African Crop Science Journal, Vol. 12. No. 1, pp. 59 - 65, 2004
Printed in Uganda.
ISSN 1021-9730/2004 $ 4.00

CONVENTIONAL BREEDING STRATEGIES TO ENHANCE THE SUSTAINABILITY OF *MUSA* BIODIVERSITY CONSERVATION FOR ENDEMIC CULTIVARS

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ABSTRACT

Africa is one of the secondary centers of diversity for the genus *Musa*. There is a wide range of genetic diversity in the bananas found in the continent with different types dominating different ecological sub-regions. Banana production in Africa is affected by a number of diseases and pests. The development of resistant cultivars is considered to be the most effective approach to controlling diseases and pests. Banana breeding at the International Institute of Tropical Agriculture (IITA) involves crossing triploid (3*x*) landraces with diploid (2*x*) accessions to produce tetraploid (4*x*) hybrids. The 4*x* hybrids are crossed with improved 2*x* accessions to produce secondary triploids. In this study, the female fertility of 70 East African highland banana landraces was assessed. This was done through hand pollination with pollen from a wild diploid banana, *Musa acuminata* spp. *burmannicoides* Calcutta 4'. Results indicate that 50% of the landraces were female fertile and produced true seeds. Seeds were germinated by embryo rescue and the hybrids obtained were assessed to exhibit increased resistance to black sigatoka. However, bunch weights of the hybrids were generally lower than the control landrace, 'Mbwazirume'. By crossing diverse genotypes, producing new populations and selecting improved genotypes, conventional breeding has the potential of increasing banana diversity at the farm level. It is thought that adoption of modern cultivars by farmers could lead to genetic erosion of landraces. However, studies in other crops showed that despite adopting modern cultivars farmers still maintain landraces. Both *Ex-situ* (off-site) and *In-situ* (on-site) conservation methods are important for *Musa*. *In-situ* conservation is appropriate for wild species in their natural habitats allowing the continuation of natural evolutionary processes and for landraces that were selected for important traits over many years by farmers. Wild species could also be conserved *Ex-situ* in field gene banks, *In vitro* culture and cryopreservation especially where their natural habitats are in prone to destruction. Field gene banks are more appropriate for breeders providing opportunities for continual assessment of the germplasm.

Key Words: Accessions, black sigatoka, cryopreservation, hybrids, land races

RÉSUMÉ

L'Afrique est un des centres secondaires de diversité pour le genre *Musa*. Il y a une large gamme de diversité génétique dans les bananes trouvées dans le continent avec des types différents dominant les différentes sous région écologiques. La production de banane en Afrique est affectée par un nombre des maladies et pestes. Le développement des variétés résistants est considéré être l'approche la plus effective pour contrôler les maladies et pestes. La reproduction de banane à l'Institut Internationale d'Agronomie Tropicale (IITA) implique le croisement triploïde (3*x*) des races de terre avec les accessions de diploïde (2*x*) pour produire les tétraploïdes hybrides (4*x*). Les hybrides 4*x* sont croisés avec les accessions 2*x* améliorés pour produire les triploïdes secondaires. Dans cette étude, la fertilité féminine des races de terre de banane de 70 régions de montagne de l'Afrique de l'est était évaluée. Ceci était faite à travers la pollination ouvrière avec le pollen d'un diploïde de banane sauvage, *Musa acuminata* spp. *burmannicoides* Calcuta 4'. Les résultats indiquent que 50% des races de terre étaient de fertilité féminine et ont produit des vraies graines. Les graines étaient germées par l'aide d'embryon et les hybrides obtenus étaient évalués pour exhiber l'augmentation de résistance au sigatoka noire. Cependant, les poids de bouquet des hybrides étaient généralement bas que les races de terre de contrôle, 'Mbwazirume'. En croisant diverses génotypes, produisant des nouvelles populations et en sélectionnant des génotypes améliorés, la reproduction conventionnelle a le potentiel d'augmenter la diversité de banane au niveau de la ferme. Il est pensé que l'adoption des variétés modernes par les fermiers pourrait mener à l'érosion génétique de la race de terre. Cependant, les études sur les autres plantes ont montré que malgré l'adoption des variétés modernes les fermiers continuent à maintenir les races de terre. La conservation des deux méthodes *Ex-situ* (sur site) et *In-situ* (sur site) est importante pour *Musa*. La conservation *In situ* est appropriée pour les espèces sauvages dans leurs habitats naturels permettant la continuation du processus évolutionnaire naturel et pour les races de terre qui étaient sélectionnées pendant beaucoup d'années par les fermiers pour les traits importants. Les espèces sauvages pourraient aussi être conservées dans les banques des gènes sur terrain *Ex-situ*, en culture *In vitro* et en cryopréservation spécialement la où leurs habitats naturels sont en destruction prône. Les banques des gènes de terrain sont plus appropriées pour les reproducteurs pourvoyant les opportunités pour l'évaluation continuelle du germplasme.

Mots Clés: Accessions, sigatoka noire, cryopréservation, hybrides, races de terre

INTRODUCTION

Banana (*Musa* spp.) is an important food and cash crop in the humid forest and mid-altitudes zones of Africa. Different types of bananas including the plantain (AAB), cooking banana (ABB), dessert (AAA) and the east African highland (AAA) are cultivated in different sub-regions. The east African highland banana comprises over 80 triploid ($2n = 3x = 33$) clones that are classified into two types: (i) 'matooke' and (ii) 'mbidde' that are used primary for cooking and beer production, respectively. These bananas are endemic to the East African highlands, which are high rainfall regions ranging between 1200 and 1900 m above sea level and do not occur anywhere else in the tropical world.

Edible bananas originated in the forests of South-East Asia and the western pacific regions (Simmonds, 1962) and spread throughout the tropical regions of the world where they have become of greater importance as a food crop. It is hypothesised that bananas were initially transported from Indonesia to Madagascar via the Indian Ocean and thereafter reached the East African coastline (Robinson, 1996). The plant diversified through an accumulation of somatic mutations (De Langhe, 1961) that seemed to have mainly influenced the bunch characteristics. Human selection, climatic and physio-geographic influences could also have played a role in broadening the crop's genetic diversity (Karamura, 1998). East Africa is now regarded as a secondary center of diversity for this *Musa* AAA group (Swennen *et al.*, 1995). There are 84 distinct cultivars of highland bananas in Uganda alone (Karamura, 1998). Banana germplasm collections are also present in Burundi, Kenya, Rwanda and Tanzania. These collections have not been fully characterised. Our DNA marker studies showed that the banana cultivars in Burundi are very different from those in Uganda. This information implies that the genetic diversity of bananas in East Africa is quite high even if one considers that a number of local names may exist for the same cultivar in the different nations surrounding the East African great Lake region (Rossel, 1998).

Genetic resources are a necessary staring point for plant improvement (Berthaud, 1997). Banana production in East Africa is affected by a small number of diseases and pests, yet they are responsible for highly devastating losses in yield and production (De Vries and Toennissen, 2001). They include the fungal diseases black Sigatoka (*Mycosphaerella fijiensis*) and Fusarium wilt (*Fusarium oxysporum*), a complex of nematodes (*Radopholus similis*, *Pratylenchus goodeyi*, *Helicotylenchus multicinctus*), the banana weevil (*Cosmopolites sordidus*) and viruses, especially banana leaf streak. The use of resistant cultivars is considered the most effective, economical and environmental friendly approach to controlling diseases and pests (Line and Chen, 1995). There is concern that adoption of cultivars that are resistant to pests and disease infestation by farmers could lead to genetic erosion of landraces or folk varieties. The purpose of this study is to show that banana breeding for the production of improved technologies can play a role in increasing banana biodiversity on farm.

Breeding strategies in *Musa*. High male and female sterility handicaps sexual reproduction in banana. The breeding approach involves crossing triploid (3*x*) landraces with diploid (2x) accessions that carry useful genes to produce tetraploid (4*x*) hybrids. The 4*x* hybrids are usually both male and female fertile and produce few to many seeds thereby reducing their consumer acceptability. To overcome this limitation, the 4*x* hybrids are crossed with improved 2*x* accessions to produce secondary triploids that are seedless (Pillay *et al.*, 2002). The hybrids are evaluated for disease resistance and other agronomic characters over a number of years firstly on station, then over multiple locations before being released as cultivars to farmers (Fig. 1).

MATERIALS AND METHODS

Genetic material and crossability. Seventy east African banana landraces, representing the five major clone sets (Karamura, 1998) were used in this study. The plants were screened for female fertility by crossing with an unrelated male fertile wild diploid accession, *M. acuminata* spp. *burmannicoides*, 'Calcutta 4'. Pollinations were carried out as described by Ortiz *et al.* (1998a). Bunches were harvested at full maturity at least 90 days after the initial pollination.

Seed set and embryo culture. The harvested bunches were stored in a ripening room containing acetylene for four days. The yellow fruits were peeled and the seeds were extracted manually from the pulp with a mechanical pressing device. The number of seeds from each bunch was recorded. The embryos from fertile seeds were excised and cultured aseptically *in vitro* as described by Vuylsteke *et al.* (1990).

Field experiments. Two-month old seedlings and parental genotypes were established in early evaluation trials. All field evaluations were carried out at IITA's research site at Namulonge (0° 32' N, 0° 32' E), in Uganda. The experimental design was an unbalanced randomised complete block design with three replications. Preliminary screenings for black sigatoka resistance, vegetative and fruit characteristics were recorded.

RESULTS

Seed set for the five major clone sets of east African highland bananas is shown in Table 1. Only 35 of the 70 landraces used in this study were shown to have female fertility. The Nfuuka and Nakabululu clone sets produced more seeds per

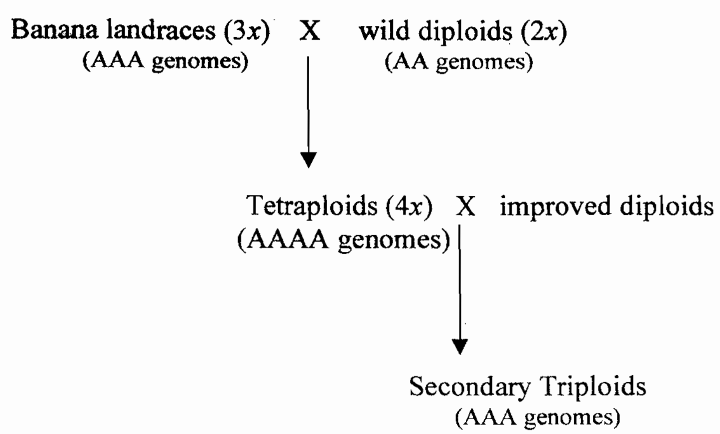

Figure 1. Simplified diagrammatic representation of breeding scheme used for banana breeding at IITA (adapted from Pillay *et al.*, 2002).

bunch than the others. The agronomic characteristics of selected hybrids are shown in Table 2.

DISCUSSION

Hybrid development. Less than 50 % of the 70 banana landraces in Uganda showed some degree of female fertility (Table 1). Majority of these landraces belonged to the Nfuuka and Nakabululu clone sets. Landraces that were fertile also had very low seed set. The fertile seeds required embryo rescue before germination. Despite these difficulties, this study showed that it is possible to obtain true seeds and hybrid plants from crossing some of the east African landraces with wild bananas. This was also shown earlier for plantains in West Africa (Vuylsteke *et al.*, 1993; Ortiz *et al.*, 1998b). The hybrids are now being tested in early evaluation and preliminary yield trails. This study showed that some of the hybrids generated in this study had an increased resistance to black sigatoka (Table 2) a disease that is generally responsible for up to 50% yield losses in banana (Mobambo *et al.*, 1993). Resistance to black sigatoka was introgressed from the wild diploid 'Calcutta 4' used as the male parent in this study. 'Calcutta 4' is also considered to be resistant to the banana weevil (*Cosmopolites sordidus*) and some nematodes species that affect the crop. The bunch weights of the hybrids were generally less than that of the control (Table 2). However, we hope to increase bunch weights and maintain resistance to sigatoka by crossing these primary selected hybrids to advanced diploids.

About 50% of the banana landraces in Uganda were infertile and could not be used directly for genetic improvement. This implies that a certain degree of genetic erosion is expected with loss of alleles from the sterile clones. It would be important to understand the factors affecting fertility in bananas so that sterility in some of the important female landraces could be overcome. However, new methods of plant improvement such as genetic transformation offers the potential of adding one or a few genes to a landrace cultivar without altering its genetic make-up. Genetic transformation can, therefore, be envisaged as a tool for increasing diversity in sterile bananas. Similarly, although identification of desirable traits

TABLE 1. Summary of seed set by clone sets in east African highland banana landraces pollinated with 'Calcutta 4' (December 1994- November 1997)

Clone set	No. of landraces	No. of seed fertile landraces	No. bunches pollinated	Pollination success (%)	Total seed set	Seed set per bunch
Musakala	10	1	66	1.52	11	0.17
Nfuuka	29	24	655	47.90	3792	5.79
Nakitembe	6	0	73	0.00	0	0.00
Nakabululu	7	5	115	60.87	705	6.13
Mbidde	10	3	100	4.00	9	0.09

TABLE 2. Preliminary evaluation of black sigatoka resistance and yield parameters of some selected hybrids compared to the landrace 'Mbwazirume' (control)

Hybrid	Female parent	Male parent	INSL[1] (%)	Bunch weight	No. of hands	No. of fingers
365K-1	Kabucuragye	Calcutta-4	45.5±12.3b	13.3±1.2bc	9.1±0.3a	165.5± 8.0a
376K-7	Nante	Calcutta-4	40.0±18.0b	10.1±1.8cd	6.2±0.5cd	100.0±11.9cd
660K-1	Enzirabahima	Calcutta-4	100.0±11.7a	15.5±1.1ab	6.9±0.3bc	117.2±7.7bc
917K-2	Enzirabahima	Calcutta-4	60.0±10.5b	12.1±1.0bc	6.7±0.3bcd	118.7±6.9bc
1201K-1	Nakawere	Calcutta-4	30.0±12.9bc	11.2±1.3cd	5.8±0.3d	93.5±8.4d
1438K-1	Entukura	Calcutta-4	51.2±10.9b	8.4±1.1d	5.8±0.3d	82.3±7.1d
Control	Mbwazirume		7.1±10.2c	17.1±1.0a	7.6±0.3b	123.3±6.6b

[1] Index of non-spotted leaves
Means with same letter (s) are not significantly different ($P < 0.05$)

in wild relatives is relatively easy, the difficulty of introgressing the desired character into improved crop germplasm without introducing undesirable characteristics associated with the wild species is problematic (Hawtin *et al.*, 1996). Genetic transformation is an opportunity of introducing specific useful genes directly into elite banana cultivars.

Conserving wild banana germplasm. Wild relatives of crop plants that have survived under strong natural selection pressures are useful as a source of genes for specific adaptive traits. In view of this, *in-situ* conservation, in the wider context, should be of paramount importance in regions where banana originated and where wild diploid species still exist. *In-situ* conservation favors evolution of plants in their natural conditions as they have been for a long time facing diseases and pests. Some species of *Musa* have already been placed on the IUCN 'Red list of Threatened Plants' (Walter and Gillet, 1998). Our edible bananas originated in the rainforests and the alarming rate of plant loss from forest clearings is threatening wild banana variability such as that of *M. acuminata* and other *Musa* species (Ingram, 1999). Therefore, *ex-situ* conservation encompassing field gene banks, *in vitro* culture and cryopreservation should also become integral components of banana biodiversity conservation. Banana field gene banks are of more value to the plant breeder than other forms of *ex-situ* conservation. Our field gene bank consists of banana accessions from a number of banana breeding programs and from the *Musa* Germplasm Transit Center, Leuven, Belgium. These accessions are being evaluated and screened for specific traits useful in breeding East African highland bananas. One of these accessions, 'Kikundi' has been found to be resistant to black Sigatoka. The plant's bunch characteristics resemble those of East African highland landraces indicating its potential for the improvement of these bananas.

Breeding increases banana diversity. By displacing highly diverse landraces with relatively few bred cultivars, plant breeding is generally perceived as the cause of genetic erosion (Simmonds, 1979; Beets, 1990). Plant improvement programs tend to produce relatively few widely adapted and often inter-related cultivars that displace diverse and narrowly adapted landrace cultivars. New cultivars created by breeders give higher yields than the highly diverse landraces and tend to replace the landraces. For example, the IRRI (International Rice Research Institute) rice cultivar IR36 once occupied over 6 million hectares around the world (Beets, 1990). These plants were nearly genetically identical and had a high level of genetic vulnerability to diseases and pests. On the contrary, by using a variety of female and male parents in our banana-breeding program, our study is expected to produce a variety of populations from which plants with useful agronomic traits are selected for the farmer. Banana breeding, therefore, has the potential for increasing genetic diversity in farmer's fields by introgressing genes from wild and related species into the landraces. The diversity present in the wild plants is generally greater than that in the landraces. Diversity in the East African highland bananas is believed to have arisen by mutations and selection by farmers over the last 1500 years (Simmonds, 1962). Previous studies have shown that the East African bananas have a narrow genetic base (Pillay *et al.*, 2001). By creating new populations through breeding the genetic diversity of these bananas could be increased by enabling new allelic recombination. Triploids and tetraploids generated from the crosses in this study are expected to contain 33 % and 25 % of wild banana alleles.

Banana breeding strategies involve tissue culture. A common feature of tissue- culture derived plants is the potential of somaclonal variation, that is, genetic variation generated during tissue culture that can be stably passed onto progeny. Therefore, somaclonal variation could be exploited as a source of increasing genetic diversity in banana. In populations of the 'False Horn' plantain, 'Agbagba' derived from tissue culture, changes in inflorescence morphology in the form of a reversion to a typical 'French' with increased female fertility was obtained (Vuylsteke *et al.*, 1997). Somaclonal variation was useful in initiating breeding in the 'False Horn' plantain pool that is completely sterile. Nwauzoma *et al.*, (2002) also identified superior somaclonal mutants in *Musa*. Ploidy level changes have been observed in banana plants stored for long periods *in-vitro*. Therefore, *in vitro ex-situ* conservation of banana could also enhance genetic variability. These examples demonstrate that routine biotechnology can be employed to create variation within existing *Musa* populations.

Adoption of modern cultivars. It was generally believed that the introduction of modern cultivars would lead to the genetic erosion of landraces or folk varieties (Hawkes, 1983). On the contrary, there is increasing evidence that farmers still retain their landraces even after introducing modern varieties into their fields. Brush (1995) outlined three instances where farmers in regions of high crop diversity maintained their landraces while still adopting high yielding crop cultivars and high-input technology. Generally, farmers retain the folk varieties for their social or cultural value, economic or nutritional importance, or environmental adaptation (Soleri and Smith, 1995). In the case of potato, landraces were regarded as superior in flavor, with better storage potential due to a higher percent of dry matter, landraces had higher market prices, landraces were prized as gifts and as special payment for exchange of labour and finally landrace mixtures are kept for potential future markets. Landraces were grown mainly for home consumption since less chemical inputs were required (Brush, 1995).

CONCLUSION

In this study, we have demonstrated that at least 50% of the East African highland banana landraces is fertile with potential for genetic improvement by conventional breeding. By creating new populations from crosses involving diverse parents, banana breeding can increase the diversity of existing bananas. In addition, biotechnological tools such as genetic transformation and *in vitro* methods can create new variation and increase diversity of existing populations.

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