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INTERSPECIFIC HYBRIDIZATION IN SUFLOWER

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CYTOLOGICAL STUDIES OF THE HYBRID BETWEEN *HELIANTHUS ANNUUS* L AND *HELIANTHUS DEBILIS DEBILIS* NUTT.

SUMMARY

Attention of researchers and plant breeders was turned to *Helianthus debilis debilis* since 1985. This species seems to have taken part in allopolyploid differentiation of *Helianthus* genus.

In order to get information on interspecific hybridization in the genus *Helianthus* we have studied four accessions, *H. debilis debilis, H. annuus* Ha 89mt, F1 hybrid and F2 selfed line.

Chromosome number analysis that revealed the ooccurrence of aneuploid chromosome number was performed; in root and shoot tips. F the parents, the occurence of diploid number is superior to thet in the hybrids.

Microdensitometric analysis of nuclear DNA content of shoot and root apices showed variability in DNA values.

INTRODUCTION

The genus *Helianthus* comprises 67 species divided into four sections and among these there are several subspecies having a very different habitats in relation to various areas of growth (Schilling and Heiser 1981).

In order to discover new sources of disease resistance, new sources of cytoplasmic male sterility and fertility restoration and, drought and salt resistance in *Helianthus* genus, interspecific hybridization between wild sunflower species and cultivated sunflowers had been conducted. The importance of wild species comes from their wide genetic variability and are used as a source of genes determining good agronomical and technological characters (Seiler 1984, Skoric et al. 1984; 1987).

Interspecific hybridization concerning transfer of genetic material from wild species into the cultivated genotypes has been studied (Leclerq 1966, 1969, Georgieva Todorova and Lakova 1979 and Georgieva Todorova, 1984). Leclerq in 1969 found cytoplasmic male sterility in *Helianthus priolaris* x *Helianthus annuus* and Vranceanu and Stoenescu in 1971, Vranceanu in 1978 and Fick et al. in 1984 found fertility restorer genes. These years mark the beginning of commercial hybrids production.

The literature is scant with genetics and cytogenetics studies on interspecific hybridization between cultivated sunflower and *Helianthus* species with different ploidy

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level (Cauderon 1965, Georgieva Todcrova 1988). In this paper we relate observations about a group of four accessions: *Helianthus annuus* L (Line HA 89 mt), *Helianthus debilis debilis* Nutt (wild specie) and F1 and F2.

Our observations concern nuclear DNA content and chromosomal variability. Nuclear DNA content variations occur in plants and are a common feature due to their inability to escape shanging enviromental conditions (Walbot and Cullis 1985, Cionini 1989 and references therein). Such variations are due, often, to differential DNA replication or DNA loss during plant cell differentiation leading to amplification or underrepresentation of specific sequences (Bassi 1984, Natali 1986, Altamura 1987). Intraspecific variations of nuclear DNA content in *Helianthus annuus* have been determined by Nagl and Capesius (1976), Olszewska and Osiecka 91983), Michaelson and Price (1985), Cavallini et al. (1986). While by cytological observations chromosome number more that diploid (2n=34) has been never demonstrated by Cavallini and Cremonini (1985), Cremonini and Cavallini (1986) have evidenced aneusomaty.

There are data in literature that chromosomal variability present in callus and in regenerated plants may be due not only to in vitro induction but also may be a condition preexistent in the primary explant. In *Orobanche gracilis* aneusomaty is due to a tendency to non exhibits disjunction during mitosis (Greilhuber and Weber 1985), also *Lathyrus sativus* instability of somatic chromosome number in root and shoot tip mitosis (Lavania 1982). Therefore it is possible to have aneusomaty both in root and shoot apices with a large variability among plants and inside the same plant.

Since knowledge of chromosome number variation may be useful in a breeding program we have investigated also this phenomenon in some detail.

MATERIALS AND METHODS

As material we have used four accessions:

- *Helianthus annuus* L (Line HA 89 mt)
- *Helianthus debilis debilis* Nutt
- Hubrid F1
- F2 selfed line

Seeds of the four accessions were germinated, under sterile conditions, in Petri dishes on filter paper (Watman n.2) soaked with distilled water. Twenty seeds with 6 ml solution for each dish were used for each accession. The dishes were placed in an incubator in the dark at 23°C and in order to avoid partial evaporation a filter paper weetted with distilled water has been put in the upper dish and sealed with laboratory film.

A first sample of root and shoot apices, fixed in ethanol-acetic acid 3:1 (v/v), were squashed in a drop of 45% acetic acid after treatment with a 5% solution of pectinase (Sigma) for 1 h at 38°C. The cover slips were removed by the dry-ice method and the preparations were Feulgen stained after hydrolysis in 1 N HCl at 60°C for 7 min. The slides were then subjected to three 10-min washes in SO2 water, dehydrated and mounted in DPX (BDH).

In order to obtain comparable results, squashes made with root tips of plantlets of *Vicia faba* were stained for each group of slides and used as standards. Feulgen-DNA absorptions in individual cell nuclei were measured at the wavelength of 550 nm using a

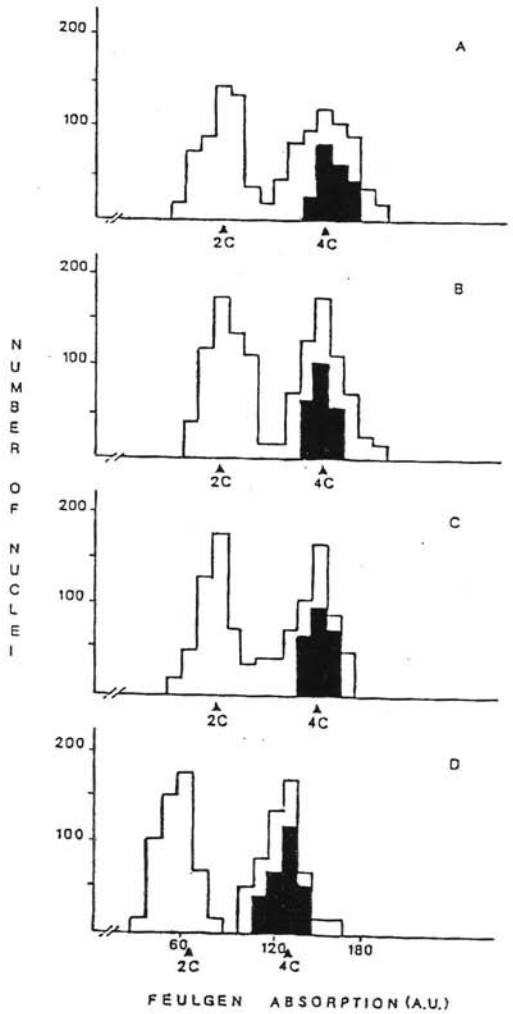

Fig. 1 - Feulgen absorption in individual cell nuclei of ten roots and shoots for each accession of *Helianthus*. Solid bars represent mitotic nuclei. For each tip fifty randomly selected nuclei were measured. The Feulgen absorption values are expressed as arbitrary units
(A: *H. annuus* HA 89 mt; B: *H. debilis debilis*; C: F1; D: F2).

Leitz MPV3 microscope photometer equipped with a mirror scanner and a HP 85 computer. For each tip fifty randomly selected nuclei were measured.

A second sample of root and shoot apices was treated for shromosome counts with a 0.3% colchicine solution for 5 h at room temperature. They were Feulgen stained and squashed and the slides were then prepared as above. The chromosomes were counted in mataphase plates.

RESULTS

DNA cytophotometry

The mean Feulgen-absorption of nuclei in the root and shoot meristems of the four accessions are reported in Figure 1. Nuclear DNA content of root and shoot of each accession does not differ significantly. The 4 C value was obtained by early prophase measurements in each accession and the mean absorption of G2 nuclei correspond to the mean Feulgen absorption of prophase nuclei.

In all the accessions it is possible to observe nuclei in the DNA presynthetic conditions (2C, G1) and nuclei in the DNA postsynthetic conditions (4C, G2). Significant differences in the mean Feulgen absorption of nuclei are found between the fourth accession and the others: in fact the three accessions, *H. annuus HA 89 mt, H. debilis debilis* and F1, as it may be seen from Figure 1, show higher values in the Feulgen absorptions. The statistical elaboration (Table 1) confirm this result. No significant differences in the Feulgen absorption of prophase and interphase nuclei among the plants of each accession were found.

Table 1. Feulgen absorption (a.u.) of individual interphase nuclei (4C) in the four accessions. For each accession shoot and root meristematic cell were measured.

Accession	Absorption	
	Mean	+S.E.
H. annuus	1329.8	10.47
H. debilis debilis	1340.6	20.04
F1	1337.6	12.81
F2	1184.2	16.21

Cytological analyses

We have also scored cells both in root and in shoot tips in order to get information on somatic chromosome number. Chromosome counts for each accession are reported in Tables 2, 3, 4 and 5. In *H. annuus HA 89 mt*, as shown in Table 2, several cells with chromosome number lower than 25-26 are present. In the other accessions: *H. debilis debilis* and F1 it is possible to observe that (Table 3 and 4) the lowest chromosome number is in the class 25-26, while in the fourth accession cells with chromosome number lower than 26-27 are not present (Table 5). Cytological analysis has also shown that there are no cells with chromosome number over the diploid one and it is not possible to observe any abnormalities in cell division as anaphase separations or aberrant mitoses with lagging chromosomes. The results of DNA cytophotometry and cytological analyses in each plant, and we have used ten plants for each accession, are the average of the orservations in shoot and root apices.

Table 2. Chromosome counts in apical' cell of *Helianthus annuus* Ha 89 mt.

PLANTS	Percentage of cells in each chromosome number class							
	19/20	21/22	23/24	25/26	27/28	29/30	31/32	33/34
A	4.7	–	6.7	–	13.3	15.3	23.3	36.7
B	–	1.5	1.5	3.0	3.0	3.0	9.2	78.5
C	–	–	–	3.8	7.7	11.5	11.5	65.4
D	–	2.6	–	–	–	–	15.6	82.3
E	–	–	3.6	3.6	3.6	7.1	3.6	78.6
F	–	–	–	–	–	27.3	18.2	54.5
G	–	–	–	5.9	11.8	11.8	25.3	45.3
H	–	6.7	–	16.7	–	–	26.7	50.0
I	3.6	–	3.6	3.6	7.1	7.1	17.6	57.4
L	–	–	4.7	3.8	5.0	18.0	25.1	43.4
Mean	0.8	1.8	2.0	4.0	5.1	10.1	17.6	59.2
+E.S.	0.5	1.6	0.8	1.8	1.5	2.7	2.4	5.1

Table 3. Chromosome counts in apical' cells of *Helianthus debilis debilis* Nutt.

PLANTS	Percentage of cells in each chromosome number class							
	19/20	21/22	23/24	25/26	27/28	29/30	31/32	33/34
A	–	–	–	–	–	27.8	33.3	38.9
B	–	–	–	–	10.8	7.1	32.1	50.0
C	–	–	–	4.0	4.0	16.1	28.0	48.0
D	–	–	–	5.0	5.0	10.0	30.0	50.0
E	–	–	–	–	8.0	20.0	32.0	44.0
F	–	–	–	4.5	6.0	18.0	28.0	43.5
G	–	–	2.0	3.5	5.0	16.3	31.0	42.2
H	–	–	–	–	8.0	20.5	32.0	39.5
I	–	–	1.9	4.0	5.0	17.5	26.5	45.1
L	–	–	–	4.0	10.5	10.5	30.0	45.0
Mean	–	–	0.4	2.5	6.2	16.4	30.3	44.7
+E.S.	–	–	0.1	0.2	0.7	1.9	0.7	1.2

Table 4. Chromosome counts in apical' cells of the hybrid *H annuus* x *H. debilis debilis.*

PLANTS	Percentage of cells in each chromosome number class							
	19/20	21/22	23/24	25/26	27/28	29/30	31/32	33/34
A	–	–	–	–	–	5.5	2.6	91.9
B	–	–	–	–	6.4	–	16.2	77.4
C	–	–	–	–	–	–	–	100.0
D	–	–	–	–	6.7	6.7	26.6	60.0
E	–	–	–	–	6.7	6.7	20.0	66.7
F	–	–	–	–	–	8.0	24.0	78.0
G	–	–	–	–	6.7	6.7	18.0	68.6
H	–	–	–	–	–	–	–	100.0
I	–	–	–	–	–	3.3	6.7	90.0
L	–	–	–	–	–	6.0	14.6	79.4
Mean	–	–	–	–	2.6	4.3	16.8	81.2
+E.S.	–	–	–	–	0.1	0.5	2.9	4.4

Table 5. Chromosome sounts in apical' cells of F2 progeny.

PLANTS	Percentage of cells in each chromosome number class							
	19/20	21/22	23/24	25/26	27/28	29/30	31/32	33/34
A	–	–	–	–	3.5	4.0	20.5	72.5
B	–	–	–	–	4.0	–	12.0	84.0
C	–	–	–	–	–	–	–	100.0
D	–	–	–	–	–	4.6	12.0	83.4
E	–	–	–	–	3.0	5.0	18.0	74.0
F	–	–	–	–	4.5	–	21.5	74.0
G	–	–	–	–	–	–	–	100.0
H	–	–	–	–	2.0	4.6	13.4	80.0
I	–	–	–	–	2.5	2.0	16.5	79.0
L	–	–	–	–	4.5	–	18.0	77.5
Mean	–	–	–	–	2.4	2.0	13.2	82.4
+E.S.	–	–	–	–	0.4	0.5	1.3	3.2

DISCUSSION

An extensive aneusomaty is present in *H. annuus* (Cavallini and Cremonini 1985; Cremonini and Cavallini 1986) both in the root and in the shoot apices of the seedlings and such aneusomaty, originated during embryo development, progressively decreases in the course of plant growth to disappear at pre-meiosis. By our observations we have found variations in the chromosome number but they are not sufficient to state the presence of a wide aneusomaty. In the shoot and root apices of hybrids variations of chromosome numbers are present and we have observed metaphases with chromosome number class 27-28, while the other accessions show wider shromosomal variability. The observed chromosomal variability present in the F2 does not justify the lower DNA content because the mean number of chromosomes per nucleus is the same in the four accessions.

Many data on variations in nuclear DNA content are present in literature; intraspecific variations in the size of the genome are often due to enviromental factors like in flax and in *Pinus* (Cullis 1983); Cavallini et al. (1986) have shown that variability of nuclear DNA content orbserved in thirty-one cultivated varieties or lines of *H. annuus* are due to the variations of repeated DNA sequences. Variations of repeated DNA sequences may be correlated with both developmental and physiological stimuli (Walbot and Cullis 1985), they also originate from differentiation and dedifferentiation processes (Bassi et al. 1984, Natali et al. 1986); at the same time such variations do not influence the phenotypic characters of the tested plants.

There are data which suggest that some hybrids are unstable for nuclear DNA content (Gerstel and Burns 1966) and the results of the research of Price et al. (1983) suggest that "some DNA sequences are unstable and can undergo deletion or amplification in a hybrid. The altered DNA content may be heritably stable and show little or no segregation in the D2 progeny".

Therefore, according to these remarks, our variations may be due to a different organization of the genomes and/or this organization, partially, may be not liable for a true diversity in the quantity but for a different staining at Feulgen reaction.

Biochemical analyses of DNA of our accessions should lead to a better knowledge of the nature of the DNA sequences which differ.

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HYBRIDISATION INTERSPÉCIFIQUE CHEZ LE TOURNESOL.

RÉSUMÉ:

Depuis 1985, l'attention des chercheurs et des sélectionneurs, a été attirée par *Helianthus debilis debilis*. Cette espèce semble avoir joué un role dans la différenciation allopoliploïdique du genre *Helianthus*.

Afin d'obtenir plus d'informations sur l'hybridisation interspécifique chez le genre *Helianthus*, nous avons étudié quatre accessions: *Helianthus debilis debilis, Helianthus annuus*, Ha 89mt, l'hybride F1 et la lignée F2.

Nous avons effectué l'analyse du nombre de chromosomes qui permet de révéler le nombre de chromosomes aneuploïdes est supérieure à celle observée chez l'hybride.

Les analyses microdensitométriques de l'ADN nucléaire au niveau des apex de racines et de rameaux révélent une variabilité de la concentration en ADN.

Mots clés: *Helianthus*, hybridisation, concentration de l'ADN nucléaire, anlayse du nombre de chromosomes.

HIBRIDACION INTERESPECIFICA EN GIRASOL.

RESUMEN

La atención de los investigadores y mejoradores se centró en *Helianthus debilis debilis* desde 1985. Esta especie parece haber tomado parte en la diferenciación del género *Helianthus*.

Para tener información sobre la hibridación interespecifica en el género *Helianthus* se han estudiado cuatro accesiones. *H. debilis debilis, H. annuus* HA 89 mt, al híbrido F1 y la F2.

El análisis del número de cromosomas, que reveló la ocurrencia de un número aneuploide de este fue llevado a cabo en los extremos de la raíz y el tallo fué superior la ocurrencia del número diploide de cromosomas es superior que en los híbridos.

El análisis microdensitometrico del contenido de ADN nuclear de los ápices del tallo y la raíz mostraron variabilidad en los valores de ADN.