

CYTOGENETIC INVESTIGATIONS IN CONNECTION WITH THE
GENE ms₁ IN SUNFLOWER

By

A.V. Vranceanu, Stefania Staicu, Fl.M. Stoenescu
(Romania)

Summary

The investigated biological material was the nuclear male sterile line AS-110 T containing the gene ms₁, the male fertile plants being marked by the gene T. The cytological analyses revealed a distinct morphology for a pair of chromosomes of the green male sterile plants (tt ms₁ ms₁) as compared to the anthocyanic male fertile plants (Tt Ms₁ ms₁).

The green male fertile cross-overs (tt Ms₁ ms₁) could be identified in this way, the study being thus useful in selection and hybrid seed production works when nuclear marked male sterile lines are used.

A comparative cytogenetic study is presented in the case of the F₁ hybrid Romsun 53 and its parents.

Introduction

Among the 68 species of Helianthus genus (Heiser, 1969) H. annuus is the most important, being cultivated as an oil crop as well as for forage and ornamental purposes. Due to its wide geographical distribution and phenotypical variability, a certain variation of chromosomal morphology is also expected to occur.

The number of sunflower chromosomes was determined as early as the beginning of the century (Trhara, 1915), but cytogenetic studies performed by now are quite limited and their results not always concordant (Georgieva-Todorova, 1967; Georgieva-Todorova et al, 1968; Raicu et al, 1976).

The main causes of difficulties encountered in sunflower chromosome studies could be:

- a high number of chromosomes as compared to the amount of DNA, sunflower chromosomes being relatively small;
- a short duration of mitotic cycle, especially in metaphase (Makino and Nakahara, 1953; Mazia, 1961; Van't Homm and Sparrow, 1963; Georgieva-Todorova and Ronchi, 1969).

The present paper reports a new study on the morphology and classification of sunflower chromosomes, in connection with the gene ms₁.

Material and Methods

The biological material under study was made up to two inbred lines differing in origin and characteristics and their F_1 hybrid Romsun 53. The female line AS-110 T was derived from the open-pollinated variety VNIIMK 8931 and contains the recessive gene ms_1 which control the male sterility character. The male fertile plants of this line were marked with the gene T for anthocyanic color using a scheme based on back-cross, selfing and selection (Vranceanu, 1974). At present, this inbred is multiplied by sibbing between green sterile plants and red fertile plants a constant proportion of 50 percent of both genotypes being maintained.

The male line P-1380 originates from the open-pollinated variety Peredovik.

Samples for microscopic analyses were prepared using primary meristems from embryonic root tips taken when radicle length was 4-6 cm. Prefixation was performed in - bromnaphthalene with saponine (30 cm^3 - Brom + 100 cm^3 water + 50 mg saponine) at 4°C for 4-1/2 hours. The material was fixed in alcohol-acetic acid (3:1) at 4°C for 7 days and then hydrolized in HCl^1N at 60°C for 15 minutes. The chromosomes were stained with a reagent of a composition similar to Schiff's. After 10 minutes treatment the root tips were transferred into ammonia acetate buffer (pH 6.8-7.00) for 30 minutes in order to get a staining augmentation. Squash type preparations were obtained in acetic carmine solution 4 percent.

Caryotypes were made up using the chromosome classification proposed by Levan et al (1964) which takes into consideration the centromere position in accordance with the arm ratio.

Results and Discussion

More than 100 microscopical analyses were performed for each entry and about 50 caryotypes studied. The chromosome groups drawn up are concordant with those reported by Georgieva-Todorova (1967):

- Group I: 7 metacentric chromosome pairs;
- Group II: 6 sub-metacentric chromosome pairs, out of which 3 with satellites;
- Group III: 4 acrocentric chromosome pairs.

The caryotypes of Romsun 53 (Figure 1) and of its parental inbreds (Figures 2 and 3) show a strict homology of chromosome pairs. The only pair differing morphologically is the first sub-metacentric chromosome pair with satellites.

The green sterile plants ($tt\ ms_1\ ms_1$) of the female line AS-110 T have a constant caryotype in 98 percent of the cases. They present specifically double-sized satellites at the first sub-metacentric chromosome pair in contrast with the male line (Figure 2). In some metaphases a secondary constriction is observed on the satellite arm of this chromosome pair. The male line P-1380 has always simple satellites, significantly smaller, lacking secondary constrictions (Figure 3).

Coming back to Figure 1, the origin of each homologous chromosome from the first sub-metacentric pair is evident. At the same time, the double amount of the constitutive chromatic material of the respective satellite, coming from the female line, is obvious.

A more accurate comparison can be made in Figure 4.

This morphological difference between the chromosomes of the first sub-metacentric pair with satellites was also observed to the single hybrid Romsun 52 and its parental lines, the female parent of this hybrid combination being the same as in Romsun 53.

The double-sized satellite of the green-sterile plants from AS-110 T may be considered as a particular case and used as a chromosomal marker for further cytogenetic studies. Thus, the present observations suggest the fact that gene ms₁ might be localized on this chromosome pair. It is possible that the two percent proportion of metaphases within the female line, similar to those specific for the F₁ hybrid (Ms₁ms₁), represent cross-overs (green fertile plants) occurring normally in a similar proportion as in AS-110 T male sterile inbred. Further investigations are being undertaken for confronting the karyotype with the actual phenotype of the individual plants.

In the course of seed multiplication, it is desirable that the female inbred has a low a cross-over proportion as possible. Microscopical identification of cross-overs in germinating seed stage would be very useful for selection. On the other hand, the percentage of non-hybrid seed in F₁ could be estimated by microscopical screening, at least in hybrids with AS-110 T as female parent.

The karyotype of the anthocyanic fertile plants of the inbred line AS-110 T (Tt Ms₁ms₁) is similar to that of the hybrids Romsun 52 and Romsun 53 in most cases (Figure 5), which supports the assumption that the gene ms₁ is located on the first sub-metacentric chromosome pair with satellite. However, 10 to 15 percent of the anthocyanic plant metaphases were different, showing both chromosomes of this pair with satellites, either of maternal or paternal type. This variation could possibly be explained on account of the repeated backcrosses of AS-110 to Fuksinka, an obsolete Russian anthocyanic cultivar, performed for obtaining the linkage between the genes ms₁ and T.

Literature Cited

- GEORGIEVA-TODOROVA, I., 1967. Cariologichno prouchivane na kultura slynciogled Helianthus annuus. Genetichni isledvaniia. Sofia, 85-90.
- GEORGIEVA-TODOROVA, I., RONCHI, V.N., 1969. The mitotic cycle time of root meristem cells of Helianthus annuus. Caryologia, Vol. 22.22,n.4:331-338.
- GEORGIEVA-TODOROVA, I., LAKOVA, M., SPIRKOV, D., 1972. Cariological analysis of Helianthus debilis. In: Remote Hibridization of Plants, Sofia.
- HEISER, C.B., 1969. The North American Sunflower (Helianthus) Mem. Torrey Bot. Club, 22, 3, N.Y.

- LEVAN, A., FREDGA, K., SANDBERG, A.S., 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, 52:201-220.
- MAKINO, S., NAKAHARA, H., 1953. Citologische Untersuchungen an Tumoren VIII Beobachtungen über den Mitosenablauf in lebenden Tumorzellen der Ascites Sarkome des Ratten. *Z. Krebsforsch.* 59:298.
- MAZIA, D., 1961. Mitosis and the physiology of cell division. *The Cell*, 3.
- RAICU, P., VRANCEANU, A.V., ALEXANDRINA MIHAILESCU, CONSTANTA POPESCU and MARICICA CHIRILOVA, 1976. Research on the chromosome complement in Helianthus L. genus, *Caryologia* Vol. 29, n.3:307-316.
- TAHARA, M., 1915. Cytological investigation on the root-tips of Helianthus annuus. *Bot. Magazine, Tokio*, 29
- VAN'T HOFF, J., SPARROW, A.H., 1963. A relationship between DNA content, nuclear volume, and minimum mitotic cycle time. *Proc. of the National Academy of Sciences*, 49:897-902.
- VRANCEANU, A.V., 1977. *Floarea-soarelui*. Editura Academiei R.S.R. Bucuresti.