MAJOR VEGETATIVE MORPHOLOGICAL CHARACTERS AND DNA FINGERPRINTS OF TWO MEDIUM CHILLING SWEET CHERRY STRAINS

EMAN SOBHY ATTALA SEIF

Hort. Res. Inst, Agric. Res. Cent, Min. Agric., Cairo, Egypt.

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Abstract

No significant differences in most of the studied characters of stem parts and leaves between the two strains of sweet cherries were detected. However, number of shoots developed on major branches was higher in strain (A) than (B), and colour of branches was slightly different in both studied strains. Shoot lenticels were well more elongated in strain (A) than (B). leaf blade base was wider in strain (B), number of serrates in strain (A) was more than (B), while upper surface in strain (B) is evidently darker in colour. Five random primers (10-mer) were used to amplify genomic DNA from combined leaf samples for each strain. Specific bands were observed with some primers.

Three primers namely: (OPA – 10, OPC – 11 and OPN - 13) gave sufficient amplificantion products with clover genome. Primer OPA – 10 gave 7 bands in strain (A) and 9 bands in strain (B), while primer OPC – 11 gave only 4 bands in strain (A) and 6 bands in strain (B), while primer OPN – 13 gave 4 bands in strain (A) and 5 bands in strain (B).

Thus, DNA fingerprints could be used for the recognization of the studied strains. However, strain (B) could not be considered as good pollenizer for strain (A) as the results show certain genetical relationships.

INTRODUCTION

Cherries belong to the family Rosaceae, sub family Prunoidease, the genus Prunus and sub – Genus Cerasus (Bailey, 1949). Sweet cherries Prunus cerasus, (Baily, 1949) are mainly produced in temprate zone countries where 800 to 1700 hours of chilling can be fulfilled. (Ruck, 1975). The Egyptian climate, which is subtropial with warm winter does not meet these requirements. However, it has been reported by Deidda et al., (1994) that some Sardinian cvs. need less than 600 hrs of chilling. Cherries are appreciated by the Egyptians and are imported from some near – by countries. Some cherry plants have been imported from Syria by the Ministry of Agriculture recently.

The french cultivar (Coeur de Pigeon) which seems to be of medium chilling requirements and an unkown pollenizer are among them. In the course of trials conducted to establish successful local production of cherries in Egypt, H_2CN_2 at different concentrations and time of application was tried to improve bud burst and accelerate vegetative growth (Attala and Stino, 1999). Traditionally, morphological characters are usually used to identify various fruit cultivars (Bailey, 1949).

In recent years, random amplified polymorphic DNA (RAPD) technique has been used by (Williams *et al.*, 1990) as such markers are not influenced by environmental conditions (Lefort – Buson *et al,* 1990). Moreover, RAPD technique can reveal polymorphism even within genetically closely related groups (Hu and Quiros, 1991) and has been recently developed as a way to generate a great number of DNA markers in many plant species (Williams et al, 1990). However, this investigation was carried out to detect the major vegetative characters and DNA (RAPDs) paterns of coeur de pigeon and the unknown recommended pollenizer. Results of such studies may help in identifican-tion of plants and further investigation to detect relationships which affects the pollen compatibility between the two strains.

MATERIALS AND METHODS

The material used for this investigation was obtained from a private orchard in Menoufia Governorate. Six – seven – years old cherry trees of two strains (A) & (B) on Maheleb stock, in the transformation phase, were used. Strain (A) has been introduced from Syria as coeur de pigeon (cv.) and strain (B) as pollenizer. All normal cultural practices were followed. The following studies were carried out at the end of the two seasons of 1999 & 2000.

1 Morphological characters

The following determinations were carried on stem parts and leaves:-

1-1 Stem parts: length of, one – year – old (shoot), two – years – old (branch) and shoot internodes were measured. Number of buds on shoots and branches number of shoots and branches were also measured. Number of spurs on shoots and branches, and shoot diameter were measured as well. General colour of stem was determined using Munsell color charts for plant tissues (Munsell color, 1977).

Gross description of lenticels was studied on enlarged photos taken to illustrate the surface structure of the branches of the two strains. Number of lenticels per square cm was counted.

1-2 Leaves

The following morphological characters were determine on mature leaves according to Bellini *et al.*, (1984). Description of shape of blade apex, base, margins and number, length and width of serrates were recorded.

Average shades of upper and lower surface leaf colour were determined by matching with various colours using the same colour chart. Impression of blade lower surface of five mature leaves from each strain were made using cellodine in acetone (at

the end of the season). Number of stomata/m2 was determined using a light microscope.

2 Genetic Finger Printing (Isolation of DNA)

2-1 Total genomic DNA isolation

Isolation of DNA was carried out according to the protocol described by Walbot (1988).

Additional chlorofrom – isoamyl alcohol extraction and ethanol precipitation were performed to improve the purity of DNA. Rnase treatment was directly conducted before amplification step.

2-2 DNA amplification and primers:

All polymerase chain reaction were performed in a reaction mix containing 20ng genomic DNA, O.5 unit Taq polymerase 200 UM each of ATP, dCTP, dGTP and dTTP (sigma), 5 PM random primer and appropriate amplification buffer.

The amplification was assembled on ice, overlaid with a drop of mineral oil. The reaction was performed in thermal cycler (coy model No. 110p) with the following temperature conditions: 94°C for 3 min. followed by 45 cycles of 92°C for 30 sec. 35°C for 60 sec. and 72°C for 2 min, for denaturation, annealing and extension, respectively. Reaction was finally incubated at 72°C for 10 min. and further, 10min. at 62°C.

The RAPD products were analyzed by electrophoresis in 2% agarose in TAE buffer, stained with ethidium bromide and photographed under UV light. Five random, 10 – mer primers (Operon Technologies) were screened for RAPD markers. Three primers which gave clear and consistent amplification products were only used to amplify a bulk sample of the plants (Table 1).

The amplified well resolved bands with different primers were recorded and their positions were detected. The molecular size of amplification products was calculated from standard curve based on known size of marker DNA fragments, Gibco 1.5 kb ladder.

Table 1. Sequence and amplified products of five arbitrary primers (Operon Technologies) used to generate RAPD markers in sweet cherries strains

Primer	Sequence	Number of bands (Strains)
OPA – 10 (Al)	5'CAGGCCCTTC3'	7 to 9
OPM – 13 (M13)	5'GGTGGTCAAG3'	(a)
OPK – 20 (K20)	5'GTGTCGCGAG3'	(c)
OPC – 11 (C11)	5'AAAGCTGCGG3'	4 to 6
OPN – 13 (N13)	5'AGCGTCACTC3'	4 to 5

a) Presence of amplified products but bands are faint and not consistent.

b) No amplification products.

Statistical analysis

Means and standard deviations (descriptive statistics) were computed and ranges were calculated for each studies character. Comparison between ranges were done to determine significant differences (Snedecor and Cochran, 1990).

RESULTS

1 Morphological characters

1-1 Stem parts

The results of characters examined during the seasons of 1999 and 2000 i.e. length of, one – year -- old (shoots), two – years – old (branches), and shoots internodes, number of, one year old shoots, shoot buds, branch buds, shoot spurs, branch spurs, shoot internodes and shoots diameter are summarized in (Tables 2&3).

Table 2 : Quantitative morphological main characters of stem parts (season1999)

characters / Strains	Descriptive Statistics	Length of shoot	Length of branches of (two years old)	Length of intermodes on shoots	Number of shoots of (one year old)	Number of buds/shoots	Number of buds/ branch	Number of spurs/shoot	Number of spurs/ branch	Number of intermodes/shoots	Shoot diameter
A	Mean	82.80	60.80	3.31	6.20	7.70	5.40	1.33	6.70	31.11	0.69
	Sd	5.75	9.90	0.60	2.30	3.90	7.50	0.37	1.60	4.96	0.13
	Range	77 → 88.5	50.9 → 70.7	2.4 → 4	3.9 → 8.5	3.8 → 11.6	2.1 → 12.9	0.96 → 2.7	5.1 → 8.3	25 → 40	0.5 → 0.9
B	Mean	70.60	53.80	3.40	2.00	10.00	7.70	2.50	6.30	25.33	0.74
	Sd	9.50	13.60	0.49	1.00	5.60	3.90	0.52	1.00	4.70	0.14
	Range	61 → 80	40.2 → 67.4	2.8 → 4.3	1.0 → 3.0	4.4 → 15.6	3.8 → 11.6	2 → 3	5 → 8	20 → 35	0.5 → 0.9
Statistical Results		Insignificant	Insignificant	Insignificant	Significant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant

Table 3 : Quantitative morphological main characters of stem parts (season 2000)

characters / Strains	Descriptive Statistics	Length of shoot	Length of branches of (two years old)	Length of intermodes on shoots	Number of shoots of (one year old)	Number of buds/shoots	Number of buds/ branch	Number of spurs/shoot	Number of spurs/ branch	Number of intermodes/shoots	Shoot diameter
A	Mean	83.50	82.00	3.34	6.40	5.80	6.40	3.10	6.40	32.22	0.71
	Sd	6.38	5.00	0.37	1.30	0.43	1.30	0.78	1.30	3.40	0.11
	Range	76.5 → 89	77 → 88	2.8 → 4	4 → 8	5.3 → 6.5	4 → 8	2 → 4	4 → 8	28 → 38	0.55 → 0.90
B	Mean	74.00	70.00	3.40	6.30	6.70	6.30	2.50	6.30	28.22	0.80
	Sd	8.54	9.00	0.40	1.00	0.66	1.00	0.52	1.00	3.37	0.06
	Range	65 → 82	61 → 80	2.8 → 4.3	5 → 8	5.8 → 78	5 → 8	2 → 3	5 → 8	23 → 35	0.7 → 0.9
Statistical Results		Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant

The differences between majority of characters of both strains (A&B) were insignificant in the two seasons. Nevertheless, the following of differences could be detected out, number of shoots ranged between (3.9→8.5) in strain (A) and (1.5→3.0) in (B) in the first season.

The main colour of the branches in the two strains was brown (Table 4). It varied between 5/2 7.5YR in strain (A) and 5/2 2.5Y in strain (B) in the two seasons respectively.

Table 4: Qualitative morphological.

Average shades of stem colour (brown)

Strains	Season(1999)	Season(2000)
A	5/27.5YR	5/27.5YR
B	5/22.5Y	5/22.5Y

By comparison with standard shades of colour represented in Munsell colour chart for plant tissues.

The stem lenticels were completely formed Figure (1) and were more elongated in strain (A) than (B).

Average number of lenticels on stem surface per cm^2 (Table 5) did not show significant results.

Table 5: Average number of lenticels on two years old (branch) per cm^2

Strains	Descriptive statistics	Number of lenticels per cm2
A	Mean Sd Range	6.4 1.3 4 → 8
B	Mean Sd Range	6.3 1 5 → 8
Statistical result		Insignificant

lenticels strain [A]

lenticels strain [B]

(X 3.3)

Figure1: Frequency and shape of lenticels on the surface of stem.

1-2 Leaves:

General characters of leaf's blade are summarized in Table 6.

Shape: Lanceolate form characterized the blade in both strains.

Apex: All the leaves in the two strains have an acute apex.

Base: Hastate base was noticed in leaves of the two strains, however, strain (B) has wider base than strain (A).

Margins: All the studied blades have a clear serrate margin.

Table 6: Qualitative morphological leaf's blade characteristics

Strains	Out lines of the blades (Blade shape)	Blade apex	Blade base	Blade margin
A	Lanceolate	acute	hastate	serrate
B	Lanceolate	acute	hastate	serrate

Number of serrates: The range of serrates number in strain (A) was (7.25→10.90), however, in strain (B) it ranged between (3.68→5.32), showing a significant difference (Table 7).

Measurments of serrates: length and width of serrates did not show any significant differences (Figure 2).

Table 7 : Quantitative morphological characters of leaf serration

characters / Strains	Descriptive Statistics	Number of serrates along 2 cm of major	Length of serrates single	Width of serrate single
A	Mean	9.10	3.10	2.75
	Sd	1.85	0.99	0.63
	Range	7.25 → 10.95	2.11 → 4.09	2.12 → 3.38
B	Mean	4.50	2.20	4.00
	Sd	0.82	0.42	0.94
	Range	3.68 → 5.32	1.78 → 2.62	3.06 → 4.94
Statistical Results		Significant	Insignificant	Insignificant

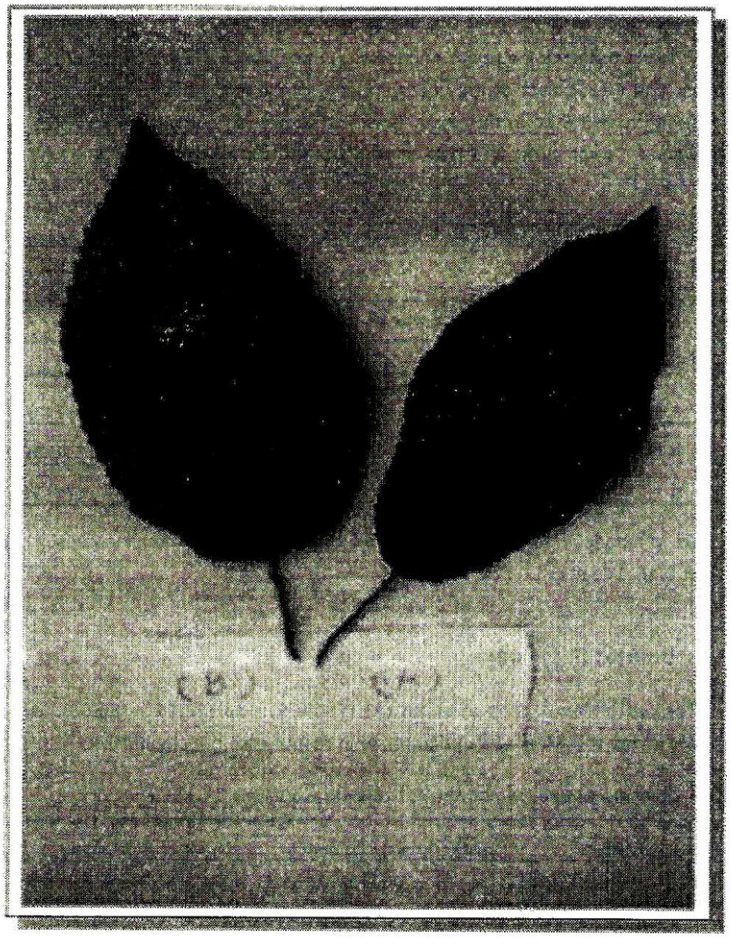


Figure 2: Shape, Apex, Base, Margins and serration of the leaf.

Blade colour:

Table 8 Shows the upper and lower surface blade shades of colour in the two seasons. The main colour of the upper surface, of the leaf blade was green, in strain (A) reffering to 4/6 5GY in the chart, however, strain (B) colour was 4/4 5GY in the two seasons. Lower surface in both strains (A) and (B) matched with 6/4 5Gy in the two seasons.

Table 8: Qualitative morphological. Average shades of upper and lower surface leaf colour

Strains	Upper surface		Lower surface	
	Season -1999	Season -2000	Season -1999	Season -2000
A	4/6 5GY	4/6 5GY	6/4 5GY	6/4 5GY
B	4/4 5GY	5/4 5GY	6/4 5GY	6/4 5GY

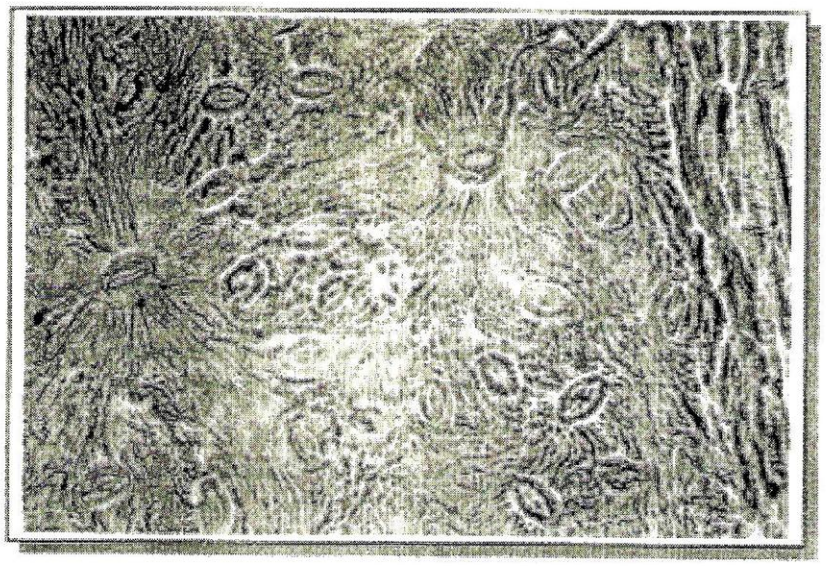
By comparison with standard shades of colour represented in Munsell colour chart for plant tissues.

Leaf stomata: (Figures 3)

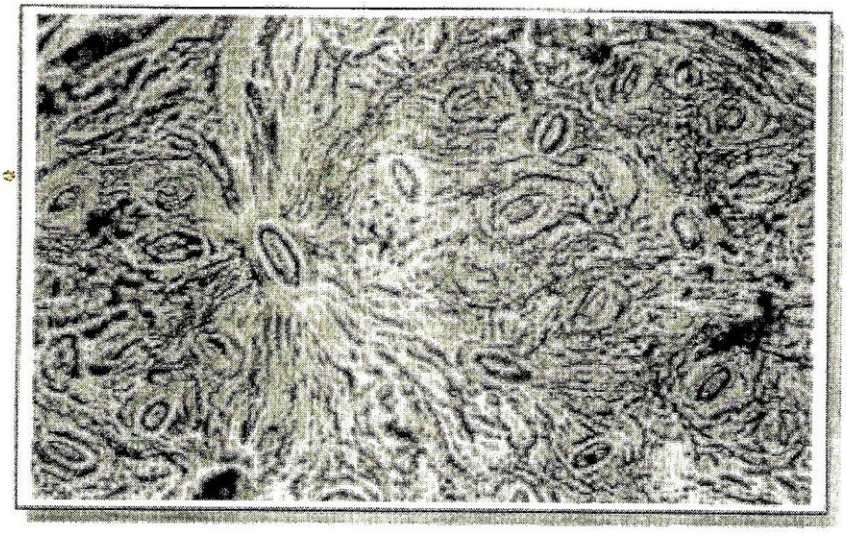
The leaf stomata rang in strain (A) (Table 9) was (39.14 → 50.06), while in strain (B) it was (37.84 → 45.76) difference in results was not significant.

Table 9: Stomata frequency per mm^2 of lower leaf surface

Strains	Descriptive statistics	Number of stomata
A	Mean	44.4
	Sd	5.6
	Range	39.14 → 50.06
B	Mean	41.8
	Sd	3.96
	Range	37.84 → 45.76
Statistical result		Insignificant



stomato strain [A]



stomato strain [B]
(X 3.3)

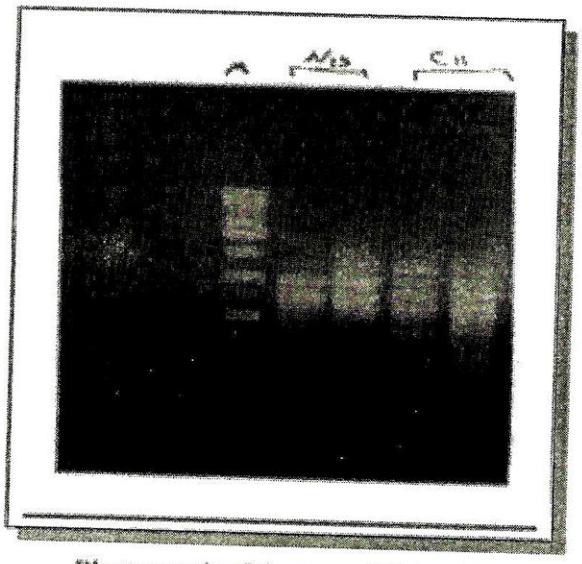
Figure 3: Frequency and shape of stomato on leaf lower surface.

2 Genetic fingerprinting

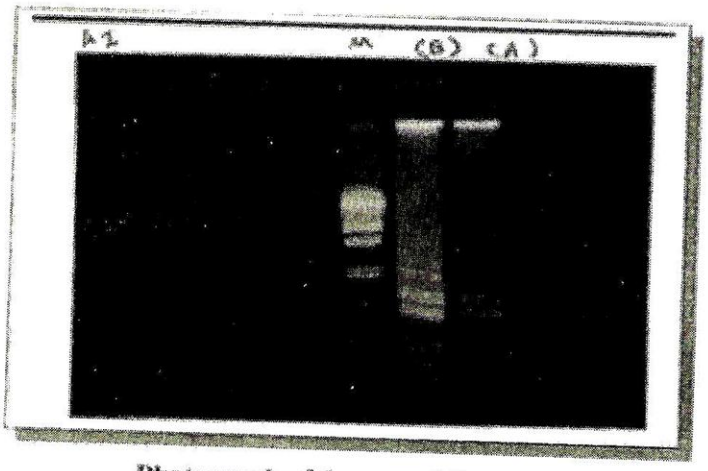
It is essential to optimize the PCR conditions to obtain reproducible results before going on routine analysis. Investigating each factor individually, such as genomic DNA quality and concentrations, primer annealing and extension temperature and denaturation time and temperature is a prerequisite. Series of preliminary experiments were conducted to select the suitable primers (Table 1) and the optimal conditions for RAPD analysis as described in materials and methods.

Five random (10 - mer) primers were screeneds for genomic DNA amplification for each strain. Three primers (OPA – 10, OPC – 11 and OPN - 13) gave clear and consistent amplification products and were used as markers for the two strains of cherries. Data presented in (Table 1) show the squence of the five primers and the number of bands resulted from studied strains (Figure 4) .

Primer OPA – 10 (Al) gave 7 bands in strain (A) and 9 bands in strain (B). Strain (A) showed the following bands (1.1, 1.25, 1.3, 1.4, 1.65, 1.75 and 5.0 kbp), nevertheless, strain (B) showed 9 bands (1.1, 1.25, 1.3, 1.4, 1.5, 1.6, 1.7, 1.9 and 5.0 kbp), (Table 10). It is evident that number of bands was more by two bands in strain (B) than in strain (A).



Photograph of Agarose OPA – 10
" Carrier Ampholyte 7 – 9 "



Photograph of Agarose OPC – 11
" Carrier Ampholyte 4 – 6 "
and OPN – 13
" Carrier Ampholyte 4 – 5 "

Figure 4: RAPD-Profiles of tow strains [A,B] genrated by PCR amplification using the random primers.

Table 10: Random DNA primers selected to distinguish RAPD fingerprints of two strains of cherries

Primer	Marker		Strain(A)		Strain(B)	
	Number of bands	Site of bands	Number of bands	Site of bands	Number of bands	Site of bands
OPA-10 (A1)	7	1.00 1.50 2.00 2.80 3.00 3.50 3.90	7	1.10 1.25 1.30 1.40 1.65 1.75 5.00	9	1.10 1.25 1.30 1.40 1.50 1.60 1.70 1.90 5.00
OPC-11 (C11)	8	1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00	4	0.70 1.30 1.60 1.80	6	0.80 1.20 1.60 1.80 1.90 2.10
OPN-13 (N13)	8	1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00	4	1.60 1.70 1.90 2.30	5	1.30 1.60 1.70 1.90 3.00

However, sites of bands were similar in positions (1.1, 1.25, 1.3, 1.4 and 5.0 kbp) in the two studied strains, but they were different in positions (1.65 and 1.75 kbp) and (1.5, 1.6, 1.7 and 1.9 kbp) in strain (A) and (B), respectively.

Concerning primer OPC – 11 (C11), Strain (A) showed 4 bands and 6 with strain (B). Detected sites of bands in first strain were (0.7, 1.3, 1.6 and 1.8 kbp), while they were (0.8, 1.2, 1.6, 1.8, 1.9 and 2.1 kbp) in the second, all bands were different in sites except (1.6 and 1.8 kbp) which were at the same site for the two strains. However, primer OPN – 13 (N13) gave 4-5 bands in strains (A) and (B) respectively. Similar bands are in positions in the two strains at (1.6, 1.7 and 1.9 kbp). Nevertheless, different bands were at (2.3 kbp) in strain (A) and (1.3 and 3.0 kbp) in strain (B).

DISCUSSION AND CONCLUSION

It is of great importance for the grower to differentiate between the grown tree cultivars in the orchard. This is difficult if the grown cultivars are genetically close. Most of close cultivars are usually incompatible and could not be used for cross pollination (Williams *et al.*, 1990). Thus, this investigation was carried out and intended to give the DNA fingerprints of the two strains (A & B) of sweet cherries as an easy identification and detection of probable genetical closeness. It is evident that the majority of studied vegetative characters did not differ mainly than what has been described in sweet cherries (Childers, 1983 and Cummins, 1991). However, slight differences could be noticed in number of shoots per branch and colour. Lenticels, which were completely formed, are more elongated in strain (A) than (B). lenticels have been described clearly by Hayward (1938). Blade base was wider in strain (B) than (A), slight differences in leaf serration could be noticed, number of serrates was more in strain (A) than (B).

Traditionally, morphological characters are usually used to identify various cultivars, however, there are narrow differences between strains (A & B) concerning those characters.

This study revealed that RAPD markers could be utilized to identify the two strains of cherries according to the position and number of bands, it can be concluded that both strains are genetically closely related to each other.

The molecular marker techniques is not influenced by environmental condition (Lefrt – Buson *et al,* 1990). The ability of DNA based molecular, markers to detect more genetic variations compared to isozyme or morphological markers was cleared (Kongkiatngam *et al,* 1995). The obtained data of molecular analysis extracted from RAPD patterns showed genetic variation by using three random primers OPA – 10, OPC 11 and OPN – 13, whereas no clear evidence of genetic variations existed with the other two primers.

The polymorphism in the amplification products may be either from changes in the sequence of the primer banding site (e.g. point mutations) or changes which alter the size or prevent the successful amplification of the target DNA (e.g. insertion, deletions and inversions).

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الصفات المورفولوجية الأساسية الخضرية والبصمات الوراثية لسلالتين متوسطى وحدات البرودة – من الكريز الحلو

إيمان صبحى عطالله

مركز البحوث الزراعية – معهد بحوث البساتين

تم دراسة الصفات المورفولوجية الأساسية لاجزاء الساق والأوراق كمياً ووصفيا كذلك الوراثية للسلاتين (أ) (Coeur de pigeon ب السلالة الملقحة) من الكريز الحلو – لم توجد اختلافات أساسية لمعظم الصفات المدروسة. كان عدد الأفرع لكل زراع اكثر فى السلالة (أ) عن (ب). كما وجد اختلاف بسيط بين لون الساق فى السلالتين . وجد ان العديسات فى السلالة (أ) أطول منها فى السلالة (ب). كما كانت قاعدة النصل اعرض فى السلالة (ب)، كما ان عدد السنون فى الأوراق كان اكثر فى السلالة (أ) عن (ب)، لون السطح العلوى للنصل اغمق فى السلالة (ب).

استخدم خمسة بوادئ عشوائية (تتكون كل منها ١٠ قواعد نيتروجنيية) مأخوذة من عينات الأوراق لكل سلالة ووجدت اختلافات وراثية على المستوى الجزيئى بين السلالتين باستخدام ثلاثة بادءات (و ب ١٠، و ب س – ١١ ، و ب ن – ١٣) أعطت نتائج موضحة لوجود اختلافات للجينوم فى السلالتين . أعطى بادئ (و ب أ – ١٠) ٧ مواقع فى السلالة (أ)، ٩ مواقع فى السلالة (ب) ، فى حين أعطى بادى (و ب س – ١١) ٤ مواقع فى السلالة (أ)، ٦ مواقع فى السلالة (ب)، كما أعطى بادى (و ب ن – ١٣) ٤ مواقع فى السلالة (أ)، ٥ مواقع فى السلالة (ب).

وقد أثبتت الدراسة انه بإمكان استخدام البصمة الوراثية بالطريقة الموضحة فى البحث للتفرقة بين سلالتين الكريز، كما أوضح انه توجد درجة من القرابة بينهم مما لا يجعل السلالة (ب) ملقح متوافق مع السلالة (أ)