Genetic Analysis of Root and Hypocotyl Color Traits in Sugar Beet

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ABSTRACT

The present research was carried out in Sabahia Agricultural Research Station, Alexandria, Egypt, (2010 - 2013) to study root and hypocotyl colors traits in sugar beet in F_2 plants derived from hybridization among sugar beet multigerm diploid American inbred line (C39) and Egyptian table beet. In addition to, Gene frequency of hypocotyl colors (Red, Pink and green) in the three multigerm sugar beet populations introduced from USA "3859 (Sp), Z120 and 3915 (Sp)". Results revealed that F_2 phenotype seedlings from (C39 X Table beets) were segregated to three groups according to leaf, hypocotyl and root colors. The main synopsis are gene frequency of hypocotyl colors in the three examined populations indicated that green hypocotyl (rr) were presented in rear percentages (eg, 0.016, 0.01 and 0.02) in populations "3859 (Sp), Z120 and 3915 (Sp)" in the three examined populations, respectively.

INTRODUCTION

Sugar, fodder, red beet, and chard belong to *Beta* vulgaris L. The first sugar beet (white Silesian beet), is considered the ancestor of all sugar beets, it had originated from crossings between typical fodder beet and chard. The F_2 obtained from fodder beet x chard segregated forms and color variants largely corresponding to sugar beet (Fischer 1989).

Two main genes, *R* and *Y,* each with a series of alleles, control the color formation of the cultivated beet *Beta vulgaris* ssp. *vulgaris.* Both genes are localized to the same linkage group and, according to various data, are located at a distance of 6.3 to 7.5% crossing over units from one another (Laursen 1972). A combination of the recessive and dominant alleles of these loci creates various coloration types of the hypocotyl, root, and aboveground part of beet plant.

Root color in beets is determined by genes at least two loci (Y and R), these genes of each constituting a multiple allelic series (Keller 1936; Owen and Ryser 1942). At the Y-locus, dominant genes e.g. the genotype "rr YY", result in yellow to orange root color, the recessive allele "y" giving white. At the R-locus, the most common dominant gene e.g. the genotype R, "RR yy", conditions white root with red hypocotyl. The recessive allele "rryy" conditions green hypocotyl and white root. Dominant genes from both loci being present in the genotype of a beet, e.g. the genotype RRYY, result in red root color. However, a gene at the R-locus designated Rh (R~) conditions only red hypocotyl even when dominant genes are present at the Y-locus (Laursen, 1972). Furthermore Keller (1936) has reported a gene at the R-locus which conditioned pale red roots only when a dominant gene was present at the (Y)-locus. The R- and Y-loci are linked with a recombination percentage of about 7 (Keller 1936); Owen and Ryser 1942). The R-locus has been located to chromosome II of the beet (Butterfass 1968).

MATERIALIS AND METHODS

Present research was carried out to study root and hypocotyl colors in F_2 plants derived from hybridization between sugar beet multigerm inbred line (C39) and Egyptian table beet (C39 X Table beets). Gene frequency of hypocotyl colors (Red, Pink and green) in the three multigerm sugar beet populations introduced from USA "3859 (Sp), Z120 and 3915 (Sp)" also. Hypocotyl color frequency in the three examined populations was compared with three sugar beet commercial multigerm varieties (Nada, Lola and Maribo poly) in this trait.

Sugar beet breeding materials used in this investigation were obtained from Egyptian Sugar Beet Breeding Program (ESBBP) by El-Manhaly (Egypt), by the breeding program from USDA Plant Introduction Office by Robert, T. Lewellen (USA) These breeding materials include three multigerm diploid sugar beet populations "3859 (Sp), Z120 and 3915 (Sp)" and one multigerm diploid inbred line (C39).

June, 2010 seeds of sugar beet multigerm inbred line C39 were snowed in germination tray. Red hypocotyl seedlings (yyRR) were observed and selected after three weeks (Fig.1). This line has segregation ratio of the three hypocotyl colors (23.2, 50.99 and 25.8 %) for the red (RR), pink (Rr) and green (rr), in respect. Selected seedlings were transplanted to plastic pots then after 6 weeks to earthen pots. At age of 8 weeks plants were exposed to cold treatment for vernalization in cold store in Sabahia Research Station. February, 2011 selected plants were transferred from cold store to green house under artificial light 18 h/day to push the plants to flower.

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Seeds of annual table beets (BB) were planted in earthen pots at November & December (2010) and January (2011) to choose the flowering plants in the hybridization. (Fig.2). Plants of C39 (parents) and table beet plants (pollen donor) were put together in isolation rooms in green house for hybridization and the seeds of F_1 were harvested from plants as seed parents in May (2011). Seeds of F_1 were planted in June (2011) to introduce F_1 plants. Red roots seedlings were selected to inshore that the hybridization is done, Selected seedlings were transplanted to plastic pots then after 6 weeks to earthen pots. F_1plants were exposed to cold treatment for vernalization in cold store and transferred from cold store to green house under artificial light 18 h/day to push the plants to flower. F_2 seeds were collected in June (2012) and in November (2012). F_2 seedlings were uprooted from the soil (three weeks age) to examine the segregation ratios in his colors.

December, 2011 seeds of the three sugar beet populations "3859 (Sp), Z120 and 3915 (Sp)", were planted in germination trays to study hypocotyl color character. In four weeks growth stage the seedlings were lifted from the soil and the hypocotyl color were observed and calculated. The Pearson Chi-square criterion was used for the statistical handling of the segregation data and heterogeneity. To calculate the chi square statistic χ^2 by the following steps according to (Snedecor 1957):

$$X^2 = \sum_{i=1}^{k} \frac{(observed\ count - expected\ count)^2}{expected\ count} \quad (1)$$

$$= \sum_{i=1}^{k} \frac{(O-E)^2}{E} \quad (2)$$

1. For each *observed* number in the table subtract the corresponding *expected* number (O- E).
2. Square the difference $[(O-E)^2]$.
3. Divide the squares obtained for each cell in the table by the *expected* number for that cell $[(O- E)^2 / E]$.

Sum all the values for $(O- E)^2/E$.

RESULTS AND DISCUSSION

Top crosses between sugar beet X table beet

Narrow genetic background was described among Egyptian sugar beet and sugar beet genetic resources from USA (Coons 1936; Bosemark 1979; Lewellen 1992 and Panella 1996). Top crosses between American sugar beet genotypes with Egyptian table beets code increase the genetic diversity and transfer adapted genes to Egyptian condition to sugar beet gene pool. Deming (1942 &1950) crossed sugar beet with red table beet and selected in subsequent generations for globe-shaped with roots. Sugar beet inbred multigerm (MM) line (C39) 2n = 18 was hybridized with Egyptian table beets. Red hypocotyl (yy RR) seedlings for (C39) line were selected and hybridized with Egyptian table beets (YYRR) after flowering.

Depending on morphological variations in Fig. 3, F_2 seedlings were segregated to three groups according to leaf, hypocotyl and root colors as flow, group (1): Red leaf, Red hypocotyl and Red roots, group (2): Green leaf with red veins, Red hypocotyl and Red roots and group (3): Green leaf, Red hypocotyl and White roots.

Figure 1. Selected seedlings of C39 inbred line, red hypocotyl seedlings with white roots (Right)

Figure 2. Parents of the top crosses (C39) inbred line (left) and Egyptian table beet (right)

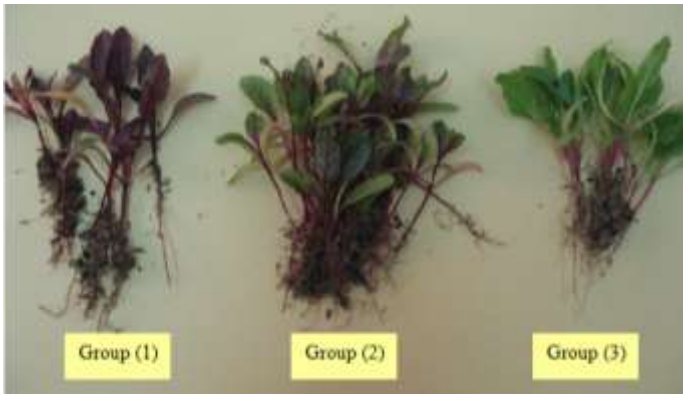

Figure 3. Seedlings of F_2 generation in the three segregated groups

Genetic analysis of F2 seedlings:

Selected seedlings for red hypocotyl sugar beet plants from C39 line had a genetic genotype of (yyRR) for hypocotyl color was hybridized with table beets had (YYRR) background (Keller, 1936). F_1 plants carry genetic background (YyRR) as shown in Figure 4. Results in Figure 4 showed the genetic description of the three sugar beet phenotypes. Data indicated that F_2 plants segregated groups and phenotype was analyzed as flowing: Group (1) has phenotypic ratio 47 plants (RRYY), Group (2) the phenotypic ratio 98 plants (RRYy) and Group (3) the phenotypic ratio 52 plants (RRyy) as shown in Table 1. Chi square distribution showed value of χ^2 =0.2589 lies between 0.103 and 0.446. The corresponding probability is 0.80 <P<0.95. This is smaller than the conventionally accepted significance level of 0.90, so the hypothesis that the three distributions are the same is expected. Since our χ^2 statistic (0.2589) exceeded the critical value for 0.95 probability level (0.103) we can expect the hypothesis that the observed values of our cross are the same as the theoretical distribution of a 1:2:1 ratio. Table (1) presented F_2 segregation seedling numbers of the three different genotypes and χ^2 values.

Hypocotyl color in sugar beet belongs to un-complete dominance traits with three genotypes (RR, Rr and rr) and three phenotypes (Red, Pink and Green hypocotyl), respectively (Keller (1936). Data of hypocotyl color ratio in the three examined populations "3859 (Sp), Z120 and 3915 (Sp)" were present in Table (2). Data indicated that the percentages ratio of hypocotyl colors were (0.75, 0.23 and 0.016) for (RR, Rr and rr), in respect in population 1 "3859 (Sp)". Population 2 Z120 had percentages of (0.73, 0.25, and 0.02) for the three genotypes while population 3 " 3915 (Sp)" had percentages of (0.75, 0.24 and 0.01) for the three hypocotyl colors. These percentages did not belong to Mendelian ratios which segregate to (1:2:1) or (0.25:0.50:0.25) in the un-complete dominance characters.

If the frequency of green hypocotyl plants are known "rr" in population 1 "3859 (Sp)" is (0.016) then: q^2 = 0.016, (q=0.13) (the gene frequency of "r"), p =1 – 0.13 = 0.87 (the gene frequency of "R").

Table 1. F_2 segregation seedling numbers and Chi square values

Genotype	Observed	Expected	(O – E)	$(O - E)^2$	χ^2
YYRR	47	49.25	-2.25	5.0625	0.1028
YyRR	98	98.50	-0.50	0.25	0.0025
yyRR	52	49.25	2.75	7.5625	0.1536
	197	197	0	-----	0.2589

Figure 4. F_1 plants green leaf with red veins (left) and Genotypic description of the F_2 sugar beet seedlings (Right)

Table 2. percentages ratio of hypocotyl colors in the three populations

Population	3859 (Sp)			Z120			3915 (Sp)		
Hypocotyl Color	Red	Pink	Green	Red	Pink	Green	Red	Pink	Green
Genotype	RR	Rr	rr	RR	Rr	rr	RR	Rr	rr
Number in sample	527	161	11	696	233	21	220	72	3
Observed frequency	0.75	0.23	0.016	0.73	0.25	0.02	0.75	0.24	0.01
Calculated frequency	0.76	0.23	0.016	0.74	0.24	0.02	0.81	0.18	0.01

The probable distribution of genotypes in this population can now be computed as follows: p^2 (RR) : 2 pq (Rr): q^2(rr) = $(0.87)^2$: 2 (0.87) (0.13): (0.13) = 0.76 RR: 0.23 Rr : 0.016 rr, Gene frequency of hypocotyl in population "3859 (Sp)" = 0.76 + 0.23 + 0.016 = 1. Compared the calculated percentages of hypocotyl colors in the examined population (Calculated frequency) with the (Observed frequency) indicated that this population in equilibrium state Table (2). Data in Table 2 indicated that the three examined populations at in equilibrium state, while present of green hypocotyl (rr) in the three examined populations at rear percentages (eg, 0.016, 0.01 and 0.02) in populations "3859 (Sp), Z120 and 3915 (Sp)" respectively.

2.2. Hypocotyl color in commercial varieties

Three sugar beet multigerm commercial varieties (Nada, Lola and Maribo-poly) were studied in hypocotyl color percentages to compare gene frequency of hypocotyl color with the three examined populations in this trait. Figure 5 illustrated different hypocotyl colors in sugar beet commercial variety Nada. Hypocotyl color percentages were presented in Table (3). Data indicated that observed frequency of green hypocotyl (rr) for Maribo-poly variety was similar to the studied frequencies of this trait in the three examined populations. While green hypocotyl values in the other tow varieties (Nada and Lola) are close to the Mendelian ratios which segregate to (1:2:1) or (0.25: 0.50: 0.25). For that. Nada frequencies were examined by Chi square for theoretical distribution ratio of (1:2:1).

Chi square χ^2 statistic presented in Table (4). The data showed that χ^2 =25.373, predetermined alpha level of significance (0.05), and degrees of freedom (df = 1). Entering the Chi square distribution table with 2 degree of freedom value of χ^2 (25.373), this value not include in table of probability which that mine reject the hypothesis and the frequency of hypocotyl color in Nada variety not belong to Mendelian ratios.

Root and hypocotyl colors in sugar beet had a great concern by many sugar beet breeders and investigators. Hypocotyl color can be used as a description tool in sugar beet registration lines and/or germplasms, (Campbell,1990; Panella, 1999 and McGrath, 2003).

Table 3. percentages ratio of hypocotyl colors in the three commercial varieties

Variety	Nada			Lola			Maribo-poly		
Hypocotyl Color	Red	Pink	Green	Red	Pink	Green	Red	Pink	Green
Genotype	RR	Rr	rr	RR	Rr	Rr	RR	Rr	rr
Number in sample	377	600	254	640	940	326	685	421	75
Observed frequency	0.31	0.49	0.21	0.34	0.49	0.17	0.58	0.36	0.06
Calculated frequency	0.29	0.50	0.21	0.35	0.48	0.17	0.56	0.38	0.06

Table 4. Hypocotyl colors frequencies and Chi square values of Nada commercial variety

Genotype	Observed	Expected	(O–E)	$(O - E)^2$	χ^2
RR	377	307.7	69.3	4802.5	15.607
Rr	600	615.6	-15.6	243.3	.395
Rr	254	307.7	-53.7	2883.7	9.372
	1231	1231	0	-----	25.373

Figure 6. Different hypocotyl colors in sugar beet commercial variety Nada

Hypocotyl color can be also used in a crosses control of sugar beet plants, to identify hybrids in the progenies obtained from the various mating. If a plant with green hypocotyl color carry double recessive allele (rr) pollinated with pollen from a plant carrying the homozygous dominant allele (RR), pink hypocotyl color identifies F_1's in the progeny Stewart *et al.* (1946). It can be used also in linkage maps as genetic marker to identify the distance between different genes in linkage groups (Abe *et al.*, 1985 & 1993 Boudry, *et al.* 1994). Root color can be used commercially as food dyes. Primary pigments in red beet are the betalains, which include the red-violet betacyanins and the yellow betaxanthins. The recent adoption of betalain pigments from red beet as an alternative to synthetic food dyes has heightened interest in genetic modification of pigment production (Clement, *et al.* 1992 and Adriana, *et al.* 2010).

CONCLUSION

Skewed percentages ratio of green hypocotyl color plants (rr) in the three examined populations "3859 (Sp), Z120 and 3915 (Sp)" could be due to the natural selection or artificial selection. Which mine that green hypocotyl plants having bad characters and eliminated by artificial selection in breeding programs or green hypocotyl seedling have week growth or delay in germination and eliminated in thinning operations by farmers, and/or green hypocotyl plants had low values in the fitness characters which are eliminated in the generative seasons by natural selection?. For that fitness characters would be studied in the progenies of the three examined populations.

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REFERENCES

Abe, J; G.P. Guan and Y. Shimamoto (1993). Linkage maps for nine isozyme and four marker loci in sugar beet *(Beta vulgaris)*. Euphytica. 66: 117-126.

Abe, J; H. Yoshikawa and C. Tsuda (1985). Genetic analysis for annual habit and self fertility in wild relatives of sugar beet using the pigmented gene and some enzyme loci as markers. Sugar Beet Research Association. Japan. 27: 44-49.

Adriana, P.V.; C. C. Dorina; P. Liviu and P. Cornelia (2010). Histo-Anatomical Specific Features of Red Beet and Sugar Beet Vitroplantlets. Analele Universităţii din Oradea, Fascicula:Protecţia Mediului Vol. XV pp. 740 – 749.

Bosemark N. O. (1979) Genetic poverty of the sugar beet in Europe. In: Zeven AC, Van Harten AM (eds), Proc Conf Broadening Genet Base Crops. PuDoc, Wageningen, the Netherlands, pp 29-35.

Boudry, P.; R. Wieber; P. Saumitou-Laprade; K. Pillen; H. Dijk and C. Jung (1994). Identification of RFLP markers closely linked to the bolting gene B and their significance for the study of the annual habit in beets *(Beta vulgaris* L.) Theor. Appl. Genet. 88: 852 - 858.

Butterfass, T.H. (1968). Die Zuordnung des Locus R der Zuckerrube (Hypokotylfarbe) zum Chromosom 11. Theuret. Appl. Genet. 38: 348 – 350.

Campbell, L. G. (1990).Sugar beet germplasm selected from the USDA collection. Journal of North Dakota Farm Research . (47) 6. 32-34.

Clement JS, Mabry TJ, Wyler H, and Dreidlng AS, (1992). Chemical review and evolutionary significance of the betalalns. In: Evolution and systematics of the Caryophyilales (Behnke HD and Mabry TJ, eds). New York: Springer-Verlag; 247-261.

Coons, G. H. (1936) Improvement of the sugar beet. In: USDA Yearbook of Agriculture. US Gov Printing O$ce, Washington, D.C., pp 625-656.

Deming, G. W. (1942). Use of Red Garden Beet in Sugar-Beet Top Crosses. American Society Sugar-Beet Technologists, Proceedings Third General. Meeting. 336 – 341.

Deming, G. W. (1950). Recent Results with Sugar X Red Garden Beet Hybrids. Proc. Amer. Soc. Sugar Beet Technol. 6: 180 – 183.

Fischer (1989). Origin of the 'Weisse Schlesische Rube' (white Silesian beet) and resynthesis of sugar beet Euphytica 41: 75-80.

Keller W, (1936). Inheritance of some major color types In beets. J Agrlc Res 52:27-38.

Laursen, L. (1972). A New Locus for Colour Formation in Beet, *Beta Vulgaris* L. Hereditas 70: 105-112.

Lewellen, R. T. (1992) Use of plant introductions to improve populations and hybrids of sugar beet. In: Shands HL, Wiesner LE (eds) Use of plant introductions in cultivar development, part 2. Crop Sci Soc Am, Madison, WI, pp 117-136.

McGrath, J.M. (2003). Registration of SR96 and SR97 smooth-root sugar beet germplasm with high sucrose. Crop Science. 43:2314-2315.

Owen, F. V. and G. K. Ryser (1942). Some Mendelian characters in Beta vulgaris and linkages observed in the Y-R-B linkage group. J. Agr. Res. 65: 155-171.

Panella, L. (1996) Report on the status of *Beta* germplasm in the United States. Sugar beet Crop Germplasm Committee, USDAARS, Crops Research Laboratory, Fort Collins, Colorado.

Panella, L. (1999). Registration of FC709-2 and FC727 Sugar beet Germplasms Resistant to Rhizoctonia Root Rot and Cercospora Leaf Spot. Crop Science. Vol. 39 No. 1, p. 298-299.

Poehlman, J.M. (1979). Breeding sugar beets. In: breeding field crops. 2nd ed. Avi. Pub. Co. Westport., Conn., U.S.A.

Snedecor, G. W**.** 1957. Statistical methods applied to experiments in agriculture and biology. Fifth Edition. - *The Iowa State College Press, Ames, Iowa*.

Stern, C. (1943). The Hardy-Weinberg Law. Science, New Series, Vol. 97, No. 2510 (Feb. 5, 1943), pp. 137-138.

Stewart, D.; J. O. Gaskill, and G. H. Coons (1946). Heterosis in Sugar Beet Single Crosses. Proceedings American Society of Sugar Beet Technologists. 210 – 222.

الملخص العربي

التحليل الوراثى لصفة لون الجذور ولون الهيبوكتيل فى بنجر السكر

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إجـرى هـذا البحـث فى محطـة البحـوث الزراعيـة بالصـبحية بالإسكندرية فى الفترة مابين 2010- 2013 والغرض الأساسى من هذا البحث هو التحليل الوراثى لصفة لون الجذور فى بنجر السكر وذلك فى الجيل الثانى الناتج من التهجين مابين السلالة الأمريكية (C39) وبنجر المائدة المصرى. كذلك تم دراسة لون الهيبوكوتيل فى ثلاثة عشائر مختلفة من بنجر السكر مستوردة من الولايات المتحدة الأمريكية عن طريق البرنامج المصرى لتربية بنجر السكر وهى: .

"3859 (Sp), Z120 and 3915 (Sp)" تم مقارنة نسب التكرار الجينى لصفة لون الهيبوكوتيل فى الثلاث عشائر تحت الدراسة مع التكرار الجينى لنفس الصفة فى ثلاث أصناف تجارية مستوردة هى (ندا- لولا- ماريبو بولى).

وقد أوضحت النتائج مايلى:-

1 بالنسبة للتحليل الوراثى للجيل الثانى الناتج من التهجين مابين السلالة (C39) وبنجر المائدة المصرى فإنه بعد زراعة البذور الخاصة بالجيل الثانى وعند تفريد البادرات انقسمت البادرات الناتجة إلى ثلاثة مجموعات تبعاً للون الأوراق ولون الهيبوكتيل ولون الجذور

- المجموعة الأولى (لون الجذرأحمر- لون الأوراق أحمر- لون الهيبوكتيل أحمر)
- المجموعة الثانية (لون الجذرأحمر- لون الأوراق أخضر بعروق حمراء -لون الهيبوكتيل أحمر)
- المجموعة الثالثة (لون الجذرأبيض - لون الأوراق أخضر - لون الهيبوكتيل أحمر)

وعند التحليل بإستخدام مربع كاى χ^2 وجد أن نسب الأشكال المظهرية المتحصل عليها تتناسب مع نسب الصفات الوراثية المندلية للسيادة الغير تامة والتى تنعزل فى الجيل الثانى بنسبة 1: 2: 1 والتى يتحكم فيها زوج واحد من الجينات

2 بالنسبة لدراسة التكرار الجينى لصفة لون الهيبوكوتيل فى الثلاث عشائر تحت الدراسة وجد الأتى:

- نسب لون الهيبوكوتيل الأخضر كانت قليلة جداً فى الثلاث عشائر تحت الدراسة حيث كانت 0.016 - 0.02 - 0.01 للعشائر (Sp) 3859 و Z120 و (Sp) 3915 على الترتيب مما يفسر أن هناك نوع من الإنتخاب يحدث ضد هذه الصفة سواء إنتخاب صناعى لوجود هذه الصفة مرتبطة ببعض الصفات الغير جيدة مما يؤدى إلى أستبعادها خلال برنامج التربية أوأن البادرات الناتجة تكون ضعيفة نسبياً فيتم أستبعادها خلال عملية الخف بواسطة المزارعين أوأن هناك إنتخاب طبيعى يحدث ضد هذه الصفة لأرتباط هذه الصفة ببعض الجينات المميتة أو الشبه مميتة أو المقللة للحيوية وهذا مايجب دراسته دراسة وراثية مستفيضة لمعرفة ذلك.