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RECONSTITUTION OF *Fragaria X Ananassa* 3- FRUIT CHEMICAL CHARACTERISTICS AND THEIR HETEROSIS AND HYBRID FINGERPRINTING

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ABSTRACT

This study was conducted during 1999/2000 and 2000/2001 seasons to investigate fruit chemical characteristics and their heterosis and fingerprinting of the new obtained strawberry hybrids from the specific hybridization between the two octaploid species *Fragaria chiloensis* and *Fragaria virginiana*. Twelve new obtained strawberry hybrids and their parents were evaluated according to chemical characterstics of fruits, viz., total soluble solids, total acidity, ascorbic acid contents and their heterosis. Protein fingerprinting for the hybrids and parents was also carried out. Results showed that most of the produced hybrids had the highest values of TSS with no significant differences between each other. Most of the hybrids showed positive heterosis for TSS content over the best parent. Concerning titratable acidity, the highest value of heterosis was exhibited in B9, B5 hybrids and B6. Most of hybrid showed negative heterosis while A4 had no heterosis for TA trait in the two seasons. A2 and B11 hybrids had the highest values of TSS/TA ratio as compared with all hybrids as well as their parents. A2 and B11 had the highest values of heterosis for TSS/TA ratio and all hybrid showed positive heterosis.A2 and A3 hybrids recorded significant increment in ascorbic acid content as compared with all hybrids. All hybrids showed negative heterosis compared to the best parent in the two seasons. SDS-PAGE of protein was successfully used for both identification and differentiation of strawberry genotypes.

The study conclude that twelve promising hybrids were obtained from reconstitution of *Fragaria X ananassa* from the specific hybridization between *Fragaria chiloensis* and *Fragaria virginiana*. Such hybrids are very important for further breeding programs

Keywords: Strawberry, hybrids, TSS, Total acidity, Ascorbic acid, Protein fingerprinting

INTRODUCTION

The content of total soluble solids in strawberry genotypes is considered one of the most important fruit quality characteristics. Okasha *et al.* (1981) found that Tioga x Balady hybrid was much better than the Fresno x Balady hybrid as well as the parents in total soluble solids. Fruits of the Fresno x Balady hybrid had higher total soluble sugars and lower acidity compared to that of the Tioga x Balady hybrid. Galletta *et al.* (1995) noticed that TSS in Northeaster strawberry fruits was 9.2% while it was higher than those of Earliglow which recorded 7.9%. Shaw (1987) reported the response to selection associated with the genetic variance for TA contents in strawberries. It was found that the genetic effects of TA content were stable to the environmental conditions and cultural systems but differed among tested cultivars and clones. Lateglow fruits had high values of total titratable acidity (0.88%) while Allstar had low value (0.51%) and Primetime gave mid

value (0.6%) as mentioned by Galletta *et al.* (1996). In a study conducted by Paydas *et al.* (2000), they recorded that the averages of total acidity were found to be 1.01% in Camrosa, 1.08% in Chandler, 0.9% in Oso Grande and 0.87% in Dorit and Douglas. The taste of strawberry fruits is expressed by the ratio between soluble solids content and total titratable acidity and this ratio is considered a very important factor for quality standardization particularly for export. Soluble solid contents/ total titratable acidity ratio was 10.82 in Belrubi cultivar (Chandel and Badiyala, 1996). The highest TSS/acid ratio was in Karola (12.6), followed by Glooscop (10.4) and Papell (9.9) and the lowest value was recorded in Blomidon (7.0) as mentioned by Haffner and Vestrheim (1997). Ascorbic acid is considered the major content and predominant vitamin in strawberry fruit, which increases their nutritive value. Sweet Charlie had higher ascorbic acid than Oso Grande (Chandler *et al.*, 1997). Wang *et al.* (1997) recorded that the value of ascorbic acid for 24 strawberry cultivars and selections ranged from 70 to 112.mg/100g. El-Shimi (1978) found that all hybrids gave positive heterosis for ascorbic acid content. Human and Low (1993) found that strawberry strains did not reveal heterosis for acidity while they recorded negative heterosis for total soluble solids.

SDS-PAGE system proved a good discrimination among some strawberry cultivars which were classified into three different groups according to their minor bands as found by Fahmy and Okasha (1992). Ahmed (1998) mentioned that the results of the electrophoretic pattern of the leaf protein of *Potentilla glandulosa,* Chandler, Selva and Balady strawberry genotypes indicated that the protein molecular weights were 70, 40, 34, and 19 KD. High similarities in protein profile bands were shown in Red lands Hope, Red lands Joy, Cabarla, Camarosa and Rosa Linda strawberry cultivars (El-Tarras *et al.*, 2001). They also concluded that the fingerprint at protein level is very important to distinguish among new strawberry cultivars. Ghazali (2002) studied some biochemical and molecular genetic markers to fingerprint eight strawberry cultivars. It was reported that the SDS-PAGE revealed many bands with different molecular weights and these cultivars were catalogued according to some unique bands which appeared clearly in most cultivars. Therefore, the aim of this work was to study fruit chemical characteristics and their heterosis and fingerprinting of the new obtained strawberry hybrids.

MATERIALS AND METHODS

This study was carried out at The Experimental Station of Strawberry and Non-Traditional Crops Center, Faculty of Agriculture, Ain Shams University at Nubaria and Cytogenetic unit, Desert Research Center, during the period from 1999 to 2002. Twelve new obtained strawberry hybrids with their parents were evaluated for chemical fruit characteristics and protine fingerprinting. A randomized complete block design, with three replicates, was used. Each replicate contained the 14 treatments (12 hybrids and the two parents). The plants were arranged in four row beds, 120 cm width and 50 cm height, at plant distances of 25 cm apart. The plot area was 12 m^2.

The beds were covered with 40 micron clear plastic as a mulching one month after planting on September 13th and 15th in the first and the seacond seasons, respectively. The plants were covered with 80 micron plastic tunnels (70 cm height) on November 1. The sprinkler irrigation was applied during the first month after planting, then the drip irrigation was used under mulching until the end of season. The soil texture of the experimental site was sandy, with pH of 7.8 and EC of 0.86. The agricultural practices concerning cultivation, fertilization, irrigation, and pest- and disease control were conducted as recommended.

Data were recorded as follows:

1. Chemical characteristics:

Random samples of twenty fruits from each experimental plot at full ripe stage was taken to measure the percentage of total soluble solids content using a hand refractometer. Total acidity and ascorbic acid contents were determined as the method described in A.O.A.C. (1990).

2. Heterosis:

Heterosis was computed according to the following equations reported by Singh and Singh (1994).

$$\text{The best -parents heterosis \%} = \frac{\overline{F_1} - \overline{B.P}}{\overline{B.P}} \times 100$$

Where: F_1 and B.P represent mean performance of hybrid and best-parents. To test the significance of differences between the F_1 means and their best-parents values, the T-test was applied. The standard error was calculated according to the formula:

$$SE = (EMS / r + EMS / 2r)^{1/2}$$

Where: EMS is the mean squares due to error from the analysis of variance and r is number of replications.

3. SDS-protein electrophoresis:

SDS-polyacrylmide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970),and modified by Studier (1973). Youngest full expanded-leaf samples were taken from each of the two parents, and their F1hybrids.

4. Statistical analysis:

Analysis of data was done by IBM computer, M-state program for statistical analysis. The differences among means for all traits were tested for significance according to Waller and Duncan (1969).

RESULTS AND DISCUSSION

1. Total soluble solids (TSS):

Data obtained in Table (1) indicate that the highest values of total soluble solids content were recorded for A2, B5, B6, B7, B8, B10 and B12 without significant differences among them. On the other hand, P1, A3 and B9 had the lowest values of TSS in the two seasons. These results are in a harmony with those obtained by Galletta *et al.* (1995). According to total

soluble solids heterosis, results in Table (2) indicate that all tested hybrids showed positive significant heterosis except A3, A4, B9 and B11 in the two tested seasons, These results are in agreement with those obtained by Okasha *et al.* (1981), they reported that most of strawberry hybrids produced fruits contained more total soluble solids content than their parents. Results of the hybrids which had negative heterosis are in agreement with those obtained by Human and Low (1993). Such hybrids with high TSS are highly needed for export as well as for local market instead of the local cultivar Balady.

Table (1): Total soluble solids (%) and total acidity (%) of two strawberry species and their 12 selected F1 hybrids.

Genotypes	TSS (%)		TA (%)	
	1999/2000	2000/2001	1999/2000	2000/2001
P1	8.27 e	7.59 h	2.393 a	2.423 a
P2	10.93 bcd	10.29 ef	1.793 bc	1.719 bcd
A1	11.17 bcd	11.86 bcd	1.403 d	1.462 cde
A2	12.67 a	13.01 ab	1.203 ef	1.300 de
A3	8.38 e	8.81 gh	1.360 de	1.389 cde
A4	10.80 cd	11.23 cde	1.793 bc	1.752 bc
B5	11.80 abc	12.54 abc	1.853 b	1.811 bc
B6	12.45 a	13.20 a	1.833 b	1.805 bc
B7	12.40 a	13.12 ab	1.447 de	1.466 cde
B8	11.85 ab	12.09 abc	1.960 b	1.989 ab
B9	9.67 e	9.56 fg	1.493 d	1.548 bcd
B10	12.24 a	12.12 abc	1.493 d	1.621 bcd
B11	10.47 d	10.76 def	1.027 f	1.108 e
B12	12.40 a	12.66 ab	1.453 d	1.584 bcd

Values in the same column by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5% .

P1= Fragaria chiloensis P2 = Fragaria virginiana

A = F. virginiana x F. chiloensis B = F. chiloensis x F. virginiana

Table (2): Heterosis of total soluble solids and total acidity of 12 F1 strawberry hybrids relative to their best-parent (h%)

Hybrids	TSS		TA	
	1999/2000	2000/2001	1999/2000	2000/2001
A1	2.19	15.25	-21.75	-41.72
A2	15.92	26.43	-32.91	-49.23
A3	-23.33	14.38	-24.15	-22.82
A4	-1.19	9.13	0.0	-27.69
B5	7.96	21.86	3.35	-25.26
B6	13.91	28.20	2.23	-25.50
B7	13.45	27.50	-19.29	-39.49
B8	8.42	17.49	9.31	-21.17
B9	-11.58	7.09	-16.73	-39.00
B10	11.99	16.62	-16.73	-40.11
B11	-4.21	4.57	-42.72	-54.27
B12	13.45	23.03	-18.96	-35.45

A = F. virginiana x F. chiloensis B = F. chiloensis x F. virginiana

2. Total acidity (TA) :

Results in Table (1) indicate that the highest values of total acidity were obtained from B5, B6 and B8 hybrids as compared with all produced hybrids in the two tested seasons. On the other hand, A2 and B11 hybrids had the

lowest values as compared with all tested genotypes. Such decrement were obtained by Shaw (1987), Galletta *et al.* (1996), and Paýdas *et al.* (2000). They reported that titratable acidity contents was differed among clones and cultivars of strawberry.

Regarding heterosis for total acidity, results in Table (2) clearly indicate that A4 had no heterosis while B5, B6 and B8 had positive heterosis in the first season. These results are in a harmony with Okasha *et al.* (1981), who mentioned that heterosis of acidity was only highly significant in some strawberry hybrids. However, all hybrids showed negative heterosis in the second season. These findings are in agreement with those of Human and Low (1993).

3. Total soluble solids /acidity ratio:

Regarding to total soluble solids to acidity ratio, it is clear from Table (3) that A2 hybrid gave the highest value as compared with all tested genotypes in the two seasons, followed by B10 and B11 with a significant difference in the first season. Chandel and Badiyala (1996) and Galletta *et al.*(1996) reported that there was a high relationship between good flavor and high total soluble solids/acidity ratio.

Results in Table (3) clearly indicated that all selected hybrids exhibited positive heterosis in the two seasons except A3 in the second season. A2 had the highest value of total soluble solids/acidity ratio heterosis in the two tested seasons.

Table (3): Total soluble solid contents/ total acidity ratio (TSS/TA ratio) and ascorbic acid of two strawberry species and their F1 hybrids

Genotypes	TSS/TA		Ascorbic acid (mg/100gfw)	
	99/2000	2000/2001	99/2000	2000/2001
P1	2.740 f	3.01 i	149.30 a	146.21 a
P2	5.653 e	5.97 g	80.15 gh	81.25 de
A1	8.153 cd	8.31 cd	92.00 ef	92.64 cd
A2	10.740 a	10.47 a	134.10 b	140.02 a
A3	6.000 e	4.70 h	110.80 c	112.10 b
A4	6.207 e	6.39f g	97.69 de	100.14 bc
B5	6.673 e	6.91 e	107.50 cd	106.86 bc
B6	6.803 de	7.21 e	75.68 gh	76.12 e
B7	8.370 c	8.91 c	83.51 fg	80.66 de
B8	6.100 e	6.21 g	70.43 hi	72.31 ef
B9	6.060 e	6.46f g	98.73 de	100.22 bc
B10	9.540 abc	8.34 cd	47.49 j	50.36 g
B11	10.220 ab	9.60 b	60.72 i	59.98 fg
B12	8.974 bc	8.08 d	92.00 ef	93.58 cd

Values in the same column by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.

P1= Fragaria chiloensis P2 = Fragaria virginiana

A = F. virginiana x F. chiloensis B = F. chiloensis x F. virginiana

4. Ascorbic acid:

In Table (3) results show that A2 had the highest value of ascorbic acid content as compared with all tested hybrids in the two seasons. However, there was no significant difference between it and P1 in the second season and with a significant decrement between it and P1 in the first season. On the

other hand, the lowest values were recorded for B6, B8, B10 and B11. In general, most of hybrids had a wide range of ascorbic acid content. Chandler, *et al.* (1997) and Wang *et al* (1997) found also a wide range of ascorbic acid content among strawberry cultivars and genotypes.

Data recorded in Table (4) showed all the tested hybrids exhibited negative heterosis for ascorbic acid trait. These findings are not in agreement with those of El-Shimi (1978), who found that all hybrids gave positive heterosis for ascorbic acid content. These contrary results may be due to the different parents used in each study.

Table (4): Heterosis of TSS/TA of 12 selected F1 strawberry hybrids relative to their best –parent (h%).

Hybrids	TSS/TA		Ascorbic acid	
	1999/2000	2000/2001	1999/2000	2000/2001
A1	44.22	39.19	-38.38	-36.64
A2	89.98	75.37	-10.18	-4.23
A3	6.13	-21.27	-25.79	-23.33
A4	9.80	7.03	-34.57	-31.51
B5	18.04	15.74	-27.99	-26.91
B6	20.34	20.77	-49.31	-47.94
B7	48.06	49.24	-44.06	-44.83
B8	7.90	4.02	-52.83	-50.54
B9	7.19	8.20	-33.87	-31.45
B10	68.76	39.69	-68.19	-65.56
B11	80.79	60.80	-59.33	-58.98
B12	58.47	35.34	-38.38	-35.99

A = F. virginiana x F. chiloensis **B = F. chiloensis x F. virginiana**

5. Protein electrophoresis:

The electrophoretic patterns revealed marked variations in the occurrence and distribution of the bands among the two parents and their F1 hybrids. It is clear from Table (5) and Fig (1) that six bands are commonly present in most genotypes and these protein fractions having molecular weights of 95.64, 58.58, 40.05, 30.78, 21.52 and 12.25 KDS. The variations in two banding patterns between the two parents indicate that these parents are genotypically differed .For example, in Table (5) the electrophoretic run of the P1 was characterized by the disappearance of band which had molecular weight 77.11 KDS and the other parent P2 had a band which had 86.37 KD.

In the meantime, the genotypes A2 and B11 were characterized by existence of unique bands which had 142 and 104.9 respectively. It is interesting to note that, the hybrids A1, B7, B8, B9, B10 and B11 were characterized by existence of unique bands which were not found in their two parents which may be play a role in the heterotic performance in strawberry hybrids. In addition, it was found that only A3 and B6 as well as P1 in existence of band which had 77.11 KD. On the other hand, B6, B7, B8, B9and B10 similar to their P2 in existence of band which had 86.37.Our results are in a harmony with those obtained by Fahmy and Okasha (1992), EL-Tarras *et al* (2001) and Ghazali (2002) who mentioned that protein fingerprinting level is very important to distinguish between the strawberry cultivars

Table (5): Protein patterns of two strawberry species and their 12 selected F1 hybrids.

Band	M.w	P1	A1	A2	A3	A4	B5	B6	B7	B8	B9	B10	B11	B12	P2
1	142.0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
2	104.9	0	0	0	0	0	0	0	0	0	0	0	1	0	0
3	95.64	1	1	0	1	0	0	1	1	1	1	1	1	0	1
4	86.37	0	0	0	0	0	0	1	1	1	1	1	0	0	1
5	77.11	1	0	0	1	0	0	1	0	0	0	0	0	0	0
6	67.84	1	1	0	1	0	1	0	0	0	1	1	0	0	1
7	58.58	1	1	1	1	1	0	1	0	0	1	0	1	1	1
8	49.31	0	0	0	0	0	0	0	1	1	1	1	1	0	0
9	40.05	1	1	1	1	1	1	0	1	1	1	1	1	1	1
10	30.78	1	1	1	1	0	1	1	1	1	1	1	1	0	1
11	21.52	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	12.25	1	1	1	1	1	1	1	1	1	1	1	1	1	1

P1= Fragaria chiloensis
P2 = Fragaria virginiana
A = F. virginiana x F. chiloensis
B = F. chiloensis x F. virginiana

Table (6): SDS-PAGE protein analysis of two strawberry species and their 12 selected F1 hybrids.

	P1	A1	A2	A3	A4	B5	B6	B7	B8	B9	B10	B11	B12
P													
A	0.933												
A	0.714	0.769											
A	1.00	0.933	0.714										
A	0.667	0.727	0.800	0.667									
B	0.769	0.833	0.727	0.769	0.667								
B	0.800	0.714	0.615	0.800	0.545	0.500							
B	0.667	0.714	0.615	0.667	0.545	0.667	0.714						
B	0.667	0.714	0.615	0.667	0.545	0.667	0.714	1.000					
B	0.824	0.875	0.667	0.824	0.615	0.714	0.750	0.875	0.875				
B	0.750	0.800	0.571	0.750	0.500	0.769	0.667	0.933	0.933	0.941			
B	0.750	0.800	0.714	0.750	0.667	0.615	0.667	0.800	0.800	0.824	0.750		
B	0.667	0.727	0.800	0.667	1.000	0.667	0.545	0.545	0.545	0.615	0.500	0.667	
P	0.875	0.933	0.714	0.875	0.667	0.769	0.800	0.800	0.800	0.941	0.875	0.750	0.667

P1= Fragaria chiloensis
P2 = Fragaria virginiana
A = F. virginiana x F. chiloensis
B = F. chiloensis x F. virginiana

The pairwise differences among strawberry genotypes calculated by (SPSS) were shown in Table (5) based on the number of markers that were different between any given pair of genotypes.The results of analysis showed two groups (Fig 2). The highest values of similarity were observed between (A4 & B12) in the first group which clustered together and closely grouped with A2. Also Fig (2) and Table (6) indicate that P1 & A3 and B7 & B8 in the second group showed the highest similarity values and they clustered with A1 and B10 respectively. Moreover, B9&P2 grouped together with high similarity values. These results are in agreement with El-Tarras *et al.* (2001).

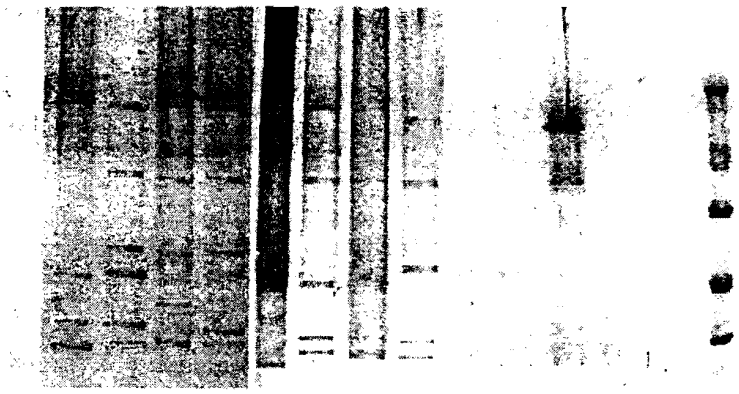

Fig. (1): SDS-PAGE for protein of leaves extracted of selected 12 F1 strawberry hybrids and their two parents.

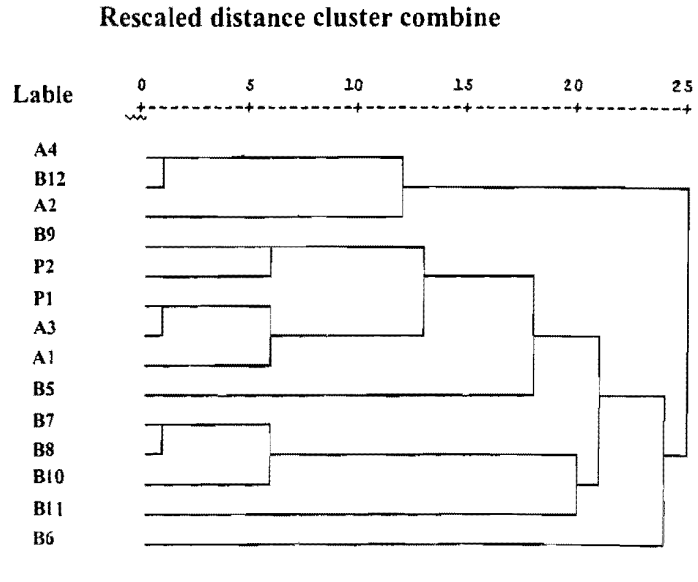

Fig. (2): Dendogram of the two strawberry species and 12 selected strawberry hybrids for SDS-PAGE similarity matrix shown in Table (6).

P_1 = *F. chiloensis* P_2 = *F. virginiana*

A = *F. virginiana* X *F. chiloensis*

B = F. *chiloensis* X *F. virginiana*

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إعادة تكوين الفراجاريا x أناناسا
٣- الصفات الكيماوية للثمار ودرجة تفوقهم والبصمة الوراثية للهجن

خليفة عطية عكاشة – رفعت محمد هلال – محمد إمام رجب – شادية عبد اللاه إسماعيل
قسم البساتين – كلية الزراعة – جامعة عين شمس – شبرا الخيمة – القاهرة

أجريت هذه الدراسة خلال موسمي ١٩٩٩ – ٢٠٠٠ و ٢٠٠٠ – ٢٠٠١ بهدف تقدير الصفات الكيماوية للثمار ودرجة تفوقهم والبصمة الوراثية بطريقة البروتين لعدد ١٢ هجين فراولة جديد تم الحصول عليها بالتهجين بين نوعى الفراولة شلونسز وفرجينيانا. وتظهر النتائج تفوق سبعة هجن هي A2, B5, B6, B7, B8, B10, B12 في محتوى ثمارها من المواد الصلبة الذائبة عن الآباء وأعطت درجة تفوق موجب عن الأب الأفضل لهذه الصفة بينما أعطت معظم الهجن درجة تفوق سالبة بالنسبة لصفة الحموضة الكلية أما نسبة المواد الصلبة الذائبة إلى الحموضة الكلية في الثمار فقد سجلت أعلي قيم لها في الهجينين A2 , B11 وقد زاد محتوى ثمار الهجينين A2 , A3 من حمض الاسكوربيك زيارة معنوية مقارنة بجميع الهجن الناتجة كما سجلت جميع الهجن قيما سالبة لقوة الهجين مقارنة بالأب الأفضل في كلا الموسمين . أوضحت النتائج المتحصل عليها من أنماط التفريد الكهربي للبروتين وجود تشابه إلى حد كبير بين الهجن B12 , A4 وكذلك بين الأب فراجاريا شلونسز و الهجين A3 وأيضا بين B7, B8 ومن ناحية أخري توضح النتائج وجود تباعد بدرجة ٥٠ % بين الهجين A4 وكل من الهجن B6 , B7 , B8 , B10 وكذلك بين B5 , B6 وأيضا بين B12 وكل من B6 , B7 , B8, B10 .

تجمل الدراسة انه أمكن إعادة تركيب الفراجاريا X أناناسا بالتهجين بين النوعين شلونسز وفرجينيانا و إنتاج ١٢ هجين مبشرة مختلفة في البصمة الوراثية لها ومتفوقة في بعض الصفات ويمكن أن تدخل في برامج تربية الفراولة لنقل مثل هذه الصفات المتميزة كصفة زيادة المواد الصلبة الذائبة وحمض الأسكوربيك في الثمرة .