IDENTIFICATION AND SELECTION OF SUPERIOR BANANA PHENOTYPES IN THE CULTIVAR DWARF CAVENDISH USING AGRONOMIC CHARACTERISTICS AND RAPD MARKERS

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Abstract

Banana production in Turkey occurs in those regions with a subtropical environment. However, there have not been any studies on the identification of superior types *via* intra-varietal selection. The aim of this study was to identify banana off-types resulting from spontaneous mutations in field and greenhouse grown 'Dwarf Cavendish' banana. Mutations were identified based on the occurrence of altered agronomic parameters and *via* genetic polymorphisms as detected by Randomly Amplified Polymorphic DNA (RAPD) analysis. Phenotypic characters evaluated included stem circumference, plant height, leaf number at the flowering stage, bunch stalk circumference, number of fruit hands and fruit number, bunch weight, and fruit circumference and length. Selection studies resulted in identification of 48 off-types; 17 of them were identified in the field and 31 in the greenhouse. Eight of the selected off-types (2 from the field and 6 from the greenhouse) showed high levels of stability for various agronomic characteristics over a 3-year period of observation. These off-types displayed higher levels of variability for morphological characters affecting yield than the control 'Dwarf Cavendish.' Genetic similarities between the types ranged from 0.550 to 0.913 and genetic differences from 0.088 to 0.413, as determined by RAPD analysis. The high levels of genetic polymorphism among banana types indicated that the RAPD technique can be useful in evaluating banana intra-varietal genetic variation. Types 'Alanya 5', 'Gazipasa 11', 'Gazipasa 15', 'Anamur 10', 'Anamur 8' and 'Anamur 12' had the greatest similarities, whereas 'Alanya 5' and the control 'Dwarf Cavendish' were the most distant types. Results indicated that selections on banana grown in subtropical conditions allowed identifying the superior types in terms of yield and quality.

Introduction

Banana production occurs extensively in the humid agroecological zones of the tropics. Many banana cultivars cannot be grown in non-tropical regions. As a result, most banana breeding takes place in tropical regions (Smith *et al*., 1998). However, some banana types can be cultivated in subtropical regions between 20° and 30° north and south of the equator. The main goals of banana improvement programs in these sub-tropical regions are the development of genotypes that are better adapted to cooler climates and that have resistance to pests and diseases with higher fruit yield and quality. The main climatic factors affecting banana production in the cooler subtropics are the greater diurnal temperature fluctuations, lower night temperatures, higher rainfall and stronger winds in the summer. Furthermore, winter leaf sunburn, underpeel discolouration and growth cessations are typical physiological problems associated with banana production in the subtropics (Robinson, 1996). Local intra-varietal selection remains an important means of overcoming these environmental constraints.

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Commercial banana cultivars within the Cavendish sub-group are triploid, seedless, sterile and parthenocarpic (Khayat *et al*., 1998). Therefore, banana production has been improved in many countries by either importing promising cultivars/selections from other geographical areas or *via* the identification of superior and stable local selections (Eckstein *et al*., 1998; Khayat *et al*., 1998; Smith *et al*., 1998). Examples of superior clones imported from other areas are numerous (Pushkaran *et al*., 1991) and include lines 49, 100, 132 and 133 which were derived *via* selection of sports from cv. Nedran (AAB) in India. The superiority of these clones was documented by Pushkaran *et al*., (1991).

The contribution of mutations in the development of new banana cultivars is significant. Sports from 'Williams' proved to be superior to 'Dwarf Cavendish' line from which cv. `Williams' was originally selected (Robinson *et al*., 1993). Hwang & Tang (1996) reported the identification of off-types within the Cavendish subgroup that were resistant to 4 races of *Fusarium*. Menon (1996) identified 20 promising off-types, and Rajamony *et al*., (1996) reported 18 new off-types, during intra-varietal selection studies in India. The 'Dwarf Cavendish' selection 'Lancefield' displayed slightly higher yields than the control cultivars 'Grande Naine Israel' and 'Williams'. 'Chinese Cavendish' selections KBC1 and KBC2 exhibited shorter cycle times than the control cultivars. Intra-varietal selection studies in Western Galilee utilizing 'Grande Naine' resulted in the development of 5 novel types (5-1, 6-6, 37-5, 42-5, and 17-1) that had higher yields than 'Grand Naine' (Khayat *et al*., 1998).

In addition to improved agronomic traits, improvement of external and internal fruit characteristics, resistance to pests and diseases, as well as tolerance to climatic stresses are the major targets for *Musa* variety development. However, banana breeding programs have been rather slow in developing new clones with these characteristics due to the complex and polyploidy nature of the *Musa* genome that results in sterility barriers and other obstacles to conventional breeding approaches to this crops improvement (Novak *et al*., 1992). During the last 50 years, only a few cultivars have been developed (Khayat *et al*., 1998). As a result, the selection of improved "dessert" banana types adapted to specific environmental conditions continues to be important in the local improvement of this crop. Selection of off-types under marginal growing conditions has resulted in clones with improved bunch weight and fruit quality (Khayat *et al*., 1998).

Production of banana is significantly influenced by local environmental conditions. Thus, it is desirable to assess genotype x environment interactions for specific characteristics in the different ecological zones within a country (Smith *et al*., 1998). An alternative to conventional breeding methods involving mutation and recombinant DNA technologies has been suggested (Novok *et al*., 1990; Sagi *et al*., 1995). It has been reported that these techniques facilitate small genetic changes as opposed to large-scale recombination (Khayat *et al*., 1998). Various laboratories have initiated banana improvement programmes using tissue culture as a means to induce genetic change. However, even after several years expectations have yet to be fulfilled since this technique are not very efficient in improving specific characters (Garcia *et al*., 2002). Consequently, local selection efforts remain an important potential for banana genetic improvement.

Until recently, morphology-based methods had been used for the characterisation of *Musa* germplasm (Ortiz, 1997; Ortiz *et al*., 1998). Morphological characteristics are

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influenced by the environment. Therefore, molecular methods including PCR-based analysis techniques such as Random Amplified Polymorphic DNA (RAPD) (Williams *et al.*, 1990), Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphisms (AFLPs) have been used to elucidate genetic relationships among different *Musa* genotypes (Shinwari, 1995). RAPD assays have been used to distinguish plantain landraces (Howel *et al.*, 1994), for the identification of dwarf mutants within the Cavendish group (Damasco *et al.*, 1996), and for classification of *Musa* clones in India (Bhat & Jarret, 1995). In addition, this method was proven to be efficient in efforts to determine genetic diversity among 76 plantain landraces (Crouch *et al.*, 2000) and for the evaluation of genetic relationships among 19 East African highland bananas (*Musa* spp.).

Banana has been grown in Turkey for over a century. However, there are few reports on the utilization of intra-varietal selection for improvement of this crop. There has not been any previous report on the selection of promising mutants from the banana production areas in Turkey as well as being one of the rare selection studies conducted in greenhouse conditions in countries with sub-tropical climate. The present report gives the result of our efforts to select desirable off-types from 'Dwarf Cavendsish' grown in Turkey that are improved in terms of their yield and fruit quality, and to further characterize these by means of RAPD-based genetic analysis. The selections identified in this study are thought to be advantageous for banana cultivation not only in Turkey but also in other subtropical regions.

Material and Methods

This study was carried out in Anamur and Bozyazi in Icel, and also in Alanya and Gazipasa, in Antalya province of Turkey. Observations of the selections of superior types that were the result of natural somatic mutations in 'Dwarf Cavendish' were carried out on suckers over a 3-year period. The survey was carried out in a total area of 1875 ha, 1000 ha of which in protected cultivation and 875 ha of which in the open field. Stem circumference and height, leaf number at the flowering stage, bunch stalk circumference, hands and fruit number, bunch weight, fruit circumference and length were measured according to methods given by Pushkaran *et al.*, (1991) and Pekmezci *et al.*, (1998).

Selections which appeared to be phenotypically stable were subjected to RAPD analysis. Total genomic DNA was extracted from fresh leaf tissue using the CTAB procedure as described by Pancholi (1995). DNA samples for PCR analysis were diluted to a final concentration of 25 ng/µl. Ten 10-mer oligonucleotides were used as primers in PCR reactions (Table 1). The primers were chosen according to Pancholi (1995). Amplification reaction volumes were 25 µl, each containing 25 ng DNA, 2.5 mM $MgCl_2$, 0.4 mM each dNTPs, 1.25 U Taq Polymerase (Promega) and 1 µM primer in a reaction buffer containing 500 mM KCI, 100 mM Tris-HCI pH 9.0 and 1% triton. Amplifications were performed in a Perkin Elmer Thermal Cyler with the following temperature cycles: an initial 1 min., denaturation at 94 ^{o}C; 1.30 min., primer annealing at 35 ^{o}C; and 2 min., primer extension at 72 ^{o}C for 1 cycle. This was followed by: 44 cycles of 2.30 min., at 94 ^{o}C; 1.30 min., at 35 ^{o}C; and 2 min., at 72 ^{o}C. The final extension step was 72 ^{o}C for 10 min., (Pancholi, 1995). Amplification products were resolved by electrophoresis on 1.5 % agarose gels in 1 x TAE buffer and stained with ethidium bromide. PCR products were visualized on a transilluminator.

Table1. RAPD markers produced by ten primers among different banana types.

Primer No.	*Sequence*	GC (%)	No. total bands	No. polymorphic bands
MP10	CACCAGGTGA	60	5	2
MP12	TTATCGCCCC	60	5	4
MP7	AGATGCAGCC	60	7	3
MP3	CCAGATGCAC	60	7	7
MP6	AAGACCCCTC	60	8	4
MP8	TCACCACGGT	60	6	3
MP17	CTACTGCCGT	60	14	10
MP5	TCAGGGAGGT	60	13	7
MP1	CCCAAGGTCC	70	7	5
MP14	TGCGGCTGAG	70	8	4
Total			**80**	**49**

Data analysis: The data obtained from the observation of the agronomic characteristics of the selections made in different locations were used to prepare graphics. For analysis of the RAPD data, only reproducible amplification products were considered. RAPD fragments were scored as present (1) or absent (0). Data were used to calculate Nei & Li's (1979) smilarity coefficients. Dice Genetic Similarity indices (GSI) were calculated as Sxy= 2nxy/(nx+ny), where nx and ny are the numbers of fragments shared between individuals X and Y according to Nei & Li (1979). The dissimilarity matrices (Dxy=1-Sxy) were analyzed by the Unweighted Pair Group Mean Average (UPGMA) or Neighbor Joining (NJ) method of Saitou & Nei (1987) using PAUP* software (Swofford, 1999).

Results

Agronomic characteristics: Considerable variation was observed in the evaluated plant materials. For example, two plants produced twin bunches under greenhouse conditions (Fig. 1). The results of the analysis of stem circumference, height, and leaf number of the selected off-types from the field and the greenhouse are shown in Fig. 2. Stem circumference and height measurements of the selections were greater than those of the 'Dwarf Cavendish' control. Selections identified in the field had more leaves at flowering than did those in the greenhouse. Among the selections in the greenhouse, only 'Anamur 10' had fewer leaves than the control. The results of the analysis of bunch stalk circumference, number of hands and bunch weight are presented in Fig. 3. Among the selections from the field, 'Alanya 5' had a greater bunch stalk circumference. 'Gazipaşa 15' had a narrower bunch stalk circumference than the control. Selections obtained from the greenhouse were greater in bunch stalk circumference than those of the control. 'Alanya 5' had more hands than the control when grown in the field or greenhouse. 'Alanya 5' in the field and 'Anamur 8 and Bozyazı 14' in the greenhouse had larger bunch weight.

Fig. 1. The general view of twin bunches in 'Dwarf Cavendish' cultivar.

The results of the analysis of fruit number, fruit circumference and fruit length are presented in Fig. 4. Field-grown 'Alanya 5' and greenhouse-grown 'Anamur 12' had a greater number of fruits per bunch than did all other types and the control. Open field selected 'Alanya 5' had the greatest average fruit length. Among the greenhouse selected types, 'Anamur 8' and 'Bozyazı 14' produced the greatest bunch weights. Field grown 'Alanya 5' was readily differentiated from the other lines due to its larger stem circumference, height, number of hands and bunch weight. Similarly, 'Anamur 8' had noticeably greater stem height, number of hands, number of fruits and bunch weight. 'Bozyazı 14' had a smaller stem circumference but greater fruit circumference and fruit length values.

RAPD analysis of different banana types: The genotypes selected from different locations for their enhanced agronomic characteristics were subjected to RAPD analysis. Data in Table 1 show the number of total and polymorphic DNA fragments obtained using different RAPD primers. A total of 80 amplification products were generated. Fourty nine (61.25%) of those amplification products were polymorphic and thirty-one (38.75%) were monomorphic. The number of fragments produced by the various primers ranged from 5 to 14. Depending on the primer used, the length of the fragments obtained ranged from 250 bp to 4507 bp. Fig. 5 is an example of RAPD amplification with the primers MP12, M17 and MP14.

A matrix of genetic similarities and genetic distances based on Nei and Li's index is shown in Table 2. Levels of similarity between the genotypes ranged from 0.550 to 0.913. The genetic differences were between 0.088 and 0.413. The dendogram constructed from the matrix of similarity coefficients, using Unweighted Pair Group

Method Arithmetic average (UPGMA) is shown in Fig. 6. High levels of genetic similarity were observed among 'Alanya 5' and 'Gazipasa 11', 'Gazipasa 15' and 'Anamur 10', and 'Anamur 8' and 'Anamur 12'. The highest genetic similarity was between 'Anamur 12' and 'Anamur 4', whereas 'Alanya 5' and the control were the most distant types.

Table 2. Similarity matrix (above) and genetic distance (below) for different banana types on the basis of RAPD markers*.

	AL 5	G 11	G 15	AN 8	AN 10	AN 12	B 11	B 18	C
AL 5**	**1.000**	0.913	0.825	0.763	0.825	0.775	0.863	0.813	0.688
G 11	0.088	**1.000**	0.838	0.700	0.863	0.738	0.850	0.800	0.675
G 15	0.175	0.163	**1.000**	0.688	0.900	0.750	0.838	0.813	0.638
AN 8	0.238	0.300	0.313	**1.000**	0.663	0.838	0.750	0.725	0.550
AN 10	0.175	0.138	0.100	0.338	**1.000**	0.700	0.863	0.813	0.638
AN 12	0.225	0.263	0.250	0.163	0.300	**1.000**	0.763	0.813	0.588
B 11	0.138	0.150	0.163	0.250	0.138	0.238	**1.000**	0.825	0.825
B 18	0.188	0.200	0.188	0.275	0.188	0.188	0.175	**1.000**	0.650
C	0.313	0.325	0.363	0.450	0.363	0.413	0.300	0.350	**1.000**

*The distance matrix was analyzed by the UPGMA method of Saitou & Nei (1987) on the basis of the RAPD markers. The calculated as Dxy= 1-Sxy, which can range from 0.0 to 1.0 when all bands in the two lines are the same (0.0), when there are no bands in common (1.0). Genetic similarity (Sxy) ranges from 1.0 to 0.0, when two lines are identical (1.0) and when there are no bands in common between the two lines (0.0).

AL 5**: Alanya 5, G 11: Gazipasa 11; G 15: Gazipasa 15, AN 8: Amamur 8, AN 10: Anamur 10, AN 12: Anamur 12, B 14:Bozyazı14, B 18: Bozyazı 18, C: Control .

Discussion

This study was conducted to determine the extent of genetic diversity for agronomic characteristics and RAPD markers in the banana germplasm of Turkey. Bananas are a major fruit crops in the humid tropics and banana improvement programmes have been largely restricted to such regions. However, many cultivars developed in the tropics do not produce well in the lower temperatures and with the larger diurnal fluctuations in temperature that commonly occur in the subtropics. Smith *et al*., (1998) stated that improvement of cold tolerant (less than 16 ^{o}C) cultivars in the tropics were not an objective of any of the conventional banana breeding programs. Consequently, local efforts continue in the sub-tropics to select for off-types that fare better under sub-tropical environmental conditions. Results obtained from the present study indicate that selections on banana which are grown in subtropical conditions allowed identifying the superior types in terms of yield and quality.

Problems associated with clonal classification, and the various ways that molecular approaches can be utilized to overcome these difficulties, have been reported previously (Bhat & Jarret, 1995). RAPD markers are less expensive and technically less complex to analyse than RFLPs. RAPD analysis does not require large amounts of DNA or prior knowledge of the genetic structure (sequence) of the genome. Large amounts of information can be obtained quickly (Ford-Lloyd *et al*., 1996). Our results confirmed that RAPD markers could be readily detected and analyzed for different banana types.
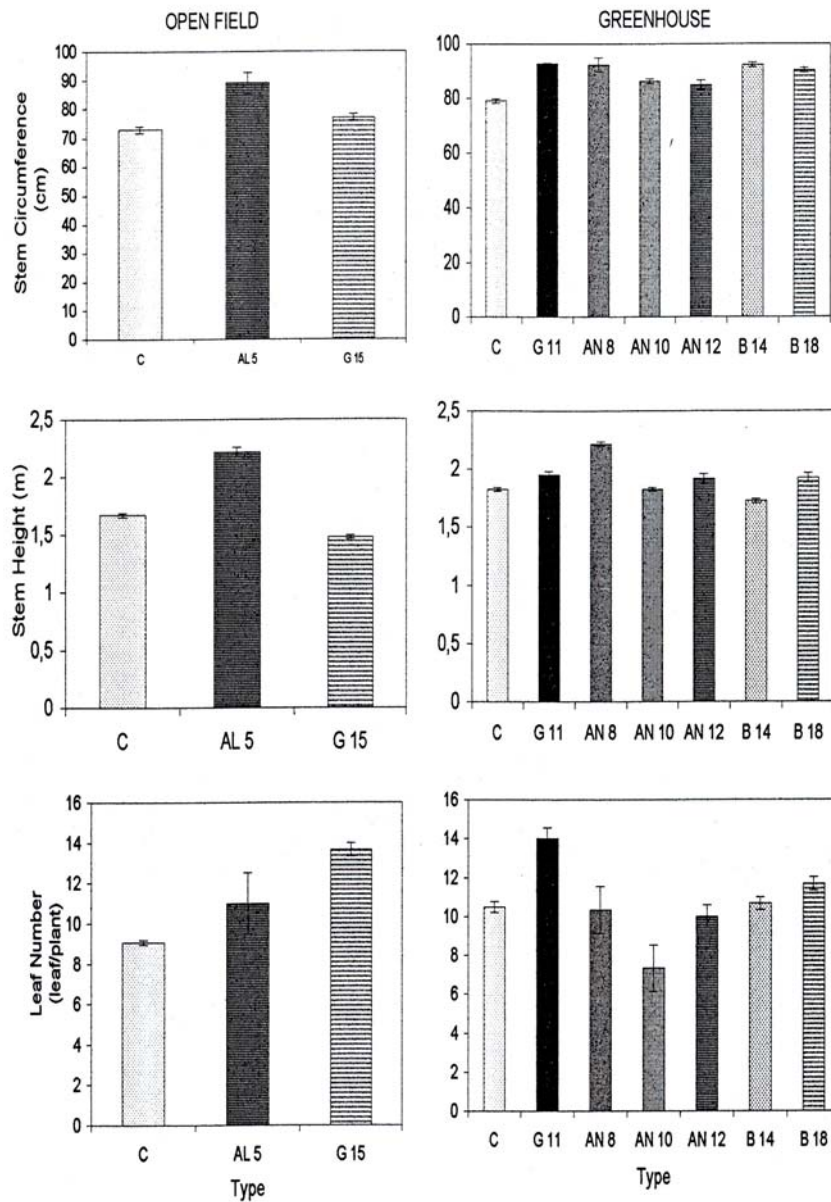
Fig. 2. The values of stem circumferences, stem heights and leaf numbers in types of 'Dwarf Cavendish' cultivar selected for open field and greenhouse growing.
Bars represent standard errors of means when larger than plotting lines.
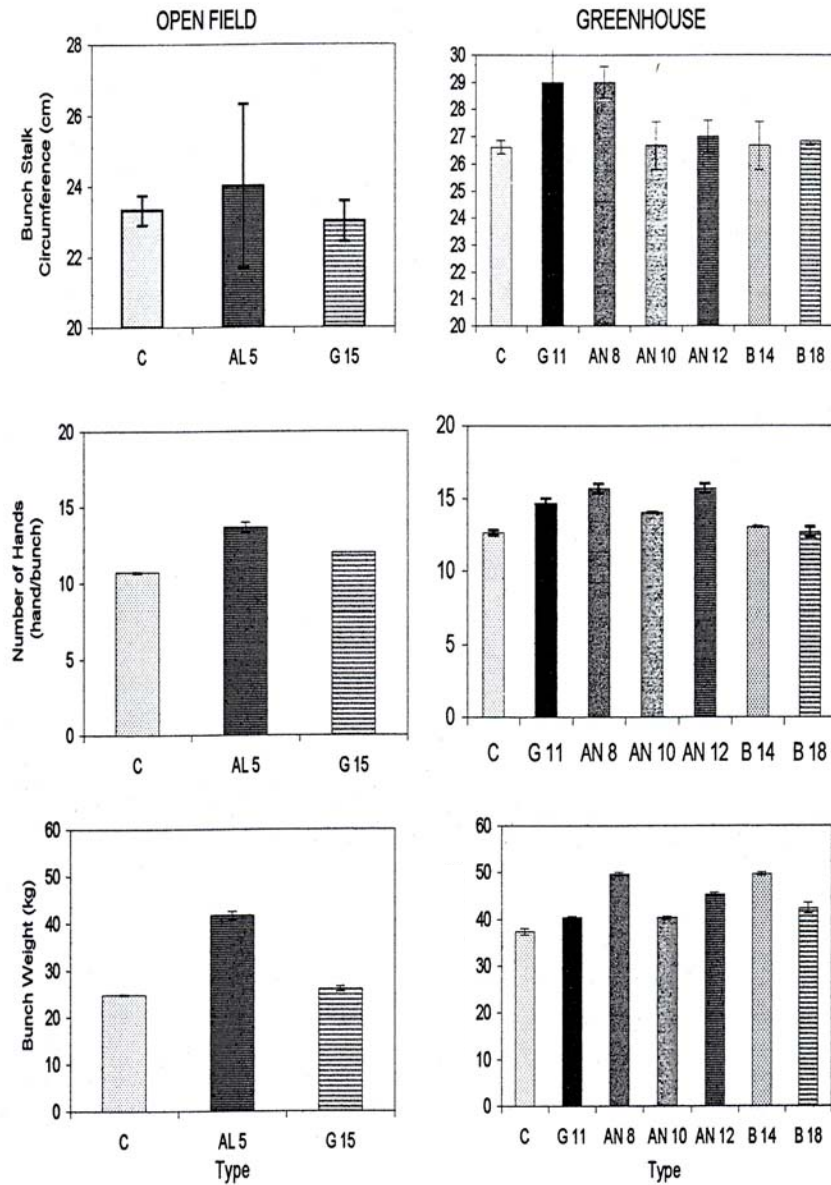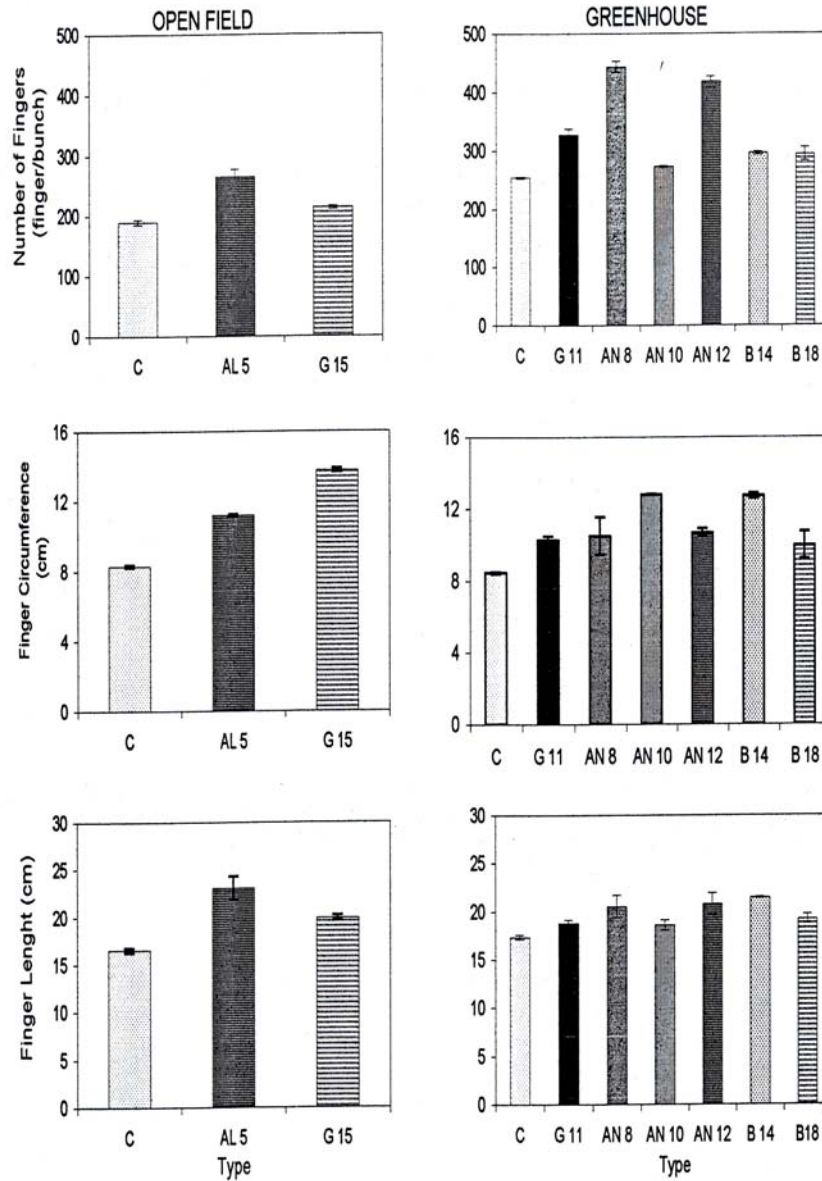
Fig. 3. The values of bunch stalk circumferences, number of hands and bunch weight in types of 'Dwarf Cavendish' cultivar selected for open field and greenhouse growing.
Bars represent standard errors of means when larger than plotting lines.

Fig. 4. The values of finger numbers, finger circumferences and finger lengths in types of 'Dwarf Cavendish' cultivar selected for open field and greenhouse growing.
Bars represent standard errors of means when larger than plotting lines.
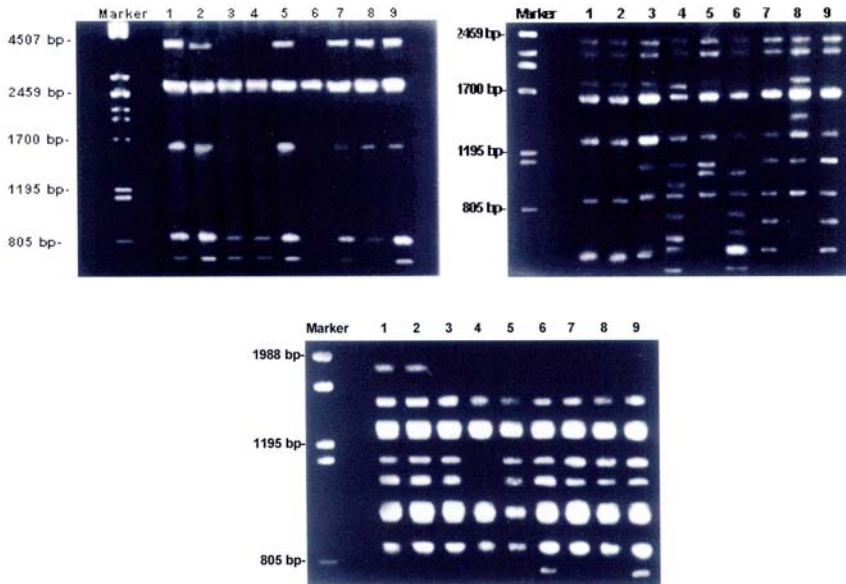
Fig. 5. Examples of RAPD fragments amplified with the primers MP12 (left) and MP17 (right) and MP 14 (below). (Marker, 1: Alanya 5, 2: Gazipasa 11, 3: Gazipasa15, 4: Anamur 8, 5: Anamur 10, 6: Anamur 12, 7:Bozyazı 14, 8: Bozyazı 18, 9:Control).
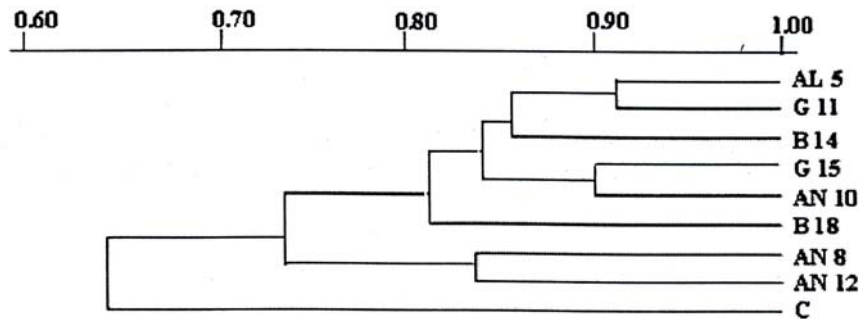

Fig. 6. The trees were generated using the distance matrix based on Nei's formula from 10 RAPD primers. (Al 5: Alanya 5, G 11: Gazipasa 11, B 14: Bozyazı 14, G 15: Gazipasa 15, AN 10: Anamur 10, B 18: Bozyazı 18, AN 8: Anamur 8, AN 12: Anamur 12, C: Control).

RAPD analysis revealed a total of 80 fragments - 49 of them polymorphic. Among the off-types, 'Alanya 5', 'Gazipasa 11', 'Bozyazı 14', 'Gazipasa 15', 'Anamur 10' and 'Bozyazı 18' were found to be more distantly related genetically to the control than 'Anamur 8' and 'Anamur 12'. The number of polymorphic bands generated *via* MP17, MP3 and MP5 primers was higher than the others. No significant correlation was found between individual molecular markers and specific agromomic characteristics. Crouch *et*

al., (2000) identified only a weak relationship between RAPD-based genetic and phenotypic similarities in study involving 76 plantain landraces. However, Engelborghs *et al*., (1999) found a significant correlation between molecular diversity and morphotype grouping.

It is probable that certain somatic mutations affecting agronomic characteristics have been selected and perpetuated by farmers over the years. In our study, we observed two plants with twin bunch (Fig. 1). This may have resulted from a common mutation in a particularly unstable region of the genome. Agronomically neutral somatic mutations have contributed to considerable random drift within genomic regions (Crouch *et al*., 2000).

Selection efforts, which were carried out on 'Dwarf Cavendish' banana cultivar both in the field and the greenhouse, were successful in the identification of 8 different types by RAPD analysis. Yield parameters and factors influencing yield were observed to be superior to 'Dwarf Cavendish' which was the control in this study. These results demonstrate that it is very important to evaluate genotype x environment interactions for specific traits in different ecological regions within a country. The selections identified in this study can be advantageous for banana cultivation not only in Turkey but also in other subtropical regions.

Acknowledgements

References

Bhat, K.V. and R.L. Jarret. 1995. Random Amplified Polymorphic DNA and genetic diversity in Indian *Musa* germplasm. *Genet. Res. Crop Evol*., 42:107-118.

Crouch, H.K., J.H. Crouch, S. Madsen, D.R. Vuylsteke and R. Ortiz. 2000. Comparative analysis of phenotypic and genotypic diversity among plantain landraces (*Musa* spp., AAB group). *Theor. Appl. Genet*, 101:1056-1065.

Damasco, P.O., G.C. Graham, R.J. Henry, W. Adkins, K. Smith and I.D. Godwin. 1996. Random Amplified Polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (*Musa* spp. AAA) bananas. *Plant Cell Reports*, 16(1-2): 118-123.

Eckstein, K., C. Fraser, J. Husselmann and N. Temple Murray. 1998. The evaluation of promising new banana cultivars. *Acta Hort*., 490: 57-69.

Engelborghs, I., R. Swennen and S. Van Campenhout. 1999. The potential of AFLPs to detect genetic differences and somaclonal variants in *Musa* ssp. *Infomusa*, 7: 3-5.

Ford-Lloyd, B.V., J.W. Kaemmer and P.J.L. Lagoda. 1996. Molecular marker assisted analysis of the *Musa* genome complex. *INIBAP, Networking Banana and Plantain, Annual Report*. 24-29.

Garcia, L.R., P.J. Perez, I.C. Bermudez, P.P. Orellana, N.R. Veitia, Y.M. Pardon and C.Q. Romero. 2002. Comparative study of variability produced by induced mutation and tissue culture in banana (*Musa* sp.) cv. 'Grande Naine'. *Infomusa*, 11(2): 4-7.

Howel, C.E., H.J. Newbury, R.L. Swennen, L.A. Withers and B.V. Ford-Lloyd. 1994. The use of RAPD for identifying and classifying *Musa* germplasm. *Genome*, 37: 328-332.

Hwang, S.C. and C.Y. Tang. 1996. Somaclonal variation and its use for improving Cavendish (AAA dessert) bananas in Taiwan. *Musarama*, 9(3): 6.

Khayat, E., A. Duvdevani, A. Shchukin and E. Lahav. 1998. Banana improvement program at Rahan Meristem. *Acta Hort.*, 490: 71-78.

Menon, R. 1996. Description and comparative evaluation of some banana clones. *Musarama*, 9(3): 8.

Nei, N. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. (USA),* 76: 5269-5273.

Novak, F.J., R. Afza, M. Van Duren and M.S. Omar. 1990. Mutation induction by gamma irradiation of *in vitro* cultured shoot tips of banana and plantain (*Musa* cvs). *Trop. Agric. (Trinidad*), 67(1): 21-28.

Novak, F.J., H. Brunner, R. Afza, R. Morpurgo, R.K. Upadhyay, M. Van Duren, M. Sacchi, J. Sitti Hawa, A. Khatri, G. Kahl, D. Kaemmer, J. Ramser and K. Weising. 1992. Improvement of *Musa* through biotechnology and mutation breeding. Biotechnology applications for banana and plantain improvement. *INIBAP*, 143-159.

Ortiz, R. 1997. Morphological variation in *Musa* germplasm. *Genet. Res. Crop Evol.*, 44: 393-404.

Ortiz, R., S. Madsen and D.R. Vuylsteke. 1998. Classification of African plantain landraces and banana cultivars using a phenotypic distance index of quantitative descriptors. *Theor. Appl. Genet.*, 96: 904-911.

Pancholi, N. 1995. *Aspects of tissue culture in relation to banana improvement and germplasm conservation*. The University of Reading, Department of Agricultural Botany, School of Plant Sciencec, 303 p.

Pekmezci, M., H. Gübbük. and M. Erkan. 1998. Investigation on growing possibilities of banana in Turkey. *Acta Horticulturae*, 490: 599-603.

Pushkaran, K., P.K. Rajeevan, N. Krishnan Nair, P.A. Varkey, A.K. Babylatha and S. Prasannakumariamma. 1991. Intraclonal variation in 'Nedran' banana. *Banana Newsletter*, 13: 4-6.

Rajamony, L., R. Menon and K.C. George. 1996. Screening banana clones of AAA group for yield and quality. *Musarama*, 9(3): 9.

Robinson, J.C., D.J. Nel and K. Eckstein. 1993. A field comparison of ten Cavendish sub-group banana cultivars and selections (*Musa* AAA) over four crop cycles in the subtropics. *Journal of Horticultural Science*, 68(4): 511-521.

Robinson, J.C. 1996. *Bananas and Plantain*. Crop Production Science in Horticulture, Series 5. CAB International, Cambridge, 238 p.

Sagi, L., B. Panis, S. Remy, H. Schoofs, K. deSmet, R. Swennen and P.A. Cammue. 1995. Genetic transformation of banana and plantain (*Musa ssp.*) *via* particle bombardment. *Biotechnology*, 13: 481-485.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4: 406-423.

Shinwari, Z.K. 1995. Introduction of new Techniques in Biological Sciences in Pakistan. I: Future Directions for Plant Systematics using RFLP. *Sci. Tech. Dev.*, 14(4): 22-30.

Smith, M.K., S.D. Hamill, P.W. Longdon and K.G. Pegg. 1998. Selection of new banana varieties for the cool subtropics in Australia. *Acta Horticulturae*, 490: 49-56.

Swofford, D.L. 1999. Paup*. Phylogenetic analysis using parsimony (*and other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research*, 18: 6531-6535.