

## CYTOMORPHOLOGICAL STUDY IN THE INTERSPECIFIC HYBRID *Helianthus annuus* L. x *Helianthus argophyllus* T. & G.

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### SUMMARY

An investigation was undertaken to study the cytomorphology of the interspecific hybrid *H. annuus* var. Morden x *H. argophyllus* along with the parents. The hybrid showed considerable vigour for plant height and stem girth. The hybrid was intermediate for seed size and test weight and exhibited reduced pollen and seed fertility. The chromosome pairing was normal in the parents while one chain or ring quadrivalent was observed in the hybrid in most of the meiotic cells scored in diakinesis / metaphase I, suggesting that the species *H. annuus* and *H. argophyllus* differ by one reciprocal translocation. The mean chiasmata frequency in the hybrid was 20.94 per cell and 1.22 per bivalent, while for *H. annuus* and *H. argophyllus* the values were 22.76, 22.16 per cell and 1.33, 1.33 per bivalent, respectively.

**Key words:** *Helianthus annuus* L., *Helianthus argophyllus* T. & G., interspecific hybrid, cytomorphology

### INTRODUCTION

The genus *Helianthus* comprises about 64 species which are mostly of North American origin and very few of these have been examined cytologically (Kulshresta and Gupta, 1979). Many wild species of this genus are known to possess agronomically desirable characters but no easy crossability of these wild species with the cultivated sunflower has nevertheless enabled transfer of many desirable genes into the cultivated species. A perusal of literature shows a need and scope for cytological investigations in the genus *Helianthus*. The present paper deals with the cytomorphology of the interspecific hybrid *H. annuus* x *H. argophyllus*.

### MATERIALS AND METHODS

The species *H. annuus* var. Morden and *H. argophyllus* were grown in the greenhouse. To facilitate easy crossing, a solution of 100 ppm gibberellic acid (GA<sub>3</sub>)

was applied at star bud stage to induce male sterility in *H. annuus* var. Morden (Seetharam and Kusumakumari, 1975). The pollen collected from *H. argophyllus* was applied and the pollination was continued for 2-3 consecutive days. Both, the species and the  $F_1$  seeds, were planted in the field along with the parents for the study of comparative morphology. For meiotic studies, flower buds of appropriate size were fixed in freshly prepared Cornoy's II mixture made up of six parts of ethyl alcohol, 3 parts of acetic acid and one part of chloroform. The flower buds were collected during 8-10 AM on bright sunny days. The anthers were smeared in one or two drops of acetocarmine and all observations were made on temporary preparations using binocular microscope.

## RESULTS AND DISCUSSION

The data on comparative morphology of the hybrid and parents are presented in Table 1. There was considerable difference for days to flowering between the two species. The cultivated species *H. annuus* var. Morden flowered early (48 days) while *H. argophyllus* was late in flowering (142 days).  $F_1$  was intermediate and flowered in 91 days. The hybrid was taller than both parents and exhibited smaller head diameter, higher stem girth and higher leaf length and width. The hybrid showed profuse auxiliary flowering and was intermediate for seed size and test weight. However, for oil content, the hybrid was nearer to the inferior parent, *H. argophyllus*.

Table 1: Salient features of the interspecific hybrid ( $F_1$ ) *H. annuus* x *H. argophyllus* and its parents for various characters

Species	Days to 50% flowering	Plant height (cm)	Head diameter (cm)	Stem girth (cm)	Number of leaves	Fifth leaf length (cm)	Fifth leaf width (cm)	Number of branches	Number of auxiliary flowers	Seed length (cm)	Seed breadth (cm)	Test weight (g)	Oil content (%)
<i>H. annuus</i>	48.0	58.2	11.6	1.2	19.2	12.8	11.2	-	-	1.03	0.51	4.68	32.7
<i>H. argophyllus</i>	142.0	151.0	3.0	1.8	33.0	5.8	4.0	22.2	-	0.44	0.20	0.54	25.3
$F_1$	91.0	228.0	9.1	2.2	35.2	17.0	11.0	10.0	85.2	0.78	0.34	2.58	24.6

The parental species pairing between the homologous chromosomes was normal at diakinesis and metaphase I with 12 II formed (Table 2). The later stages of meiosis were normal, resulting in good pollen and seed fertility. On the contrary, in the hybrid, usually 15 II + 1 IV (Figure 1) was observed in 90 percent of PMC's. Narkhede *et al.* (1986) reported the formation of quadrivalents in the interspecific hybrid *H. annuus* and *H. argophyllus*. Occasionally, the chromosome association of 13 II + 2 IV (Table 2) was also observed. The quadrivalent observed was either a chain or ring (Figures 1 and 2). This indicates that translocation might be the cause

of quadrivalent formation and the higher frequency of chain quadrivalents might be due to unequal size of segments exchanged between non-homologous chromosomes.

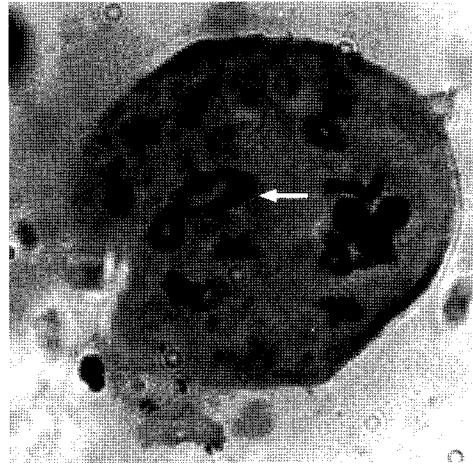
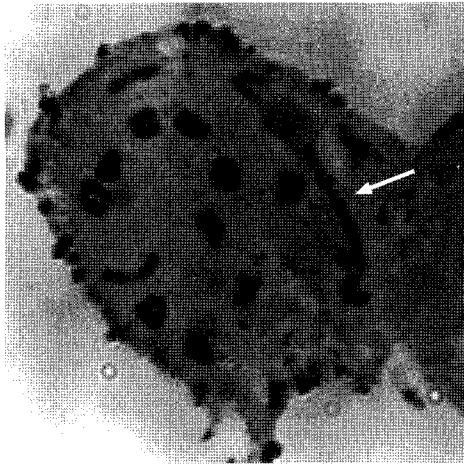


Figure 1: Diakinesis in the interspecific hybrid *Helianthus annuus* x *Helianthus argophyllus* showing 15 II + 1 IV - see the chain quadrivalent

Figure 2: Metaphase I showing 15 II + 1 IV in *Helianthus annuus* x *Helianthus argophyllus* - see the ring quadrivalent (1000 x)

The latter stages of meiosis (anaphase I and telophase I) also showed abnormality with chromosome bridges and lagging chromosomes. The presence of single quadrivalent at diakinesis / metaphase I indicates that the genome of *H. argophyllus* differed from that of *H. annuus* by at least one reciprocal translocation.

Table 2: Chromosome association and chiasma frequency in the interspecific hybrid (*H. annuus* x *H. argophyllus*) in comparison with the parents

Species	Cell scored	Number of cells with chromosomal association			Mean chiasmata/bivalent	Mean chiasmata/cell
		17 II	15 II+1 IV	13 II+2 IV		
<i>H. annuus</i>	25	25	-	-	1.33	22.76
<i>H. argophyllus</i>	25	25	-	-	1.30	22.16
F <sub>1</sub>	25	-	24	1	1.22	20.94

Study on chiasma frequency (Table 2) revealed that the mean chiasmata frequency in the hybrid was 20.94 per cell and 1.22 per bivalent which was lower compared with either of the parents, *H. annuus* and *H. argophyllus*, with the values 22.76, 22.16 per cell and 1.33, 1.30 per bivalent, respectively. The lower chiasma frequency in the interspecific hybrid is suggestive of the fact that the genome of both species might be differing for small structural changes in the form of deletions and inversions, in addition to one major translocation.

Table 3: Comparison between pollen and seed fertility of the interspecific hybrid *H. annuus* x *H. argophyllus* and its parents

Species	Pollen fertility (%)	Seed fertility (%)		
		Main head	Branches	Auxiliary flowers
<i>H. annuus</i>	99.6	93.13	-	-
<i>H. argophyllus</i>	95.8	91.13	1.27	-
F <sub>1</sub>	20.2	20.90	2.10	0.008

The pairing abnormalities in terms of quadrivalent observed in first division of meiosis and abnormal disjunction of chromosomes in latter stages of first and second division of meiosis, resulted in low pollen and seed fertility of the hybrid compared with the parents. The pollen fertility was around 20.2 percent in the hybrid against 99.6 percent in *H. annuus* and 95.8 percent in *H. argophyllus*. The seed fertility was as low as 20.9 percent in the hybrid as against more than 90 percent (Table 3) in the parental species.

#### REFERENCES

- Kulshresta, V.D. and Gupta, P.K., 1979. Cytogenetic studies in the genus *Helianthus*. *Cytologia*, 44, pp. 325-334.
- Narkhede, M.N., Dharmaraj, V. and Meshram, L.D., 1986. Meiotic studies in interspecific hybrids of *Helianthus*. *PKV Res. J.*, 10 (2), pp. 85-87.
- Seetharam, A. and Kusumakumari, P., 1975. Induction of male sterility by GA<sub>3</sub> in sunflower. *Indian J. Genet.*, 35, pp. 136-138.

### EL ESTUDIO CITOMORFOLÓGICO DE HÍBRIDOS INTERSPECIE *Helianthus annuus* L. x *Helianthus argophyllus* T. & G.

#### RESUMEN

La investigación ha sido emprendida para estudiar la citomorfología de híbridos interspecie *H. annuus* var. Morden x *H. argophyllus* y sus padres. El híbrido manifestó un vigor híbrido considerable con respecto a la altura de planta y el volumen de tronco, siendo intermediario con respecto a la grandeza de semilla y el peso de grano, y manifestó también la fertilidad reducida de polen y semilla. Mientras la copula de cromosomas era normal a los padres, un cuadrivalente de cadena y de anillo fué notado en los híbridos en todas las células durante la diaquinesis/metafase I. Eso indica que las especies *H. annuus* y *H. argophyllus* se diferencian por una translocación recíproca. Las frecuencias medias de chiasmata eran en los híbridos 20,94 por célula y 1,22 por bivalente, mientras esos valores en *H. annuus* y *H. argophyllus* eran 22,76 y 22,16 por célula y 1,33 y 1,33 por bivalente.

**ETUDE CYTOMORPHOLOGIQUE DE L'HYBRIDE  
INTERSPECIES *Helianthus annuus* L. x *Helianthus  
argophyllus* T. & G.**

RÉSUMÉ

Il s'agit d'une étude sur la cytomorphologie de l'hybride interspecies *H. annuus* var. Morden x *H. argophyllus* et de ses parents. L'hybride a montré une grande vigueur en ce qui concerne la hauteur de la plante et le pourtour de la tige. Il était intermédiaire pour ce qui concerne la taille de la semence et le poids de 100 graines et il présentait une fertilité réduite du pollen et de la semence. L'appariement des chromosomes était normal chez les parents, on a pu remarquer un quadrivalent annulaire ou en forme de chaîne chez l'hybride dans toutes les cellules méiotiques au cours de la diakinésie/métaphase I. Ceci indique que les espèces *H. annuus* et *H. argophyllus* diffèrent d'une translocation réciproque. Les fréquences moyennes de chiasma chez l'hybride étaient de 20,94 par cellule et de 1,22 par bivalent alors que pour l'*H. annuus* et l'*H. argophyllus*, les valeurs étaient de 22,76, 22,16 par cellule et 1,33 et 1,33 par bivalent.

