Early Detection and Characterization of Dwarf off-Types in Micropropagated Banana

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Key Words : *In vitro* selection, Dwarf off-types , RAPD markers, Banana .

ABSTRACT

The present work was carried out in Pomology Department and Biotechnology Laboratory, Crop Science Department, Alexandria University, during the period from 2000 to 2003.

Early detection by gibbrellic acid (GA3) was used to assess the percentage of dwarf off-types of four banana cultivars namely, Maghrabi, Williams, Grand Nain and Valery after 25 subcultures. Simultaneously, selecting the dwarf variant (s) of the giant cultivar 'Maghrabi'. Four hundred plantlets at deflasking stage were sprayed with 0 or 300 µM l^{-1} GA3, and were incubated under controlled–temperature glasshouse for one month. Sheath–length of the first formed leaf after spaying was measured for each plantlet. The percentage of dwarf off–types were 18, 16, 10, 2 % for Williams, Maghrabi, Grand Nain and Valery, respectively. The dwarf off-types were characterized with short pseudostem height and petioles length, narrow distance among the petioles and leaf index (leaf length /width) was less 2 as a morphological marker associated with the dwarfism in banana as compared to normal plants.

The dwarf off-types clones of the gaint cultivar " Maghrabi " were identified by RAPD markers. Seventy three amplification products were obtained, out of which 95 % showed polymorphism . Primer OPJ-04 detected the band (1500 bp) which was reported in previous study as DNA marker for dwarfism in banana. Primer OPC-15 revealed a band (936 bp) which present in normal plant only and may be linked to the dwarfism in this banana cultivar. Primer OPA-06 detected a band (1334 bp)which present in all dwarf off–types only, also, may be related to the dwarfism in banana. The bands, which produced by these primers, were used to construct a similarity matrix. The genetic similarity among these tested variants ranged from 0.09 to 0.50. These variants were classified to six clusters.

The use of the dwarf specific RAPD marker at the *in vitro* stage could be particularly useful for *in vitro* selection in banana breeding programme and subsequently obtaining a new genotypes in Maghrabi cultivar.

INTRODUCTION

Micropropagation is a rapid method of producing large quantities of disease – free banana for commercial production. However, widespread use of such materials is hindered by the high percentages of tissue culture-induced dwarf variants especially with Cavendish banana (Smith, 1988). The dwarfism is the most common off-types in the Cavendish banana with almost 75% of the total variants (Stover, 1987; Cote *et al.*,1993; Sandoval *et al.*, 1995; Damasco *et al.*,

1996a; Damasco *et al.*, 1998). Nevertheless, these variants offer considerable opportunity for banana improvement (Mak *et al.*, 1998), and can be incorporated in plant breeding progammes (Jain, 2001).

Economically, Smith and Drew (1990) indicated that sometimes dwarf off–types can not be detected until the flowering or fruiting stage. At this stage, these dwarfs can have a choked appearance, have bunches with closely packed hand and shorter fingers. Detection of dwarf off – types at this stage is too late, since is very costly to remove these plants and replace them with new plants. Also, Damasco *et al.*, (1996a) revealed that fruit bunches produced by the dwarf off – types are of inferior commercial value, causing serious economic losses to the growers. Thus, early detection and elimination of those from planting materials is highly desirable, but not easy to undertake (Smith and Hamill, 1993 ; Damasco *et al.*, 1998).

The objectives of this study were to (1) Detect dwarf off-types in micropropagated banana by gibbrellic acid (GA_3) and applying this detection tool for selecting the useful variants in the giant banana cv. ' Maghrabi' and (2) Investigate the efficiency of RAPD markers in determining, accurately, the genetic relationship between the selected dwarf clones and the normal plants banana cv. ' Maghrabi' .

MATERIALS AND METHODS

The present work was carried out in Pomology Department and Biotechnology Laboratory, Crop Science Department, Alexandria University, during the period from 2000 to 2003.

Plant materials and culture preparation

Shoot apices of 4 banana cultivars Williams (W), Grand Nain (G), Valery (V) and Maghrabi (M) were used in this study . The basal medium of Murashige and Skoog (1962), supplemented with 80 mg/L adinine sulfate, 0.5 mg/L BAP, 0.5 mg/L NAA, 30 g/L sucrose and 5 g/L agar was used for the initial culture and the multiplication stage (Al-Murish, 2003). The pH was adjusted to 5.8 before autoclaving. The medium was dispensed into screwtopped glass jars (350 ml), each receiving 60 ml. Cultures were incubated at 25 ± 2° C under 16 h illumination (2000 lux, daylight fluorescent tubes) .

Dwarf Screening

Multiplied shoots derived from shoot apices of 4 banana cultivars; namely, Maghrabi, Williams, Grand Nain and Valery were established and subcultured for 25 subcultures. Shoots were transferred to rooting medium for root induction (Al-Murish, 2003).

To screen dwarf plants, one hundred plantlets of each cultivar were planted in plastic pots (300 ml) containing a peat moss : perlite mixture (3:1, v/v). These plants were immediately, watered, and then sprayed with either GA_3 (300 µM)

solution (Sigma Chemical Co. St. Louis, USA) or distilled water (as control). Following spraying, plants were covered with a clear plastic to maintain a high humidity around them for 1 month, the GA_3 treatment was repeated at the 3rd and 5th day from planting *ex vitro* . The plants were daily fertigated, at rate 50 ml water + 1g l^{-1} Kristalon fertilizer pot $^{-1}$.

One month later, plantlets leaf–sheath length of the formed newest leaf (base of shoot to start of leaf lamina) were measured for dwarf screening according to Damasco *et al.*, (1996b).

Dwarf clones characterization

Eight dwarf ramets as well as four normal plants of banana cv. Maghrabi, were replanted in black plastic bags (22 cm x 30 cm) containing a peat moss : perlite (3:1, v/v). Plants were incubated in a controlled–temperature glasshouse for 5 months. A qualitative description for both variants and normal plants were made. Plant height; number of leaves; petioles length; laminae length and width of 4 upper leaves and the distance between 8 consecutive leaves from the top of the plant were measured.

RAPD markers for dwarfism detection in banana.

Plant materials

Analyses of PCR were carried out by using the genomic DNA from six dwarf off - types clones of the cultivar 'Maghrabi' as well as 3 normal plants .

DNA extraction

Frozen young leaves (1g) were ground to a powder in a mortar with liquid nitrogen. The powder was poured into tubes, containing 9.0 ml of warm (65 °C) CTAB extraction buffer . The tubes were incubated at 65 °C for 60 - 90 min. An additive of 4.5 ml chloroform / octanol (24:1) was used and tubes were rocked to mix for 10 min. and centrifuged for 10 min. at 3200 rpm. The supernatants were pipetted into a new tubes and 6 ml isopropanol was added. After 60 min, the tubes were centrifuged for 10 min. and the pellets obtained were put in sterile Eppendrof tubes, containing 400 µl of TE buffer of a pH 8.0 (10 mM Tris- HCl, pH 8.0 + 1.0 mM EDTA, pH 8.0) (Sagahi-Maroof *et al.*,1984). Thus, the DNA's of the given genotypes were extracted and stored at –20 °C until use.

PCR amplification

Five primers (Table 5), obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, England HP79 NA), were tested to amplify the templated DNA of dwarf and normal plants.

Amplification reaction volumes were 25 µl, each containing 1X PCR buffer with $MgCl_2$ (50 mM KCl, 10 mM Tris-HCl. pH = 9.0), 2 mM $MgCl_2$ and 1% trition X-100), 200 µM each dATP, dCTP, dGTP and dTTP, 50 PM primer, 50 ng template DNA and 1.5 µl of taq polymerase. Reaction mixtures

were exposed to the following conditions : 94 °C for 3 min, followed by 45 cycles of 1 min. at 94 °C, 1min. at 63 °C, 2min. at 72 °C, and a final 7 min. extension at 72 °C.

Amplified products were vizualized with DNA marker on 1.6 % agarose gel with 1X TBE buffer and detected by staining with an ethidium bromide solution for 30 min. Gels were, then, destained in deionized water for 10 min. and photographed on Polarid films under UV light.

Data handling and cluster analysis

Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. If a product was present in a genotype, it was designated as "1", if absent it was designated as "0" after excluding the unreproducible bands. Pairwise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients, according to Jaccard (1980). The similarity coefficients were, then, used to construct dendrograms, using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program Rohlf, 1993).

Statistical analysis

Dwarf off–types detection by GA_3 was carried out in a 2–factor experiment (cultivar and GA_3 concentration), in a randomized complete block design (RCBD) with 5 replicates (each treatment = 5 rep x10 plantlets).The analysis of variance of the data as well as the mean comparisons by least significant difference test (LSD) in all the above studies were performed using Genstat program 5 Release 3.2 (1995). Concerning RAPD data were analyzed with *Totl*Lab. Software V. 1.11.

RESULTS AND DISCUSSION

Dwarf off – types detection of microprpagated banana by GA

The ultimate objectives of the present work were the early detection of dwarf off-types after 25 subcultures for four important banana cultivars being planted in Egypt, namely Maghrabi, Williams, Grand Nain and Valery to select the dwarf somaclones of the giant and superior cultivar Maghrabi for breeding programme as well as to exclude these off-types from commercial banana production.

The plantlets of this cultivars were treated with GA_3 (300 µM) at deflasking stage and the sheath- length of the first formed leaf of each plantlet was measured, as the best discriminating parameter in identifying dwarf from normal plants according to Damasco *et al.*, (1996b).

The analysis of variance presented in Table (1) indicated that the leaf–sheath length was highly significantly influenced by differences in GA_3 treatments. However, it was not significantly influenced by differences in banana cultivars. The GA_3 treatment x cultivar interaction was significant.

Results in Table (2) showed that the leaf-sheath length was increased with GA_3 treatment (300 µM) and was significantly different than the control (0.0 µM) across the four cultivars.

The interaction between cultivars and GA3 treatments was significant. The leaf-sheath length of the cultivar Valery gave the highest response with the GA3 treatment and was significantly different than the other treated cultivars (Table 2).

Figure (1) illustrates number of GA_3–treated and non–treated plantlets in relation to their sheath–length of the first formed leaf after GA_3 application, as well as number of detected dwarf off–types from the four studied cultivars after 25 subcultures. The range of this elongation was 1.5 to 2.5, 2.6 to 3.2 and 6.4 to 8.5 (cm) for control, dwarf off–types and treated plants (normal), respectively (Figure 2).

These results agree with those obtained in banana by Damasco *et al.*, (1996 b). Similar effect of GA was ,also, observed in other crops such as : *Pisum sativum* (Reid *et al.*,1992), barely (Borner *et al.*,1999), rice (Min *et al.*, 2001).

Production of banana plants has been accelerated in recent years using *in vitro* culture. However, this technique results in a significant percentage of morphological variants among the new plants, and this can be commercially prejudicial (Carvalho *et al.*, 1998)

The dwarfism is the most common off-types in the Cavendish banana subgroup, with almost 75 % of the total variants (Stover, 1987; Cote *et al.*, 1993; Sandoval *et al.*,1995; Damasco *et al.*,1998).

Sometimes dwarf-off-types can not be detected until the flowering or fruiting stage. At this stage, the plants produce bunches with closely packed hand and shorter fingers. Thus, removing these plants is very costly and the inferior of commercial value of these fruits causing serious economic losses of to the growers.

Regarding the screening of dwarf off-types, the plantlets which exhibited high response to GA_3 and produce leaf-sheath length 2-fold greater than other treated plantlets were separated as a normal plants according to Damasco *et al.*, (1996b). The percentage of dwarf off-types after 25 subcultures were 18, 16, 10, and 2 % for the cultivars William, Maghrabi, Grand Nain and Valery, respectively (Table 3). Previously, different percentages of dwarf off-types in micropropagated banana were recorded in several studies on banana : 2.4 % (Hwang, 1986), 25 % (Stover, 1987), 0-70 % (Smith,1988), 0.5 - 69.1 % (Vuylsteke *et al.*, 1991), 0 – 90 % (Cote *et al.*, 1993) .

The results in Table (3) showed that the percentage of dwarf off-types after 25 subcultures in Valery cultivar is very low (2 %) as compared with

Williams (18 %) or those previously mentioned which reached in some cases to 70 % (Smith, 1988) and 90 % (Cote *et al.*,1993). The low percentage of dwarfs in the Valery cultivar in this study revealed that the number of subculture may be was not an essential factor to induce this phenomenon. These results agreed with those obtained by Reuveni *et al.*, (1993) who reported that the rate of variants obtained in micropropgated banana was not affected by medium composition or by the rate of multiplication. They ,also, reported that the initial explant was found to be the main factor that determines the occurrence of clonal variants in forming 'stable' and 'non-stable' families obtained from these explants. Moreover, Damasco *et al.*, (1998) demonstrated that dwarf off-types can arise early in the multiplication process and were detected as early as the 4^{th} subculture after initiation of shoots tips. Their results ,also, strongly indicated that adventitious shoot multiplication is the main factor contributing to the formation of dwarf off-types. Adventitious buds are promoted by higher concentration of the phytohormone BAP, splitting of propagules longitudinally during micropropagation and by preferentially selection bulbil-like structures as propagules for further multiplication. The inherent instability of the cultivar being micropropgated was another major factor influencing the production of dwarf off-types. They ,also, reported that it was found that New Guinea Cavendish produced dwarfs at a higher frequency than Williams. Rodrigues *et al.*, (1998) found that the somaclonal variation in micropopagated banana cv. Nanico appeared after 5, 7, 9 and 11 subcultures at rate of 1.3, 1.3, 2.9 and 3.8 %, respectively.

Ortiz and Vuylsteke (1992) suggested that the dwarfism in diploid and tetraploid offspring derived from the AAB plantain 'Bobby Tannap' is due to a recessive gene (dw). They also found that the substitution of the dominant Dw by its recessive allele only reduced the height of the plant by 36 cm.

The causes of the somaclonal variation in *Musa* still remain unknown. Many of the somaclonal variations induced by tissue culture may be caused by disturbances in gibberillin metabolism (Sandoval *et al.*,1995). They observed that GA_3 concentration in dwarf plants was 811 ng g^{-1} dry weight. For normal and giant plants, the endogenous GA_3 levels were respectively 3.6 and 4.6 times higher. The GA_{20} concentration in the giant plant was 68 ng g^{-1} of dry weight. This concentration was, respectively, 4.6 and 7.3 times higher than those of normal and dwarf plants. These results suggested that the somaclonal variations affecting banana plant height are associated with modification in GA metabolism.

This hypothesis is supported by the demonstration of the particular response of dwarf and giant variants of banana to exogenous application of GA_3 during the *in vitro* stage (Reuveni, 1990; cote *et al.*, 1993) and the acclimatization period (Cote *et al.*,1994). In other dwarf mutant, a reduction of endogenous GA_3 level has also been reported, e. g. in *Lycopericon* sp. (Bohner

et al., 1988), *Zea mays* (Fujioka *et al.*,1988), *Lathyrs odoratus* (Ross *et al.*, 1992).

Recently, Veilleux and Johnoson (1998) and Jain (2001) concluded that there is not a single mechanism or a factor, which controls somaclonal variation. Plant genotype is a major factor that determines the type and frequency of these variants. The number of subcultures, the length of time of the culture, the components of culture medium and the age of the donor plant are ,also, important in reducing this phenomenon.

Breeding dwarf clones for 'Maghrabi' cultivar

One of the goals of the present investigation was to obtain dwarf clones of the giant 'Maghrabi' cultivar. It has a psedudostem high over 3.5 meter (Abd El-Kader, 1995). This cultivar is important and superior as compared with the other ones in Egypt. Its characteristics as follows : high production (bunch weight 30 Kg and fruit length 18 cm), high quality (excellent test and aroma), more ability to handling and export , more resistance to bunchy–top virus, and more tolerance to low temperature, but its cultivated area was ,dramatically, reduced by the wind's damage (Abd El-Kader, 1995; Ibrahim and Kholief,2000).

Eight dwarf clones of this cultivar were selected after 25 subcultures by GA_3 application and abbreviations for each clone was gave (D1 to D8). The morphological characteristics of this clones, after 6 month of growth under greenhouse condition were illustrated in Table (4) . The data in the table ,cleary, indicate that plant height of all dwarf-off types has shorter pseudostem height (Mean = 28.75 cm) as compared with normal plants (mean = 46 cm). The dwarf off-types of all clones had a leaf index of less than 2 while normal plants had a leaf index more than 2. The petiole length and the distance between the petioles were in normal plants two times than in dwarf off-types (Table 4). These morphological characteristic were also observed in previous studies (Stover, 1987; Smith and Draw 1990, Smith *et al.*, 1994; Grillo *et al.*, 1998), and the leaf index which was noted by Damasco *et al.*, (1998) was less than 2 in dwarf off-types of Williams cultivar, while normal plants had a leaf index of 2.2 or higher.

A dwarf growth habit is a goal of many breeding programs and has already contributed to important yield advances (Welander, 1988). Sarwar *et al.*, (1998) reported that dwarf trees have several advantages : they can be planted close together to give good yield, due to reduced vegetative growth; they require minimal pruning and training, so, labor cost is reduced; it is easier and less expensive to apply pesticide ; and they produce uniform sized and colored fruits. Robinson *et al.*, (1993) recommended that, in the subtropics, the ideal banana should have a short and sturdy pseudostem but an absence of choke throat, a large cylindrical bunch and a short cycle. Janick and Moore (1996) mentioned that most banana are grown in areas that have periodic strong winds, so any new cultivar must be a dwarf plant. This dwarf plant has a shorter pseudostem prevents it from toppling during tropical storms in these areas (Elsen, 2002). Other variants were observed during the dwarf screening process in the

greenhouse. These variants include : (a) mosaic-like lamina deformed lamina (c) mosaic and deformed lamina (d) rosette shape leaves (e) long and narrow lamina (f) heart-shaped lamina . These variants were also previously observed (Stover, 1987; Smith and Draw 1990; Damasco *et al*, 1998).

RAPD markers for early detection of banana dwarf off–types

Screening for polymorphic primers

DNAs were extracted from six dwarf somaclones derived after 25 subculture and 3 normal plants. These plants were previously screened by GA_3 spraying and morphologically characterized after 6 month under glasshouse conditions. Five primers (Table 5) were used in this study according to Damasco *et al.*, (1996a) and Grajal–Martin *et al.*, (1998).

A total of 73 DNA fragments were amplified by the five primer, in which 72 bands were polymorphic. The number of bands for each primer varied from 7 (OPJ-10) to 20 (OPJ-04) with an average of 14.6 bands per primer. This high amplification can be attributed to four of these primers were chosen from 66 random primers were used in the initial screening by Damasco *et al.*, (1996a). They reported that these primers not only generated high polymorphisms, but also had the ability to differentiate between normal and dwarf in the banana cultivars New Guinea Cavendish and Williams.

The primer OPJ-04 revealed that the DNA fragment (1500 bp) was absent in all tested dwarf clones (D1 to D6) and present in 2 normal clones (N1and N3) as shown in Figure (3). This result is consistent with the observation of Damasco *et al.*, (1996a) who reported that the primer OPJ–04 ($5'$ – CCGAACACGG – $3'$) was found to amplify an approximately 1.5 kb band which was consistently present in all normal but absent in all dwarf plants of both cultivars. They also reported that reliable detection of dwarf plants was achieved using this marker, providing the only available means of *in vitro* detection of dwarfs.

However, figure (4) shows the amplification profiles, generated by primer OPC–15 across the banana dwarf off–types and normal plants. All of the 19 scorable bands were polymorphic across the banana variants. The primer OPC–15 was also found to consistently amplify an approximately 936 bp band present in all normal plants (N1 to N3) but absent in all dwarf plants (D1 to D6). This results has been observed previously with the results of Grajal–Martin *et al.*, (1998) who reported that polymorphism using OPC–15 was found among some dwarf variants of Grand Nain.

Polymorphisms among normal plants and dwarf off–types was also detected using the primer OPA – 06 (Figure 5). The RAPD band, OPA–061334, was consistently present in all normal plants. Previously, the primer OPA – 06 was able to differentiate between normal and dwarf New Guinea Cavendish (Damasco *et al.*,1996a).

Generally, the obtained results of RAPD technique in this study were clearly confirmed the previous results which observed either by GA_3 spraying of

plantlets at deflasking stage or by the morphological characteristics of plants after 6 moths under controlled conditions (Exp.III). Furthermore, this study certainly proved that the OPJ–04 primer (5′ – CCGAACACGG –3′), which identified the band 1500 bp, is a RAPD marker specific to the dwarf off-type in micropagated banana as reported by Damasco *et al.*, (1996a).

It can be concluded that, the use of this marker as well as the markers which obtained by the primers OPC-15 and OPA-6 in this study could be facilitate the early detection and elimination of dwarf from batches of micropropagated bananas and may be a useful tool in breeding program such as the case of 'Maghrabi' cultivar in this work.

Cluster analysis

One of the goals of the present study was to investigate the efficiency of RAPD markers in determining, accurately, the genetic relationship between the selected dwarf clones and the normal plants banana cv. 'Maghrabi'.

The RAPD markers, produced by 5 primers, were used to construct a similarity matrix (Table 6). Simple matching coefficient, ranging from 0.09 to 0.50 suggested a broad genetic base for these variants of Magharbi cultivar. The high range of diversity which observed may be due the high rate of somaclonal variation induced in this cultivar after 25 subcultures. Table (6) indicates the genetic similarity estimates of the 36 pairwise comparisons among the dwarf off–types and normal plants of Maghrabi cultivar, based on the 72 polymorphic bands. Figure (6) represents the clustring of Maghrabi somaclones generated by UPGMA analysis. Six culsters could be observed. The first cluster included only D1 while the second one include D3, D4 and D5. The third cluster included only D6 while the fourth include N1 and N3. The fifth cluster included only D2 and the last one included N2.

The absence of some bands in the present investigation as well as the low amplification may be due to several factors in RAPD technique. Samec (1993) reported that RAPD is known to be highly sensitive to the reaction conditions. Samec and Nasinec (1996) reported that the problems can arise with the RAPD pattern reproducibility and the evaluation of fingerprints when the assays are performed in various labs. Therefore, it is always necessary to perform control reactions with standard genomic DNAs. The evaluation of electrophoretic gels should be performed with an automatic computer system, which would circumvent doubts with the detection of very faint bands. Recently, Korbin (2000) reported that quality of DNA is well–known as a factor limiting genetic analysis. They also reported that the high concentration of chemical compounds in the plant sap such as proteins, polysaccharides and polyphenolic compounds as well as the contamination of DNA with the remains of Tris – HCl and EDTA may be are the critical points for RAPD analyses.

The results of the present investigation demonstrate that RAPD analysis can be used to detect genetic variation in micropropagated Maghrabi cultivar of banana. The results also demonstrate that the use of the dwarf specific RAPD

marker at the *in vitro* stage affords a reliable means for early detection of dwarf off–types allowing for the elimination of dwarfs before planting of micropropagated plants in the filed for the other cultivars such as Williams and Grand Nain. However, with the Maghrabi cultivar, the use of the dwarf specific RAPD marker at the *in vitro* stage could be particularly useful for *in vitro* selection in banana breeding programme and subsequently obtaining a new genotypes in Maghrabi cultivar.

Table 1 . Analysis of variance for leaf–sheath length in the four tested cultivars of banana plants after 40 days from GA_3 treatment.

Source of variance	D.F.	MS	
Replicates	4	1.1960	
GA3 (A)	1	2281.9	**
Cultivars (B)	3	2.5020	
A x B	3	3.1170	*
Error	388	1.105	

*, ** ; Significant at 0.05 and 0.01 levels of probabilities, respectively.

Table 2 . Means of leaf–sheath length (cm) as influenced by cultivars, GA_3 treatments and their interactions.

GA_3 conc. µM	Cultivar				GA_3 conc. mean.
	M	W	G	V	
0	2.07	1.98	1.99	2.00	2.01 B
300	6.50	6.61	6.78	7.25	6.79 A
Cultivar mean	4.29 A	4.30 A	4.39 A	4.63 A	

$LSD_{0.05}$ for GA3 treatment x cultivar interaction = 0.4133
Means within a column or a row followed by the same letter are not significantly different at the 0.05 level of probability.

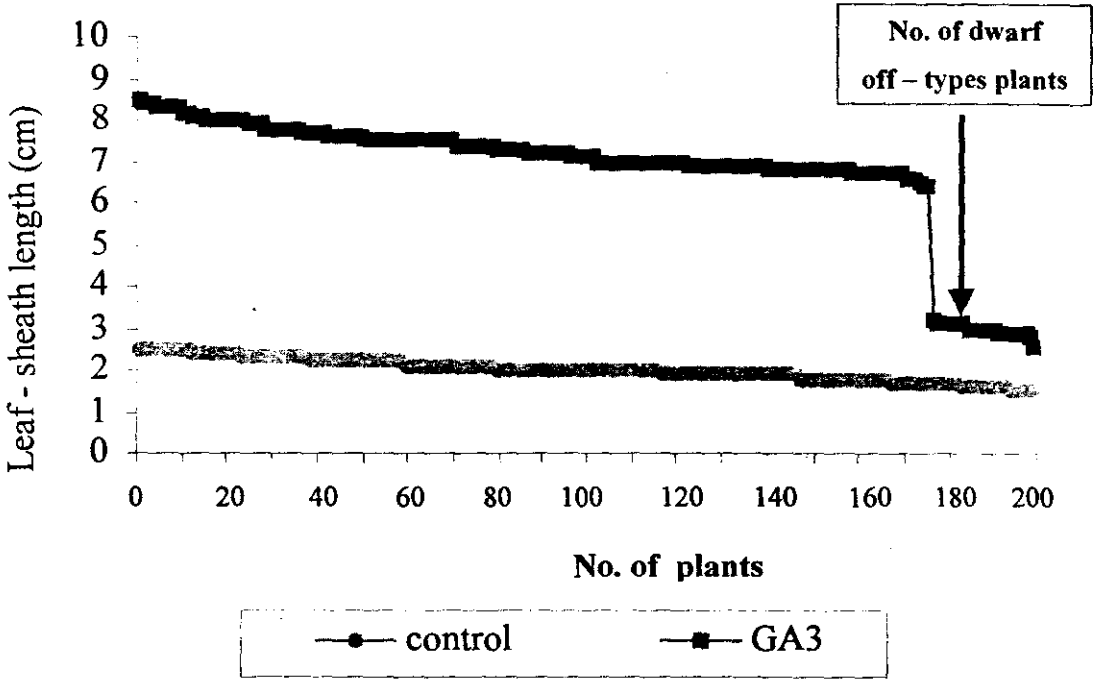

Figure 1. Number of banana plants and their sheath – lengths of the first formed leaf after GA_3 application as compared with control. The arrow indicates to the number of detected dwarf off-types of four banana cultivars after 25 subcultures.

Table 3. Percentages of normal plants and dwarf off-types for four banana cultivars after 40 days from GA_3 application at deflasking stage (No. of treated plants with GA_3 = 50).

Cultivars	Normal plants		Dwarf off-types	
	No. [(a)]	%	No.	%
M	41	82	8	16
W	42	84	9	18
G	45	90	5	10
V	49	98	1	2

(a) Number of normal plants = number of plants which their sheath – length of the first formed leaf after GA_3 application were 2- fold greater than observed in dwarfs according to Damasco *et al.*, (1996b).

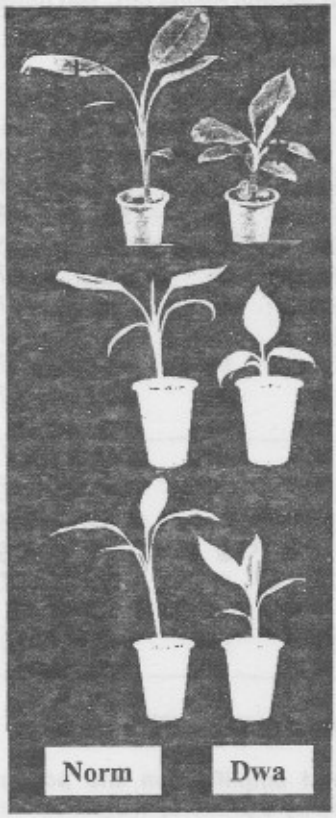

Figure 2 . Sheath-length of the first formed leaf after GA_3 application. (A) Maghrabi (B) Grand Nain (C)Williams. The arrows indicate to the first formed leaf.

Table 4 .Vegetative characteristics of dwarf banana derived-clones. 'Maghrabi' (D1- D8) and normal plants (N1- N4) generated after 25 subcultures.

Characters / Clones	Plant Height (Cm.)	No.of leaves plant $^{-1}$	Leaf			Petiole Length (Cm.)	Distance between petioles (Cm.)
			Length (Cm.)	Width (Cm.)	Index *		
D1	28	9	31.8	17.4	1.83	6.0	9
D2	29	9	32.2	18.2	1.77	6.2	10
D3	29	9	29.6	16.8	1.76	6.6	10
D4	27	9	30.8	17.0	1.81	5.8	9
D5	28	9	31.4	17.4	1.81	6.4	9
D6	28	9	30.6	17.4	1.76	6.6	10
D7	30	9	30.8	17.0	1.81	7.2	10
D8	31	9	31.6	17.4	1.82	6.8	11
Means	28.75	9	31.1	17.33	1.80	6.45	9.75
N1	46	9	45.2	20.2	2.23	11.2	18
N2	46	9	44.4	19.4	2.28	11.2	19
N3	45	9	44.4	19.2	2.31	12.0	19
N4	47	9	45.6	20.2	2.28	12.2	20
Means	46	9	44.9	19.75	2.28	11.65	19

* Leaf index = length / width ratio .

Table 5. Number of amplification and polymorphic products (bands), generated by 5 primers, in banana normal plants cv. ' Maghrabi' (N1 to N3) and their dwarf variants (D1 to D6).

Primers	Sequence 5' – 3'	No. of amplification bands (a)	No. of ploymorphic bands (b)	Polymorphism % (b / a)
OPJ- 04	CCGAACACGG	20	19	95
OPC- 15	GACGGATCAG	19	19	100
OPA- 06	GGTCCCTGAC	18	18	100
OPH- 13	GACGCCACAC	9	9	100
OPJ- 10	AAGCCCGAGG	7	7	100

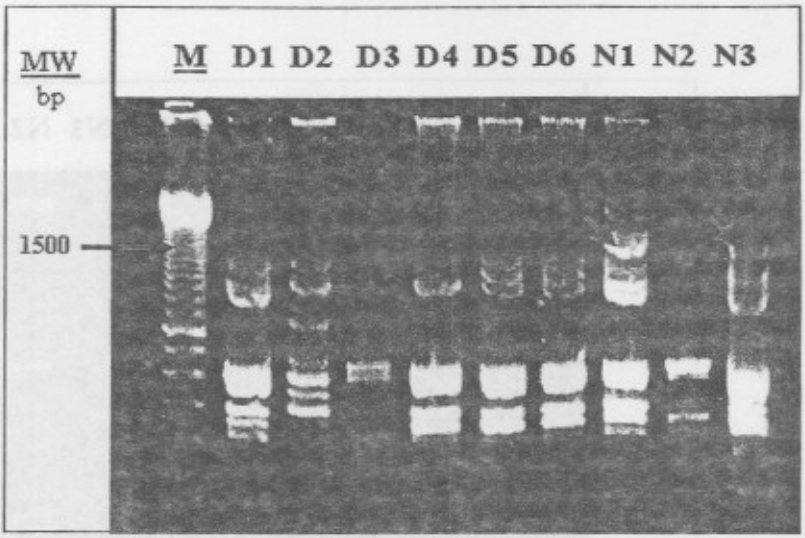

Figure 3 . RAPD profiles, with primer OPJ–04, of banana variants (dwarf D1 to D6) and normal plants (N1 to N3). The band (1500 bp) was absent in all dwarf clones.

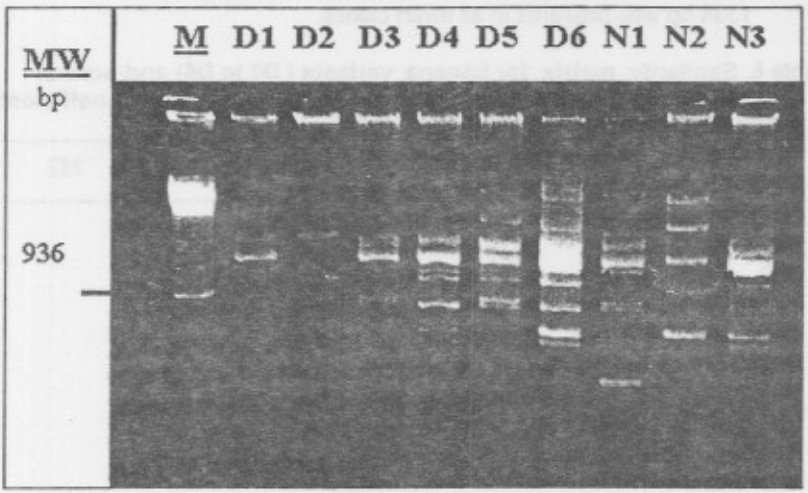

Figure 4. RAPD profiles, with primer OPC–15, of banana variants (dwarf D1 to D6) and normal plants (N1 to N3). One band (936 bp) was present in normal plants, while absent in all dwarf clones.

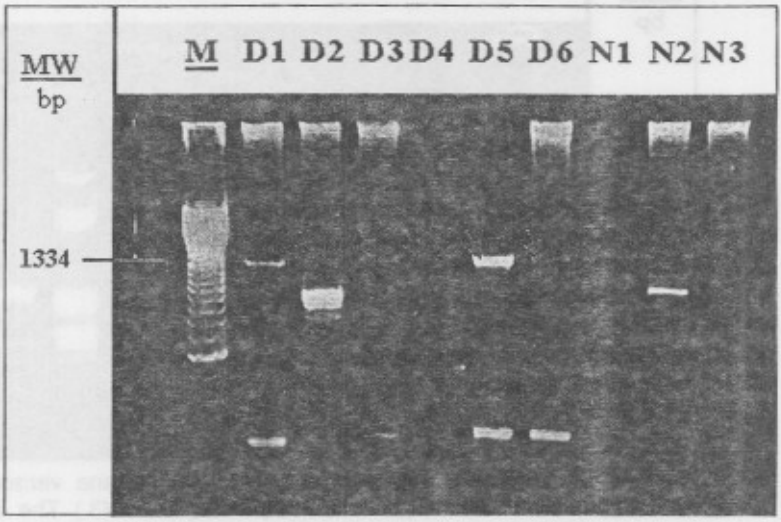

Figure 5. RAPD profiles, with primer OPA–06, of banana variants (dwarf D1 to D6) and normal plants (N1 to N3). The band 1334 bp was presents in all dwarf clones.

Table 6. Similarity matrix for banana variants (D1 to D6) and normal plants (N1 to N3), constructed by Jaccard's similarity coefficients and RAPD data.

	D1	D2	D3	D4	D5	D6	N1	N2	N3
DI	1.00								
D2	0.21	1.00							
D3	0.29	0.21	1.00						
D4	0.39	0.26	0.40	1.00					
D5	0.27	0.24	0.45	0.50	I.00				
D6	0.29	0.29	0.35	0.40	0.45	1.00			
N1	0.30	0.26	0.29	0.33	0.34	0.32	1.00		
2	0.30	0.09	0.14	0.19	0.16	0.21	0.21	1.00	
N3	0.18	0.18	0.22	0.33	0.31	0.32	0.47	0.18	1.00

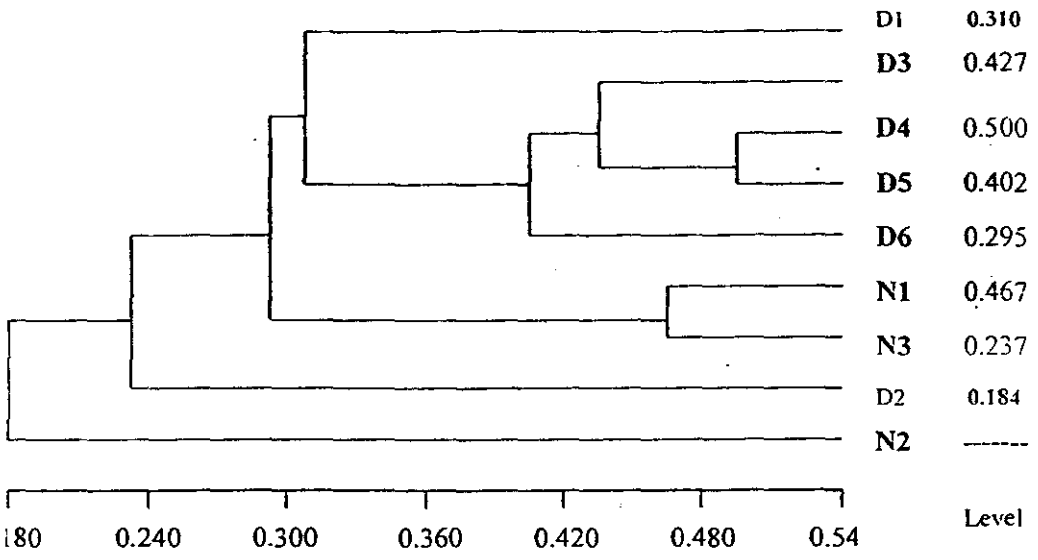

Figure 6. Dendrogram of genetic distances, constructed using RAPD data and the UPGMA method of clustring, shows DNA similarity between dwarf clones (D1 to D6) and normal plants (N1 to N3) of banana cv.'Maghrabi'

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الملخص العربي

الكشف المبكر عن صفة التقزم في نباتات الموز الناتجة من الإكثار المعملي الدقيق

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أجريت هذه الدراسة خلال الفترة من عام ٢٠٠٠ حتى عام ٢٠٠٣ . تم الكشف المبكر عن صفة التقزم في أربعة أصناف من الموز هي المغربي ووليامز وجراندناين وفاليري بعد ٢٥ نقلة (subculture) وذلك باستخدام حمض الجبريلليك (GA_3) . فكانت أعلى نسبة مئوية لظهور صفة التقزم (Dwarfism) في الصنف وليامز Williams ١٨% يليه الصنف المغربي Maghrabi ١٦% والصنف جراندناين Grand Nain ١٠% ثم الصنف فاليري Valery ٢% .

تم الكشف والتعرف على النباتات المتقزمة للصنف المغربي (طويل الساق الكاذبة) وذلك باستخدام تقنية RAPD markers وعليه تم الحصول على ٧٣ حزمة (band) ٩٥% منهم Polymorphic . وأظهر البادئ OPJ-04 غياب الحزمة (1500 bp) في السلالات الست المتقزمة مقارنة بالسلالات الطبيعية . كما أظهر البادئ OPC-15 أن حزمة (936 bp) موجودة في السلالات الطبيعية فقط وأظهر البادئ OPA-06 وجود حزمة (1334 bp) في السلالات المتقزمة فقط . وكذلك تم عمل التماثل الوراثي Similarity matrix من نتائج البادئات primers المستخدمة وتراوحت قيمته ما بين ٠,٠٩ إلى ٠,٥٠ . وبناءا على تحليل التجمع Cluster الناتج فقد قسمت تلك السلالات إلى ست مجاميع Six clusters .

يتضح من هذه الدراسة أن استخدام تقنية الـ RAPD markers والمتخصصة في الكشف المبكر عن صفة التقزم في مرحلة الإكثار الدقيق (*In vitro*) كان له أهمية كبيرة في عملية الانتخاب للحصول على سلالات جديدة ومرغوبة لصنف الموز المغربي .