Morphological, anatomical and biochemical characterization of Syrian pear (*Pyrus syriaca Boiss*) genotypes

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ABSTRACT

Characterization of Syrian pear genotypes was achieved using morphological, anatomical and isozyme markers. Morphological studies discriminated between the six Syrian pear genotypes. Abu-Satel genotype was the most vigorous compared with other genotypes and produced fruits with high quality and quantity. Anatomical studies, depending on leaf thickness and midrib differentiated between the six pear genotypes, especially midrib and vascular bundle thickness. Abu- Satel revealed the highest midrib thickness, while Meskawi genotype revealed the highest vascular bundle thickness. Biochemical markers (isozymes) were used to discriminate between the six pear genotypes using peroxidase and esterase. Peroxidase showed high polymorphism among the six pear genotypes (90%), Abu- Satel revealed the highest number of bands (9 bands), three of which were unique bands, while esterase revealed two monomorphic bands.

Key words: *Pear, Pyrus syriaca, morphological characters, anatomical characters, biochemical markers, isozymes, peroxidase, and esterase.*

INTRODUCTION

The pear genus- *Pyrus*- probably originated during the tertiary period in the mountainous regions in Western and Southern China (Rubstov, 1944).The genus Pyrus belongs to the subfamily *Pomoideae*, in *Rosaceae* family. The basic chromosome number of *Pyrus* (x=17) (Chevereau *et al.,* 1989). Most of the pear cultivars are diploid (2n =2x = 34). By natural mutation and artificial selection, a few of triploid and tetraploid cultivars emerged and vegetatively propagated by growers (Cao *et al.,* 2002). *Pyrus syriaca* (Boiss.) is one of the main pear species that widely distributed in Palestine, Lebanon, Turkey, Iraq, Jordan, and Syria. Many genotypes of *P. syriaca* and related cultivars are distributed in different regions of Syria from semi arid to humid areas within different altitudes from 200 to 1800 m above the sea. Pear cultivars are planted in south and west of Syria, and occupy the third crop of deciduous tree fruits after apple and grape. The total number of trees, 1.809 million, produces 20000 tons of pear fruits. Morphological studies are necessary to build up an important role for breeding programs (morphological traits and adaptation to a wide range of environments). Biochemical markers such as isozymes have proved to be useful for cultivar identification in pear. The first study of pear isozyme was carried out in 1980 to identify 6 ornamental varieties of *P.calleryana*

by peroxidase (Chevereau *et al.,* 1997). Recently, DNA fingerprinting was applied to pear species.

The present study was carried out to identify 6 Syrian pear genotypes depending on morphological and anatomical traits, in addition to isozyme markers.

MATERIALS AND METHODS

Plant material was obtained from the Agricultural Scientific Research Committee of Syria. Six Syrian pear genotypes were used (Table1). Morphological studies were achieved depending on pear descriptors (Thibault *et al.,* 1983 and Muzher, 1999).

Table (1): The six Syrian pear genotypes, climatic regions, and end use.

Genotype	Climatic region	End use
Meskawi	South of Syria	Cultivar (scion)
W.T1 (*P.syriaca* Boiss)	South and middle of Syria	Rootstock
Abu-Satel	South of Syria	Cultivar (Scion)
W.T2 (*P.syriaca* Boiss)	South and middle of Syria	Rootstock
W.T3 (*P.syriaca* Boiss)	South and middle of Syria	Rootstock
Romi	South of Syria	Cultivar (Scion)

Anatomical studies

The leaves were fixed and preserved in FAA (40% formalin, 100% glacial acetic acid, and 70% ethanol, 5:5:90 v/v/v, respectively. The materials were taken and dehydrated through a series of chloroform-ethyl alcohol and placed in paraffin wax (60-62°C, melting point at 30°C) for 12-14 hours. They were then vacuum filtered for 24-36 hr, the chloroform-paraffin mixture was then replaced with fresh paraffin for embedding (Johanson, 1940). The paraffin blocks containing the leaves were sectioned at 17µ thick in cross-section with rotary microtom. The prepared slides were then deparaffinied and stained with safranin and malachite green. The sections were examined and photographed to study the differentiation between the six pear genotypes by leaf, midrib, mesophyll thickness, xylem, phloem and vascular bundle diameter.

For isozyme analysis, extraction was performed using the youngest green leaves from two weeks old grafted pear. Analysis was performed using PAGE according to the method of Chevreau *et al.* (1997). Leaf extracts were prepared by grinding 200 mg fresh leaves in 1.5 ml of the following extraction buffer: KH_2PO_4 (0.1M), sucrose (7%), egg albumin (0.1%), ascorbic acid (0.4%). β-mercabtoethanol (0.15%), TritonX100 (0.02%),polyvinyl polyrrolidone (8%), pH=7.5, centrifugation at 18000g for 1 hr was applied.

A polyacrylamide standard gel was prepared by dissolving 8.55 g acrylamide and 0.45 g bisacrylamide in 150 ml Tris-borate buffer (0.125 M Tris, pH 8.9). After filtration, 145 ml of this monomer solution were used to prepare the gel by adding 50 mg Sodium Thiosulfate, 0.1 ml TEMED, and 2.8 ml ammonium persulfate. PAGE electrophoresis

was performed in Biometra apparatus using slab gels (11x12 cm). Electrophoresis was performed in acrylamide gels for peroxidase (PRX) and esterase (EST). Staining was performed according to Wendel and Weeden (1989). The stain composition used for the isozymes included:

A. Peroxidase (PRX): 85 ml (100 mM) potassium acetate pH 4.76, 15 ml of 10% benzidine and 1 ml H_2O_2 1%.

B. Esterase (EST): 100 ml phosphate (0.15 M) pH7.2, 20 mg 6-naphthylacetate dissolved in 2 ml acetone, and 50 mg fast blue RR salt.

The gels were fixed in 50% glycerol for one hour and then soaked in water for several hours prior to scoring and photography.

RESULTS AND DISCUSSION

Morphological characters

Growth habit

Growth habit based on high and spread measurements of adult trees on their own roots, or relative to reference species on a common rootstock. Table (2) shows that tree vigor of all Syrian pear genotypes were small to intermediate except Abu-Satel genotype which was more vigorous than the other genotypes. The shoot length ranged between 18 cm ($W.T_1$) and 40 cm (Abu-Satel), while the shoot length of the other genotypes ranged between 25-35 cm. Ramification of trees differs from erect to intermediate. Some genotypes were thorny such as $W.T_2$, while the other genotypes were thornless. The leaves are serrated, and the leaf index (leaf width/leaf length) ranged from 0.35 for $W.T_3$ (leaf length 5.5 cm and leaf width 2 cm) to 0.78 for Abu-Satel (leaf length 7 cm and leaf width 5 cm). Stipules were present in four genotypes while absent from the others.

Flowers and fruits

The perfect flowers bloom on 2 years or older spurs between April and May in the south of Syria. The inflorescence consists of 4-12 flowers occurring in umbel-like racemes. Petals are white, 20 to 30 anthers, and 2-5 free styles which are closely constricted at the base. The fruit shape differs from wild types to cultivars which ranged from globose to pyriform, the highest length of fruits is revealed by Abu-Satel (12 cm), while the lowest is revealed by $W.T_2$ (3.5 cm), the ground color of differs from green through yellow or red during maturation, stone cells were present with high density in wild genotypes, while the other cultivars were juicy. The ripening season starts in late July ($W.T_2$ and Meskawi) to September for other genotypes, fruits of wild genotypes are not used for eating except $W.T_2$, but fruits of some cultivars can be eaten directly from trees, whereas others may require a period of storage to ripen. Each fruit contains 5-up to10 seeds, wild types produced a large number of seeds compared with cultivars. Approximately, number of seeds per Kg ranged between 8925 ($W.T_1$) and 23800 (Meskawi) (Table 3). The size of seeds in wild genotypes was larger than cultivated genotypes; this result was in agreement with Westwood and Bjornstad (1968) who found that seeds of Syrian pear (*Pyrus syriaca* Boiss) were the largest seeds compared with other species.

Most of morphological characters discriminated Abu-Satel genotype from the other genotypes.

Table (2): Tree vigor, ramification, shoot length, thorns, leaf index, leaf serration and stipules of the six pear genotypes.

Genotype	Tree vigor	Ramification	Shoot length/ cm	Thorns	Leaf index	Leaf serration	Stipules
Meskawi	Intermediate 3-4 m length	**Erect**	35	-	0.64	Serried	+
W.T1	Intermediate 3-4m length	Intermediate	18	-	0.36	Serried	-
Abu-Satel	Vigorous 3-5m length	Intermediate	40	-	0.78	Serried	+
W.T2	Small to intermediate 2-3m length	Erect	25	+	0.37	Serried	+
W.T3	Intermediate 3-4m length	Erect	25	-	0.35	Serried	-
Romi	Intermediate 3-4m length	Intermediate	30	-	0.71	Serried	+

+: Present
-: Absent

Table (3): Flower, fruit, and seed characters of the six pear genotypes.

Genotype	Flowers			Fruits				Seeds			
	Bloom season	Inflorescence	Color	Ripening season	Fruit shape	Fruit size		Fruit skin color	Size			# of seeds/kg
						Length/cm	Width/cm		Length/ cm	Width/ cm	L/W	
Meskawi	M	5-12	White	M	Pyriform Elongate*	10	6	Yellow with red	0.7	0.35	2	23800
W.T1	M	5-12	White	ML	Globose	4	5	green	0.7	0.6	1.16	8925
Abu-Satel	M	5-12	White	ML	Pyriform Elongate*	12	8	Yellow	0.7	0.3	2.33	21500
W.T2	EM	4-12	White	EM	Pyriform very short straight**	3.5	4	Yellow	0.6	0.4	1.5	9520
W.T3	M	5-12	White	ML	Pyriform very short straight**	5.5	4.5	green	0.6	0.4	1.5	9100
Romi	L	5-12	White	ML	Pyriform to globose**	9	8.5	Yellow with red	0.6	0.4	1.75	16670

* Relative position of the maximum diameter towards the eye.
** Relative position of the maximum diameter towards the middle.
Bloom season: EM = March 20-April 1, M = April 2-April 8, ML = April 9- April 17, L = April18- April 30.
Ripening season: EM = July 6- August 8, M= August 9- August 25, ML = August 26- September 28.

Table (4): Anatomical analysis of the six pear genotypes, Leaf thickness, mesophyll thickness, midrib thickness, xylem, vascular bundle, collenchymas thickness, and collenchymas layer.

Genotype	Leaf thickness/ µ	Mesophyl thickness/ µ	Midrib thickness/µ	Xylem		Vascular bundle/ µ	Cuticle thickness / µ		# Cuticle layers	
				Layers	Thickness/M		Lower	Upper	Lower	Upper
Meskawi	12	8	61	11	21	34	16	21	10	10
W.T1	11	8	45	13	14	22	10	12	10	9
Abu-Satel	14	10	62	12	17	25	17	19	12	11
W.T2	16	14	50	14	14	21	12	13	12	12
W.T3	12	10	39	12	10	17	8	11	8	10
Romi	15	12	54	11	16	27	10	14	9	8

µ: Micron

Anatomical characters

Anatomical characters were illustrated in Figure (1) and Table (4).

Leaf thickness

The leaf thickness was measured for the six pear genotypes. The highest thickness is revealed by W.T2 (16 µ), while the lowest thickness revealed by W.T1 (11 µ). Leaves covered on both surfaces by single layered epidermis, which are covered by a waxy substance called cutin. Mesophyll tissues of leaf lie between the upper and lower epidermis are consisting of palisade and spongy parenchyma. The palisade parenchyma is composed of elongated cells close together, which means that pear genotypes receive direct light (distributed in sunny places). Mesophyll thickness ranged between 8 µ (Meskawi and W.T1) and 14 µ of W.T2.

Midrib

The function of midrib is to strengthen the leaves and the tissues of the conducting system are situated near /or at the center of the midrib. Midrib thickness varies among pear genotypes, Abu-Satel genotype showed the highest midrib thickness (62 µ), followed by W.T1 (61 µ), while W.T3 revealed the lowest thickness (39 µ). The cuticle thickness varied between the six pear genotypes due to the number and the size of cuticle layers; Abu-Satel genotype revealed the highest thickness of lower cuticle (17 µ), while Meskawi genotype revealed the highest thickness of upper cuticle (21 µ). In addition, Meskawi revealed the lowest number of cuticle layers with high thickness, but W.T3 revealed the lowest number and lowest thickness of lower and upper cuticle layers (12/12 layers and 8/10 µ thickness, respectively). The vascular bundle consists of phloem and xylem. Phloem is always found towards the lower side and the xylem towards the upper side of the leaf. Vascular bundle thickness of the six pear genotypes was measured; Meskawi genotype revealed the highest thickness (34 µ), followed by Romi (27 µ), while W.T3 revealed the lowest thickness. Xylem is one of the important tissues giving the mechanical strength of the leaf. The xylem elements are composed of dead and lignified cells. The number of xylem layers of the six pear genotypes ranged from 11 layers (Meskawi and Romi) to 14 layers for W.T2. Meskawi revealed the highest xylem thickness (21 µ), while W.T3 showed the lowest thickness (10 µ). Table (4) showed that Meskawi has the highest xylem thickness and the lowest number of layers, while W.T3 revealed the lowest xylem thickness with high number of layers.

Polymorphism detected by biochemical markers (Isozymes)

The term "Isozyme" was introduced in 1959 to designate different molecular forms of the same enzyme occurring either in a single individual or in different members in the same species. According to the recommendation of the Commission of Biological Nomenclature of IUPAC-IUB, isozymes are defined as genetically determined multiple molecular forms of an enzyme. Isozymes are used for solving numerous problems of systematic as will as for reconstruction of phylogenetic relationships between related species (Manchenko, 1994).

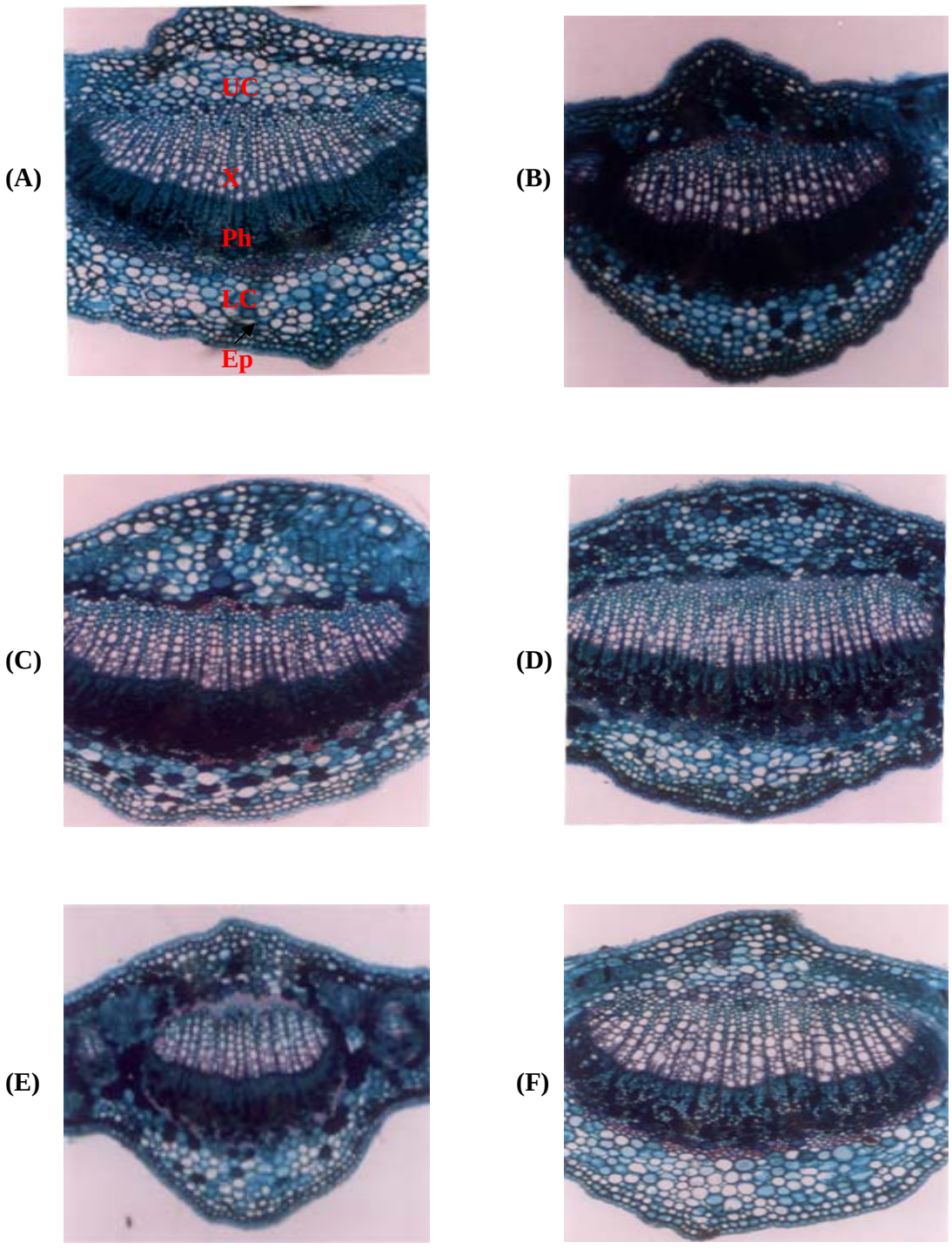

Fig.(1). Anatomical characterization of (mid rib) in the six Syrian pear genotypes. A: Meskawi; B: W.T1; C: Abu-Satel; D: W.T2; E: W.T3; and F: Romi genotypes.Ep: Epiderm, U C: Uper cuticle, L C: Lower cuticle, X: Xylem, and Ph: Phloem.

Peroxidase (PRX) Polymorphism

Peroxidase banding patterns of six pear genotypes are presented in Figure (2) and Table (5). Ten bands were detected with R.M values: 1.5, 2.5, 3.3, 3.5, 3.7, 4.2, 4.5, 4.7 and 5.Only one band was monomorphic (R.M = 2.5) and all others were polymorphic (polymorphism = 90%). Abu-Satel revealed the highest number of bands (9 bands), 3 bands of them were unique at R.M: 1.5, 3.5 and 4.7, respectively. While Romi genotype revealed only one unique band at R.M = 5. W.T1 (*Pyrus syriaca* Boiss) revealed the lowest number of bands (2).

Previous Studies indicated that peroxidase produced enough polymorphic bands (Chung *et al.*, 1995). Also, Fachinello *et al.* (1999) used three isozyme patterns of enzyme systems (beta glucosidase, esterase and peroxidase) in flower buds and shoot bark extracts of 2 sets of pear graft combination. Peroxidases bands were present in the rootstocks and were absent from the scion cultivars before grafting, but subsequently appeared weakly after grafting. Fachinello *et al.* (2000) found that peroxidase showed high variability among all pear genotypes with isozyme extracted from buds and other tissues showed enough variability compared with esterase and beta-glucosidase. Manganaris *et al.* (2002) found that peroxidase revealed high polymorphic bands, which can be used for pear cultivar identification.

Esterase Polymorphism

The esterase banding patterns of six pear genotypes were illustrated in Figure (3) and Table (6). Two monomorphic bands were detected with R.M values at 8 and 8.7, respectively. There was no differentiation between all investigated pear genotypes detected.

Previous studies showed that esterase produced low polymorphic bands (Maass and Klass, 1995; Ipek and Ipek, 2003), while Fachinello *et al.* (2000) found that esterase is the most reliable enzyme for identification of pear cultivars.

In this study esterase was not active to differentiate between the six Syrian pear genotypes.

Table (5): Peroxidase banding patterns of seven pear genotypes.

Band No.	R.M	W.T1	Meskawi	W.T3	Abu-Satel	Romi	W.T2
1	1.5				+		
2	2.5	+	+	+	+	+	+
3	3.3			+	+	+	+
4	3.5				+		
5	3.7	+	+	+	+	+	+
6	3.9		+		+		
7	4.2			+	+	+	
8	4.5			+	+	+	
9	4.7				+		
10	5					+	
Total		**2**	**3**	**5**	**9**	**6**	**3**

R.M: Relative mobility

Table (6): Esterase banding patterns of seven pear genotypes.

Band No.	R.M	Meskawi	$W.T_1$	Abu-Satel	W.T2	W.T3	Romi
1	8	+	+	+	+	+	+
2	8.7	+	+	+	+	+	+
Total		2	2	2	2	2	2

R.M: Relative mobility

It is concluded from these results that the different marker systems (morphological, anatomical and biochemical markers) are appropriate to differentiate between the six pear genotypes and discriminated Abu-Satel genotype from the other pear genotypes. Also, these marker systems could be complementary to each other and should be followed by molecular characterization using PCR-based markers to establish an integrated data about the Syrian pear genotypes.

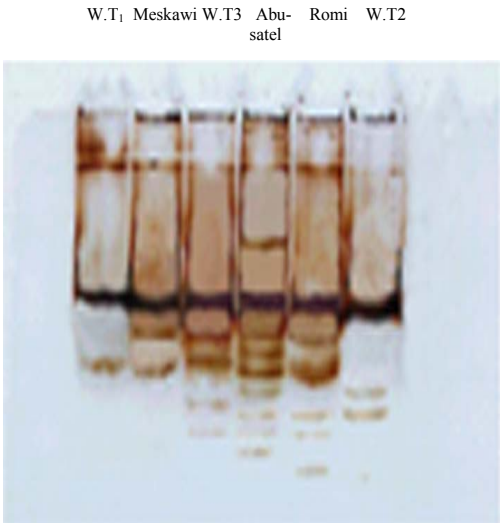

Fig. (2): Zymogram of peroxidase isozyme from leaves extracts of pear genotypes.

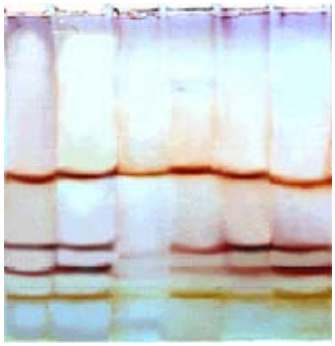

Fig. (3): Zymogram of esterase isozyme from leaves extracts of pear genotypes.

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الملخص العربي

التوصيف المورفولوجي و التشريحي و البيوكيميائي لستة أصناف من الكمثرى السورية

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تم إجراء توصيف مورفولوجي و تشريحي وبيوكيميائي لستة تراكيب وراثية من الكمثرى السورية. أظهر التوصيف المورفولوجي تفوق الصنف أبو سطل على باقي التراكيب الوراثية من حيث قوة النمو و إعطاء ثمار بمواصفات نوعية وكمية عالية، كما أوضح التوصيف التشريحي الفروق بين التراكيب الوراثية الستة من الكمثرى السورية حيث تميز الصنف أبوسطل بأعلى سمك للعرق الوسطي بينما أعطى الصنف مسكاوي أعلى سمك للأوعية الناقلة. أظهر التوصيف البيوكيميائي (شبيهات الانزيمات) الفروق بين التراكيب الوراثية المختلفة للكمثرى السورية حيث أعطى البيروكسيديز تبايناً كبيراً بين التراكيب الوراثية (٩٠ %)، كما تميز الصنف أبوسطل بأكبر عدد من الحزم (٩) منها ثلاثة واسمات فريدة مقارنة مع باقي التراكيب الوراثية فيما لم يعط الاستريز أي تباين بين التراكيب الوراثية المدروسة .