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CYTOGENETIC RELATIONSHIP BETWEEN HELIANTHUS ANNUUS L. AND H. TUBEROSUS L.

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Abstract

Eleven populations of Helianthus tuberosus L. were used in crosses with the Helianthus annuus L. inbred line HA89 in order to study the cytogenetic relationship of the two species and the fertility of the F_1 hybrids and their backcross progeny. Pollen fertility of 180 F_1 hybrids and 170 BC_1 progeny was studied. Samples of pollen were placed in acetocarmine for three days and only well-colored grains with well stained sperm nuclei were scored as fertile. The mean fertility of the F_1 hybrids was 17.2% with a range of 1.2 to 34.2%. The mean fertility of the BC_1 progeny was 3.0% with a range of 0.0 to 11.6%. In most hybrids it was not possible to obtain good preparations allowing a clear chromosome analysis and only 27 F_1's were analyzed cytologically. The bivalent mean of the F_1 was 21.2 per cell with a range of 8 to 34. The pairing configurations were scored at diakinesis and the number of bridges and fragments was recorded at anaphase I and early telophase I. The largest chromosome association was a chain of eight chromosomes and the maximum number of bridges observed was six. According to these results the two species may differ by three reciprocal translocations and by six paracentric inversions, but homoeologous pairing is likely to account for much of the multivalent formation because of the amount of pairing observed. The mean of bivalents in the BC_1 progeny was 15.19 with a range of 10 to 24. Trivalents averaged 1.57 per cell. In this hexaploid X diploid cross the pairing is very good and suggests that introgression of genes from H. tuberosus to the genome of the cultivated H. annuus may not be difficult.

Introduction

Interspecific hybrids of sunflowers have not been as widely studied as in other crops (1, 3, 5,), however, some have been reported using diploid, tetraploid, and hexaploid species (2, 4, 6,10, 11, 12, 13). Interspecific hybrids using the cultivated sunflower, Helianthus annuus L. (2n = 34), and the wild hexaploid, Helianthus tuberosus L. (2n = 102), were reported as early as 1937 by Pustovoit, cited by Stoenescu (9) in breeding programs aiming to obtain disease resistance.

The most recent report concerning the use of H. tuberosus was published by Cauderon (2) in 1965. In this experiment the author used one clone of the hexaploid species in crosses with two open pollinated varieties of the cultivated sunflower. The number of bivalents ranged from 6 to 34, the largest chromosome association was a chain of six, and the maximum number of bridges was five with a frequency of 0.04 per cell. The pairing was very good but some hybrids could not be analyzed because of the tendency of the chromosomes to stick together preventing accurate analysis. The presence of multivalents was suggested to be due to both reciprocal translocations and homoeology between the genomes of the two species.

Materials and Methods

Eleven populations of H. tuberosus were sampled in the summer of 1981 and were used as male parents in crosses with the H. annuus inbred line HA89. The hexaploid condition of the wild species was verified by PMC observations. F_1 hybrids from crosses were verified by root tip and PMC chromosome analysis.

In the summer of 1982, only nine populations of H. tuberosus were used and reciprocal crosses were produced. Hybrids obtained the previous year were backcrossed to HA89. All nine populations plus the inbred line used as parents were sampled for pollen stainability and microsporogenesis study. Root tips were collected and placed in ice water for 10 to 14 hours, fixed in 3:1 ethanol-glacial acetic acid solution and stained with Feulgen stain for two hours after hydrolysis for ten minutes in 1N HCl at 60 C. Buds were collected and fixed in the same solution for 24 hours and transferred to 70% ethanol. Individual anthers were squashed in a drop of acetocarmine for cytological observation and, if the proper stage was found, the other anthers in the floret were stained by the procedure explained by Snow (8).

In the summer of 1983, tubers were collected from the wild populations and planted in the green house together with part of the F_1 seed to produce additional BC_1 progeny. Chromosome associations and meiotic configurations were studied at diakinesis and anaphase I of the F_1 .

Backcross seed was planted in the fall of 1983 and the plants were sampled for pollen stainability and PMC analysis.

Results

Eight hundred sixteen seeds were obtained from the cross H. annuus X H. tuberosus in the summer of 1981. All plants produced from the crossed seed that germinated had 34 chromosomes with the exception of one plant that was profusely branched, male and female sterile and did not produce tubers. A large number of univalents were observed in the PMC's of the exceptional plant. The univalent mean was 51.3 per cell and ranged from 34 to 62 and the bivalent mean was 5.3 and ranged from 0 to 14 over a total of 15 cells examined. The pollen stainability was 0.5% reflecting the failure of chromosome pairing. The largest chromosome association was a chain of six.

Five seeds were obtained from the reciprocal cross of H. tuberosus population 6 X HA89 and one seed germinated to produce a mature plant. This plant had 68 chromosomes, produced many tubers and had pollen stainability of 11.7%. Pairing was greater in this hybrid with a mean of 23.41 bivalents and ranged from 17 to 32 per cell over a total of 22 observed. Seed was produced when backcrossed to HA89.

A total of 93 hybrids were obtained from the cross HA89 X H. tuberosus (cross 1) and 117 were obtained from the reciprocal cross (cross 2), during the summer of 1982 and 1983. Pollen fertility was scored for 180 plants. No differences were found in pollen stainability of reciprocal crosses. Mean pollen fertility was 17.21% and ranged from 1.2 to 34.2%. Nine plants out of 180 did not produce tubers.

PMC analysis of 27 hybrids was performed. The number of cells scored at diakinesis was variable for each hybrid and ranged from 8 to 22. Most of the sterile pollen grains had one or two spherical nuclei instead of the two thread-like sperm nuclei that are clearly visible in normal gametes.

Pollen size was scored for the parental types and for the hybrids. The mean size of HA89 was 31.00 u with a variance of 0.161. The pollen size for the wild populations ranged from 30.96 to 39.83 u. The variance ranged from 0.318 to 4.499 with the less fertile populations having the largest variances. Pollen stainability ranged from 52.3 to 99.1%.

The most frequent chromosome associations in the F_1 were bivalents; however, univalents and multivalents were also present in variable frequencies. The univalent mean per cell was 18.6 and ranged from 0 to 48. The bivalent mean was 20.6 and ranged from 8 to 34. The largest chromosome association found was a chain of eight.

Study of 27 hybrids at anaphase I allowed scoring of bridges and fragments. The number of fragments was usually larger than the number of bridges. Fragments were detected as early as diakinesis and metaphase I. Some of the cells could not be analyzed because of the presence of complex meiotic configurations. The maximum number of fragments scored at anaphase I was 22 and the maximum number of bridges was six.

One hundred and eighty seven BC_1 progeny were obtained and 170 were grown to maturity. Sixty two BC_1 progeny were obtained from the cross HA89//H.tuberosus/HA89 and HA89//HA89/ H.tuberosus and 125 from crosses using the reciprocal F_1 as the female parent. Tubers were present in 58 BC_1 plants. The mean pollen stainability for the progeny was 3.7 and ranged from 0.0 to 11.6%. The sterile pollen grains were mostly large and binucleate while a few were small and colorless. Pollen formation among the BC_1 progeny was variable and ranged from 0 (no pollen) to 5 (100% pollen formation). Thirty plants scored 0 and fifteen scored 5. PMC analysis of plants scored zero and many that scored 1 or 2 was not possible because of the few PMC produced.

Meiotic analysis of the 27 BC_1 progeny studied was difficult due to the overlapping of chromosomes. The meiocytes observed had 51 chromosomes with a univalent mean per cell of 12.55 and a range from 9 to 34. The mean of bivalents per cell was 15.19 and ranged from 10 to 24. Trivalents were present in a larger frequency than observed for the F_1 hybrids and averaged 1.57 per cell. The plants with no pollen and a selected group of the others were used as female parents in backcrosses to cultivated sunflowers. The largest chromosome association was a chain of six.

Discussion

Many F_1 hybrids could not be analyzed cytologically because of the tendency of the chromosomes to stick together and the formation of complex meiotic configurations. However, the hybrids studied indicated that a relatively large amount of pairing occurs in the interspecific cross. The pollen fertility of the hybrids ranged from 0 to 34% . Telophase II cells were found with up to seven micronuclei, some of which apparently resulted in micro-

spore production. Formation of pentads instead of normal tetrads was reported by Jackson (7) in 1973. Extra microspores were formed by the micronuclei and/or fragments present in the nucleus. In future experiments, the frequency of micronuclei which result in microspore production will be scored to get a better estimate of sterility due to meiotic irregularities of the F_1 . According to the chromosome pairing observed, greater fertility in the F_1 is expected than was observed. The study of the pollen size indicates that uniformity of pollen is a better criteria for assessing pollen fertility than pollen size. The variability in pollen size in the most fertile parental populations was small in comparison to the variances observed in the F_1, the backcrosses, and in the less fertile populations of the wild species.

The largest association observed in the interspecific F_1 at diakinesis was a chain of eight chromosomes, however, such large configurations occurred at a low frequency. The multivalents were usually present in chains and rarely in rings and may be derived from either interchanges or homoeologous pairing.

The number of fragments was larger than the number of bridges which may be due to abnormalities observed in diakinesis and metaphase I. A detailed study of diakinesis and MI, scoring the frequency of fragments, may help in determining the number of fragments originating at anaphase I accompanying bridge formation. The maximum number of bridges scored was six which may be underestimated because of the elimination of cells with complicated meoitic figures from the analysis.

The results obtained indicate that the two species may differ by as many as three reciprocal translocations and six paracentric inversions. However, homoeology between the genomes of the two species is likely to be responsible for much multivalent formation. In a previous report by Cauderon (2) the largest chromosome association scored was a chain of six. The larger sample of genotypes used in this study could account for different observations in the two experiments. In the previous experiment Cauderon used one clone of the wild species as female parent with two varieties of the cultivated sunflower, while in the present work nine different populations of the hexaploid parent were studied and used in the crosses. Cauderon also indicated that microsporogenesis in the clone used was normal with no major abnormalities observed. In the present study seven of the nine populations had meiotic abnormalities and in two of them the effect on pollen fertility was noticeable. Population 4 had a ring of four chromosomes in 37 of 98 cells observed and had pollen stainability of 52.3%. Populations 5 and 12 had chains of six chromosomes in 10 out of 42 and 50 cells observed, respectively.

On the whole the BC_1 progeny showed less meiotic abnormalities than the F_1 . The frequency of trivalents was higher than observed for the F_1 as should be expected in triploid plants. The mean fertility of the BC_1 hybrids was very low due to the triploid condition. The observations indicate that abnormalities observed in the F_1 may be reduced in further backcrosses which in turn may result in better pollen fertility for the progeny. The BC_2 seed obtained will be planted for future study.

Pollen formation was very variable among the BC_1 progeny. Plants with no pollen were all crossed as the female parent to a normal line and to a restorer line. Plants scored zero and some that scored 1 or 2, had too few PMC's to make a reliable chromosome analysis.

Observations from this study suggest that introgression of genes from H. tuberosus into H. annuus should not be seriously impeded by a lack of chromosome pairing and low fertility in the progeny of the interspecific and backcross hybrids. Fertility of progeny should improve with backcrossing.

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References

1. BEARD, B.H. and CHANDLER, J. M. 1982. Utilization of wild Helianthus in germplasm development. Poceedings Sunflower 10th International Conference, Surfers Paradise, Australia. p 207-209.

2. CAUDERON, Y. 1965. Cytogenetic analysis of hybrids between Helianthus tuberosus and H. annuus . Consequences on selection (in French). Annales de L'amelioration des plantes. Institut National De La Recherche Agronomique, Paris. 15(3):243-261.

3. FICK, G.N., ZIMMER, D. E. and THOMPSON, T. E. 1976. Wild species of Helianthus as a source of variability in sunflower breeding. Proceedings Sunflower Forum, p 4-5.

4. GEORGIEVA,T.Y. and LAKOVA, M. 1979. Hybridization of Diploid sunflower, H. annuus (2n = 34), with some Tetraploid Helianthus species. Z. Pflanzenzuchtg. 83: 340-349.

5. HADLEY, H.H., and OPENSHAW, S. J. 1980. Interspecific and Intergeneric Hybridization. p 133-159. In W.R. Fehr and H.H. Hadley, Hybridization of Crop Plants. p 133-159. Am. Soc. of Agron., Madison, Wis.

6. HEISER, C. B. 1951. Hybridization in the annual sunflowers: Helianthus annuus X H. agrophylus . The American Naturalist. 85(820):65-72.

7. JACKSON, R. C. 1973. Chromosomal evolution in Haplopappus gracilis: a centric transposition race. Evolution 27:243-256.

8. SNOW, R. 1963. Acoholic hydrochlóric acid-carmine as a stain for chromosomes in squash preparations. Stain Technology 38(1):9-13.

9. STOENESCU, F. 1974. Breeding. p 125-179. In A. V. Vranceanu (ed.) Sunflower. The Academy of Romania Socialist Republic. Translated from Rumanian for the Agricultural Research Service U.S.D.A. and the National Science Foundation.

10. WHELAN, E. D. 1978. Hybridization between annual and perennial diploid species of Helianthus . Can. J. Genet. Cytol. 20:523-530.

11. WHELAN, E. D. 1978. Cytology and Interspecific Hybridization. p 339-369 In J.F. Carter (ed.) Sunflower Science and Technology. Am. Soc. of Agron., Madison, Wis.

12. WHELAN, E. D. 1979. Interspecific hybrids between Helianthus petiolaris and H. annuus : Effect of backcrossing on meiosis. Euphytica 28: 297-308.

13. WHELAN, E. D and DORRELL, D. G. 1980. Interspecific hybrids between Helianthus maximiliani Schrad. and H. annuus L.: Effect of Backcrossing on Meiosis, Anther Morphology, and seed Characteristics. Crop Sc. 20: 29-34.