Comparison of the Effect of X-rays (Soft X-rays) on the Production of Mutation in Diploid and Tetraploid Species of *Phalaris*

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Since the artificial production of mutation by irradiation of biological materials was reported by Muller (1928) in X-rayed *Drosophila* and Stadler (1928, a, b) in maize and barley, wide range of mutations varying from almost undetectable changes to drastic easily observable morphological changes, and also from lethal to economically useful, have been produced by different kinds of radiation.

Much of the irradiation work has been done in many self-fertilizing grasses, the chief being barley. The present material, i.e. *Phalaris* species, has been selected following the work of Singh (1961) who observed the chromosomes to be favourable for study from the point of view of size and number. One previous worker, Bremer-Reinders (1962, 1963), has also worked on *Phalaris canariensis* and its colchicine derived tetraploid.

**Materials and methods**

Dormant dry seeds of *Phalaris canariensis* Linn (2n = 12) and *Phalaris minor* Retz (2n = 28) were irradiated at the M.R.C. unit, Hammersmith Hospital, London. A brief description of the method of irradiation has been described by Prasad and Godward (1965).

**General description of mutants observed at *M₂* generation**

*Phalaris canariensis*  All phenotypic *M₂* mutants for the sake of convenience have been divided into two major groups:

A) Chlorophyll mutants  
B) Morphological mutants

**Table 1. Relationship between *M₁* fertility and frequencies of chlorophyll mutation per 100 *M₂* seedlings**

<table>
<thead>
<tr>
<th>% Fertility classes per spike</th>
<th>Number of seedlings per class</th>
<th>Number of chlorophyll mutants per class</th>
<th>Frequency of mutation per 100 <em>M₂</em> seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>122</td>
<td>3</td>
<td>2.40</td>
</tr>
<tr>
<td>11-20</td>
<td>354</td>
<td>16</td>
<td>4.50</td>
</tr>
<tr>
<td>21-30</td>
<td>439</td>
<td>9</td>
<td>2.10</td>
</tr>
<tr>
<td>31-40</td>
<td>570</td>
<td>13</td>
<td>2.30</td>
</tr>
<tr>
<td>41-50</td>
<td>917</td>
<td>2</td>
<td>0.22</td>
</tr>
<tr>
<td>51-60</td>
<td>682</td>
<td>19</td>
<td>2.80</td>
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<tr>
<td>61-70</td>
<td>775</td>
<td>5</td>
<td>0.64</td>
</tr>
<tr>
<td>71-80</td>
<td>356</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>81-90</td>
<td>107</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
A) Chlorophyll mutants

**Frequency of chlorophyll mutants and relationship with fertility.** Table 1 shows the relationship between range of fertility determined per spike and frequency of mutation per 100 M₂ seedling (Gaul 1957).

It seems from Table 1 that the frequency of mutations per 100 M₂ seedlings is higher in the low fertility groups in comparison to the higher fertility groups. No chlorophyll mutations have been recorded in the fertility classes 71–80% and 81–90%. The frequencies in different fertility classes vary from 0.22 to 4.5 and in view of the sporadic distribution of high and low frequencies in all classes where mutants occur, no conclusion regarding this distribution is possible. The mean of all the frequencies of the different classes is 1.66 per 100 M₂ seedlings.

**Mutant spectrum.** The method of Gustafsson (1940) has been used to classify the mutants. The histograms represented in the Fig. 1 show viridis to be most common and maculata to be rare. When viridis is broken up into different types on the basis of colouration, lutescens is commoner than virescens and chlorina which are present in almost equal numbers.

1. **Viridis.** For the purpose of description this mutant type has been divided into three varieties:
   a) **Virescens.** Yellowish-green at seedling stage. The colouration gradually changes from yellowish-green to light green and finally normal. These mutants survived till maturity although the rate of growth in comparison to the control and the irradiated M₂ generation plants was very slow.
   b) **Chlorina.** Yellowish-green like virescens at seedling stage. Its subsequent leaves were also yellowish-green. The rate of growth was very slow even in comparison to virescens. It survived up to three months after germination and died long before the flowering period.
   c) **Lutescense.** This was the most common type observed in the canary grass by the writer. There was a gradual disintegration of the yellowish-green colour present at the seedling stage, and the seedlings died within 20 days of germination.

2. **Albino.** This was the next common type. Two kinds of albino were seen i) seedlings of pinkish-white colour showing the presence of anthocynin. It survived up to the three-leaved stage. All leaves were of pinkish-white colour. The leaf
shape was like that of the control which it resembled except for the absence of a green colour. Death occurred within 23–25 days after germination, ii) white, showing the absence of carotenoids. The mutants had very narrow leaves and died within 15–20 days of germination. They were rare.

3. **Tigrina.** This is also common. The distinguishing character of this mutant is the presence of transverse zones of normal green alternating with yellow-green or whitish-yellow bands. The yellow-green or white-yellow band is narrow and constricted. The presence of constrictions distinguishes this from the maculata type. There was a gradual degeneration of pigments and finally all seedlings died between 25–35 days after germination.

4. **Virido-albino.** This is characterized by the presence of long white base to the leaves, (like albino) with a yellowish-green upper part. Sometimes only the tip of the leaf was seen to be yellowish-green. This type was fairly common. The plants died within 20–25 days of germination.

5. **Maculata.** This was a rare type in canary grass. It resembles tigirina in the presence of transverse zones of normal green alternating with yellow-green or white-yellow. They are devoid of constrictions however.

**P. minor (4n+2).** A total of 19,708 seedlings was tested for chlorophyll mutants in the M₁ generation, but no mutants were observed.

**B) Morphological mutants**

Morphological mutants have been classified into three groups, according to the organ involved, which may be:

1) **Leaf**
2) **Rachis**
3) **Spike**

**P. canariensis:**

1) **Leaf.** Mutants of this group have been observed before flowering stage. The mutants are not found in plants showing chlorophyll mutation.

   a) **Split and appendage present on the margin of the leaf.** This type has been observed most frequently. The leaf sheath is slightly rolled up and elongated. The appendage is present at the end of the sheath and beginning of the leaf blade. The split is in some cases restricted to the middle or almost the middle of the leaf and in some cases is nearer to the appendage.

   b) **Split present on the margin of the leaf.** The split is restricted to only one side of the margin. One, two or many incisions covering almost 2/3 of the margin will be present. Incisions may be deep, extending up to the midrib, or almost superficial. They may be very close, looking like saw’s teeth, or at a little distance.

   c) **Split on the lamina of the leaf.** This type was very rare and has been observed in only one plant. The split is restricted to the wider portion of the lamina. The leaf emerging next to it is rolled up and shows a tiny appendage.

   d) **Appendage on the margin of the leaf.** The general shape and size of the leaf was like mutants which showed an appendage with split. It is characterised by the
absence of split on either margin or lamina of the leaf. This was rare in comparison to mutants having both appendage and split.

e) **Trifid apex of the leaf.** One third of the apical portion of the leaf is rolled up and is split into three parts, a central thin, narrow, long section and two small ones on either side, thus forming a trifid apex. Each of the split portions is also rolled up.

f) **Bifid apex of the leaf.** This leaf is almost rudimentary and has been split into a narrow elongated, and a short wider section. Both sections show a blunt apex. Splitting first starts at the apex and then gradually descends downward, dividing the leaf. This mutant is very rare.

g) **Rudimentary leaf.** The rudimentary rolled and green leaf was enclosed in the sheath of an old normal-looking leaf. It gradually withers away and dies earlier than other leaves on the same plant.

h) **Leafless.** This form observed at seedling stage was characterized by the complete absence of the leaf.

2) **Rachis.** These mutants appeared at the time of flowering and are from the progenies that failed to show any chlorophyll mutants.

a) **Twisted.** Rachis may be short or long. Twisting may be present all along the rachis or only just near the base, forming a hook shaped structure.

b) **Pendulate rachis.** Here the rachis is bent at the point of its origin, and thereby it produces pendulate inflorescence. There may be a slight twisting of the rachis also.

3) **Spike.** White head, tweaky and white-green were observed among the progeny which segregate for chlorophyll mutants at the seedling stage.

a) **White head (cf. Lawrence 1955).** The growth of this mutant was normal until the time of flowering. The plant may be tall or short. The leaf accompanying the appearance of the inflorescence is rolled, the next leaf which encloses it is straight and narrow. The inflorescence is completely sterile, the glumes are white, the whiteness continuing until the plant withers.

b) **Tweaky (cf. Lawrence 1955).** The spike shows missing florets at one or more places on the rachis. In one case a crippled and curled small spike was seen at the base of the rachis of the older spike. The small spike showed impeded emergence due to rolled leaf sheath.

c) **Multiflorous (cf. Lawrence 1955).** There is a supernumerary inflorescence which remains partly enclosed in the elongated sheath. It is developed at the base of the older spike whose rachis has elongated. The older spike may show normal glumes or the glumes may be broader than the normal.

d) **Impeded inflorescence.** There is a curled spike which fails to emerge from the leaf sheath.

e) **White green.** Only a few florets at the apex of the head were normal green, the rest being white.

f) **Spikeless.** The plant showed the absence of spike. In the place of the
spike there is the development of very long, thin, narrow and light green thread-like structure.

*P. minor* No morphological mutants have been observed in spite of 19,708 seedlings tested.

**Discussion**

Bremer-Reinders (1962) has worked on the effect of X-rays and neutrons on diploid canary grass and its colchicine-derived tetraploid and had reported various chlorophyll and morphological mutants.

All chlorophyll mutants reported in the present case are similar to those of Bremer-Reinders (1962). The frequency is, however, 4.5% compared with her 10%. It seems difficult to compare the two results as he has grown seeds in different batches and has not distinguished frequency classes, whereas in the present case, all seeds at M2 were grown together and frequency classes have been given.

So far as morphological mutants are concerned, a great difference is noticed between Bremer-Reinders' (1962) observations and the present ones. She observed splitting of the leaf apex at seedling stage, whereas the writer has seen it only in the mature plant. She has not distinguished the different kinds of splitting, e.g. at the apex, margin, and across the lamina.

The present observation of leafless, tweaky, whitehead, multiflorous and elongated-rachis mutants may be compared with Lawrence's (1955) observation of leafless (X-rays); tweaky (Co60), multiflorous (Betatron), whitehead (Radium-berryllium and Co60) and elongated-rachis (X-rays) and (Co60) in Montcalm barley.

From their nature-deformation, splitting, rolling and constriction of the organs concerned, it seems possible that the phenotypes themselves are produced by irregularities in meristems; splitting seems probably due to elimination of abnormal cells following mitosis. Why these changes should be confined to late appearing organs such as inflorescence, when all other parts of the plant have not shown them, is hard to explain.

The failure of appearance of mutants in *P. minor* (2n=28) might be due to the presence of duplicate sets of genes. The lack of appearance of mutants in autotetraploid barley, Müntzing (1942); autotetraploid flax, (Levan 1944); and tetraploid barley, (Bhaskaran and Swaminathan 1962) at M2 general is similar to the present observation.

**Abstract**

Dry dormant seeds of *Phalaris canariensis* Linn. (2n=12) and *Phalaris minor* Retz. (2n=28) were irradiated with 10 Kr. soft X-rays at the M.R.C. Unit, Hammersmith Hospital, London and its effect on the production of chlorophyll, and morphological mutants at M2 generation have been studied and discussed in both the species.

In *Phalaris canariensis* Linn., the usual chlorophyll mutants, virescens, chlorina,
lutescens, albino, tigrina, virido-albino and maculata were seen at seedling stage. Viridis was found to be most common and maculata to be rare. When viridis was broken up into different types on the basis of colouration, lutescens is commoner than virescens and chlorina which are present in almost equal numbers.

The frequency of mutants varies from 0.22-4.5. This was determined per 100 M$_2$ seedlings (Gaul 1960) and was found to be higher at the low fertility range. No mutants were seen between the 71 and 90% fertility classes.

Morphological mutants of the type, split and appendage on the margin of the leaf, split on the margin and lamina, appendage on the margin, trifid and bifid apex, rudimentary leaf, leafless, twisted and pendulate rachis and whitehead, tweaky, multiflorous, impeded, white-green and spikeless inflorescence have been observed in *P. canariensis*.

No chlorophyll and morphological mutants were seen in *P. minor* inspite of 19,708 seedlings tested.

The appearance of chlorophyll and morphological mutants at M$_2$ generation in the diploid species *P. canariensis* (2n=12) suggest that they are more sensitive to radiation than the tetraploid *P. minor* (2n=28).

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References


