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NEW PROMISING SQUASH INBRED LINES DEVELOPMENT AS A MAJOR STEP TO DEVELOP SUPERIOR HYBRIDS

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ABSTRACT

The inbreeding strategy for five successive seasons began from 2014 to 2017 at Gemmezia Experiment Station, Gharbya governorate was conducted. Seventeen promising squash inbred lines were selected. The selection focused on plant length, number of female flowers, and the average fruit weight. The coefficient of variance (C.V. %) values showed that most of the lines were highly homogeneous. The inbreeding did not cause depression on most studied traits. All the seventeen developed inbred lines and commercial hybrid F1 Sama (control) were evaluated for the most important characteristics during the two seasons 2017 and 2018 under the open field conditions. Significant differences were observed among all evaluated genotypes in all studied characteristics. The highest values were found in S 36 A for plant length, number of leaves/plant, number of days to the first female flower, and number of female flowers. Among the seventeen lines, S 35, S 27 B, and S 36 A gave the highest values for fruit characteristics and yield components. Also, the previous three promising lines gave the highest early and total yield. These inbred lines are potentially promising as a good source for developing new hybrids characterized by outstanding yield quantity and quality.

Key words: ***Squash, Cucurbita pepo, Breeding, Selection, Inbred lines, Yield, Vegetative growth.***

INTRODUCTION

Summer squash is one of the most important vegetable crops in Egypt. The zucchini type of squash *C. pepo* is a high-value vegetable and cash crop. Egypt occupies eighth place within the producing countries in the world for squash as reported by FAOSTAT (2010). Squash production in Egypt of two seasons in 2017 was 462,654 tons, cultivated area was 56,696 feddans, and productivity was 8.160 tons/feddan, (Agriculture Directorates of Governorates, Ministry of Agriculture and Land Reclamation, Egypt, 2017). Summer squash is native to North America and can be found growing wild in northeastern Mexico and southern, southeastern, and central USA. (Whitaker 1947). The highest production of summer squash was found in Turkey, Italy, Egypt, Spain, USA. and Mexico (Paris 1996 and 2008). Summer squash belongs to the *Cucurbitaceae* family. Squash is a short-season crop, fruits are consumed in an immature stage, so it is considered one of the most famous cash crops for smallholder farmers. Nowadays, Egypt faces a big challenge to develop a strategy of sustainable agriculture. It is well known that the seeds are one of the most important inputs in the agricultural process. Unfortunately, Egypt imports most of its needs from squash seeds, hence the importance of the role of plant breeding. Inbreeding approach is the best ideal breeding strategy for isolating promising inbred lines for the production of the local hybrid and directly contribute to solve the seed crisis. There are many methods of plant breeding such as back cross and self-pollination methods for several generations. Self-pollination is a suitable method for squash breeding, which is not negatively affected by inbreeding methods. The main goals for squash breeders are earliness, productivity, fruit quality, tolerance or resistance to biotic and abiotic stress Whitaker and Davis (1962) and (Loy 2004). Cucurbit crops do not usually exhibit inbreeding depression, a factor that may be related to the reduced heterosis (Rubino and Wehner 1986). Inbreeding depression is not an important factor for producing seed of most hybrid cucurbit cultivars (Wehner 1999). Although *Cucurbita* species are monoecious, they are often listed along with self-pollinated species in books of breeding because they are easily self-fertilized, and recent studies indicated that inbreeding depression was either nonexistent or detectible (Cummings and Jenkins 1928; Haber 1928 and Scott 1934). Cardoso (2004) reported that self-pollination increases plant homozygosity, which is not the natural genetic state of cross-pollinated species, and it can cause "inbreeding depression". Squash, being a cross-pollinated crop is an example of a group of species, where lines seem to lose little vigor by inbreeding (Whitaker and Robinson, 1986 and Robinson, 1999). Self-pollination for ten generations of *Cucurbita maxima* did not badly affect vigor and reproductive capacity (Cummings and Jenkins 1928) and Bushnell (1922) Studied the effects of inbreeding for *C. pepo*, and observed that vigor loss during self-pollination did not necessarily occur.

Although several researchers assume the hypothesis of reduced inbreeding depression in *Cucurbita*, other researchers observed inbreeding depression for several characteristics of *C. pepo* and *C. maxima* (Borghi *et al* 1973). Johannsson *et al* (1998) showed decreased fruit yield and pollen performance by inbreeding of *C. Texana*. Inbreeding may also affect pollen quality of *C. pepo* (Stephenson *et al* 2001). Inbreeding depression is not a limiting factor for hybrid seed production of cucurbits, in spite of being cross-pollinated. However, some lines can have decreased vigor and yield (Robinson, 1999 and Robinson and Decker-Walters 1999). Grisales *et al* (2009) evaluated six genotypes of *Cucurbita moschata* Duch. from open pollination (S_0) and their respective inbreeding lines (S_1 and S_2). The inbreeding that accompanied selection did not cause negative effects on yield and economic traits like fruit weight, fruit color, and fruit dry matter. Inbreeding depression does not occur in *Cucurbitaceae* (Allard 1960) although it has been registered in advanced lines of cucumber *Cucumis sativus*, the 'ahuyama' squash *Cucurbita moschata* Duch, the melon *Cucumis melo* and watermelon *Citrullus lanatus* (Robinson, 1999; Cardoso, 2004; Berenji, 1986 and Hallauer, 1999).

In Egypt, Mohamed *et al* (2003) developed five squash inbred lines (*C. pepo)* from the open-pollinated population of cv. Eskandrani. They reported that the number of leaves/plant, main stem length, and number of internodes below the first female flower were reduced compared with the original cultivar. All developed lines greatly surpassed cv. Eskandrani in total fruit yield. Line 12-127-219 gave the highest total yield in summer and winter seasons. Line 18-136-222 was comparable to line 12-127-219 in summer season only. However, line 18-136-222 gave a significantly larger portion of the total yield that was harvested early than line 12-127-219 and cv. Eskandrani in both seasons. Eight genotypes of summer squash and their hybrids evaluated for some vegetative and yield characters. Significant differences for these traits were found among the evaluated genotypes (Al-Jebory 2006). In addition, Ghobary and Ibrahim (2010) used the inbreeding followed by selection for three generations for improving squash cv. Eskandarani. The estimation of the variability in the original population (P_0) and the selected population of third-generation (P_3) in the studied traits indicated that the coefficient of variation decreased from P_0 to P_3 for all studied traits.

The present study aimed to producing promising inbred lines of squash as a first step for developing successful hybrids, hypothesizing that accurate choice of the best parental lines is an extremely important phase. The inbred lines should be characterized by economically important traits such as productivity and quality. Self-pollination method for six successive generations was chosen to fix the favorable traits as inbred lines.

MATERIALS AND METHODS

This work was conducted for producing some inbred lines characterized by desirable horticultural characteristics using self-pollination for six generations. The study was carried out from (2014 to 2018) under field conditions at Gemmeiza Experiment Station, Gharbia governorate, Horticultural Research Institute, Agricultural Research Center. This study was divided into two major parts, the first experiment was conducted for producing homozygous inbred lines and estimating the homogeneity values within inbreeds. The second one was the evaluation experiments of the different inbred lines.

Homogeneity estimation:

The inbreeding program was started by seeds of four populations from landraces, two populations of commercial squash hybrids were obtained from the commercial F_1 hybrids Azyad (Sakata seed Co.) and Riviera (Seminis U.S.A. seed Co.) and an open-pollinated variety *Cucurbita pepo* L. that were collected from some regions of Egypt in the summer season 2014.

The best plants were selected and were self-pollinated manually. These populations were sown for five successive seasons on 22 February 2015, 18 August 2015, 4 March 2016, 30 August 2016, and 28 February 2017, respectively. Two generations were developed a year. The inbreeding program was continued from 2015 to 2017. Five hundred plants from each population (S_0) were grown. The best fifty individual plants were selected and the seeds separately collected (S_1). In the next season, the progeny of these fifty plants were planted (S_2). Selection was made within the S_3 populations to select the best plants with the best horticultural characteristics and discarding exclude the undesirable characters. Seventeen populations were selected according to the inbreeding program and seeds of the best plants from each population were separately collected as S_4 generation. Selection was continued during early and late summer to develop S_4 through S_6 generations, respectively. In the early summer of 2017, the F_6 populations of the seventeen selected populations were sown with the hybrid Sama F_1 as a check (control) to estimate the coefficient of variation (C.V. %) for the most commercial traits plant length (cm) number of female flowers/plant and fruit weight/plant(kg). The degree of homogeneity in these genotypes was estimated according to the next formula:

$$\text{Coefficient of variation} = \frac{\text{Standard deviation(Sd)}}{\text{Mean(M)}} \times 100$$

Evaluation experiments

The seventeen inbred lines F_6 (selected genotypes) of summer squash (*Cucurbita pepo* L). were evaluated for some vegetative, flowering and yield traits in comparison with Sama F_1 during 2017 and 2018 under open field conditions. The genotypes seeds were sown directly in the soil on 28 August 2017 and 3 March 2018, respectively. All plants were grown with space of 40 cm apart within a row, 120 cm in width, and 6 m length. The common cultural practices, *i.e.*, irrigation, fertilization, and pest control procedure for commercial squash production under field conditions were followed. The horticultural traits (vegetative, flowering, fruits, and yield attributes) were determined. Total number of plants/plot were 15 plants. The experiments were designed as a randomized complete block design with three replicates.

Trait measurements

Data were recorded for the following characteristics on ten randomly chosen plants from each genotype:

1. Vegetative growth.

a. Main stem length (At the end of the season), b. The number of leaves /plant. (At the end of the season)

2. Flowering characteristics

a. Number of days to first female flower anthesis. b. Number of female flowers. (All the season). c. Number of male flowers. (The whole season). d. Sex ratio (number of the female: male flowers)

3. Fruit characteristics

a. Fruit length. b. Fruit diameter. c. Average fruit weight. d. The percentage of fruit set (total number of harvested fruits divided by the total number of the female flowers and multiplied by 100).

The above-mentioned characteristics were measured for 20 marketable fruits from each genotype and the averages were recorded.

4. Yield and yield components

a. Early yield was determined as the weight of fruits /plant harvested during two weeks from the beginning of harvesting. b. Total yield was determined as the total weight of harvested fruits /plant.

Results were subjected to analysis of variance according to statistical analysis as described by Gomez and Gomez (1984). Treatment means were compared by using the Least Significant Difference Test (LSD) at 0.05 probability level.

RESULTS AND DISCUSSION

Homogeneity estimation

Data in Figure 1. showed coefficient of variance (CV%) percentages among genotypes for three economical important characteristics i.e. plant length, number of female flowers, and average of fruit weight. These results for the second-generation S_2 for all inbred genotypes population in all studied characteristics average were ranging from 45 to 52% compared with 8% only for Sama F_1 (as a control). These results indicate high heterogeneity among the populations compared to control. Regarding S_3 most genotypes C.V% values reached to around 39% in this generation all genotypes still have heterogeneity compared with the F_1hybrid Sama as control. The values of CV% for S4 generation ranged from 18 to 18.9%. These percentages are close to the homogeneity. The selection was continued to develop the S_5 generation where the values of CV% became (10-12%), *i.e.* lower than the previous generation.

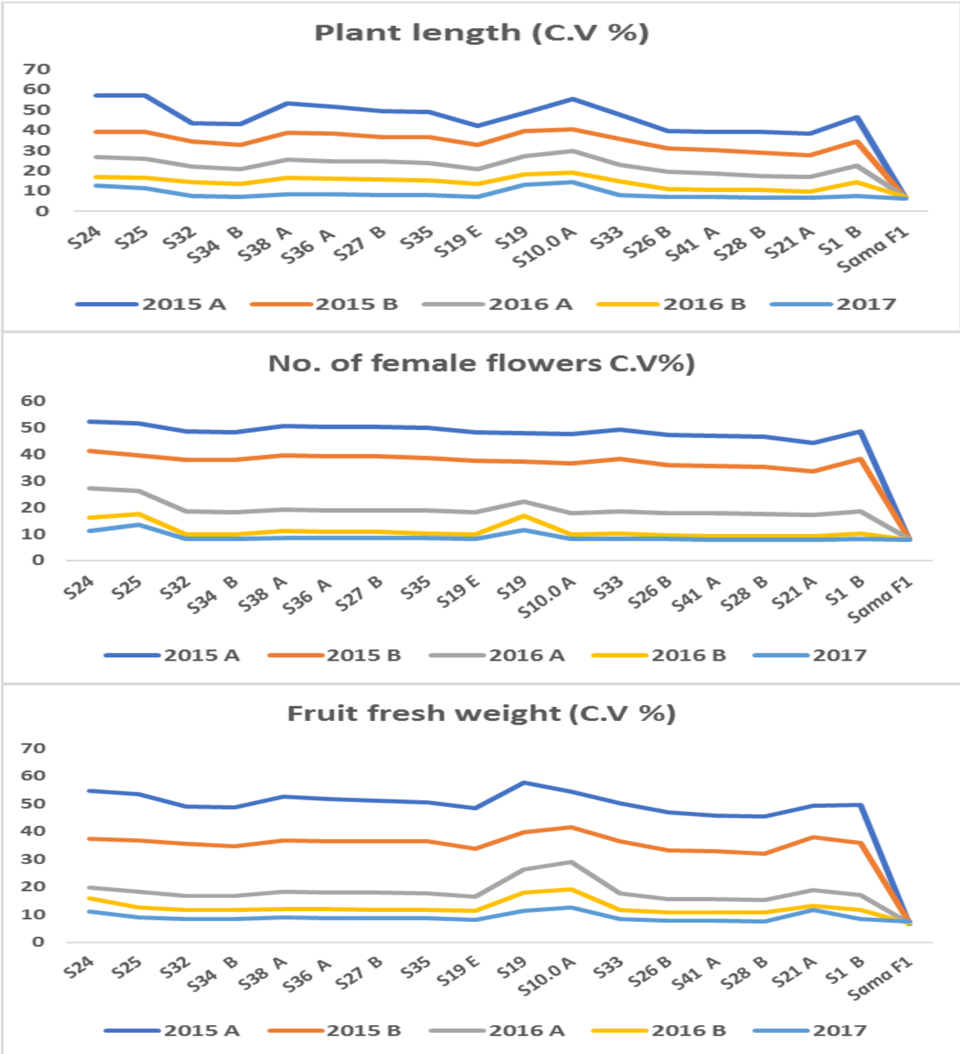

Fig. 1. Development of coefficient of variance (C.V%) values across five generations of inbreeding *i.e.*, 2015,2016 and 2017 spring and late summer seasons for plant length, number of female flowers and fruit fresh weight of 18 genotypes.

By the sixth generation of inbreeding, the C.V% values were less than 9% for most genotypes and control. Fortunately, there were no differences between Sama F_1 (control) and the developed inbred lines for coefficient of variance (CV%). Regarding the number of female flowers, all inbred lines populations were high homogeneous, except, S19, S24, and S25. On the other hand, the lowest homogeneity was observed in S19, S10A, and S21 inbred lines in average fruit weight character. Finally, the highest homogeneity was observed in thirteen out of seventeen S_6 genotypes (S 32, S 34 B, S 38 A, S 36 A, S 27 B, S 27 B, S 35, S 19 E, S 33, S 26 B, S 41 A, S 28 B and S 1 B) compared to the squash hybrid Sama F_1 (control). These results are in line with many results from a lot of researches. Inbreeding for six generations in squash *Cucurbita pepo* did not affect plant vigor and yield component characters. These results agreed with Cummings and Jenkins (1928) who found that self-pollination for 10 generations of *Cucurbita maxima* did not affect vigor or reproductive capacity. In addition, Bushnell (1922) studied the effect of inbreeding for *C. pepo* and observed that vigor loss during self-pollination did not necessarily occur.

Evaluation experiment

The most important vegetative and flowering characteristics data are presented in Table 1. According to plant length trait, there were significant differences among all selected inbred lines and control, except for S 36 A, S 41 A, S 19, and S 25. S36A exhibited the highest plant length compared with all inbred lines and the commercial hybrid Sama F1 followed by S41A. In previous studies, significant differences for this trait were found among the evaluated genotypes (Al-Kummer *et al* 2009; Kasrawi, 1995 and Hassan *et al*, 2016).

As for the number of leaves, S36A exhibited the highest number of leaves with significant differences compared with other inbred lines and control. In addition, three inbred lines S32, S25, and S19 were higher in this trait than the control of a commercial squash hybrid Sama F_1. After six-generations of inbreeding and selection, four inbred lines showed superiority in earliness.

Table 1. Mean values of the squash inbred lines compared with the commercial hybrid Sama F_1 as a control for vegetative and flowering traits (combined data across 2017 and 2018 seasons).

Inbred lines and control	Plant length (cm)	No. of leaves	No. of days to flowering	No. of flowers		Sex ratio	Fruit set%
				Female	Male		
S 24	46.93	34.25	21.3	10.3	16.0	1.55	71
S 25	52.07	48.85	21.3	9.0	22.0	2.44	64
S 32	49.60	49.63	19.0	15.0	21.3	1.42	82
S 34 B	37.40	37.22	18.0	17.7	11.7	0.66	91
S 38 A	38.60	39.20	18.7	16.7	14.0	0.84	86
S 36 A	57.93	55.64	18.0	22.7	18.3	0.81	78
S 27 B	47.30	41.60	18.0	20.7	15.3	0.74	87
S 35	43.23	40.44	18.0	19.7	13.3	0.68	91
S 19 E	36.23	35.60	19.0	13.0	14.3	1.1	93
S 19	53.00	48.00	21.0	11.3	16.3	1.44	76
S 10 A	34.67	26.33	20.0	13.7	17.0	1.24	74
S 33	37.67	30.33	19.0	20.3	18.3	0.9	71
S 26 B	34.00	42.43	20.7	13.7	16.7	1.22	72
S 41 A	57.67	28.33	20.0	14.3	16.7	1.17	67
S 28 B	42.67	29.67	20.3	15.0	16.0	1.07	69
S 21 A	34.33	34.33	20.7	11.7	15.7	1.34	77
S 1 B	28.33	35.67	19.3	18.0	15.7	0.87	80
Sama F_1 (control)	57.00	44.40	18.0	18.0	19.0	1.06	94
LSD at 0.05	6.44	8.37	1.45	2.47	2.83	0.217	10.6

Data obtained on the number of days to flowering showed significant differences among the evaluated inbred lines. This trait values ranged from 18 to 21.3 days. The lowest number of days to flowering was shown by S 34 B, S 36 A, S 27 B, S 35 equally with control.

The highest value of sex ratio was recorded in inbred lines S 34 B, S 38 A, S 27 B, and S35 because they have the highest female flower number. Fruit formation is presented in Table (1) as fruit set percentage. Fruit set values ranged from 67 to 94%. Fruit set percentage values for evaluated inbred lines had significant differences. The hybrid Sama F_1 (control) was the highest value compared with all inbred lines for fruit set percentage. Fortunately, no significant differences were found among the inbred lines S 19 E, S 35, and S 34 B and the control. These results agree with Wien *et al* (2002) who reported that, there appears to be genotypic variation in the capacity of plants to produce female flowers.

Flowering and fruiting patterns are a key attribute in breeding squash and pumpkin. Important aspects of flowering are: (1) time of the first appearance of staminate and pistillate flowers, (2) ratio of staminate and pistillate flowers, and (3) environmental effects on flowering patterns and fruit set. In early maturing cultivars of summer squash, female flowering commences as early as 30 to 40 days from sowing.

Data obtained on average fruit weight for studied genotypes are presented in Table (2). Data showed significant differences in this trait among the studied inbred lines. The largest significant average fruit weight was found in inbred lines S 36 A, S 27 B, S 19 E, S 41 A, S 35, and the commercial squash hybrid Sama F_1 without significant differences among them. While inbred line S 10 A had the lowest average fruit weight. Fruit length values ranged from 10.67 to 14.83 cm. The highest significant fruit length was occurred for S 35, while S 33 had the lowest fruit length. The highest significant fruit diameter was found in S 1 B, while the control Sama F_1 had the lowest fruit diameter. In former studies, significant differences in these traits were found among the evaluated genotypes (Ghobary and Ibrahim 2010 and Rakha *et al* 2012).

Table 2. Mean values of the squash inbred lines compared with the commercial hybrid Sama F_1 as a control for yield and fruit traits (combined data across 2017 and 2018 seasons).

Inbred lines and control	Average fruit weight (g)	Fruit (cm)		Yield (kg/plant)		Number of fruits/plant	
		Length	Diameter	Early	Total	Early	Total
S 24	68.09	12.40	2.83	0.122	0.496	1.79	7.28
S 25	82.67	11.33	3.39	0.127	0.475	1.54	5.74
S 32	82.00	12.33	3.17	0.261	1.005	3.18	12.26
S 34 B	99.67	14.00	3.33	0.422	1.605	4.23	16.10
S 38 A	101.67	14.33	3.38	0.391	1.455	3.85	14.31
S 36 A	116.67	12.67	3.98	0.562	2.034	4.82	17.43
S 27 B	113.32	14.13	3.61	0.569	2.025	5.02	17.87
S 35	108.67	14.83	3.45	0.541	1.951	4.98	17.95
S 19 E	115.33	11.83	4.14	0.431	1.393	3.74	12.08
S 19	68.16	13.07	2.72	0.149	0.588	2.18	8.62
S 10 A	64.33	11.33	2.89	0.167	0.656	2.59	10.20
S 33	82.33	10.67	3.49	0.285	1.189	3.46	14.44
S 26 B	91.67	12.42	3.55	0.237	0.903	2.59	9.85
S 41 A	109.83	12.33	4.14	0.315	1.055	2.87	9.61
S 28 B	100.83	13.67	3.66	0.287	1.045	2.85	10.36
S 21 A	82.43	14.00	3.13	0.201	0.738	2.44	8.95
S 1 B	102.33	11.32	4.19	0.304	1.464	2.97	14.31
Sama F_1 (control)	119.14	14.67	4.05	0.574	2.016	4.82	16.92
LSD at 0.05	18.553	1.181	1.31	0.087	0.224	0.874	2.571

Yield component values for the evaluated inbred lines are divided into two parts, early (number and weight fruit) yield and total (number and weight fruit) yield. Early yield ranged from 0.122 to 0. 57 kg/plant.

The highest significant early yield was found in inbred lines S 36 A, S 27 B, S 35, and the squash hybrid Sama without significant differences among them, while S 24 and S 25 had the lowest early yield without significant differences among them. The highest significant early and total fruit number was found in inbred lines S 27 B, S 35, S 36 A, and the commercial squash hybrid Sama without significant differences among them. In previous studies, significant differences in these traits were found among the evaluated genotypes of summer squash (Al-Jebory 2006; Ghobary and Ibrahim 2010; Marie *et al* 2011 and Mohamed *et al* 2003).

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انتاج سلالات كوسة جديدة واعدة كخطوة رئيسية لإنتاج هجن فائقة

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١. قسم بحوث انتاج الخضر تحت ظروف الزراعة المحمية – معهد بحوث البساتين –مركز البحوث الزراعية

٢. قسم بحوث الخضر خلطية التلقيح (القرعيات) – معهد بحوث البساتين – مركز البحوث الزراعية

تم تنفيذ إستراتيجية التربية الذاتية لمدة خمسة مواسم متتالية من ٢٠١٤ إلى ٢٠١٧ في محطة تجارب الجميزة – التابعة لمركز البحوث الزراعية بمحافظة الغربية. حيث تم اختيار سبعة عشر سلالة مرباة تربية داخلية واعدة. اعتمد الاختيار على عدة صفات اهمها طول النبات، وعدد الازهار المؤنثة، ومتوسط وزن الثمرة. أظهرت قيم معامل الاختلاف (CV٪) أن معظم السلالات كانت متجانسة بدرجة عالية. لحسن الحظ لم تتسبب التربية الذاتية فى احداث اية تدهور في معظم الصفات المدروسة. تم تقييم جميع السلالات السبعة عشر المنتخبة بالمقارنة مع الهجين التجاري Sama F_1 حيث استخدم كصنف مقارن لدراسة اهم الصفات خلال الموسمين ٢٠١٧ و ٢٠١٨ تحت ظروف الحقل المفتوح. لوحظت فروق معنوية بين الطرز المقيمة الأخرى في جميع الصفات المدروسة. تميزت السلالة S 36 A بأعلى القيم لصفة طول النبات وكذا عدد الأوراق على النبات و عدد الأيام لانتاج اول زهرة مؤنثة (التبكير) وعدد الأزهار المؤنثة على النبات. من بين السلالات السبعة عشرالمرباة داخليا أعطت السلالات S 35 و S 27 B و S 36 A أعلى القيم لصفات الثمار ومكونات المحصول. كما أعطت السلالات الواعدة السابقة أعلى محصول مبكر ومحصول كلي.من المحتمل أن تصبح تلك السلالات مصدرا جيدا لإنتاج هجن جديدة عالية الانتاج والجودة.

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