Efficiency of the gamma irradiation in the induction of in vitro somatic mutations

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Abstract

This study has been performed to induce somatic mutations in sugar beet (Beta vulgaris L.). Meristem cultures of sugar beet (line E-145) were established and irradiated with 0, 10, 25, 50, 75 and 100 Gy of gamma radiation. In irradiated cultures, average fresh and dry weights of the regenerated plants were determined. From the results obtained, GR_{50} value was calculated. For the E-145 line, 20 Gy was defined as the optimum dosage. At first, the haploid sugar beet plants (line E-145) that were obtained by culturing unfertilized ovules were irradiated with this dosage (20 Gy). Irradiated haploid plants were vegetatively reproduced and M_{1}V_{1}-M_{1}V_{3} generations were obtained. Mutant plants in these generations were then determined according to their rooting capabilities.

Key words: Somatic mutation, sugar beet, meristem culture, gamma radiation

In vitro somatik mutasyon oluşturulmasında gama radyasyonunun etkisi

Özet

Bu çalışmaya şeker pancarı (Beta vulgaris L.) bitkisinde somatik mutasyonları teşvik etmek amacı ile başlandı. E-145 şeker pancarı hattına ait meristem kültürleri kuruldu ve bu kültürler 0, 10, 25, 50, 75 ve 100 Gy lik gama radyasyonu ile işlandı. İşlenmiş meristem kültürlerinden rejenere olan bitkilerin ortalama taze ve kuru ağırlıkları tespit edildi. Bu sonuçlara göre GR_{50} dozu saptandı. E-145 hattı için optimum doz olarak 20 Gy belirlendi. E-145 şeker pancarı hattına ait bitkilerin döllenmemiş ovüllerinden in vitro koşullarda elde edilen haploid şeker pancarları bu dozla (20 Gy) işlandı. İşlenmiş haploid bitkiler vejetatif olarak çoğaltıldı ve M.V_{1}-M.V_{3} generasyonları elde edildi. Bu generasyonlarda köklenme yeteneklerine göre mutant bitkiler belirlendi.

Anahtar sözcükler: Somatik mutasyon, şeker pancarı, meristem kültürü, gama radyasyonu

Introduction

Utilization of in vitro plant tissue culture techniques increased the benefiting of agriculture and agricultural industries from biotechnological applications. Therefore, the rapid development of biotechnology in recent years accelerated the studies on development of modernized improvement methods and increased the genetic variability (Ahloowalia,1998). With traditional methods, plants with desired characteristics could be produced in periods as long as 10-20 years, utilization of the new technologies in plant improvement programs significantly shorten the time is required. Among the factors that play a role in increasing the plant variability, hybridization, recombination, spontaneous or induced mutations are used most frequently. The rate of spontaneous mutations is too low to be considered for practical purposes. Therefore, physical and chemical mutagens might be used together with
in vivo or in vitro techniques to increase the mutation frequency (Ahloowalia, 1998; Donini and Sonnino, 1998).

In vegetative growing plants like sugar beet, a spontaneous or induced mutation in axillary meristem cells or in somatic cells located in cell layers of shoot meristems is reflected in somatic clones. Growing is slower in in vitro tissue cultures than in vivo, which allows mutant sectors to be displayed by competing with wild types (Constantin, 1983). In vitro clonal production is a preferred application to defeat the problems in determination and separation of the induced somatic mutants. Isolation of somatic mutations in M1, M1 V1, M1 V2, M1 V3 generations is easily accomplished by this technique (Donini, 1982; Donini and Sonnino, 1998).

This investigation has been started to induce formation of mutants by applying physical mutagens in haploid individuals of a sugar beet that has significance in plant improvement studies. Besides, in this study, the sensitivity of this plant to irradiation was investigated by determining the optimum dosage of gamma irradiation to induce somatic mutation in sugar beets in vitro.

Materials and methods

Meristem cultures of sugar beet E-145 line were set up to determine the optimum irradiation dosage for the formation of somatic mutations. Seeds were from E-145 line, and kindly provided by improvement division in the Sugar Institute of Türkiye Sugar Factories.

Meristem cultures

With the help of a needle, hard protective tissue that surrounds the outer surface of the sugar beet line E-145 seeds was removed, leaving the seed only. Seed surface was sterilized. Then the seeds were left in petri dishes that contain 0.8% sterilized agar solution and kept at 26°C in incubation chambers that have a 16 hrs day/8 hrs night lightning period to germinate. The primary leaves that were formed after six days of germination were cut aseptically on the level of epicotil nodes. Tips of the primary leaves were also cut away and the explants were inoculated into regeneration medium (MS, 1mg/l MS vit, 1mg/l BAP and 30g/l sucrose) (Detrez et al.,1988). The leaf explants were then irradiated with 0, 10, 25, 50, 75 and 100 Gy of gamma rays from a Cs-137 source (Donini and Sonnino, 1998). On the 28. day, fresh and dry weights of the control and irradiated plants regenerated in meristem cultures were measured and GR_{50} dosages were determined (Welander, 1976; Özbek and Atak, 1984; Detrez et al., 1989).

Induction of somatic mutations in haploid cultures

Haploid sugar beet plants had been obtained from the unfertilized ovules by in vitro ginogenesis (Alikamanoğlu, 2000). Among these, R1, R2, R3, and R5 plants were irradiated with 20 Gy of gamma rays which was defined as the optimum dosage. Growing medium of irradiated plants was supplemented with MS 1/2 and 30g/l sucrose to induce rooting (Zhongxian et al., 1993). Rooting capabilities of M1 V1 and M1 V3 generations were determined.

Results and Discussion

In this study, seeds of sugar beet line E-145 were used and meristem cultures were formed to determine the optimum gamma irradiation dosage that is required to induce in vitro mutations in sugar beet plants. Data on regeneration capabilities in control and irradiated explants were documented in Table 1. The response against the different irradiation dosages varies in different genetic varieties of the plants (Conger et al., 1977) hence, determination of the GR_{50} values that are obtained by mutagen applications gains great

<table>
<thead>
<tr>
<th>Dosage (Gy)</th>
<th>Number of explants</th>
<th>Regenerated plants</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>7 Days</td>
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<tr>
<td></td>
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<td>Number</td>
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<tr>
<td>0</td>
<td>30</td>
<td>30</td>
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<tr>
<td>10</td>
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<tr>
<td>25</td>
<td>30</td>
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<tr>
<td>50</td>
<td>30</td>
<td>21</td>
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<tr>
<td>75</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>14</td>
</tr>
</tbody>
</table>
importance. Therefore in our study, sensitivity of the seeds of sugar beet that exhibits ovule regeneration in haploid culture conditions against the irradiation was determined. In the control and irradiated meristem cultures, regeneration was observed in all dosages. However, although there is no significant difference observed between the control group and plants that were irradiated with low dosage (10 Gy), plant regeneration gradually decreased with increasing dosages. In the control group, all explants cultured in medium were regenerated at the end of 7 days. Plant regeneration in meristem cultures formed from irradiated explants was retarded with increasing dosages. Number of regenerated plants irradiated with lower dosages approached the number in control plants, and was almost equalized in some cases. It is known that cells that were affected by irradiation might self-repair and restore their cellular functions (Özalpan, 2001). Atak and Alikamanoğlu (1994) reported that in meristem cultures from irradiated explants of 3 different type of soybean plants, gamma irradiation affected regeneration capabilities. They stated that gamma irradiation of 25 Gy clearly decreased the regeneration in all 3 types of soybean plants, while 50 Gy completely inhibited regeneration.

In the meristem cultures from irradiated explants of sugar beets, leaf counts were also determined to demonstrate the effect of gamma irradiation on plant development (Figure 1). In meristem cultures of control plants, leaf formation proceeded in a regular fashion, and leaf counts in some regenerated plants exceed 12. In the plants regenerated after irradiation with 10 and 25 Gy, there was a slight decrease in leaf count. However plants irradiated with dosages equal to or above 50 Gy, leaf count remained at 4 in the regenerated plants. According to our results, this dosage caused considerable physiological damage, and were found to be excessive for a mutation study in this line of sugar beets. The detrimental effect of gamma irradiation on M1 generation of plants appears to be chromosomal or non-chromosomal and the most important effect is the retardation in growth and finally, death (Gaul, 1977). It was also reported earlier that this retardation was a result of irradiation, meristematic and leaf primordia were affected, and morphological corruptions in young leaves also

Figure 1: Leaf count of the regenerated plants in 28-days meristem cultures that were set up from control and irradiated sugar beet E-145 line.
occurred. Guzman et al. (1982), considered the main stem length and leaf count on the stem and shoot formation as an indication of growth and also stated that in irradiated (with 1.0 and 2.5 Gy) cultures formed from banana plant shoot tip explants, irradiation had an effect on leaf formation.

The effect of mutagens on plant growth and development can be determined by studying the cytological and morphological damage (Siddiqui and Javed, 1982). In mutation improvement studies, determination of growth capability upon calculation of GR$_{50}$ values aid in finding the irradiation dosages for other possible mutation studies. For this purpose, we used meristem cultures set up from irradiated explants of sugar beet line E-145 to investigate the effect of gamma irradiation on plant fresh and dry weight (Table 2). In 28-day-old cultures, a gradual decrease in average plant fresh and dry weights was observed in accordance with the increase in irradiation dosages starting with 25 Gy. In meristem cultures, dosages that cause a 50% decrease in plant fresh and dry weight, i.e. the GR$_{50}$ dosages were also assessed. It was calculated that, for the average plant fresh weight, GR$_{50}$ dosage was 52 Gy while for the average plant dry weight was 56 Gy. Özbek and Atak (1984) reported that they applied 0-70 krad irradiation to two different type soybean plants, and determined the effect of the irradiation in respect to the plant length and dry weight, calculating the ED$_{50}$ values for each plant type. In the mutation studies in vegetatively reproducing plants, LD$_{50}$ values were accepted as an upper limit to prevent the unwanted mutations (Sanada and Amano, 1998). Considering the physiological damage caused by the irradiation with GR$_{50}$ dosages obtained for both fresh and dry weight of plants, in our study, 20 Gy was applied to induce somatic mutations in haploids, which was the dosage that decreased the growth 30% in comparison with controls. Although it exhibits variations among different genotypes, 20 Gy is a generally accepted value to induce mutations in in vitro plant tissue culture studies (Ahloowalia, 1998).

Clones that are asexually formed from the mother plant contain genetically identical cells and have the same genotype in either homozygous or heterozygous form. However, via spontaneous mutations or mutation inducing mutagens, genetical variations may occur and a new clone may arise by separating the mutated section of the plant from the mother plant. Genotypes of such clones do not change in the following generations since no sexual reproduction takes place. Plant tissue culture techniques are successfully utilized in in vitro selection of mutants. Significant amount of shoots may be obtained via micro amplification techniques. Thus it is possible to identify the M. V. or M.V. generation plants with desirable characteristics formed with somatic mutations after irradiation (Sanada and Amano, 1998). In the plants that are capable of reproducing with seeds in addition to vegetative reproduction (including sugar beet), new populations that display idiovariability which is very valuable for improvement studies may arise. Root formation capabilities of M.V. generations of the irradiated

### Table 2: Average dry weight and ratio of the regenerated plants after 28 days.

<table>
<thead>
<tr>
<th>Dosage (Gy)</th>
<th>Number of explants</th>
<th>Average plant fresh weight (mg)</th>
<th>Average plant dry weight (mg)</th>
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<tr>
<td></td>
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<td>Number of plants</td>
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<tr>
<td>0</td>
<td>30</td>
<td>592.5 a*</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>572.7 a</td>
<td>96.65</td>
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<tr>
<td>25</td>
<td>30</td>
<td>393.8 b</td>
<td>66.46</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>309.5 c</td>
<td>52.23</td>
</tr>
<tr>
<td>75</td>
<td>30</td>
<td>151.8 d</td>
<td>25.62</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>133.8 e</td>
<td>22.58</td>
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*Means with the same letter are not significantly different by Student-Newman-Keuls test (p<0.01)
cultures were documented in Table 3. At Table 3, root formation occurs in the irradiated plants that were regenerated from unfertilized ovules. Although in irradiated haploid plants labelled R₂, R₅ and R₆, root formation was clearly visible, no root formation occurred in the others. The applied dosage might have a positive or negative effect on root formation (Fujita and Wada, 1982). In irradiated haploid plants, root formation incapabilities might be an indication of a negative mutation of the genes that affect the root formation. Plant growth and development are under the common control of many different genes (polygenic control). Similarly, it is known that the root shape and size of the sugar beet plant are also controlled by the action of various genes (Gökçora, 1973). In our study, root formation disability of M₁V₁ generation of R₁ sugar beet plant haploid mutant was also observed in M₁V₃ generation, which suggested that this negative mutation might be transferred to the subsequent generations. It was also observed that while the R₁ plant did not root in M₁V₁ generation, it rooted in M₁V₃ generation. According to our results, it can be speculated that R₁ plant was more susceptible to damage by irradiation and although it lost its rooting ability due to physiological damage, in the subsequent generations this ability was restored. In their in vitro mutation improvement studies in potato plant, Sommio et al. (1986) observed the root formation and determined the node count 40 days after forming the M.V₁ generation by applying 30 Gy of gamma irradiation to the single node sections. Moreover, they determined 158 mutants among 1094 plants and reported that 36 of those exhibited abnormalities in leaf size and shape, 39 exhibited abnormal leaf color, 24 exhibited abnormal flower color, besides 5 plants were dwarf, 46 plants had unusual tuber color. However, control plants did not exhibit such varieties and maintained their phenotypes in the clone.

In this work, somatic mutations were induced by applying gamma irradiation to haploid sugar beet plants grown in culture medium and it was noted that mutants formed could be selected in a short time period in tissue culture systems.

### Acknowledgements

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