# CHIMERISM IN $M_1$ PLANTS OF SUNFLOWER AND ITS SIGNIFICANCE FOR MUTATION BREEDING

T. HERMELIN [1], S. DASKALOV [1], A. MICKE [2], F. STOENESCU [3], MONICA IUORAŞ [3]

## INTRODUCTION

Mutation breeding requires the handling of large populations as a mutation in a particular gene is a rare event even with efficient mutagen treatment. Therefore it is of interest to handle the mutagen treated plant material in a rather economic way. This refers to planting density in the $M_2$ generation but also the harvest of seeds from $M_1$ plants and the cultivation of the $M_2$ generation for effective mutant screening.

$M_1$ plants derived from seed irradiation are chimeric for any induced mutation. A particular mutation can be present only in a part of the $M_1$ plant, e.g. spike, branch, sector, group of flowers. The chimeric pattern differs strongly with the ontogenetic system of the plant species in question. In order to decide about the most efficient system of collecting seeds from mutagen treated plants for subsequent mutant screening, one should know the chimeric pattern. The present investigation is part of our ongoing work to clarify the $M_1$ chimerism in dicotyledonous species (Hermelin *et al.*, 1983). We hope that the results will help sunflower breeders to make efficient use of induced mutations.

## MATERIALS AND METHODS

The experimental work was carried out at the Research Institute for Cereals and Industrial Crops of Fundulea, Romania, and at the F.A.O./I.A.E.A. Agricultural Biotechnology Laboratory in Seibersdorf, near Vienna, Austria, during the period 1983—1984.

$F_1$ seeds were obtained by crossing the inbred lines CA-83-199 (white seeds) and CG-83-2 974 (black seeds). These seeds were irradiated with 0, 100 or 200 Gy gamma rays at a dose rate of 11 Gy $min^{-1}$ and planted in the fields at Fundulea and Seibersdorf.

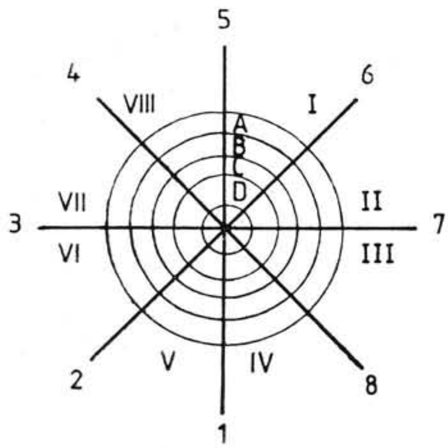


*Fig. 1* — Template for simulated harvesting of mutated seeds in the sunflower capitulum

*1—8* : Angles, for non-random selection ; *I—VIII* : Sectors, for random selection ; *A—D* : Zones. Relative areas 3.24 : 2.52 : 1.8 : 1.10. for random selection

Mature capitula of the $F_1M_1$ plants were investigated for occurrence of chimeric mutated "sectors" consisting of seeds deviating in colour from the intermediate heterozygote of the grey phenotype. The "mutant sectors" were studied as to size and shape described by drawing actual patterns in form of a standard size capitulum (Fig. 2). The relative areas covered by mutant sectors were then calculated (Table 2).

To simulate different harvesting systems in a sunflower mutation breeding project, two series of experiments were carried out : (a) simulated non-random and (b) simulated random sampling of seeds from the $F_1M_1$ capitula. The idea behind was to find out how many seeds would have to be harvested to pick all mutations and whether there would be an advantage in one or the other harvesting system in terms of economic efficiency.

[1] F.A.O./I.A.E.A. Agricultural Biotechnology Laboratory, Seibersdorf, A-1 400 Vienna, Austria.

[2] Joint F.A.O./I.A.E.A. Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development, A-1 400 Vienna, Austria.

[3] Research Institute for Cereals and Industrial Crops, 8 264 Fundulea, Romania.

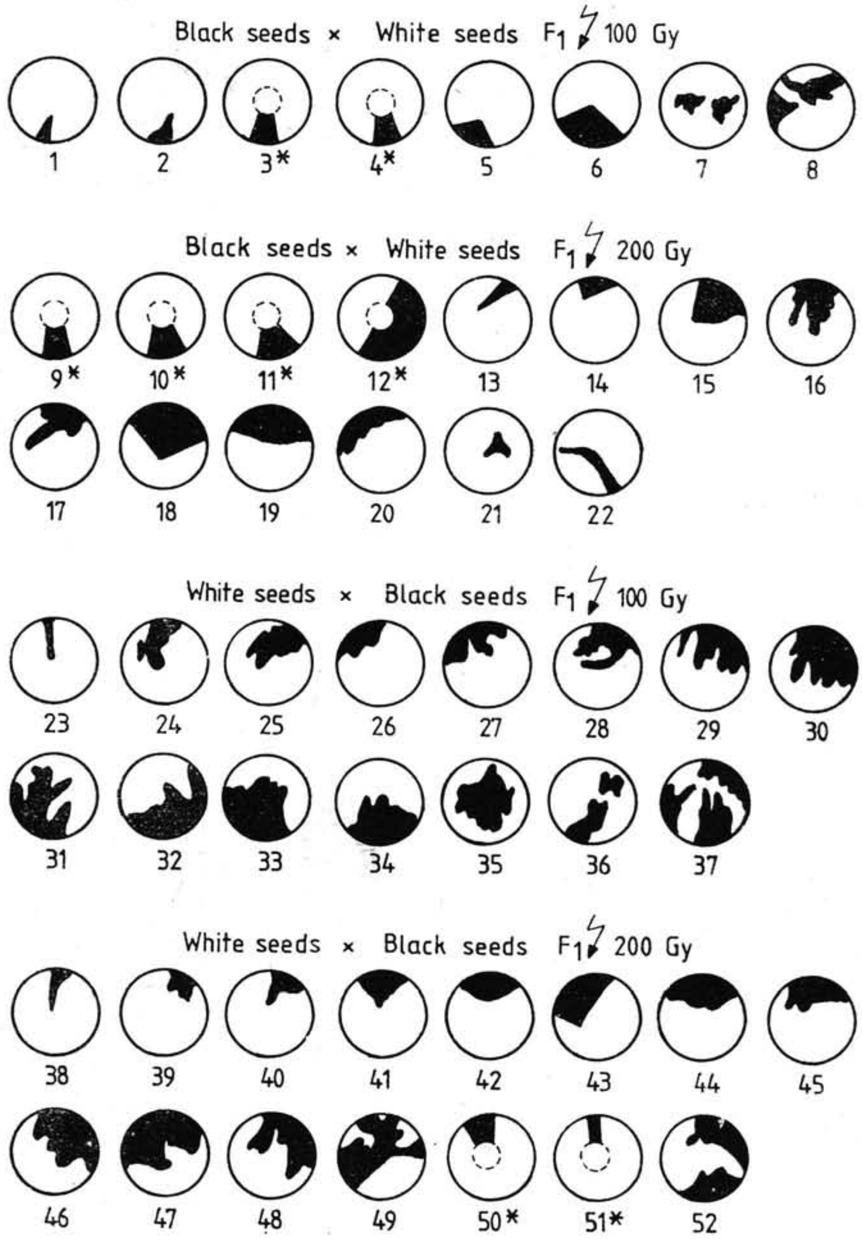


*Fig. 2* — Chimeric patterns of $F_1M_1$ sunflower capitula. Black areas = black seeds, white areas = grey seeds

A template was used in the experiments and applied to the capitula showing visible chimerism. It consisted of eight sectors (I—VIII) and four circular zones (A—D) as shown in Figure 1. The central zone, representing about 4% of the total capitulum area, was not considered worth harvesting because of pour seed development especially in the material grown at Seibersdorf. The seeds to be collected were identified by using the template on which the exact positions of selected seeds were indicated. Grey and "mutant" seeds were recorded individually.

Each capitulum was subjected to eight simulated samplings with 1, 2, 3, 4, 6, 8, 12, 16 and 24 seeds. Two different selection systems were applied :

a) *Non-random sampling*. The seeds to be collected were all situated in the outer zone (A) and at equal distances, e.g. 120° apart when 3 seeds and 15° apart when 24 seeds were collected. The template was turning eight times and oriented at the angles 1 to 8 for each number of seeds to be collected to obtain sample replicates.

b) *Random sampling*. Eight different templates were constructed for each number of seeds to be collected. The sampling of seeds was arranged in such a way that probability was equal to collect seeds in any of the four zones and in any of the eight sectors shown in Figure 1.

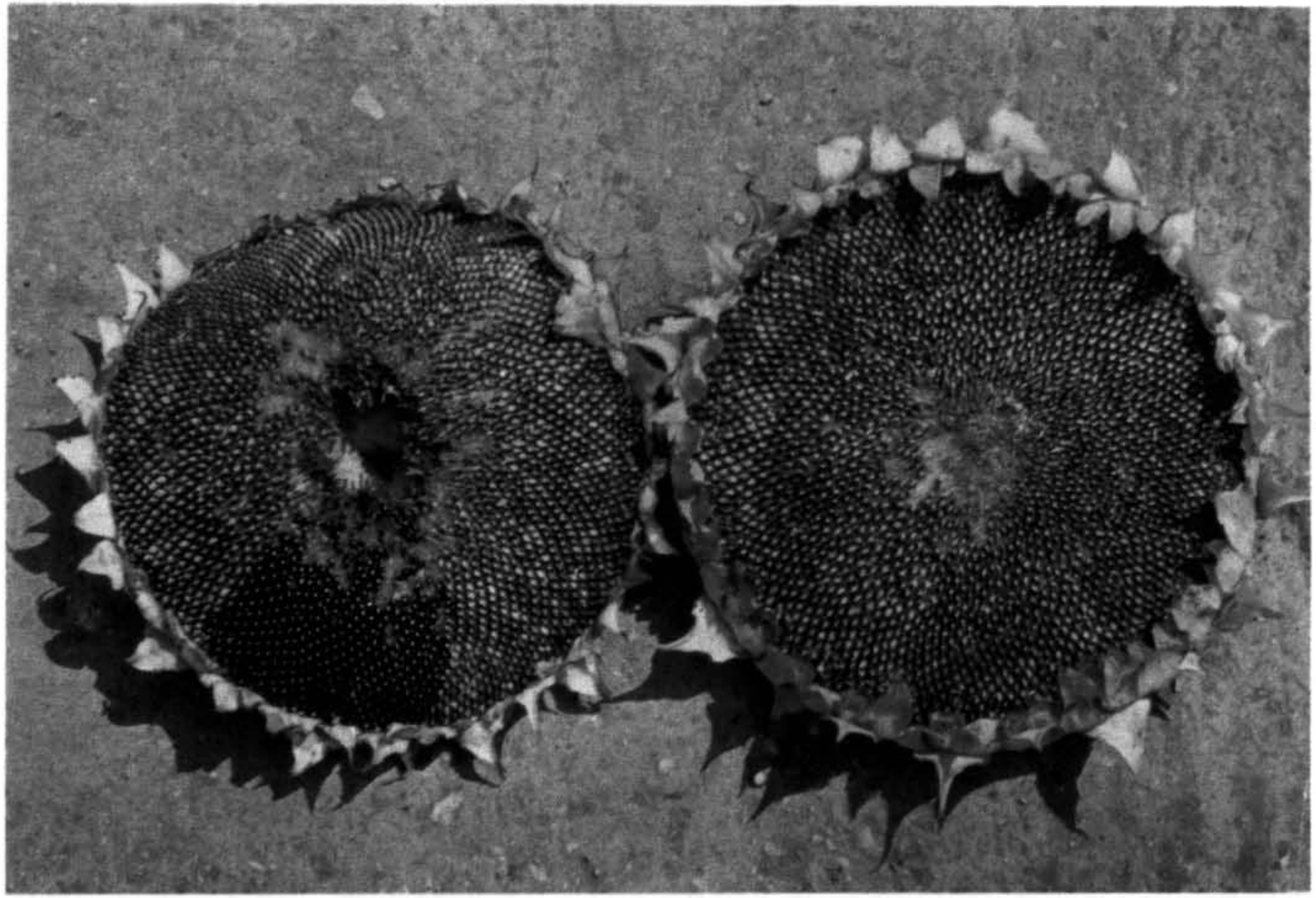

*Fig. 3* — **Capitula of sunflower $F_1M_1$ plants with a "mutant sector" to the right**

*Table 1*

**The frequency of chimeric $F_1M_1$ sunflower plants after gamma irradiation. $F_1$ seeds derived from crosses between the lines CA-83-199 (W = white seeds) and CG-83-2 974 (B = black seeds)**

| Kind of $F_1$ seeds treated | Dose Gy | Experimental site: Seibersdorf — $M_1$ plants screened | Chimeric plants found | % chimeras | Fundulea — $M_1$ plants screened | Chimeric plants found | % chimeras |
|---|---|---|---|---|---|---|---|
| | 0 | 218 | 0 | 0 | a) | 0 | 0 |
| B x W | 100 | 487 | 2 | 0.4 | 1.159 | 6 | 0.5 |
| | 200 | 550 | 4 | 0.7 | 556 | 10 | 1.8 |
| | 0 | 237 | 0 | 0 | a) | 0 | 0 |
| W x B | 100 | 619 | 0 | 0 | 456 | 15 | 3.3 |
| | 200 | 446 | 2 | 0.4 | 454 | 13 | 2.9 |

a Control plants screened but the number not recorded

*Table 2*

**Distribution of relative mutated area in 52 chimeric $F_1M_1$ sunflower capitula**

| Relative mutated sector area, % | Number of capitula: 100 Gy | 200 Gy |
|---|---|---|
| —10 | 3 | 7 |
| 11—20 | 6 | 6 |
| 21—30 | 5 | 7 |
| 31—40 | 5 | 3 |
| 41—50 | — | 4 |
| 51— | 4 | 2 |
| Total No. | 23 | 29 |
| Range | 4—56 | 4—54 |
| Median | 24 | 22 |

## RESULTS AND DISCUSSION

A number of 4,727 $F_1M_1$ plants from seeds treated with 100 or 200 Gy were examined for chimerism in addition to a large number of control plants. Fifty-two capitula showed a portion of seeds deviating in colour from the normal grey phenotype of the heterozygotic material used, so the incidence of chimeric plants was 1.1%. The "seeds" of the "mutant sectors" were in all cases black (Table 1 and Fig. 3). No chimeric plants were observed among the non-irradiated control. The frequency of chimeric plants was much higher than the mutation rate expected for a recessive change in a single gene, but in agreement with the probability to induce a break in a chromosome, being about $10^{-2}$ (B r o c k, 1979). The observed black seeded sectors in the $F_1M_1$ plants were thus probably due to deletions. Considering the fact that seed setting was not affected by the gamma-irradiation, these deletions were evidently rather small.

Most of the 52 chimeric capitula (Fig. 2) were sectorial, but some (e.g. 7, 21 and 35) were obviously different. The interpretation of the complex configurations of nos. 8, 37, 49 and 52 is rather difficult. One may assume a simultaneous mutation in more than one meristem cell, but the type could also be due to a rearrangement within the meristem and therefore the four cases are considered each as a single mutational event. An irregular border line between the black and the grey sectors, as in nos. 16, 31 and 49 might be the result of a selective advantage of either the non-mutated or the mutated heterozygous tissue which will yield larger or smaller "mutated sectors" than expected from the number of meristematic cells normally destined to form the inflorescence. In any case, these capitula confirm that there is not a strict predestination of meristem cells and the sector they are responsible for. As seen in Table 2 there is no significant difference as to the relative size of black seeded (mutated) sectors between the two doses. An increased sector size after higher mutagen doses, as described by W e i l i n g (1960) for peas, could not be observed in the material presented here. Assuming that all the 52 cases are due to a single mutational event, the "Genetically Effective Cell Number" as defined by L i and R é d e i (1969) is between 2 and 5.

Results of the two systems are shown in Table 3. The recovery of black seeds is proportional to the sample size in the range of 1—24. A sample size of 24 yielded about 20 times more black seeds than a single seed sample. The original orientation of the template in the non-random series is decisive for the number of black seeds when only 1 or 2 seeds were selected as most of the capitula with black sectors were oriented towards the top or bottom positions (Fig. 2). The heterogeneity incurred for the random sample sizes of 1 to 4 seeds is likewise due to the non-random orientation of the black sectors. Capitula 7, 21 and 35 yielded no black seeds in the non-random experiment. Out of 24 seeds harvested from capitula nos. 21, 23, 38 and 51 (all with small sectors), only up to 2 seeds were black in the random selection series.

The number of successful chimeric recoveries and distribution of black seeds recovered per capitulum in the two series of experiments are summarized in Table 3.

*Table 3*

**Number of chimeric $M_1$ sunflower plants with black seeds, and the number of black seeds harvested per capitulum following simulation of different sampling systems (52 capitula were subjected to this simulated seed sampling)**

| No. of seeds harvested per capitulum | Number of plants identified as having black seeds (a) | | | No. of seeds harvested (b) | No. of black seeds harvested | | | Expected $M_2$ mutants per capitulum (c) |
|---|---|---|---|---|---|---|---|---|
| | Mean | Range | $X^2$ (1 d · f) | | Total | Per capitulum | Range | |
| **Non-random sampling** | | | | | | | | |
| 1 | 12.4 | 4—28 | 48.5 *** | 416 | 99 | 0.24 | 0—1 | 0.06 |
| 2 | 22.9 | 12—37 | 33.1 *** | 832 | 191 | 0.46 | 0—2 | 0.12 |
| 3 | 31.8 | 26—35 | 1.62 | 1,248 | 290 | 0.70 | 0—3 | 0.18 |
| 4 | 37.5 | 34—41 | 2.61 | 1,664 | 401 | 0.96 | 0—3 | 0.24 |
| 6 | 41.8 | 34—44 | 0.61 | 2,496 | 582 | 1.40 | 0—4 | 0.35 |
| 8 | 45 | 45 | 0 | 3,328 | 784 | 1.88 | 0—5 | 0.47 |
| 12 | 46 | 45—47 | 0.17 | 4,992 | 1,094 | 2.63 | 0—7 | 0.66 |
| 16 | 48 | 48 | 0 | 6,656 | 1,543 | 3.71 | 0—10 | 0.92 |
| 24 | 49 | 49 | 0 | 9,984 | 2,317 | 5.57 | 0—14 | 1.39 |
| **Random sampling** | | | | | | | | |
| 1 | 15.2 | 3—29 | 45.1 *** | 416 | 122 | 0.29 | 0—1 | 0.07 |
| 2 | 19.1 | 9—32 | 23.8 *** | 832 | 177 | 0.42 | 0—2 | 0.10 |
| 3 | 27.4 | 16—35 | 12.0 | 1,248 | 303 | 0.73 | 0—3 | 0.18 |
| 4 | 29.6 | 15—40 | 19.4 ** | 1,664 | 404 | 0.97 | 0—4 | 0.24 |
| 6 | 39.6 | 34—45 | 2.17 | 2,496 | 621 | 1.49 | 0—5 | 0.37 |
| 8 | 41.8 | 38—47 | 2.24 | 3,328 | 800 | 1.92 | 0—7 | 0.48 |
| 12 | 46.0 | 36—50 | 3.70 | 4,992 | 1,252 | 3.01 | 0—9 | 0.75 |
| 16 | 47.4 | 42—51 | 1.52 | 6,656 | 1,516 | 3.64 | 0—12 | 0.91 |
| 24 | 50.2 | 48—52 | 0.23 | 9,984 | 2,605 | 6.26 | 0—28 | 1.56 |

a) Out of 52 capitula with black seeds, mean of 8 angles or templates
b) 52 capitula, 8 angles (non-random), or 8 templates (random selection)
c) Assuming monogenic segregation.

*Table 4*

**Mean number of cases (out of 52) in which black seeds could be recovered after non-random (n) or random (r) sampling**

| Seeds per capitulum | | No. of black seeds selected | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12—18 |
| 1 | n | 39.6 | 12.4 | — | — | — | — | — | — | — | — | — | — | — |
| | r | 36.8 | 15.2 | — | — | — | — | — | — | — | — | — | — | — |
| 2 | n | 29.1 | 21.9 | 2.0 | — | — | — | — | — | — | — | — | — | — |
| | r | 32.9 | 16.1 | 3.0 | — | — | — | — | — | — | — | — | — | — |
| 3 | n | 20.2 | 27.5 | 4.0 | 0.2 | — | — | — | — | — | — | — | — | — |
| | r | 24.6 | 18.1 | 8.0 | 1.2 | — | — | — | — | — | — | — | — | — |
| 4 | n | 14.5 | 26.5 | 9.6 | 1.5 | 0 | — | — | — | — | — | — | — | — |
| | r | 22.4 | 13.5 | 11.5 | 4.5 | 0.1 | — | — | — | — | — | — | — | — |
| 6 | n | 10.2 | 18.9 | 16.0 | 5.6 | 0.1 | 0 | 0 | — | — | — | — | — | — |
| | r | 12.4 | 16.6 | 10.9 | 9.8 | 1.9 | 0.5 | 0 | — | — | — | — | — | — |
| 8 | n | 7.0 | 15.0 | 10.0 | 2.0 | 3.0 | 0 | 0 | 0 | — | — | — | — | — |
| | r | 10.2 | 13.8 | 12.1 | 7.5 | 4.4 | 2.4 | 1.2 | 0.4 | 0 | — | — | — | — |
| 12 | n | 6.0 | 11.9 | 9.1 | 7.1 | 10.1 | 4.2 | 1.0 | 2.5 | 0 | 0 | 0 | 0 | 0 |
| | r | 6.0 | 9.8 | 9.8 | 7.0 | 5.9 | 4.8 | 5.0 | 2.4 | 1.1 | 0.4 | 0 | 0 | 0 |
| 16 | n | 4.0 | 7.0 | 10.0 | 7.0 | 4.0 | 8.0 | 5.5 | 1.5 | 2.0 | 0.6 | 2.4 | 0 | 0 |
| | r | 4.6 | 7.4 | 8.5 | 8.0 | 6.8 | 4.9 | 3.5 | 3.4 | 2.5 | 1.0 | 0.8 | 0.4 | 0.4 |
| 24 | n | 3.0 | 1.5 | 6.5 | 10.0 | 1.4 | 6.6 | 2.0 | 6.0 | 3.5 | 5.5 | 1.0 | 2.0 | 3.0 |
| | r | 1.8 | 4.1 | 6.0 | 6.0 | 4.1 | 3.4 | 3.6 | 2.8 | 3.1 | 3.6 | 3.6 | 3.6 | 6.2 |

## CONCLUSIONS

Hybrid seeds heterozygous for a marker gene were irradiated with the objective to form of a triangular sector of the $M_1$ capitu-mutated tissue appeared in several cases in from of a triangular sector of the $M_1$ capitulum. When seeds are treated, the $M_2$ seeds harvested on the $M_1$ plants are usually derived from nonmutated and mutated $M_1$ embryo cells. Few seeds from the mutant sector have to be harvested to assure a recovery of a mutant. The last column of Table 3 gives the numer of such $M_2$ mutants that can be expected per chimeric capitulum. About 20 seeds have to be collected to assure harvesting from a mutant sector and to recover at least one mutant plant in the $M_2$ generation.

The data indicate that the two approaches non-random and random sampling yield similar numbers of mutant seeds. From a breeding point of view, however, the random selection procedure has some advantages as it needs less labour : any desired number of seeds can be sampled easily from the threshed $M_1$ capitulum.

## REFERENCES


Brock R. D., 1979, *Mutation plant breeding for seed protein improvement,* Seed Protein Improvement in Cereals and Grain Legumes, IAEA, STI/PUB/496, Vol. I, 43—55.

Hermelin T., Brunner H., Daskalov S., Nakai H., 1983, *Chimerism in $M_1$ plants of Vicia faba, Capsicum annuum and Linum usitatissimum. Chimerism in Irradiated Dicotyledonous Plants,* IAEA, TECDOC — 289, 35—42.

Li S. L., Rédei G. P., 1969, *Estimation of mutation rate in autogamous diploids,* Radiat, Bot. *9,* 125—131.

Weiling F., 1960, *Über die Häufigkeit der nach Röntgenbestrahlung von Samen überlebended Corpusinitialen,* Biometr. Zeitschr. 2, 145—163.


### LE CHIMÉRISME CHEZ LES PLANTES $M_1$ DE TOURNESOL ET SON IMPORTANCE POUR L'AMÉLIORATION PAR LA MÉTHODE DES MUTATIONS

*Résumé*


Le modèle chimérique des plantes $M_1$ diffère en fonction du système ontogénique de l'espèce respective. La connaissance du modèle permet le prélèvement efficient des graines des plantes $M_1$.

Dans l'étude présente, on a utilisé un gène marqueur de la graine. Les graines $F_1$ obtenues par le croisement des lignées autofécondées CA—83—199 (graines blanches) et C 6—83—2 974 (graines noires) ont été irradiées avec 0, 100 et 200 Gy rayons gamma.

Les capitules mûres des plantes $F_1M_1$ ont été examinés concernant l'apparition des "secteurs" chimériques. Deux séries d'expériences ont été effectuées pour simuler différents systèmes de prélèvement des échantillons de graines des capitules $F_1M_1$ : simulé sans-hasard et simulé au hasard. Pour simuler le prélèvement des échantillons on a utilisé un modèle divisé en 8 secteurs et 4 zones circulaires.

On a examiné un nombre de 4 727 plantes $F_1M_1$, parmi lesquelles 52 capitules ont présentés le chimérisme. Plusieurs chimères ont eu une forme de secteur mais on a observé aussi quelques configurations complexes. En ce qui concerne le prélèvement au hasard ou sans-hasard des échantillons, les données ont indiquées que les deux procédés permettent l'obtention d'un nombre similaire de graines mutantes. Du point de vue de l'amélioration, le procédé de prélèvement au hasard des échantillons présent toutefois l'avantage de nécessiter moins de travail manuel.


### QUIMERISMO EN LAS PLANTAS $M_1$ DE GIRASOL Y SU IMPORTANCIA EN LA MEJORA POR EL MÉTODO DE LA MUTACIÓN

*Resúmen*


El modelo quimérico de las plantas $M_1$ difiere en función del sistema ontogenético de la respectiva especie. El conocimiento del modelo permite la colección eficiente de las semillas de las plantas $M_1$.

En el presente estudio se ha empleado un gene marcador de la semilla. Las semillas $F_1$ obtenidas por el cruce de las línes cosanguineas CA—83—199 (semillas blancas) y C 6—83—2 974 (semillas negras) se iradiaron con 0, 100 0 200 Gy rayos Gamma.

Los capítulos maduros de las plantas $F_1M_1$ fueron examinados can respecto a la aparición de los "sectores" quiméricos. Se han efectuado dos series de experiencias para esimular diferentes sistemas de tomar las pruebas : de los capítulos $F_1M_1$ esimulado casualmente y esimulado nocasualmente. Un modelo dividido en ocho sectores y cuatro zonas circulares se ha empleado para esimular la toma de los pruebas.

Se han examinado 4 727 plantas $F_1M_1$, de las cuales 52 capítulos mostraron quimerismo. Varias quimeras fueron de tipo sectorial, pero a la vez se notaron algunas configuraciones complejas. En cuanto a la toma casual y no casual de las pruebas, los datos indicaron que los dos procedimientos permiten obtenere un número similar de semillas maduras. Desde el punto de vista de la mejora el procedimiento de tomar casualmente las pruebas presenta sin embargo la ventaja de necesitar menos trabajo manual.