiz and Vuylsteke (1998) reported that yield potential was negatively associated with days to harvest, and fruit weight was correlated positively with fruit girth in (AAA) bananas. It is not clear how these traits are inherited in triploid progenies from tetraploid–diploid crosses. Information on the possible associations of traits is important in designing better banana improvement strategies. The objective of the study was to determine the influence of tetraploid and diploid parents on black Sigatoka resistance and agronomic traits in the triploid progenies generated from tetraploid–diploid crosses.

2. Materials and methods

2.1. Progenies for genetic studies

The male parents (AA) were selected based on their pollen fertility, bunch weights and black Sigatoka resistance while the selection of female parents (AAAA) was based on their acceptability and yield in terms of bunch weight. Female and male flowers were bagged to prevent contamination with unwanted pollen. Crosses were designed using a 4 × 5 North Carolina II mating design. The females that were selected were 199k-4, 365k-1, 376k-7, 401k-1 and 660k-1. The selected males were Calcutta 4, 8075, 9719 and Pitu. Hand pollinations were performed between 6.30a.m. and 7.30a.m. according to Shepherd (1960). Clusters of anthers with pollen from the male parent were rubbed on the stigmas of designated female parents. The female flowers are arranged in clusters. The clusters open in succession on several days. The pollinations were carried out as flowers opened. On average it took 3–4 days to pollinate a 5–6 cluster bunch. The plastic bag was removed a day after pollination of the last cluster. The pollinated bunches were labelled with tags indicating the cross number, parents and the date when pollination was started. The same information was recorded in a field book. In addition, the field book had information on the day each cluster was pollinated. At physiological (stage when the banana fingers have completely filled) maturity the pollinated bunches were harvested, ripened in an enclosed room and seeds extracted. Immediately after extraction, seeds were taken to the laboratory and germinated in vitro using the embryo culture method (Vuylsteke and Swennen, 1992).

2.2. Experimental design and management

The triploid progenies together with their parents were planted in a randomised complete block with two replicates at each site. Each replicate had eight plants of each cross (family) and the parents. The test families were exposed to natural black Sigatoka infestation. To ensure that each test plant had an equal exposure to the disease, each family was planted between rows of a black Sigatoka susceptible local check 'Mbwazirume' which served as a spreader for the disease. Plant spacing was at 3 m × 3 m.

The two sites were Kawanda Agricultural Research Institute (KARI) and Masaka District Farm Institute, Kamenyamigo. Kawanda Agricultural Research Institute is located at 00′22″N, 00′32″E at an altitude of 1193 m above sea level. The strip where the experiment was laid out at Kawanda site has a sandy clay loam texture with a pH range of 5.4–5.9 (determined in water). Kamenyamigo is located at 00′18″S, 00′33″E at an altitude of 1240 m above sea level. The soil has a sandy clay texture. The soil pH was about 5.1 (determined in water). The trials were planted on 26th April 2007 and 3rd May 2007 at KARI and Kamenyamigo sites, respectively. All the experimental plots both in Kamenyamigo and KARI had low levels of Nitrogen (critical level 0.2 mg/kg). About 10 kg of kraal manure (containing 9.2% organic matter, 0.42%N, 1093 mg/kg of phosphorus, 4134 mg/kg of potassium, 4860 mg/kg of calcium, and 1476 mg/kg of magnesium, and pH of 9.7) was applied per hole at planting and 6 months after planting.

Two and six months after planting the trials were mulched with swamp grass to a thickness of about 10 cm. A mulch thickness of