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GENE ACTION OF *IN VITRO* TRAITS FOR CANOLA DOUBLE HAPLOID LINES

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ABSTRACT

Four double haploid lines (DHs) and their 12 F_1 hybrids, which produced according to complete diallel crosses were evaluated for *in vitro* traits to determine the gene action which influence these traits. The results revealed that the mean squares for genotypes were highly significant for all *in vitro* traits. Furthermore, the partition of these genotypic variation to its components revealed mean squares of general combining ability were not significant for all studied traits except for shoot ratio. Although, the SCA mean squares were significant in the case of embryoid induction and shoot ratio. However, the reciprocal effects were significant for embryoid induction and number of embryoid/anther responded. These results indicated that embryoid induction may influenced by cytoplasmic factors in addition to nuclear factors, while number of embryoid / anthers responding may influenced by cytoplasmic factors only. However, shoot ratio traits my controlled by both additive and non-additive genes. DH_3 line showed the positive largest magnitudes for general combining ability effect (gi) in embryoid induction, number of embryoid/responded anthers and shoot ratio. Thus, it could be concluded that the DH_3 line was the best general combiner among this set of lines for *in vitro* traits.
Keywords: Gene action, embryoid induction, Double haploids

INTRODUCTION

Canola (*Brassica napus L.*) was developed from rapeseed by Canadian plant breeder for its superior nutritional qualities. The main types oilseed rape currently grown were classficated to two groups: the first group involved double low (00) varieties which are originally grown for food and contain low levels (typically less than 1%) of erucic acid and low levels of glucosinolates. It also contain high protein meal for animal feed. While, the second high erucic acid rape (HEAR) varieties which are grown specifically for their erucic acid content (typically 50 – 60% of oil). It is used to produce phenyl alcohol, which is added to a waxy crude mineral oil to improve its flow. Also, it is used in plastics, lubricants, lacquers and detergents. Thus, the improvements of canola for their yield components is considered one of the major aim of canola breeders.

Double haploids plants (DH) are produced by doubling the chromosome number of a haploid plant, which regenerated from anther culture method, whereas conventional inbred lines are developed by selfing in successive generations (Stoskopf *et al.*, 1993). With the DH method, homozygous plants are produced in one generation and homozygosity is about 100% compared to the conventional method which results in an average level of homozygosity of 96.9% after five generations of seifing (Briggs and Knowles, 1967). During DH production only one recombination

event takes place and selection is possible only after DHs are produced. While, the production of inbred lines through conventional method, one recombination event can take place in every generation of selfing and selection can be practiced in each generation. In addition, recessive genes in DH are more readily expressed at plant level.

Thus, the present investigation aimed to evaluate the performance of doubled haploid (DH) lines and their potential use as parents in hybrid cultivars to present further information dealing with nature of gene action for the *in vitro* androgenetic response traits. These estimates could be obtained through the evaluation of a set of diallel crosses.

MATERIALS AND METHODS

Genetic materials:

In this investigation four double haploid (DH) lines belong to the species (*Brassica napus, L.*) which derived from anther culture technique as described in our previous investigation (Abd El-Maksoud *et al.*, 2004) were used as started genetic materials. The procedure and pedigree of produced lines could be summarized in the following diagram:

During 2005 / 2006 season, selfed seeds from each DH line were planted at the Experimental station, Faculty of Agriculture, Mansoura University. At the flowering time, all single crosses including reciprocal were made handly among these DH lines according to Griffing's method I (1956) which outlined by Singh and Chaudhary (1985).

At maturity, hybrid's seeds were obtained from each cross. The used mating design yielded 12 hybrids which included 6 F_1 hybrids and 6 F_1r reciprocal hybrids. In addition, the four parental lines were self pollinated to obtain additional amounts of seeds for further investigation. Therefore, the genetic materials used in this investigation included four DH lines, six F_1 hybrids and six F_1 reciprocal hybrids.

During the season 2006 / 2007, the four double haploids lines (DH_1, DH_2, DH_3, DH_4) and their 12 F_1 hybrids were sown for anther culture purpose at Faculty of Agriculture, Experimental Station, Mansoura University. At the suitable morphological stage of anther culture inoculation, 12 flowerscent from each genotype were selected. The *in vitro* experimental design was a randomized complete blocks with four replications. In each replication, the anthers of three flowerscent were distributed over three Petri dishes 10 cm in diameter containing induction medium. Each Petri dish, contain about 60 – 80 anthers was considered to be one of experimental unit. Subsequently, the produced embryoids and/or calli were transferred to regeneration medium in the same manner as described in the following anther culture procedure. The buds were collected at the early to late uninucleate microspore stage. The optimal microspore stage was determined based on the buds and anther morphology. Buds were selected when they were approximately 2-3 mm in length. At this stage, the length of the petals was between 0.25 and 0.50 of the length of anthers which correlated with the early to late uninucleate stage of development as suitable stage for anther inoculation (Keller *et al.*, 1983).

In addition, some anthers were squashed in acetocarmen for cytological exam to insure the suitable stage. Sterilization of buds was carried out under sterile conditions in 1% $HgCl_2$ solution with the addition of two drops of Tween-20 as a wetting agent for 10 minutes and rinsed three times with sterile distilled water. Subsequently, the anthers were excised from each bud and placed on the induction medium under sterile conditions. The induction medium used in this study was the recommended modification B5 medium for rapeseed anther culture according to Keller *et al.* (1975), containing 12% sucrose and 2.0 mg/l dichlorophenoxy acetic acid (2,4-D).

The cultures were incubated in darkness at 25°C ± 2°C for four weeks. Then, the total number of responding anthers (which gave one or more calli and/or embryoids) and the total number of calli and/or embryoids (number of embryoids) were recorded. The produced embryoids and/or calli were transferred to regeneration medium for shoot development. The cultures were kept under 16- hours illumination (fluorescent light) at 22°C ± 2°C for four weeks. The green shoots were subcultured on the same fresh regeneration media with 0.5 mg/l ά naphthylacetic acid (NAA) and 0.5 mg/l kinetin for four weeks at 22°C ± 2°C to good rooting. Then the total green plantlets were recorded.

Therefore, The data were recorded on each replicate for the following traits: Responding anthers (R.A): this trait was determined as the ratio of the number of responded anthers (producing at least one embryoid or callus) to total number of anthers plated. Embr yoid induction (E.I): the ratio of the number of embryoids and/or calli originating from the responding anthers to the total number of anthers cultured. Number of embryoid/responded anthers (N.E/R.A): the ratio of the number of embryoid and/or calli originating from the responding anthers to the number of anthers responded. Shoot ratio (Sh. R): this was calculated as the ratio of the number of shoots to the total number of embryoid transferred to regeneration medium.

Statistical analysis:

In order to normalized the distribution of the percentage data which fall between 0.00 to 1.00 were transformed by using arcsin $x^{1/2}$ function prior to statistical analysis for all studied traits. Analysis of variance were analyzed using the general linear models procedure (GLM) of SAS (1996), in order to test the significance difference among four parental lines, 6 F_1 hybrids and 6 F_1r reciprocal hybrids. Differences among genotypic means for all studied traits were tested for significance according to the regular F test.

Heterosis estimates were determined as a deviation of F_1 hybrids than their Mid-parental and/or better parental values as described in the following equations:

Heterosis from the mid-parents was calculated as :

$$H.(M.P)\ \% = [(F_1 - M.P) / M.P] \times 100$$
$$= [(F_1r - M.P) / M.P] \times 100$$

Heterosis from the better parents was calculated as:

$$H.(B.P)\ \% = [(F_1 - B.P) / B.P] \times 100$$
$$= [(F_1r - B.P) / B.P] \times 100$$

The significance of Heterosis was determined using least significance differences value (LSD) at 0.05 and 0.01 levels of probability according to Steel and Torri (1960).

General combining ability (GCA) and specific combining ability (SCA), as well as reciprocal effects (Rec) variances were determined according to Griffing's method I (1956) as described by Singh and Chaudhary (1985).

The estimations of σ^2gca and σ^2 sca could be expressed in term of additive (σ^2A) and non-additive (σ^2D) genetic variance according to Matzinger and Kempthrons (1956).

Estimates of heritability in both broad and narrow senses were calculated as follows:

Heritability in broad sense (h^2_b):

$$h^2_b = [\sigma^2A/(\sigma^2A+\sigma^2D+ \sigma^2r+ \sigma^2e/k)]x\ 100$$

Heritability in narrow sense (h^2_n):

$$h^2_n =[(\sigma^2A+\sigma^2D)/(\sigma^2A+\sigma^2D+ \sigma^2r+ \sigma^2e/k)]x\ 100$$

where; σ^2e: is the error variance and k: is the number of replications.

RESULTS AND DISCUSSIONS

The genetic materials used in this investigation included four DH_s lines which were involved in complete diallel crosses mating design to produce six F_1 hybrids and six F_{1r} reciprocal hybrids. All genotypes were evaluated to determine the nature of gene action for *in vitro* traits. The analysis of variance and the mean squares of genotypes for *in vitro* traits are presented in Table 1. The magnitudes of the mean squares for genotypes were highly significant for all *in vitro* traits indicating that the presence of real differences among these genotypes and the partition of this genotypic variation to it is components are valid. Therefore, the planned comparisons for the understanding of the nature of variation and the determination of the amounts of heterosis for all traits are valid. However, the magnitudes of the mean squares for replications were not significant for all traits except for responding anthers which was significant, indicating to the responding anthers percentage influenced by the buds situations.

In this respect, many authors agree with these results such as Abd El-Maksoud *et al.* (2004) reported that the significance on the mean squares revealed that the genotype mean squares were highly significant for all studied *in vitro* traits, Burbulis *et al.* (2004) found that the embryogenesis formation frequency differed for the tested genotypes, Wang *et al.* (2007) reported that the genotypes had remarkable effect on embryoid induction.

Table 1: Analysis of variance and the mean squares of parents, F_1 hybrids and F_{1r} reciprocals for *in vitro* traits

S.O.V	d.f	R. A.	E. I.	N.E / R.A	Sh.R
Replicates	3	138.87*	0.111	0.243	33.37
Genotypes	15	90.53*	0.249**	0.309**	332.9**
Error	45	39.46	0.053	0.085	106.7

Note: *, significant at 0.05 and 0.01 levels of probability, respectively.**

Note: The data were transformed using arcsin $x^{1/2}$ prior to statistical analysis.

Mean Performance of Genotypes:

The means of four parental lines and their F_1 hybrids (Table 2) showed that no specific parent and/or cross was superior or inferior for all studied traits. However, of the four parental lines, the greatest mean for responding anthers was observed in DH_2 with mean of 66.28 transformed value. Although, the greatest overall value for embryoid induction and number of embryoid/anther response were observed in DH_3 and the greatest value of shoot ratio was observed in DH_4. While, DH_1 line was the inferior one with respect to the *in vitro* traits with transformed means of 59.63, 1.00,1.38 and 29.40 for responding anthers(R.A.), embryoid induction (E.I), number of embryoid/responded anther (N.E/R.A.) and shoot ratio, respectively.

Regarding F_1 hybrid and their reciprocal hybrids, the means showed that the greatest mean of embryoid induction and number of embryoid/anther response were observed in the cross $DH_4 \times DH_1$ with means of 1.99 and 2.29, respectively. On the other hand, the lowest mean value of the same traits were observed in the cross $DH_3 \times DH_2$ with means of 1.12 and 1.33, respectively. The means value for responding anther were ranged from 49.80 ($DH_4 \times DH_1$) and 68.80 ($DH_2 \times DH_1$). Although, the best combination for shoot ratio was observed in $DH_1 \times DH_3$ with transformed value of 61.45.

Heterosis:

The estimated amount of heterosis were determined for all *in vitro* traits as a deviation of each F_1 hybrid than their mid-parental and better parental values and the obtained results are shown in Table 3. The results revealed that the cross $DH_4 \times DH_1$ exhibited positive significant heterosis for embryoid induction and number of embryoid/anthers response when the average of hybrids were compared to the mid-parental value and better parental value and these value of heterosis were 85.98%, 74.565, 57.93% and 50.66%, respectively. However, $DH_1 \times DH_3$ and $DH_3 \times DH_1$ as well as $DH_4 \times DH_3$ exhibited positive significant heterosis for shoot ratio when the averages of the hybrids were compared to the mid-parental and better parental values with heterotic values 73.71%, 60.4% and 37.63% relative to mid-parents for those combinations, respectively, while it was 48.61%, 37.32% and 30.40%, relative to better parent for these combinations, respectively. The positive heterosis relative to better parents indicates that the dominant genes may be controlling these characters . In this respect, Barro and Martin (1999) as well as Zhang and Yoshihito (2001) agree with the present results and concluded that most of sixteen studied rapeseed hybrids showed superiority over their high responsive parent for microspore culture ability, Abd El Maksoud *et al.* (2004) found the heterotic values relative to mid-parents for responding anthers and number of embryoid/responded anthers were 18.06% and 19.69%, respectively. However, it was 18.63% for shoot ratio relative to better parent.

Table 2: Mean performance of parental lines, F_1 hybrids and F_{1r} reciprocals for all *in vitro* traits

Genotypes	R. A.	E. I.	N.E / R.A	Sh. R
DH_1	59.63	1.00	1.38	29.40
DH_2	66.28	1.2	1.41	43.73
DH_3	64.65	1.72	2.16	41.35
DH_4	60.00	1.14	1.52	46.28
$DH_1 \times DH_2$	61.45	1.35	1.82	38.88
$DH_2 \times DH_1$	67.80	1.27	1.49	42.08
$DH_1 \times DH_3$	61.83	1.25	1.64	61.45
$DH_3 \times DH_1$	60.03	1.29	1.73	56.78
$DH_1 \times DH_4$	60.43	1.22	1.55	35.33
$DH_4 \times DH_1$	58.58	1.99	2.29	47.45
$DH_2 \times DH_3$	67.48	1.36	1.76	55.03
$DH_3 \times DH_2$	66.23	1.12	1.33	41.38
$DH_2 \times DH_4$	63.15	1.06	1.35	50.53
$DH_4 \times DH_2$	64.60	1.42	1.76	51.48
$DH_3 \times DH_4$	54.98	1.14	1.72	54.68
$DH_4 \times DH_3$	49.80	1.32	1.91	60.35
LSD 5%	8.95	0.328	0.414	14.70
1%	11.82	0.433	0.547	19.43

Note: The data were transformed using arcsin $x^{1/2}$ prior to statistical analysis

Table 3: Estimates of heterosis from the mid-parent (M.P) and better parent (B.P) for *in vitro* traits

	R. A.		E. I.		N.E / R.A		Sh. R	
	M.P	B.P	M.P	B.P	M.P	B.P	M.P	B.P
$DH_1 XDH_2$	-2.39	-7.29	22.73	12.5	30.47*	29.08	6.331	-11.09
$DH_2 X DH_1$	7.7	2.293	15.45	5.83	6.81	5.674	15.13	-3.773
$DH_1 X DH_3$	-0.5	-4.36	-8.09	-27.3**	-7.34	-24.07*	73.71**	48.61*
$DH_3 X DH_1$	-3.41	-7.15	-5.15	25.00*	-2.26	-19.91*	60.4**	37.32*
$DH_1 X DH_4$	1.03	0.717	14.02	7.02	6.897	1.974	-6.63	-23.66
$DH_4 X DH_1$	-2.04	-2.37	85.98**	74.56**	57.93**	50.66**	25.36	2.53
$DH_2 X DH_3$	3.08	1.811	-6.85	-20.90*	-1.4	-18.52	29.36	25.84
$DH_3 X DH_2$	1.11	-0.08	-23.30*	-34.90**	-25.5*	-38.43**	-2.75	-5.37
$DH_2 X DH_4$	0.02	-4.72	-9.400	-11.70	-7.85	-11.18	12.28	9.18
$DH_4 X DH_2$	2.3	-2.53	21.37	18.33	20.14	15.79	14.4	11.24
$DH_3 X DH_4$	-11.8	-15.00*	-20.30	-33.70**	-6.52	-20.37*	24.8	18.15
$DH_4 X DH_3$	-20.1**	-23.00**	-7.69	-23.30*	3.804	-11.57	37.63**	30.40
L.S.D 0.05	7.85	9.06	0.288	0.332	0.363	0.419	12.90	14.89
0.01	10.58	12.22	0.388	0.448	0.489	0.565	17.39	20.08

*,** significant at 0.05 and 0.01 levels of probability, respectively.

Combining ability analysis:

Analysis of combining ability and the mean squares of complete diallel crosses for *in vitro* traits are presented in Table 4. Tests of significance of the mean squares of general and specific ability showed that the GCA significant for all *in vitro* traits except for embryoid induction. Although, the SCA mean squares were significant in all *in vitro* traits. However, the reciprocal effects were highly significant for embryoid induction and number of embryoid/anther responded. These results indicated that embryoid induction and number of

embryoid/anther responded may influenced by cytoplasmic factors in addition to nuclear factors. However, responding anthers and shoot ratio traits my controlled by both additive and non-additive genes.

Table 4: Analysis of combining ability variance and mean squares of F_1 hybrids and F_{1r} reciprocals for *in vitro* traits

S.O.V	d.f	R. A.	E. I.	N.E / R.A	Sh. R.
GCA	3	35.49*	0.019	0.081*	120.78**
SCA	6	32.36**	0.075**	0.068*	114.52**
Rcp	6	6.47	0.069**	0.088**	33.19
Error	45	9.87	0.0133	0.0211	26.69

*,** Significant at 0.05 and 0.01 levels of probability, respectively.
Note: The data were transformed using arcsin $x^{1/2}$ prior to statistical analysis.

General combining ability effects (g_i) for each paternal line:

Positive or negative general combining ability effects (g_i) estimates would indicate that a given inbred is much better or poorer than the average of the group involved with in the diallel crosses mating system

The estimates of general combining ability effects (g_i) for each parental line for *in vitro* traits are presented in Table 5. It could be seen from this table there are no lines exhibited significant for any traits. However, DH_3 showed positive largest magnitudes for embryoid induction, number of embryoid/responded anthers and shoot ratio. Thus, it could be concluded again, the best general combiner among this set of lines for *in vitro* traits is DH_3 line.

Table 5: General combining ability (g_i) effects of parental lines for *in vitro* traits.

Parents	R. A.	E. I.	N.E / R.A	Sh. R.
DH_1	1.15	-0.008	-0.028	-4.67
DH_2	2.06	-0.055	-0.124	-1.41
DH_3	-0.48	0.063	0.114	4.29
DH_4	-2.73	0.000	0.038	1.79
S.E	1.18	0.043	0.055	1.94

Note: The data were transformed using arcsin $x^{1/4}$ prior to statistical analysis.

Specific combining ability (SCA) effects for each cross:

Estimates of specific effects (S_{ij}) for *in vitro* traits were presented in Table 8. The results revealed that the best specific combinations for improving embryo inductions and shoot ratio were DH_1 x DH_3 and DH_1 x DH_4, respectively. While, only 2 out of 6 crosses in the cases of responding anthers and number of embryoid/responding anthers exhibited positive specific combining ability.

Table 6: Specific combining ability effects (S_{ij}) of each cross for *in vitro* traits

Crosses	R. A.	E. I.	N.E / R.A	Sh. R.
$DH_1 \times DH_2$	-0.276	0.07	0.141	-0.71
$DH_1 \times DH_3$	-1.431	-0.09	-0.156	12.23*
$DH_1 \times DH_4$	-0.581	0.31*	0.245	-2.99
$DH_2 \times DH_3$	3.587	-0.07	-0.110	-1.94
$DH_2 \times DH_4$	2.867	-0.01	-0.024	3.36
$DH_3 \times DH_4$	-6.078	-0.14	-0.001	4.18
S.E	2.89	0.106	0.134	4.74

* Significant at 0.05 level of probability
Note: The data were transformed using arcsin $x^{1/2}$ prior to statistical analysis.

Estimates of reciprocal (r_{ij}) effects for each cross:

The estimates of reciprocal (r_{ij}) effects for *in vitro* traits of each cross are presented in Table 7. It could be seen from this table that there are no crosses exhibited significant for any traits. However, the cross $DH_2 \times DH_3$ showed positive largest magnitudes for number of embryoid / responding anthers and shoot ratio. Also, the crosses $DH_2 \times DH_4$ and $DH_3 \times DH_4$ showed positive largest magnitudes for responding anthers and embryoid induction, respectively. This results illustrated that the reciprocal differences for *in vitro* responses are generally attributed to cytoplasmic factors, the physiological characteristics of the maternal plants, or specific interactions between cytoplasmic and genetic factors. In this respect, Abd El–Maksoud (1993) reported in wheat that cytoplasmic factors have an influence on the heredity of embryoid induction as well as of green and albino plantlet regeneration.

Table 7 : Reciprocal Effects (r_{ij}) for *in vitro* traits

Crosses	R. A.	E. I.	N.E / R.A	Sh. R.
$DH_1 \times DH_2$	-3.18	0.04	0.165	-1.60
$DH_1 \times DH_3$	0.90	-0.02	0.045	2.34
$DH_1 \times DH_4$	0.94	-0.39*	-0.370*	-6.06
$DH_2 \times DH_3$	0.63	0.12	0.215	6.83
$DH_2 \times DH_4$	-0.73	0.18	-0.205	-0.475
$DH_3 \times DH_4$	2.59	-0.09	-0.075	-2.84
S.E	2.72	0.099	0.126	4.47

*Significant at 0.05 level of probability
Note: The data were transformed using arcsin $x^{1/2}$ prior to statistical analysis

Nature of gene action and heritability:

The additive (σ^2A), dominance (σ^2D) and reciprocal (σ^2r) variances in addition to heritability in broad ($h^2_b\%$) and in narrow ($h^2_n\%$) sense for all *in vitro* studied traits were presented in Table 8. The results revealed that the magnitude of both additive and non-additive (including dominance) genetic variance were positive for all traits except for embryoid induction which exhibit negative value for additive variance, indicating the contribution of both components in the inheritance of these traits except for embryoid induction. However, the relative magnitudes of non-additive genetic variance for all *in vitro* traits were importance than other two components. These results

suggesting the predominance of non-additive gene effects in the inheritance of these traits. These could be verified by the ratio $(\sigma^2D/\sigma^2A)^{1/2}$ which were more than one, revealing the importance of over dominance in the genetic control of these traits.

Table 8: The relative magnitudes of different genetic parameters for *in vitro* traits

Genetic parameters	R. A.	E. I.	N.E / R.A	Sh. R.
σ^2A	1.22	-0.013	0.004	3.26
σ^2D	13.84	0.038	0.029	45.06
σ^2r	-1.69	0.028	0.033	3.26
$(\sigma^2D/\sigma^2A)^{1/2}$	3.37	>1.00	2.69	3.91
H^2_b%	60.41	47.96	37.70	68.25
H^2_n%	4.88	0.0	4.82	3.88

The data were transformed using arcsin $x^{1/2}$ prior to statistical analysis.

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الفعل الجيني للصفات المعملية في سلالات الكانولا المتضاعفة العدد الكروموسومى الاحادى

ممدوح محمد عبد المقصود ، علي ماهر العدل ، محمد سعد حمادة و رحاب محمد حبيبة
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في هذه الدراسة تم تقييم أربعة سلالات من الكانولا المتضاعفة العدد الكروموسومى الاحادى بالإضافة إلى الهجن الناتجة منها باستخدام نظام التزاوج الدائري الكامل وذلك للصفات المعملية لتحديد طبيعة الفعل الجيني لهذه الصفات.

وكانت النتائج المتحصل عليها تتلخص في الآتي:

لوحظ أن هناك اختلاف معنوي جدا بين التراكيب الوراثية المستخدمة في هذه الدراسة لكل الصفات. وبتقسيم التباين الوراثي لمكوناته أشار إلى أن القدرة العامة على التآلف كانت معنوية فقط لصفة معدل استحداث النباتات الخضراء بينما كانت القدرة الخاصة على التآلف معنوية في حالة معدل استحداث أشباه الأجنة ومعدل استحداث النباتات الخضراء بالإضافة إلى ذلك كان التباين الراجع للتهجينات العكسية معنوي لكل من معدل استحداث أشباه الأجنة و معدل أشباه الأجنة لكل منك مستجيب. ومن هذه النتائج يمكن الإشارة إلى أن معدل استحداث أشباه الأجنة يتم التحكم فيه بعوامل سيتوبلازمية بالإضافة إلى العوامل الوراثية بينما معدل أشباه الأجنة لكل منك مستجيب يمكن أن يكون محكوما بعوامل سيتوبلازمية فقط. وعلى الجانب الآخر فان معدل استحداث النباتات الخضراء يكون محكوما بكل من العوامل المضيفة والغير مضيفة.كما أظهرت النتائج أيضا أن السلالة المسماة DH_3 كانت أفضلهم قدرة على التآلف في معظم الصفات. ولذلك يمكن أن نستخلص من خذه النتائج أن أفضل طريقة لتحسين الصفات المعملية لهذه المجموعة من السلالات هي إنتاج الهجن خاصة التي تحتوى على DH_3 كأحد أبائها.