Plant Cell, Tissue and Organ Culture **42:** 269–274, 1995.

Variability in storage potential of banana shoot cultures under medium term storage conditions

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Received 27 July 1994; accepted in revised form 23 June 1995

Key words: Conservation, low temperature, *Musa*

Abstract

Shoot cultures of 401 banana clones were conserved under slow growth conditions (16 ± 1 °C, 25 μmol m^{-2} s^{-1}). Storage duration - defined as 60 % survival time of 20 shoot cultures of a clone - averaged 334 days. However, large differences occurred among the different genomic (sub)groups and even within the same (sub)group. East-African highland bananas and non-plantain AAB bananas can be stored for significantly longer periods. Shoot tip cultures of another 41 banana clones conserved at higher ambient temperature (22 ± 3 °C) needed to be subcultured sooner (every 220 days on average).

Abbreviations: BA – 6-benzyladenine, CIRAD – Centre de Coopération Internationale en Recherche Agronomique pour le Développement, IAA – indole-3-acetic acid, IBPGR – International Board for Plant Genetic Resources, INIBAP – International Network for the Improvement of Banana and Plantain, PPF – photosynthetic photon flux, QDPI – Queensland Department of Primary Industries

Introduction

Bananas and plantains (*Musa* spp.) are among the most important food crops in the world. They are a staple food for at least 400 million people and an important part of the diet of another 600 million people in the tropics (estimations based on FAO 1992). Moreover, they are a substantial export commodity for several tropical countries.

There exists a wide range of genetic variability in *Musa* in morphological and physiological characteristics and in culinary uses (for cooking, roasting, raw consumption or beer production) (Simmonds 1966). Inter- and intraspecific hybridizations between the wild species *Musa acuminata* Colla (AA genome) and *Musa balbisiana* Colla (BB genome), both originating from Southeast Asia, have generated the genomic constitutions of the edible cultivars, with the AA diploids and the AAA. AAB and ABB triploids being the most important ones (Simmonds & Shepherd 1955). Within the AAA group, the East-African highland bananas constitute a very distinct subgroup. Plantains, on the other hand, form a well-defined subgroup among the AAB bananas (Simmonds 1966).

It is generally agreed that the preservation of the naturally occurring variability in crops is of tremendous importance for mankind (Ford-Lloyd & Jackson 1986). Since bananas do not normally set seed, they are often conserved in field gene banks, which require much land and labour due to the large size of these plants. Due to their exposure to pests and diseases, and thus constant risk of loss, the establishment of *in vitro* collections consisting of shoot cultures started from meristems is an attractive alternative for working collections or collections for long-term conservation. In our working collection, shoot cultures need to be subcultured at 2–5-month intervals if maintained at 28 °C and 63 μmol m^{-2} s^{-1}. It has been shown that a reduced temperature (15 °C) and PPF (25 μmol m^{-2} s^{-1} for 24 h) significantly increased the subculturing

interval (Banerjee & De Langhe 1985; De Smet & Van den houwe 1991). However, these growing cultures are permanently threatened by the occurrence of somaclonal variation (Vuylsteke *et al.* 1991; Côte *et al.* 1993), which might ultimately result in the loss of valuable germplasm. The arrest of growth by freeze preservation would simultaneously overcome this limitation and reduce the workload. Methodologies for cryopreservation in liquid nitrogen of *Musa* embryogenic cell suspensions have been established (Panis *et al.* 1990), but the production of embryogenic cell suspensions takes from 6 up to 12 months and is genotype dependent (Dhed'a *et al.* 1991; Escalant & Teisson 1993). Cryopreservation of *Musa* apices is under investigation and has shown a survival rate of 7–58% only (Panis *et al.* 1994; Panis 1995). Cryopreservation of zygotic embryos of banana (Mora 1990) and freeze-preservation of DNA-rich materials in general (Adams 1994) could be envisaged.

INIBAP (Montpellier, France) currently maintains its *Musa* working collection consisting of 1015 accessions (INIBAP 1992) at the INIBAP Transit Centre at the Catholic University of Leuven. All germplasm is kept *in vitro* to allow a quick supply of germplasm upon request. Currently nearly one accession per working day is supplied to collaborators worldwide.

Growth of banana shoot cultures can be slowed down by changing osmotic conditions of the culture medium (Mora *et al.* 1988). However, the most successful and most widely applied approach to slow *in vitro* growth in crops is the reduction of temperature (Withers 1992). Several crops have shown genotypic differences (both on the species and on the cultivar level) in storage potential at low temperature, such as coffee (Bertrand-Desbrunais *et al.* 1991), strawberry (Reed 1991), beet (Miedema 1982), apple (Wilkins *et al.* 1988), grape (Barlass & Skene 1983) and yam (Malaurie *et al.* 1993).

Here we report the performance of more than 400 *Musa* accessions stored as shoot cultures under limiting growth conditions. The effect of genotype on storage duration is discussed.

Materials and methods

Cone-shaped shoot tips of 8–10mm were isolated from small sword suckers that arrived at the INIBAP *Musa* Germplasm Transit Centre. For each accession shoot cultures were initiated from one single meristem on a semi-solid (2 g l^{-1} Gelrite) Murashige & Skoog (1962) medium with the exception of a double phosphate concentration (400 mg l^{-1} KH_2HPO_4) and supplemented with 10^{-6} M IAA and 10^{-5} M BA and subsequently multiplied to obtain a clone of 20 cultures. Accessions reacted nevertheless differently. Some accessions produced mainly single plants, while others consisted of clusters of many small buds. In the storage room, individual cultures were inspected monthly and those that had become necrotic were eliminated. As soon as only 12 clean and viable cultures of an accession remained, the most vigourous and green ones were multiplied to produce, 20 new cultures and grown in normal growth conditions (30 ± 2 °C, 63 μmol m^{-2} s^{-1}) for 1 to 2 weeks. Storage time is thus defined as the period between the transfer of 20 cultures of an accession into the cold storage room and the removal of 12 cultures for subsequent multiplication.

The storage facilities consisted of two compartments, which differed in ambient temperature : 16 ± 1 °C (compartment A) and 22 ± 3 °C (compartment B). In both compartments the PPF was 25 μmol m^{-2} s^{-1}. In compartment A, 401 accessions with known genomic configuration were stored. Their storage duration was calculated by averaging two subsequent storage periods. In compartment B, only one storage time was determined for 41 other accessions. The age of the shoot cultures (i.e. the time after their introduction *in vitro*) ranged from 1 to 8 years.

It should be noted that contamination was sporadic and thus could have shortened storage time. However, since it appeared at random, no effect on genotypic differences nor on differences due to ambient temperature was anticipated.

Results

Temperature may play a crucial role in storage duration (Table 1). Indeed, average storage duration per genotype was extended from 57 days (for the AA edible cultivars) to 175 days (for the plantains) when stored at 16 ± 1 °C instead of 22 ± 3 °C. However, these values are indicative, since different accessions per genotype are involved at both temperatures. Therefore no statistical comparison was made.

Under the best storage temperature (16 °C) large differences were noted among the accessions evaluated (Fig. 1). Some accessions can be stored up to a maximum of 615 days (Lady Finger - Pome, AAD), whereas others needed to be subacultured every 60

Table 1. Storage duration (days) as influenced by genome configuration and ambient temperature.

Genome configuration	Storage temperature 16 ± 1 °C		Storage temperatur e 22 ± 3 °C		Difference in storage duration days
	N[1]	Storage duration (days)[2]	N[1]	Storage duration (days)[3]	
BBw	10	275 ± 110 d[4]	–	–	–
AAw	40	300 ± 113 cd	–	–	–
AA	69	331 ± 115 bc	16	274 ± 60	57
AAA	49	343 ± 105 b	–	–	–
AAA*	32	390 ± 77 a	–	–	–
AAB	25	386 ± 111 a	6	303 ± 19	83
AAB**	146	324 ± 73 bc	19	149 ± 59	175
ABB	30	345 ± 101b	–	–	–
All genotypes	401	334 ± 79	41	220 ± 87	114

[1] number of observations.
[2] each observation is the average of 2 storage times per clone.
[3] each observation is one storage time per clone.
[4]mean and standard deviation ; means are highly significantly different following GLM (General Linear Model) ($p > f: < 0.0001$) ; means followed by the same letter are not significantly different based on the Duncan multiple range test at the 5% level.
w wild.
* East-African highland bananas (Lujugira-Mutika subgroup).
** plantain subgroup.

days (SF 215, a parthenocarpic AA derivate of *Musa acuminata* spp. *banksii*). These large differences may be due to

- the genomic configuration (Table 1 and Fig. 2) and
- genetic variability within the same genome subgroup (Fig. 2).

The variability in storage potential within a subgroup, e.g. the plantain subgroup, is surprising, since this subgroup is botanically very homogeneous (Tezenas du Montcel *et al.* 1983; Swennen 1990).

The classification of the storage results for the different accessions according to their genome configurations (Simmonds & Shepherd, 1955) indicates that wild bananas are more difficult to store for a long period than edible bananas, although differences were not significant in the case of wild *Musa acuminata* (AAw) accessions versus parthenocarpic AA bananas and plantains (Table 1). Wild *Musa balbisiana* (BBw) accessions could be stored successfully for significantly shorter periods than any genomic (sub)group of parthenocarpic bananas.

Among the triploids, East-African highland bananas and AAB bananas (non-plantain) could be stored significantly longer periods than any other subgroup.

The same trends in genomic differences were also noted at higher ambient temperatures.

Discussion

The results presented here support the conclusion of Banerjee & De Langhe (1985), who had shown that a lower ambient temperature increased storage duration considerably in *Musa*. However, these authors had based their results on seven accessions only. This shorter storage capacity at higher temperature results in increased expenses in labour and consumables. In addition more subculturing increases the risk of contamination and somaclonal variation and thus loss of germplasm.

Large differences for storage duration of *Musa* germplasm under medium term storage conditions were found both among different genome (sub)groups and within genome (sub)groups. Triploids and edible diploids could be stored for longer periods than wild diploids, although differences were not significant for

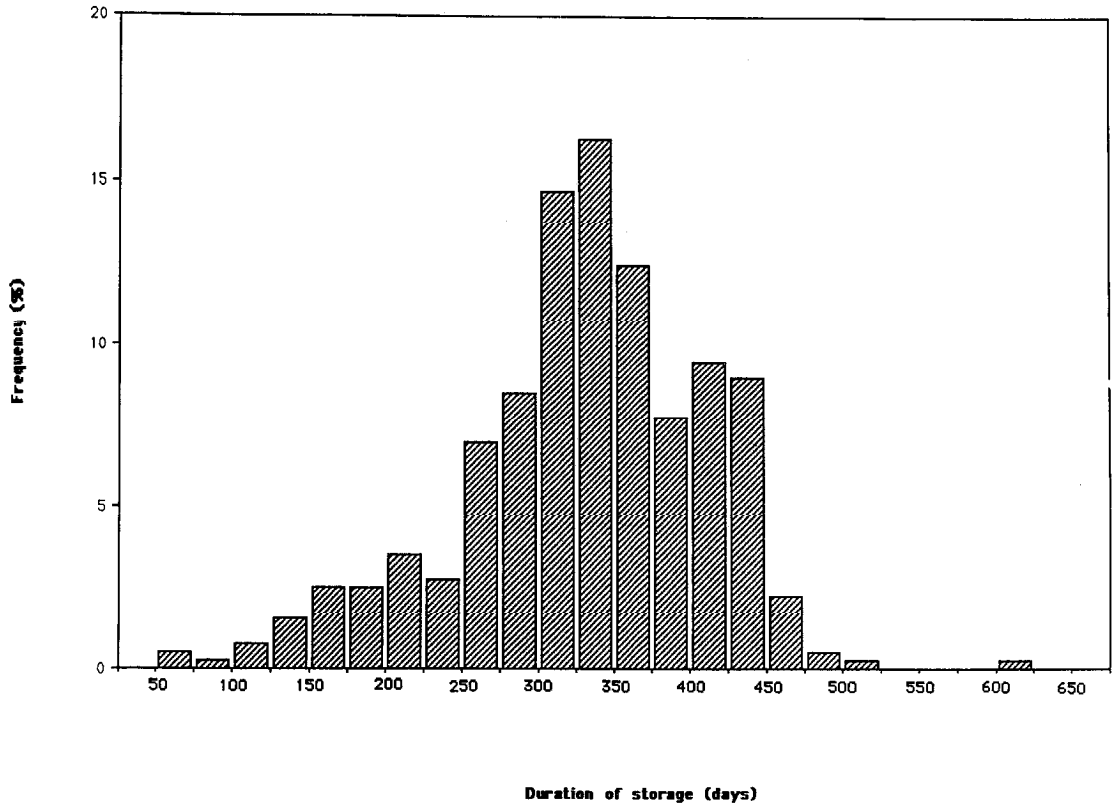

Fig. 1. Storage duration of 401 *Musa* accessions at an ambient temperature of 16 ± 1 °C (mean of 2 replications per accession).

all (sub)groups. The conservation of both wild and edible diploids is important, since they form the basis of most breeding programs (Rowe & Rosales 1993; Stover & Buddenhagen 1986; Swennen & Vuylsteke 1993; Vuylsteke *et al.* 1993; Vuylsteke & Ortiz 1995; Tezenas du Montcel 1990). Since edible diploids tend to be replaced by triploids, which produce larger fruits and thus higher yields (Simmonds 1966), emphasis in any *Musa* collection should therefore be also put on the conservation of the former material. This fact has prompted banana exploration missions in the recent past (IBPGR/INIBAP/QDPI/CIRAD collecting missions in 1987–1988).

The extended storage duration achieved with AAA highland bananas looks somehow surprising, since these cultivars are cultivated in the highlands of East Africa, where they thrive well under cool temperatures. In the East-African highlands, situated at an altitude of 1100–1800 m, air temperature fluctuates between 11 and 27 °C (FAO, 1984). Due to their adaptation to lower temperatures, a more rapid growth *in vitro* at 16 °C, and thus earlier necrosis, could have been expected for these clones.

Both AAA highland bananas and AAB non-plantain bananas, both of which can be stored for the longest periods, often produce cultures that consist mainly of large buds with swollen cormlike tissues. A clear-cut classification of the other (sub)groups according to their behaviour *in vitro* can not yet be made, and thus not yet be linked with their storage potential. A systematic *in vitro* characterisation of the entire collection might be useful in identifying the appropriate *in vitro* appearance for longer storage periods.

Acknowledgements

The authors wish to thank Dr. M.-C. Peeters (K.U. Leuven, Belgium) and Dr. Perea-Dallos (Universidad Nacional, Colombia) for reviewing the article. The maintenance and operation of the INIBAP *Musa* Germplasm Transit Centre is supported. by INIBAP

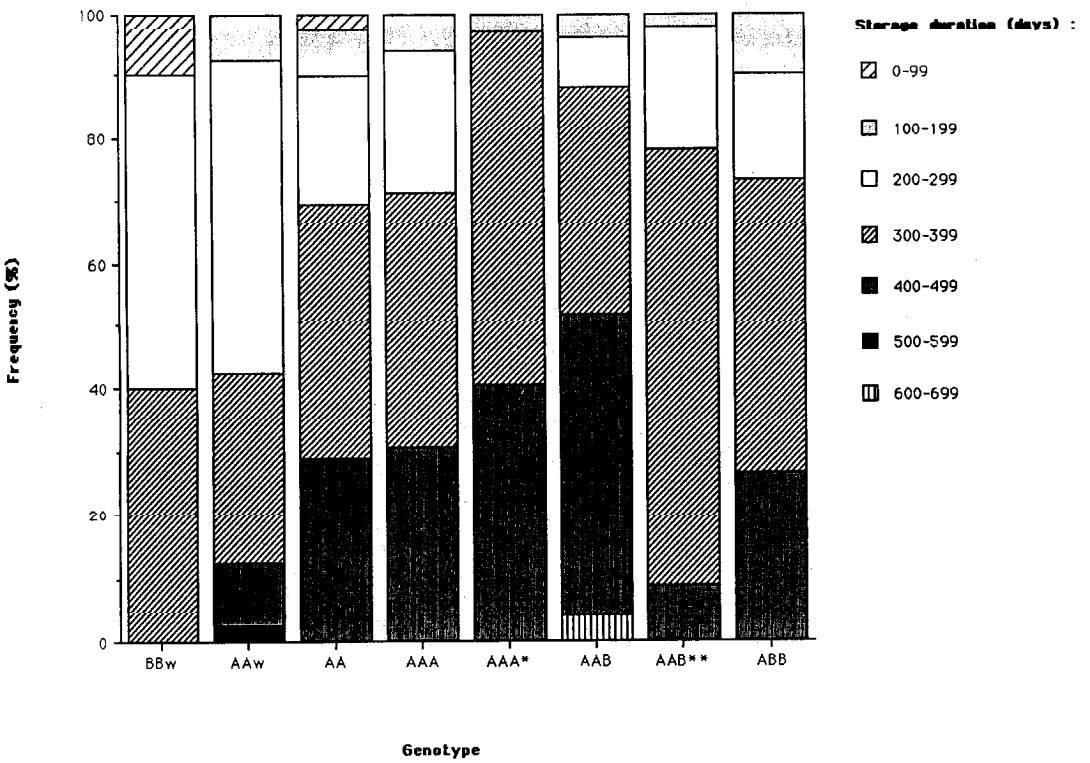

Fig. 2. Frequency distribution of storage duration of eight different genomic (sub)groups of *Musa* at an ambient temperature of 16 ± 1 °C (averages of 2 replications per accession). *East-African highland bananas (Lujugira Mutika subgroup); **plantain subgroup.

through a grant from BADC (Belgian Administration for Development Cooperation).

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