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LINKAGE STUDIES BETWEEN MS AND MARKER GENES IN SUNFLOWER

Numerous papers have announced the discovery of 1-3, or even more genes controlling sunflower male sterility. The identification of the ms genes by their phenotypic effect is rather relative because the pollen and stamen atrophy due to different genes could be very similar (Putt, Heiser, 1966; Leclercq, 1968; Anashchenko, 1969; Vrânceanu, Stoenescu, 1970; Burlov, 1970; Kovačik, Škaloud, 1973). The ms genes can be identified by cross studies or, which is even more efficient, by detecting the linkage between these genes and different marker genes.

The linkage between ms and marker genes in sunflowers proved to be very useful both in theory and practice. In 1966, Leclercq found that the ms gene and the T gene, which produces plant anthocyanic colour, lie on the same chromosome. He also suggested the utilization of this linkage in hybrid seed production.

Test studies carried out at the Research Institute for Cereals and Industrial Crops - Fundulea emphasized that only the dominant allele of one gene, namely ms_1, is linked with the gene T (Vrânceanu, Stoenescu, 1970). The frequency of ms_1 is much lower in comparison with the other ms genes.

This linkage has been used successfully for producing the first commercial sunflower hybrids in Romania (Vrânceanu, Stoenescu, 1974; Vrânceanu, et al., 1974).

Five independent genes ms were identified at Fundulea between 1965-1969. This paper is concerned with the detection and measurement of linkage between these genes and four markers. Some particularities of ms_1 and T gene linkage are specified too.

The ms and marker genes are presented in the Table.

The gene T, reported by Satsyperov in 1974 (F. Satsyperov, 1974) controls the anthocyanic colour of the entire plant.

The recessive gene y, described by Hockett and Knowles in 1970 (E. Hockett, P. Knowles, 1970), produces a temporal yellow-xanta colour of the terminal leaves.

The gene la, announced by Vrânceanu et al. in 1974 determines the white-yellow colour of ray flowers.

The fl gene controls the presence of small tubular ray flowers. It was found by Vrânceanu et al. in 1974.

Mating and selfings were performed in the breeding field and greenhouses.

The classification of the fertile and sterile plants was done visually, with the exception of plants with small amounts of pollen the fertility of which was examined by microscope.

In order to measure the strength of the linkage, the χ^2 values were broken down into components (Mather K., 1963). This was possible because the inheritance of the factors involved in crosses were known:

$$\chi^2_A \text{ (for the first pair of genes)} = \frac{[a+b-3(c+d)]^2}{3N};$$

$$\chi^2_B \text{ (for the second pair of genes)} = \frac{[a+c-3(b+d)]^2}{3N};$$

$$\chi^2_L \text{ (for A/B linkage)} = \frac{(a-3b-3c+9d)^2}{9N}$$

Each χ^2 has one degree of freedom. N is the total number of the offsprings.

The recombination fraction has been estimated by means of product method, initiated by Owen (F. Owen, 1928) and Fisher and Balma-

Table

Symbols and Origins of Five ms and Four Marker Genes in Sunflowers

Genes for male sterility			Marker genes		
Symbol	Inbred's name	Origin	Symbol	Inbred's name	Origin
ms_1	AS-110	VNIIMK 8931	T	F-300	Fuxinka-3
ms_2	AS-47	Armavir 3497	y	V-1327	VNIIMK 8931
ms_3	AS-73	VNIIMK 8883	la	GG-71-623	Synthetic variety 3
ms_4	AS-610	Armavir 3497 x Zelenka 368	fl	V-589	VNIIMK 8931
ms_5	AS-28	Morocco local variety			

kund (R. Fisher and I. Balmakund, 1928) and improved by Baily (N. Baily, 1961) and Fisher and Yates (A. Fisher, F. Yates, 1963).

Standard error (SEp) has been used as the test of significance. SEp (repulsion) = $\sqrt{\frac{(1-p^2)(2+p^2)}{N}}$; in coupling, p is substituted for $1-p^2$; p represents recombination fraction for repulsion phase, except the parental combination fraction for coupling.

The combined value of more recombination fractions (p) was calculated employing "the weighted average amount of information method" proposed by Immer and Henderson (N. Baily, 1961): $pc = \frac{\Sigma p.I}{\Sigma I}$; I represents information amount of the experimental error ($I = \frac{1}{SEp^2}$). The standard error of the new value pc is equal to $\sqrt{\frac{1}{\Sigma I}}$.

Tests of heterogeneity were carried out. χ^2's were analysed for the separate segregation ratios and for the existence of linkage to find out if the data were uniform. χ^2_L heter = χ^2_L total - χ^2_L gen. The degrees of freedom have been found out by means of substraction too.

The data obtained show clearly the independence of the ms1 gene and the marker genes y, la and fl. In one case, ms1 x la, χ^2 for heterogeneity calculated for the marker gene indicates a probability between 5% and 1%. Thus, the two offsprings appear to be not homogeneous. The main cause seems to be the small numbers of the second offspring.

The results of the cross ms1 x T give a strong evidence of the linkage. The combined amount of the recombination was 1.3±0.2 and the crossingover value for the whole F_2 offspring group was 1.0±0.5. The crossingover fractions varied from 0.0 to 3.7±0.5. All these data have been very closed to 1% obtained by Leclercq (P. Leclercq, 1966). The values obtained afterwards at Fundulea in the process of hybrid seed production, were also very close to 1%.

The specific feature of $\underline{TMs_1}/\underline{tms_1}$ linkage is the existence of $\underline{tt\ Ms_1}\ \underline{ms_1}$ crossovers (nonanthocyanic fertile plants) and not the expected 50% $\underline{tt\ Ms_1}\ \underline{ms_1}$ + 50% $\underline{Tt\ ms_1}\ \underline{ms_1}$. In order to explain this phenomenon, one can suppose the existance of a lower viability of $\underline{Tms_1}$ gamets or $\underline{Tt\ ms_1}\ \underline{ms_1}$ zygotes. Our data suggest, however, an evident incomplete penetration of $\underline{ms_1}$ when located on the same chromosome with $\underline{T}$. The partially fertile plants, usually classified as fertile, should be considered as $\underline{Tt\ ms_1}\ \underline{ms_1}$ crossovers. The manifestation of $\underline{ms_1}$ linked to $\underline{T}$ seems to be influenced by the genotype. The $\underline{ms_1}$ penetrance is higher in inbreds AS-1294 and AS-V-3319 T.

The $\underline{Tt\ ms_1}\ \underline{ms_1}$ crossovers are not difficult to detect in crossing fields, being discarded in the first vegetation stages because they are of antocyanic colour.

The hypocotyl colour proved to be very important for a clearcut elimination of the fertile anthocyanic plants at the beginning of vegetation. The gene $\underline{T}$ determines a strong violet colour. Besides this, a large scale of pink-red tints, especially on the southern part of the hypocotyl is visible to the most of genotypes, producing confusion in deciding which plant is anthocyanic and which is not. It is thus desirable that the inbreds slated for transformation into male sterile lines marked with anthocyanin have a completely green hypocotyl in different environmental conditions. The frequency of such genotypes is rather low. Among 6852 inbreds studied at Fundulea only 11.3% showed uncoloured hypocotyl.

Data accumulated in the course of obtention and multiplication of the lines with monogenic male sterility marked with anthocyanin have indicated that repeated selection of offspring with the lowest percentage of crossovers leads to the maintenance of a minimum recombinant fraction level or even to its reduction. After 5 generations yielding progenies with the lowest proportion of recombinant types, the inbred AS-116 had an almost halved crossingover fraction.

Our data show clearly an independent segregation or no linkage between ms2 and T, V and la marker genes. The ms2 and fl genes appear to be located on the same chromosome in the repulsion phase, because the d group of offspring is much smaller than the value claimed by 9:3:3:1 ratio, and ad/bc fraction is also less than 1. The combined crossingover value 19.3±5.3 is very close to the 19.1±7.5 value calculated for the whole progeny group. This linkage could not be used successfully in hybrid seed production process because of the too high recombination fraction.

The correlation of the actual and expected 3:1 and 9:3:3:1 ratios proves the independence of ms3-ms5 genes from the marker genes.

Conclusions

In the case of the already known linkage between ms1 and T, the combined amount of the recombination was 1.3±0.2 and the crossingover value for the whole F_2 offspring group was 1.3±0.5. The repeated selection of progenies with the lowest percentage of crossovers leads to the maintenance of the same crossingover fraction, or even to its reduction.

The genes ms2 and fl proved to be located on the same chromosome in the repulsion phase.

All the other ms genes proved to be independent from the respective marker genes.

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