Genetic Analysis of Root and Hypocotyl Color Traits in Sugar Beet
EL-Sayed A.K., 1 Nader R. Abdelsalam 1 and Hayam, E. A. Ibrahim 2

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 F2 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 F2 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 F2 obtained from fodder beet × 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 R Ry YY, 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).

MATERIALS AND METHODS
Present research was carried out to study root and hypocotyl colors in F2 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 sowed 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.
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 F1 were harvested from plants as seed parents in May (2011). Seeds of F1 were planted in June (2011) to introduce F1 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. F1 plants 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. F2 seeds were collected in June (2012) and in November (2012). F2 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 $\chi^2$ by the following steps according to (Snedecor 1957):

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

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 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, F2 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 F₂ 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). F₁ 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 F₂ 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 $\chi^2=0.2589$ 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 $\chi^2$ 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 F₂ segregation seedling numbers of the three different genotypes and $\chi^2$ 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. F₂ segregation seedling numbers and Chi square values

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed</th>
<th>Expected</th>
<th>(O – E)</th>
<th>(O – E)²</th>
<th>χ²²</th>
</tr>
</thead>
<tbody>
<tr>
<td>YYRR</td>
<td>47</td>
<td>49.25</td>
<td>-2.25</td>
<td>5.0625</td>
<td>0.1028</td>
</tr>
<tr>
<td>YyRR</td>
<td>98</td>
<td>98.50</td>
<td>-0.50</td>
<td>0.25</td>
<td>0.0025</td>
</tr>
<tr>
<td>yyRR</td>
<td>52</td>
<td>49.25</td>
<td>2.75</td>
<td>7.5625</td>
<td>0.1536</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Population</th>
<th>3859 (Sp)</th>
<th>Z120</th>
<th>3915 (Sp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypocotyl Color</td>
<td>Red</td>
<td>Pink</td>
<td>Green</td>
</tr>
<tr>
<td>Genotype</td>
<td>RR</td>
<td>Rr</td>
<td>rr</td>
</tr>
<tr>
<td>Number in sample</td>
<td>527</td>
<td>161</td>
<td>11</td>
</tr>
<tr>
<td>Observed frequency</td>
<td>0.76</td>
<td>0.23</td>
<td>0.016</td>
</tr>
<tr>
<td>Calculated frequency</td>
<td>0.76</td>
<td>0.23</td>
<td>0.016</td>
</tr>
</tbody>
</table>

The probable distribution of genotypes in this population can now be computed as follows: p² (RR) : 2pq (Rr) : q² (rr) = (0.87)² : 2 (0.87)(0.13) : (0.13)² = 0.76 : 0.23 : 0.016. 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 is in equilibrium state (Table 2). Data in Table 2 indicated that the three examined populations at 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 two 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 χ² statistic presented in Table (4). The data showed that χ² = 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 χ² (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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed</th>
<th>Expected</th>
<th>(O–E)</th>
<th>(O – E)^2</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR</td>
<td>377</td>
<td>307.7</td>
<td>69.3</td>
<td>4802.5</td>
<td>15.607</td>
</tr>
<tr>
<td>Rr</td>
<td>600</td>
<td>615.6</td>
<td>-15.6</td>
<td>243.3</td>
<td>.395</td>
</tr>
<tr>
<td>Rr</td>
<td>254</td>
<td>307.7</td>
<td>-53.7</td>
<td>2883.7</td>
<td>9.372</td>
</tr>
<tr>
<td></td>
<td>1231</td>
<td>1231</td>
<td>0</td>
<td>-----</td>
<td>25.373</td>
</tr>
</tbody>
</table>

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 F1’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.
ACKNOWLEDGEMENTS

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REFERENCES


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الملخص العربي

التحليل الوراثي لصفة لون الجذور ولون الهايوبكوتيل في بنجر السكر
أحمد السيد محمد خالد، نادر رجب عبد السلام وهيام عيد عبد القادر إبراهيم

- المجموعة الثالثة (لون الجذريين - لون الأوراق أخضر - لون الهايوبكوتيل أخضر)
وعند التحليل باستخدام مربع كاي X² وجد أن نسب الأشكال المظهرية المتحصل عليها تناسب مع نسب الصفات الوراثية المندلية للسيدة العمبرة تامة والتي تعزى في الجيل الثاني بنسبة 1:2:1 والتي يتحكم فيها زوج واحد من الجينات

2 عشائر تحت الدراسة وجد الأنى:
- نسب لون الهايوبكوتيل الأخضر كانت قليلة جداً في العشائر (Sp) 3859 و Z120 و 3915 (Sp) على الترتيب مما يفسر أن هناك نوع من الإختيار يحدث ضد هذه الصفة سواء
- إذ أن تقلب العشائر لوجود هذه الصفة مرتبطة ببعض الصفات الغير جيدة مما يؤدي إلى استبعادها خلال برنامج الوراثية أولو البدادات الناتجة تكون ضعيفة نسبياً فين يتم استبعادها خلال عملية الخف
وبواسطة المزروعين كان هناك اختيارات طبيعية يحدث ضد هذه الصفة لأرتباط هذه الصفة بعض الجينات الممكنة أو الشبه متينة أو المقللة للحيوية وهذا ما يسبب دراسة وراثية مستفيدة لمعرفة ذلك.

إجري هذا البحث في محطة البحوث الزراعية بالصحبية بالإسكندرية في الفترة من 2010 - 2013 والغرض الأساسي من هذا البحث هو التحليل الوراثي لصفة لون الجذور في بنجر السكر وذلك في الجيل الثاني الناتج من التهجين مابين السلالة الأمريكية (C39) وبنجر المالة المصري. كذلك تم دراسة لون الهايوبكوتيل في ثلاثة عشائر مختلفة من بنجر السكر مستوردة من الولايات المتحدة الأمريكية عن طريق البرنامج المصري لتربيه بنجر السكر وهي:

(ندا- ماريو بولى).

وقد أوضحت النتائج مايلي:

1- بالنسبة للتحليل الوراثي للجبل الخاصل الناتج من التهجين مابين السلالة (C39) وبنجر المالة المصري فإنه بعد زرع البذور الخاصة بالجبل الشام وعند ترفيه البذور اقتسمت البذور الناتجة إلى ثلاثة مجموعات تبعاً للون الأوراق ولون الهايوبكوتيل ولون الجذور

- المجموعة الأولى (لون الجذريين - لون الأوراق أخضر - لون الهايوبكوتيل أخضر)
- المجموعة الثانية (لون الأوراق أحمر - لون الجذريين أخضر - لون الهايوبكوتيل أحمر)
- المجموعة الثالثة (لون الأوراق أحمر - لون الجذريين أحمر - لون الهايوبكوتيل أحمر)