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MAINTENANCE OF THE BIOLOGICAL AND GENETIC VALUE OF INBRED LINES IN SUNFLOWER HYBRID SEED PRODUCTION

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The parental inbred lines of the commercial hybrids are selected during a long breeding process both for the homozygous state of their morphological characters and for the general and specific combining ability.

The main task of inbred seed production is to maintain the biological value of these lines at such a level that will permit to obtain the same heterosis intensity. As narrow genetic populations, inbred lines are very often subject to genetic deviation, gene frequency modification and therefore to alteration of their combining ability.

The biological value of sunflower inbred lines is maintained by alternating self and sib pollination with the purpose of avoiding the negative effects of strictly repeated inbreeding. This work is done in special nurseries under bag so that the authenticity of inbreds could be easily controlled and maintained. In the next stages, open pollination is used for inbred multiplication on large fields, where the individual variations cannot be controlled, being possible to identify only phenotypically distinct biological and mechanical impurities.

An important difficulty in sunflower inbred multiplication is space isolation for avoiding outcrosses. Our observations and experimental data showed the 3000 m isolation as the minimum for maintaining the biological purity of inbred lines at the highest level ((99.5—99.9%). Space isolation of only 1000 m can produce 10—15% outcrosses.

The normal inbred lines are multiplied on the basis of a scheme including the selfing and sibbing nurseries, the field for seed increase under bag and the open pollination field for multiplication.

Seeds from one selfed plant are planted in the sibbing nursery, where sib pollination by pairs of plants, with artificial emasculation of the female, are made under bag. Following laboratory analyses, the best sibs are selected and they will be planted next year in the field for seed increase under bag, one sib by row, for being able to discard

the uneven progenies. Group pollination is performed between the best progenies and seeds obtained under bag will be planted next year in the open pollination field for multiplication. Before blooming, two or three biological purifications are accomplished in this field, removing all untypical or diseased plants. For starting a new cycle of seed production, seed reserve from the sibbing nursery is planted in selfing nursery.

Except the maintenance of the authenticity and uniformity of inbred lines, seed production has some specific peculiarities connected with the unaltered preservation of certain genetic characteristics such as male sterility, pollen fertility restoration and maintenance of the genes for resistance to downy mildew in homozygous status.

Seed production from genetic male sterile lines. The main task is to maintain the segregation ratio and the coupling phase linkage of ms_1 and T genes. In this respect selfing and selecting works are carried out in alternation with sib pollinations. An important objective is to keep the proportion of crossovers (male fertile unmarked plants) at a minimum level, bellow 1%. Work is done after the following scheme (figure 1) :

1. Selfing nursery (1-st year). Different sources of the male sterile line which segregates after the 1 : 1 ratio (anthocyanic male fertile plants : green male sterile plants) are planted individually.

By flowering the numbers free of crossovers are selected and 3—5 anthocyanic male fertile plants within each number selfed. After field and laboratory examination, only one selfed typical plant is retained and its seeds are planted next year in the sibbing nursery.

2. Sibbing nursery (2-nd year). Seeds from one single selfed plant are planted in this nursery in rows with one seed per hill. The segregation ratio will be 3 male fertile anthocyanic plants : 1 male sterile green plant. By flowering the typical male sterile green plants are isolated by cotton bags and the typical fertile anthocyanic plants by paper bags, sib pollination being performed afterwards between individual plants (MS x MF). The seed from each female sterile plant is harvested and analysed individually and the best sibs are selected for planting next year in the field for seed increase under bag.

3. The field for seed increase under bag (3-rd year). Each sib is planted on a 100 plant plot, with 3—4 seeds per hill, keeping as a reserve 75—100 seeds from each sib. In the stage of two pairs of leaves the thinning is made, keeping only the male fertile anthocyanic plants on one half row and the male sterile green plants on the other half. This field will contain a proportion of 33% plots with only male fertile anthocyanic plants, which must be totally eliminated. Sterile heads of the typical plants are isolated by cotton bags ; paper bags are applied on the fertile anthocyanic plants. Pollen from all isolated anthocyanic plants is collected within each plot and the male sterile green plants are pollinated with it. Before harvesting all undesirable plots and plants are removed. Seeds from each male sterile plant are taken separately. Following laboratory analyses, a bulk is made by putting together the

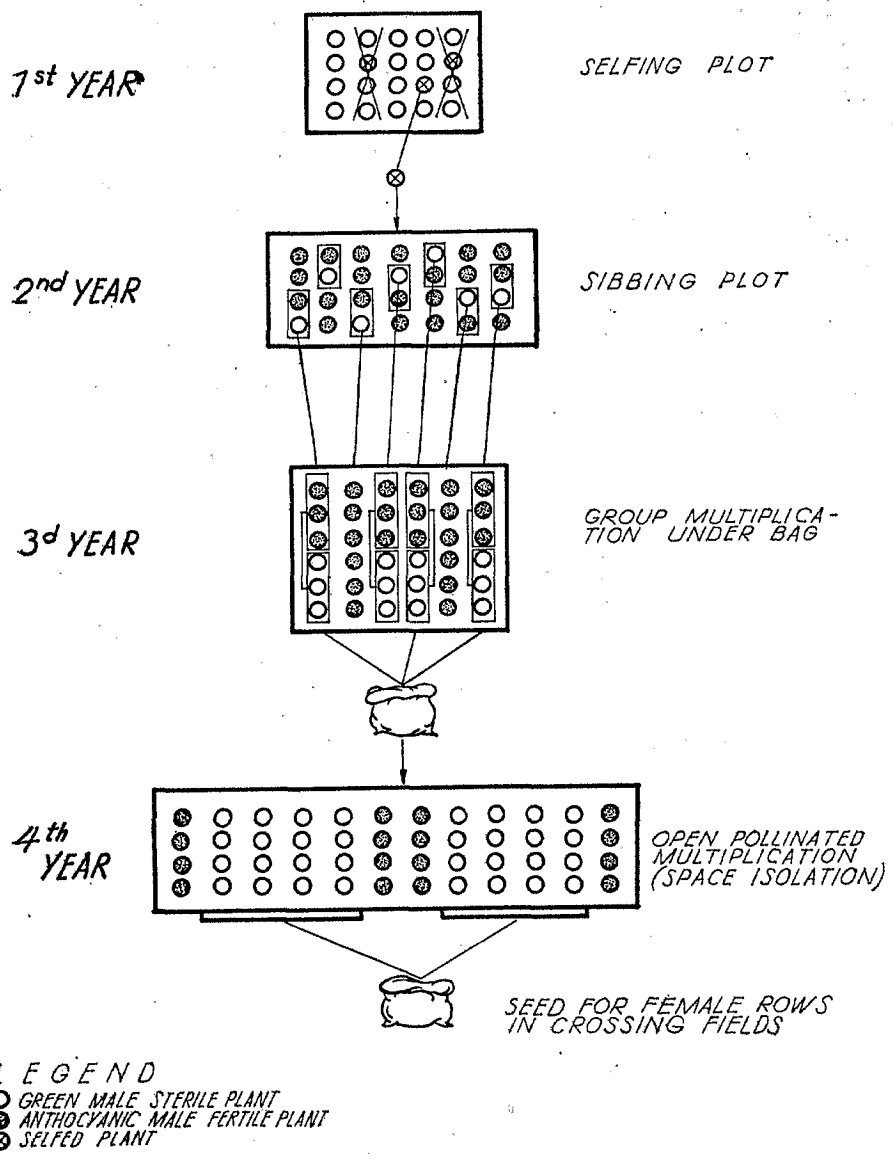

Fig. 1 — The scheme of seed production from monogenic marked male sterile lines.

seeds from all selected plants. This seed bulk will be planted next year in the open pollination field for multiplication. The seed reserve of the best sibs obtained in the second year will be planted next year in the selfing nursery, starting in this way a new seed production cycle.

4. Open pollination field for multiplication (4-th year). In this field, the segregation ratio will be 1 MS : 1 MF. Thinning is done differently, keeping two rows with only male fertile anthocyanic plants and the next four rows with male sterile green plants and so on. During flowering period, the female sterile rows must be daily checked in order to discard the fertile green plants (crossovers). Before flowering and harvesting all untypical (outcrosses) and diseased plants must be carefully removed. Only the green sterile rows will be harvested for seed, getting so the multiplicated seed of the female parent which will be planted next year in commercial crossing fields.

Seed production from cytoplasmic male sterile lines is much simpler and cheaper. Such lines consist of the A male sterile form and B male fertile analogous. The problem of inbred maintenance arises in relation to B male fertile analogous and this is achieved on the basis of the scheme presented in figure 2.

1. Selfing nursery. The fertile analogous is planted in this nursery where a certain number of typical plants are selfed.

2. Sibbing mursery. Seed from one of the best selfed plant of the previous year is planted in rows alternating with the A male sterile line. Pair pollinations A x B are made under bag, utilizing only typical plants with perfect analogy. The B plants are selfed.

3. Field for seed increase under bag. The best combinations resulted from the individual pollination A x B are planted separately, on several rows. The male fertile analogous is multiplied under bag by group pollination serving at the same time as male parent for pollinating the male sterile A line. The unsuitable A x B combinations are discarded.

4. Open pollination field for multiplication. The best A groups as well as the best B groups are mixed separately, obtaining the A and B seeds which are necessary for multiplying the cytoplasmic male sterile line by open pollination, in space isolated fields. The two forms are planted alternately in proportion of 4 A rows : 2 B rows. Because the A and B analogous lines can be distinguished phenotypically only at flowering, the rows with B analogous must be labelled as early as planting. At the maturity these rows are harvested separately and the obtained seeds sent to crushing plants. The A rows supply the seeds of the cytoplasmic male sterile line utilized for planting the female rows of the commercial crossing fields.

Seed production from pollen fertility restorer lines (C) is performed following a scheme based on the same principle of self and sib pollination alternation, for avoiding the undesired consequences of the prolonged inbreeding (figure 3). To this basic objective another one relative to the maintenance of pollen fertility restorer gene in homozygous status is added. For this, the genotype of the restorer line is kept in male sterile cytoplasm S, so that one be able to identify and eliminate all plants which do not contain the *Rf* genes.

The homozygous status *Rf Rf* is checked and maintained just from the second stage of the scheme — the sibbing nursery — where only

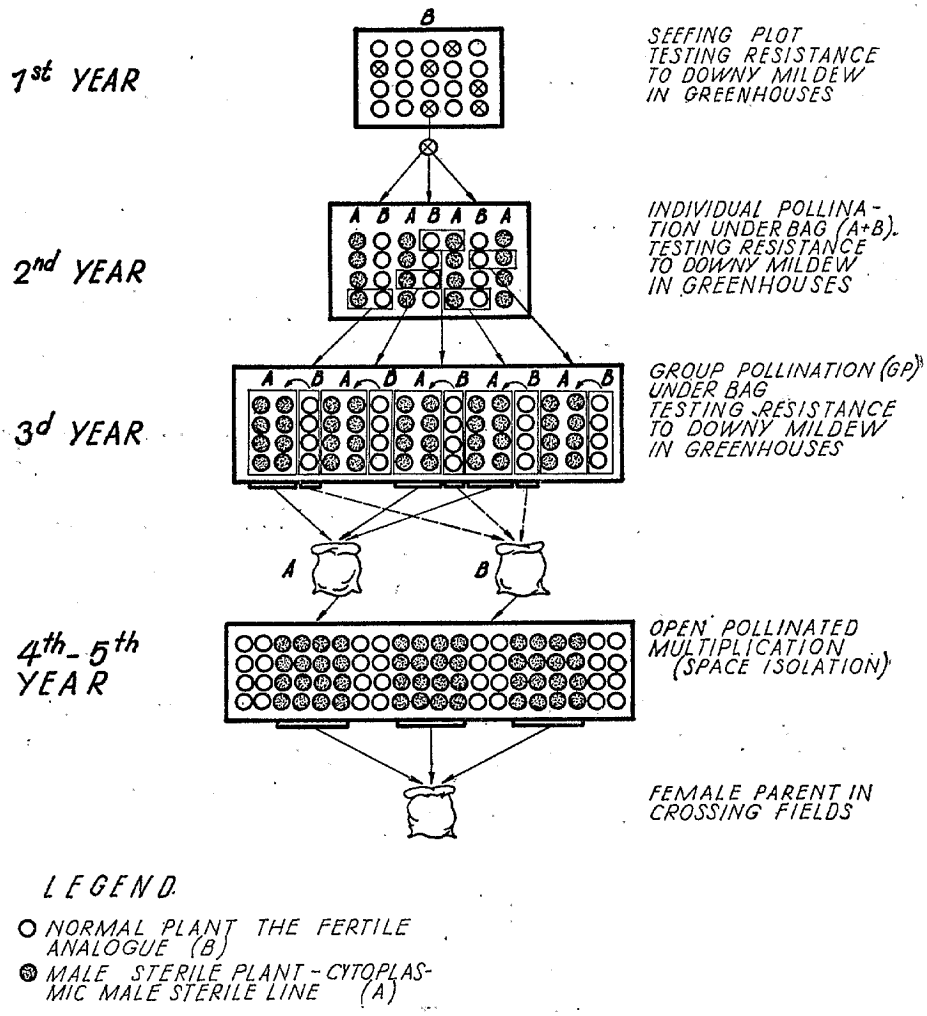

Fig. 2 — The scheme of seed production from cytoplasmic male sterile lines.

unsegregating progenies are considered. Simultaneously with sib pollination under bag, with emasculation, crosses with cytoplasmic male sterile plants are performed and the restoring capacity of the obtained hybrids is tested in greenhouses.

In the field for seed increase under bag only the female plants of the best sibs are planted and pollinated separately. Seed from the typical groups are put together and planted next year in the isolated open pollination field.

Maintenance of the Pl genes in homozygous status. The prospective sunflower hybrids will contain factors for resistance to downy mildew

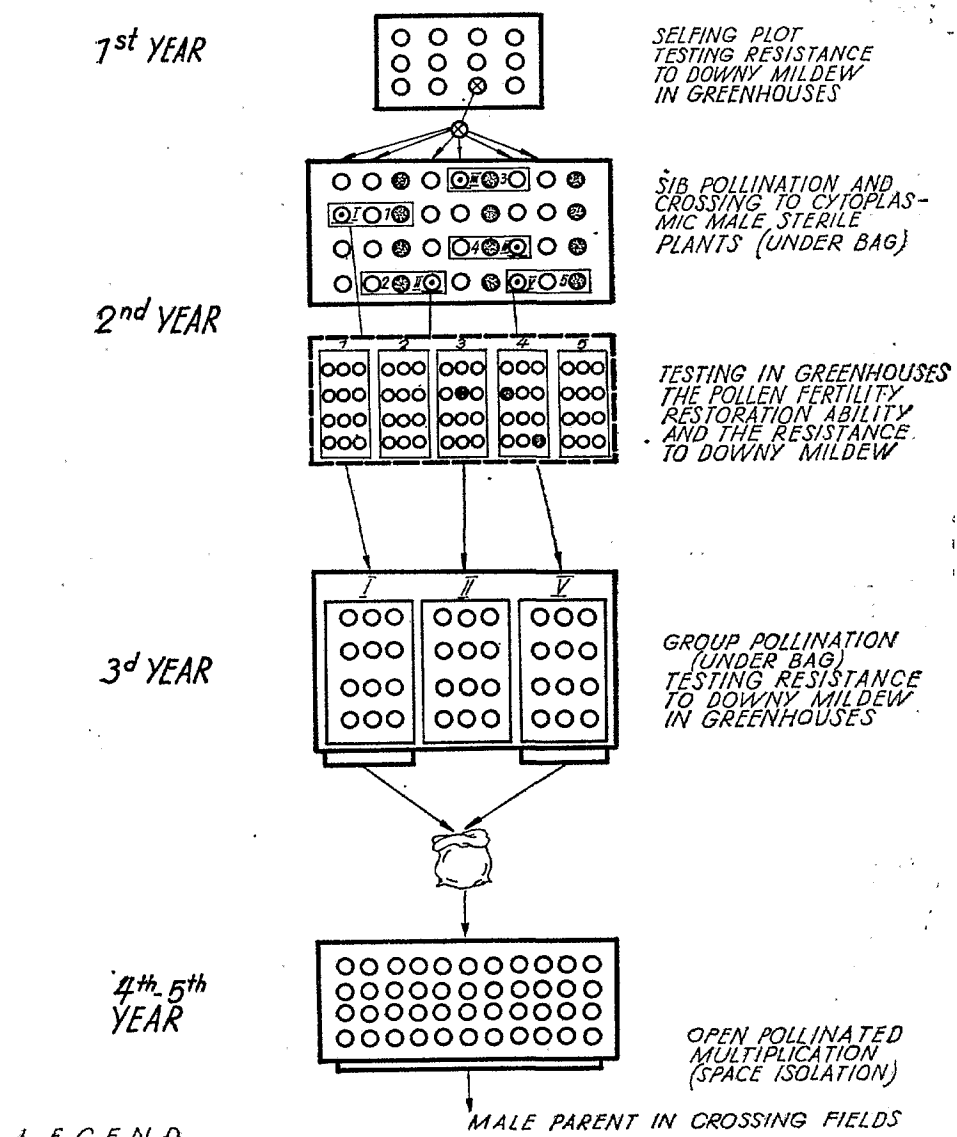

Fig. 3 — The scheme of seed production from pollen fertility restorer lines.

(*Plasmopara helianthi* Novot.) in both parental forms. That is why an important aspect of inbred seed production is to verify the homozygoosity of *Pl* genes by artificial inoculation in greenhouses, in winter time.

Both cytoplasmic male sterile lines (A and B lines) and pollen fertility restorer lines (C lines) are tested for resistance to downy mildew in first stages of seed production schemes, using the seed material obtained under bag. Such a testing assures not only the genetic purity of inbred lines for *Pl* genes, but also the stability of resistance to the local downy mildew races. making possible to detect at once any new race.