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PROGRESS REPORT

(For the period January 1989 - April 1992)

In Vitro Germ Plasm Preservation of Musa Spp.

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Introduction

The project was initiated in Jan. 1989 following its approval but the major experimental work in Israel was done following the visit of Dr. Ludwig Muller in Israel on May 1989. Fruitful discussions and an experimental program was planned during this visit. It was concluded that the work in Israel will be done on the following major lines:

1. Conservation at temperatures below 15°C.
2. Effect of plant growth inhibitors as a slow growth mean.
3. Development of monitoring methods to detect variants and reduce their occurrence among in vitro cultured plants.

Different experiments were performed on these lines and a part of them which are representing typical repeated results are brought below.

Materials and Methods

Banana *in vitro* cultures were established of the following cultivars: 'Valery' 'Williams', 'Grand Nain', 'Nathan' (an Israeli selection of a Dwarf Cavendish type) and dwarf mutants obtained in *in vitro* multiplied plants of 'Williams', 'Grand Nain' and 'Nathan'. Basically two media were used; one for the multiplication of stock plants and the other one for growing and keeping the plants in culture for conservation purposes (Table 1). Before applying differential treatments plantlets were subcultured

from the multiplication to the conservation medium and grown in the light for 6-8 weeks. After that period their leaves and roots were cut back and they were subcultured on the same medium with or without other additives and were grown in different combinations of low or regular incubation temperature (28°C) and in the dark or under light.

Study of molecular markers for detecting differences between normal and somaclonal variants was done in leaf extracts taken from mature plants grown in a greenhouse.

Plants derived from different families selected in a previous study were *in vitro* propagation and evaluated in the field for genetic stability.

Results

1. Conservation at temperatures below 15°C

From published data and experience in Israel it is known that banana cultures can be kept for 18 months without transfer to fresh medium at 15±2°C. For absolute safety it is recommended not to extend it over 12 months at this temperature. And temperatures below 11-12°C were found to be determent for many genotypes. Though so it was sought that if by preconditioning or by different treatments it would be possible to conserve *Musa* cultures at a temperature of 6°C it will enable to do the transfer once in a few years.

Out of different experiments done on this line, two main conclusions were done 1) cultures that were transfered stright from 28°C to 6°C survived at a reasonable rate if kept at the lower temperature no longer than 1 to 3 weeks (Tables 2 and 3). 2) cultures survived for a longer period at 6°C if they were transferred first to an intermediate low temperature or activated charcoal was added to medium (Table 2 and 3).

2. Effect of plant growth inhibitors as a slow growth mean

The following plant growth inhibitors namely ABA, Alar, CCC, PCIB, Uniconazole, Cultar and Ancymedol were included in the media at different concentrations. At the first stage all experiments were done under light (Tables 4 and 5) and at a later stage in the dark (Table 6) and also a comparison between growing under light or in the dark (Table 7). The main conclusions of these experiments were: 1) a significant reduction in growth

as expressed in plantlet's height and especially in the dark was achieved with relatively high concentrations of the following growth retardents: PCIB CCC, ABA and Alar. 2) the anigibberellin compounds: Uniconazole, Cultar and Ancymedol were found to be most effective in reducing plantlets height and size of their leaves. Both, under light and in the dark compact plants were produced. Of the three antigibberellins Cultar and Ancymedol seems to be more promising since they act in a wide range on concentrations while the desired activity of Uniconazole is limited to a narrow range.

3. Development of monitoring methods to detect variants and reduce their occurence among in vitro cultured plants.

Different approaches were tried to develop moniotoring methods to detect somaclonal variants. One approach was to develop selective media. It included media with different ratios between the major elements (Table 8), plant growth regulators which only part of them are reported here (Table 5), but these were not found to be effective except gibberellic acid (Table 9). As reported before plantlets of the dwarf mutant of 'Williams' were less responsive to GA_3 as compared to normal plantlets of that cultivar. In another experiment the response to GA_3 was studied in another 3 cultivars ('Valery', 'Grand Nain' and 'Nathan') and in dwarf somaclonal variants of two of them (Grand Nain' and 'Nathan'). It was found that all the tree cultivars responded to GA_3 in the same way like 'William' in reduction of leaf production and the dwarf somaclonal variants of the two cultivars were less responsive (Table 10) like the dwarf somaclonal variant of 'Williams' (Table 9).

The technique of protein composition analysis of leaf extracts by two demensional gel electrophoresis did not give satisfactory results in regard to be able to detect somaclonal variants. The ability of using DNA markers was studied. In a preliminary study RAPD markers were tried to determine polymorphic DNA fingerprints to distinguish between normal and dwarf plants of 'Williams'. For this prupose PCR amplification was conducted using 10 mer RAPD primers. So far 40 primers were tested and four of them amplified DNA bands that were polymorphic with respect to normal versus dwarf plants (Fig. 1).

In order to reduce the occurrence of somaclonal variants the possibility of selecting stable families was studied. For this purpose

plants derived from different families selected in a previous study were *in vitro* propagated and evaluated in the field. Data brought (Table 11) confirm the possibility for selecting stable families.

Plans for continuation of the study

It is planned to continue the studies in the main following lines:

1) Conservation studies of plantlets at 6°C with different combinations of media and sequential temperatures which were found to elongate survival. Another approach will be to grow plantlets before and during conservation on media with Ancymedol which was found to produce compact plants. It is expected that such plants will be more tolerant to the low temperature.
2) Reponse studies of different cultivars and their somaclonal dwarf variants to different gibberellins (except GA_3).
3) Continuation of studies with RAPD markers in different cultivars and their somaclonal dwarf variants (except 'Williams').
4) Field evaluation of plants grown on media with plant growth retardants, mainly Ancymedol.

TABLE 1: BASIC MEDIA USED FOR MULTIPLICATION AND FOR CONSERVATION STUDIES

Chemicals	Multiplication	Conservation
Macroelements	MS	MS
Microelements	MS	MS
$NaH_2PO_4.2H_2O$	380.0 mg/l	380.0 mg/l
Inositol	300.0 mg/l	100.0 mg/l
Adenine sulfate	160.0 mg/l	160.0 mg/l
Thiamine HCl	0.5 mg/l	0.5 mg/l
BA	5.0 mg/l	- -
Kinetin	- -	5.0 mg/l
IAA	2.0 mg/l	2.0 mg/l
Sucrose	3%	3%
Agar	0.65%	0.65%
pH	5.8	5.8

TABLE 2: SURVIVAL RATES OF 'WILLIAMS' PLANTLETS AFTER DIFFERENT SEQUENTIAL COMBINATIONS OF TEMPERATURES AT DIFFERENT WEEKS IN CULTURE.

Week No.							Survival
1	2	3	4	5	6	7	
Temperature							(%)
28	28	28	28	28	28	28	100
6	28	28	28	28	28	28	77
6	6	28	28	28	28	28	0
6	6	6	28	28	28	28	0
6	6	6	6	28	28	28	0
16	6	6	28	28	28	28	40
16	16	6	6	28	28	28	25
16	16	16	6	6	28	28	10
16	16	16	16	6	6	28	13
28	6	6	28	28	28	28	100
28	28	6	6	28	28	28	0
28	28	28	6	6	28	28	0
28	28	28	28	6	6	28	0

TABLE 3: SURVIVAL RATES OF 'WILLIAM' PLANTLETS CULTURED ON MEDIA WITH OR WITHOUT ACTIVATED CHARCOAL FOR DIFFERENT PERIODS AT 6°C THEN TRANSFERRED TO 28°C.

Weeks at 6°C	Activated charcoal	
	-	+
1	100	100
3	93	87
4	7	80
5	0	47
6	0	0
7	0	0

TABLE 4: EFFECT OF GROWTH REGULATORS (GR) ON NORMAL (NL) AND DWARF (DW) 'WILLIAMS' BANANA PLANTLETS GROWN IN VITRO FOR SIX WEEKS.

GR		Plantlet type					
		NL			DW		
Kind	mg/l	Height (H)*	Leaves (L)*	H/L	Height (H)	Leaves (L)	H/L
0	0	2.3	3.0	0.8	2.0	4.0	0.5
ABA	1	1.0	1.1	0.9	1.1	1.4	0.8
ABA	5	1.0	0.7	1.4	1.0	1.1	0.9
ABA	10	1.2	2.1	0.6	0.8	0.8	1.0
Uniconazole	0.1	1.5	5.1	0.3	1.3	5.7	0.2
Uniconazole	0.5	1.2	5.6	0.2	1.1	5.9	0.2
Unizonazole	1.0	0.9	2.9	0.3	1.0	4.2	0.2
Average		1.3	2.9	0.6	1.2	3.3	0.5

* H= Height of plantlets in cm, L= No. of new leaves.

TABLE 5: EFFECT OF GROWTH REGULATORS (GR) ON 'NORMAL (NL) AND DWARF (DW) 'WILLIAMS' BANANA PLANTLETS GROWN IN VITRO .

GR		Plantlet type					
		NL			DW		
Kind	mg/l	Height (H)*	Leaves (L)*	H/L	Height (H)	Leaves (L)	H/L
0	0	4.4	3.9	1.1	3.1	3.0	1.0
CCC	0.1	4.9	4.2	1.2	3.4	3.5	1.0
CCC	0.5	5.8	3.5	1.7	4.0	4.0	1.0
CCC	1.0	4.5	4.0	1.1	3.7	3.7	1.0
CCC	5.0	4.5	3.8	1.2	3.2	3.5	0.9
ALAR	0.1	5.0	4.3	1.2	4.2	3.8	1.1
ALAR	0.5	5.0	3.7	1.4	4.1	3.8	1.1
ALAR	1.0	4.6	3.9	1.2	3.9	3.2	1.2
ALAR	5.0	4.6	3.8	1.2	3.7	3.5	1.1
Average		4.8	3.5	1.3	3.7	3.6	1.0

* H= Height of Plantlets in cm, L= No. of new leaves.

TABLE 6: FFECT OF GROWTH REGULATORS (GR) ON 'WILLIAMS' BANANA PLANTLETS GROWN IN VITRO FOR SIX WEEKS IN THE DARK.

GR		Height		Leaves		H/L	
Kind	mg/l	(H)*		(L)*			
0	0	6.9	abc	3.0	c	2.3	abcd
IAA+KIN	2+5	8.1	a	3.4	c	2.6	abc
Ancymedol	1	4.8	defgh	6.8	a	0.7	gh
Ancymedol	5	3.8	fgh	6.7	a	0.7	gh
Cultar	1	5.1	cdefg	5.1	b	1.1	fg
Cultar	5	4.8	defgh	6.5	a	0.8	fgh
Uniconazole	1	4.0	efgh	6.6	a	0.8	fgh
Uniconazole	5	1.1	i	2.0	cd	0.3	h
ABA	1	6.2	abcd	2.7	cd	2.3	abcd
ABA	5	3.1	h	1.4	d	1.9	cde
PCIB	50	5.8	bcde	2.7	cd	2.2	bcd
PCIB	100	5.2	cdefg	3.2	c	1.8	de
PCIB	200	3.6	ch	2.2	cd	1.4	ef
ALAR	5	7.2	ab	2.5	cd	2.8	ab
ALAR	25	8.1	a	2.7	cd	3.0	a
CCC	5	5.7	bcdef	3.2	c	2.0	cde
CCC	25	7.6	ab	2.7	cd	2.9	ab

* H= Height of plantlets in cm, L= No. of new leaves.

TABLE 7: EFFECT OF GROWTH REGULATORS (GR) ON 'WILLIAMS' BANANA PLANTLETS GROWN IN VITRO FOR DIFFERENT PERIODS UNDER LIGHT AND IN THE DARK.

GR		Days under light								
		65			98			128		
Kind	Mg/l	H*	L*	H/L	H	L	H/L	H	L	H/L
0	0	4.9	11.0	0.4	6.0	14.4	0.4	6.0	15.0	0.4
Uniconazol	1	3.2	16.0	0.2	3.8	22.2	0.2	3.7	23.0	0.1
Uniconazol	5	2.8	0.0	0.0	0.5	1.9	0.1	0.0	0.0	0.0
Uniconazol	10	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cultar	1	4.1	16.5	0.3	5.0	20.3	0.2	5.1	27.0	0.2
Cultar	5	4.7	15.0	0.3	5.5	18.0	0.3	3.1	15.1	0.1
Cultar	10	4.5	15.0	0.3	5.5	19.9	0.3	4.8	17.2	0.2
Ancymidol	1	4.0	16.5	0.2	6.2	19.0	0.3	3.2	17.7	0.1
Ancymidol	5	4.2	17.1	0.2	4.1	20.7	0.2	1.0	5.5	0.0
Ancymidol	10	3.4	18.1	0.2	3.6	20.1	0.2	1.5	6.3	0.1
		Days in the dark								
0	0	7.1	4.7	1.4	8.6	7.4	1.2	14.4	23.1	0.6
Uniconazol	1	4.0	10.2	0.4	4.0	15.5	0.2	4.7	23.1	0.2
Uniconazol	5	2.9	0.4	0.1	2.1	4.2	0.3	0.3	1.0	0.0
Uniconazol	10	2.9	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0
Cultar	1	3.9	9.9	0.4	5.1	15.5	0.3	5.6	18.8	0.3
Cultar	5	4.4	10.2	0.6	7.8	14.4	0.5	3.1	17.1	0.5
Cultar	10	3.5	8.2	0.3	4.9	12.7	0.3	5.5	14.4	0.3
Ancymidol	1	4.2	10.3	0.4	5.6	18.3	0.3	5.8	20.1	0.3
Ancymidol	5	3.7	10.6	0.2	4.4	16.4	0.2	5.5	22.1	0.2
Ancymidol	10	3.7	13.1	0.3	4.2	15.3	0.2	4.1	17.3	0.2

* H= Height of plantlets in cm, L= No. of new leaves.

TABLE 8: EFFECT OF DIFFERENT NITROGEN SOURCE (N^{+5}/N^{T}), NITROGEN TO POTASSIUM RATIO (N/K) AND MONOVALENT TO BIVALENT CATIONS (Na+K/Ca+Mg) ON NORMAL (NL) AND DWARF (DW) 'WILLIAMS' BANANA PLANTLETS GROWN IN VITRO FOR NINE WEEKS.

Medium description			Plantlet type					
			NL			DW		
$\frac{N^{+5}}{N^{T}}$	$\frac{N}{K}$	$\frac{Na+K}{Ca+Mg}$	Height (H)**	Leaves (L)**	H/L	Height (H)	Leaves (L)	H/L
1.0	0.4	5.1	7.3	4.9	1.5	6.0	5.5	1.1
1.0	1.7	0.8	7.0	4.8	1.5	5.8	5.6	1.0
1.0	2.6	0.3	6.1	5.1	1.2	4.7	5.3	0.9
*0.7	1.1	5.1	6.4	5.2	1.2	5.0	5.6	0.9
0.7	2.6	0.8	5.6	5.0	1.1	4.4	6.5	0.7
0.1	2.6	5.1	6.0	4.5	1.3	4.8	5.9	0.8
Average			6.4	4.9	1.3	5.1	5.7	0.9

* MS medium

**H= Height of plantlets in cm, L= No. of new leaves.

TABLE 9: EFFECT OF GIBBERELLIC ACID (GA) AND KINETIN (KIN) ON NORMAL (NL) AND DWARF (DW) 'WILLIAMS' BANANA PLANTLETS GROWN IN VITRO FOR NINE WEEKS.

PARAMETER	KIN (mg/l	GA (mg/l)							
		0		1		5		10	
		Plantlet type							
		NL	DW	NL	DW	NL	DW	NL	DW
Height (H)*		4.1	3.2	3.4	1.5	3.6	2.1	3.0	2.0
Leaves (L)*	0	4.8	4.9	3.1	3.8	2.2	3.5	1.9	3.4
H/L		0.9	0.7	1.1	0.4	1.6	0.6	1.6	0.6
Height (H)*		5.0	5.0	4.1	3.9	3.2	2.9	4.9	2.7
Leaves (L)*	1	5.5	4.9	2.9	3.9	2.3	2.9	1.6	2.8
H/L		0.9	1.0	1.4	1.0	1.4	1.0	3.1	1.0
Height (H)*		2.4	3.4	1.6	3.0	1.5	2.3	2.1	1.6
Leaves (L)*	5	4.0	4.2	1.6	2.8	1.0	1.5	0.9	2.3
H/L		0.6	0.8	1.0	1.1	1.5	1.5	2.3	0.7
Height (H)*		3.0	2.5	1.9	1.0	0.9	1.8	0.8	1.7
Leaves (L)*	10	2.7	3.5	0.7	0.8	0.3	2.0	0.4	1.8
H/L		1.1	0.7	2.7	1.3	3.0	0.9	2.0	0.9

*H= Height of plantlets in cm, L= No. of new leaves.

TABLE 10: NEW LEAVES PRODUCED BY NORMAL (NL) AND DWARF (DW) PLANTLETS IN RESPONSE TO GIBBERELLIC ACID (GA) OF 'VALERY' (VAL) 'GRAND NAIN' (GRN) AND 'NATAN' (NAT) CULTIVARS AFTER 23 AND 46 DAYS IN CULTURE.

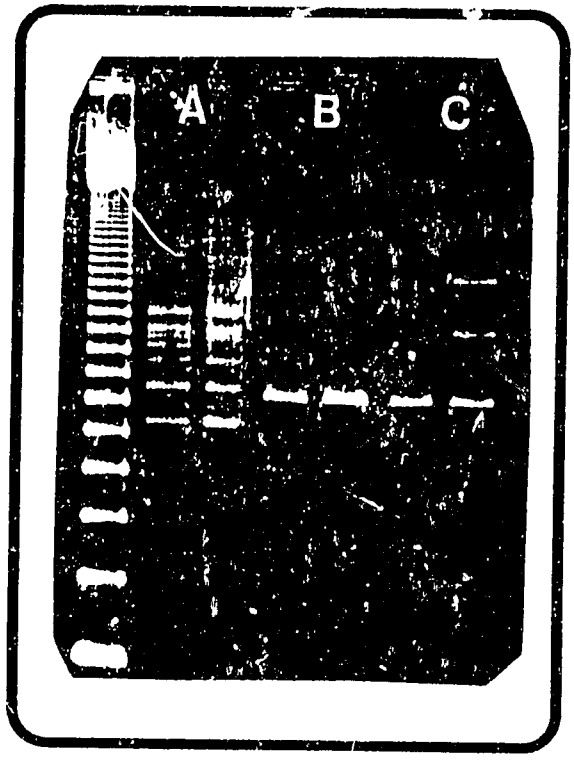
GA	23 days					46 days				
	VAL	GRN		NAT		VAL	GRN		NAT	
(mg/l)	NL	NL	DW	NL	DW	NL	NL	DW	NL	DW
0	1.3	1.4	1.5	1.8	1.4	3.1	2.9	3.4	2.8	4.1
1	0.2	0.3	0.6	1.1	0.5	0.8	0.9	1.8	1.3	2.7
5	0.1	0.4	0.6	1.5	0.6	0.6	0.9	1.4	1.5	3.0
10	0.2	0.2	0.4	1.0	0.3	0.5	0.6	1.5	1.0	2.5

TABLE 11: RATE (%) OF DWARF (DW) MOSAIC (MO) AND OTHER (OT) SOMACLONAL VARIANTS EVALUATED IN THE FIELD IN BANANA FAMILIES MUTLIPLIED BY IN VITRO TECHNIQUES.

Family	Evaluation		Variants (%)			
	Years	No.	DW	MO	OT	Total
6/32	1984-89	523	6.50	0.38	3.6*	10.51
8/19	1984-87	167	7.18	0.60	0.60	8.38
10/32	1984-90	1197	5.93	0.58	0.08	6.59
3/28	1984-87	148	4.05	0.68	1.35	6.08
17/34	1984-90	1101	3.36	0.45	0	3.81
10/10	1984-90	472	2.97	1.06	0	4.03
13/20	1984-90	760	1.97	0.53	0	2.50
10/17	1984-90	731	1.50	0.55	0	2.05
17/27	1984-90	887	1.35	0.68	0	2.03
13/7	1984-91	1600	0.13	0.25	0.06	0.44
6/3	1984-91	12562	0.02	0.37	0.06	0.45

* Many varigated in 1988.

Fig. 1: PCR AMPLIFICATION OF BANANA DNA CV. WILLIAMS NORMAL (LEFT) AND DWARF (RIGHT) USING RAPD PRIMERS, A,B AND C.



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