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CYTOGENETIC STUDY OF HEXAPLOID SPECIES *HELIANTHUS TUBEROSUS* AND ITS F_1 AND BC_1F_1 HYBRIDS WITH CULTIVATED SUNFLOWER, *H. ANNUUS*

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Helianthus tuberosus is a potential source of resistance to many disease-provoking pathogens. Three accessions of *H. tuberosus* were used in this research and they were crossed with cultivated sunflower. Six F_1 and two BC_1F_1 hybrid combinations were obtained. Analysis of meiosis was performed using aceto-carmine method (GEORGIEVA-TODOROVA, 1976) and pollen viability was determined by staining method of ALEXANDER (1969). Meiosis was regular in cultivated sunflower and the pollen viability was high (96.8-98.9%). Low percent of irregularities was found in the meiosis of *H. tuberosus*. Pollen viability was high (97.2-98.7%). Chromosome pairing was mostly regular in F_1 hybrids (34 bivalents), but some meiocytes contained 28-32 bivalents with uni- and quadrivalents present. The percent of meiocytes with fast chromosomes in metaphase was 24.6-87.2, with lagging chromosomes in anaphase I 10.5-81.0 and in telophase 25.0-33.3. Chromosome bridges were detected in 0-9.9% of meiocytes in anaphase. Pollen viability in F_1 hybrids ranged from 27.0 to 47.9%. In BC_1F_1 hybrids, number of bivalents

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was 16-25, univalents 2-18 and multivalents 0-1. Although a triploid set of chromosomes (51) was expected in BC_1F_1 hybrids, number of chromosomes was 45-57. Pollen viability varied from 0 to 54.3%.

Key words: Cultivated sunflower, *Helianthus tuberosus*, interspecific hybridization, meiosis, pollen viability.

INTRODUCTION

Sunflower belongs to the genus *Helianthus*, family *Asteraceae*. The classification and taxonomy of the *Helianthus* genus has continuously been changed and rewritten. Many authors described large number of species: WATSON (1929), HEISER et al. (1969), ROBINSON (1979) and others. Perennial species *H. tuberosus* belongs to the section *Divaricati*, series *Atrorubentes. H. tuberosus* is a hexaploid species with 2n(6x)=102 chromosomes (SCHILING AND HEISER, 1981).

H. tuberosus is known to be a potential source of resistance to several sunflower pathogens. Large number of authors used *H. tuberosus* as a source of resistance to downy mildew (P*lasmopara helianthi*). Interspecies hybridization was used to transfer resistance to cultivated sunflower. Resistance to different forms of white rot and resistance to rust have also been found in *H. tuberosus* and successfully transferred to cultivated sunflower. ŠKORIĆ (1989) found that resistance to *Phomopsis helianthi* is present in *H. tuberosus* and some other wild sunflower species. Author assumed that the resistance to *Phomopsis helianthi* and *Alternaria helianthi* is under the influence of linked genes. Sources of resistance to broomrape are present in several wild species, but the largest frequency of those genes can be found in *H. tuberosus* (ŠKORIĆ and JOCIĆ, 2005).

The use of hexaploid species *H. tuberosus*, in the crosses with cultivated sunflower is accompanied by many difficulties because of different number and structure of chromosomes.

Disease resistance is a ″desired″ trait that is transferred to cultivated sunflower by interspecies crossing, unfortunately, some ″undesired″ traits are also transferred (branching, small inflorescences, low oil content, etc.). Backcrossing is used to overcome this problem (F_1 interspecies hybrid X cultivated sunflower) but it also affects the ″desired″ traits. Cytogenetic analysis (meiosis and pollen viability) and molecular markers (RAPD), can be used to estimate the quantity of the parental genomes present in F_1 and BC_1F_1 interspecies generation (ATLAGIĆ et al., 2003).

The objective of this study was to perform cytogenetic analysis of the wild species *H. tuberosus* and its interspecies hybrids with cultivated sunflower, obtained with the goal of transferring the ″desired″ genes for disease resistance.

MATERIALS AND METHODS

The experimental material consisted of: cultivated sunflower (Pic. 1.) (inbred lines in sterile (A) and fertile (B) form), hexaploid species *H. tuberosus* (Pic. 2.) represented with three populations (6, 20, 1698), hybrid combinations obtained by direct and reciprocal crosses of *H. tuberosus* populations and the lines of cultivated sunflower in F_1 generation (Pic. 3.) (**1.** *H. tuberosus* 1698 x L-1; **2.** *H. tuberosus* 1698 x OCMS 22; **3.** *H. tuberosus* 1698 x OCMS 74; **4.** HA-26A x *H. tuberosus* 1698; **5.** HA-26A x *H. tuberosus* 6; **6.** HA-26A x *H. tuberosus* 20) and the first backcross (Pic. 4.) (F_1 interspecies hybrid x cultivated sunflower line), **1.** (HA-26A x *H. tuberosus* 6) x HA-26B; **2.** (HA-26A x *H. tuberosus* 1698) x HA-26B.

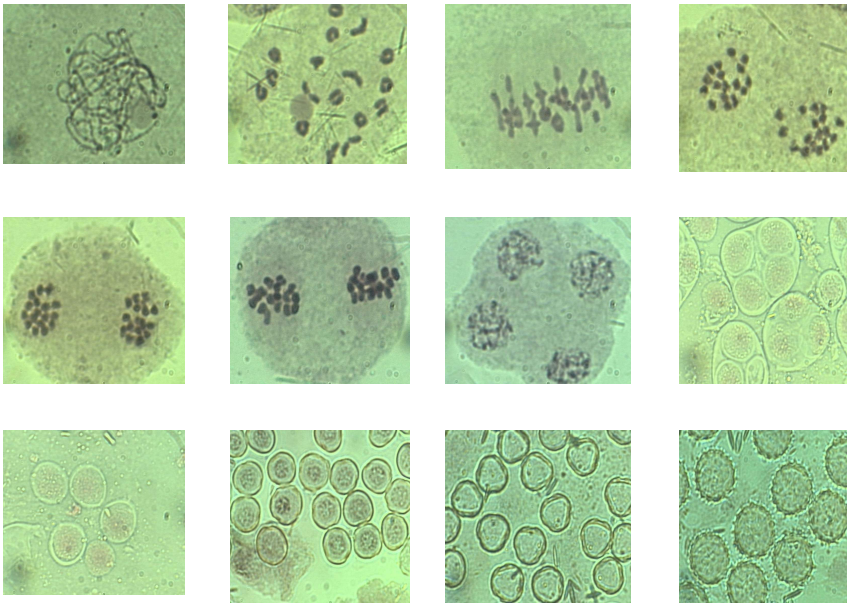
Cytogenetic methods were used to analyze the pollen viability and regularity of meiosis. Pollen viability was determined using the Alexander staining method (ALEXANDER, 1969). Meiosis was analyzed by the aceto-carmine method (GEORGIEVA-TODOROVA, 1976).

Pic. 1. Lines of cultivated sunflower

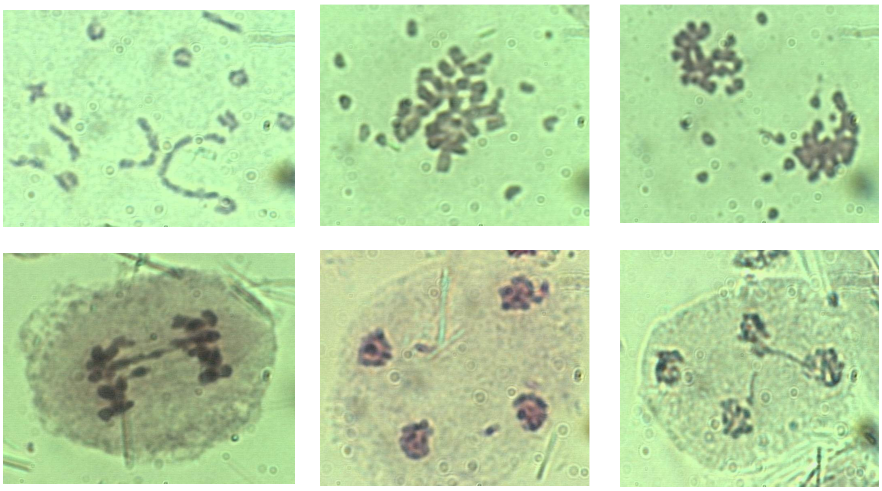
Pic. 2. *Helianthus tuberosus*

Pic. 3. F_1 (HA26 x *H.tuberosus*)

Pic. 4. BC_1F_1 (HA26x*H.tuberosus*)xHA26



Pic. 5. Regular phases of meiosis in cultivated sunflower: a) pahiten, b) diakinesis, c) metaphase I, d) anaphase I, e) telophase I, f) metaphase II, g) telophase II, h) tetrads, i) uninuclear microspore, j, k) microspores, l) pollen grains



Pic. 6. Irregular phases of meiosis in sunflower: a) diakinesis with quadri- and hexavalent, b) metaphase I with fast chromosomes, c) anaphase I with lagging chromosomes, d) anaphase I with a chromosome bridge, e) telophase II with lagging chromosomes, f) telophase II with chromosome bridges

Regularity of meiosis was examined by analyzing diakinesis, metaphase I, anaphase I and telophase II. All phases were analyzed, either normal (Pic. 5.), or with different irregularities (Pic. 6.).

RESULTS

Meiosis proceeded normally in the lines of cultivated sunflower. All analyzed samples had 17 bivalents in diakinesis. Analysis of meiosis in the populations of the wild species *H. tuberosus* indicated that it is a hexaploid species, with 51 bivalent found in diakinesis.

Regular pairing of chromosomes with 34 bivalents was found in diakinesis of F_1 hybrids from the cross between population *H. tuberosus* 6 and the line of cultivated sunflower. Although hybrids from the cross between population *H. tuberosus* 20 and the line of cultivated sunflower most often had 34 bivalents, meiocytes containing 28 to 31 bivalents were also found, with quadri- and univalents present. Diakinesis of F_1 hybrid with population *H. tuberosus* 1698 was similar to the one with *H. tuberosus* 20. Majority of meiocytes had 34 bivalents in diakinesis (regular chromosome pairing), but configurations with 30 to 32 bivalents and quadri- and univalents were also found (Tab 1.).

Table 1. Chromosome configuration in parent species, F_1 and BC_1F_1 interspecies hybrids

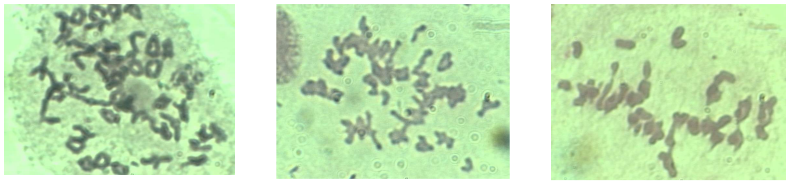
Population	Configuration in diakinesis
HA-26	34^{II}
H. tuberosus	51^{II}
F_1 *H.tuberosus* 6	34^{II}
F_1 *H.tuberosus* 20	$34^{II}(3)$; $28^{II}2^{IV}4^{I}(1)$; $29^{II}1^{IV}6^{I}(1)$; $30^{II}2^{IV}2^{I}(1)$; $31^{II}1^{IV}2^{I}(1)$
F_1 *H.tuberosus* 1698	$34^{II}(8)$; $30^{II}2^{IV}(1)$; $31^{II}1^{VI}(1)$; $32^{II}1^{IV}(1)$
F_1BC_1 *H.tuberosus* 6	$23^{II}8^{I}(2)$; $16^{II}1^{IV}1^{III}6^{I}$+4F(1); $24^{II}4^{I}(1)$; $20^{II}12^{I}$+2F(1); $19^{II}9^{I}(1)$ $25^{II}6^{I}(2)$; $21^{II}9^{I}(2)$; $23^{II}8^{I}(2)$; $20^{II}13^{I}(1)$; $21^{II}12^{I}(1)$; $21^{11}10^{I}(1)$;
F_1BC_1 *H.tuberosus* 1698	$19^{II}14^{I}(1)$; $24^{II}6^{I}(1)$; $18^{II}12^{I}(1)$; $20^{II}1^{III}$ $7^{I}(1)$; $18^{II}18^{I}(1)$; $23^{II}4^{I}$+2F(1); $16^{II}17^{I}(1)$; $19^{II}6^{I}(2)$; $23^{II}3^{I}$+1F(1); $20^{II}16^{I}$+2F(1); $20^{II}1^{IV}9^{I}$+2F(1); $25^{II}6^{I}(1)$; $21^{II}15^{I}(1)$; $16^{II}13^{I}(1)$; $18^{II}9^{I}(1)$; $22^{II}7^{I}$+2F(1); $21^{II}6^{I}(1)$; $24^{II}2^{I}$+2F(1); $17^{II}14^{I}(1)$

( ) – Number of meiocytes with indicated chromosome configuration

BC_1F_1 hybrid plants from the cross F_1 TUB6 x cultivated sunflower most often had 16-24 bivalents with large number of univalents (4-12). Some meiocytes contained tri- and quadrivalents as well as fragments (2-4). Chromosome number varied between 45 and 55 (Tab. 1., Pic. 7.).

Large number of meiocytes with different irregularities in diakinesis and metaphase I was found in hybrid plants from the backcross F_1 TUB1698 x cultivated sunflower. Bivalents (16-25) and univalents (2-18) were most frequent. Number of chromosomes varied between 45 and 47. Analyzed phases of diakinesis

and metaphase I showed bivalents and univalents, but also quadri- and trivalents, as well as one or two fragments.



Pic. 7. Chromosome configuration in a) *H.tuberosus*, b) F_1 *H.tuberosus*, c) BC_1F_1 *H.tuberosus*

Metaphase I with fast chromosomes was found in 24.59-87.20% of meiocytes in F_1 hybrid combinations and 21.43-57.90% of meiocytes in BC_1F_1 hybrids (Tab. 2.). Large percent of lagging chromosomes was typical for anaphase I of F_1 (10.53-81.00%) and BC_1F_1 hybrid plants (39.39-57.14%). Anaphase I with chromosome bridges was found in 0-9.9% of meiocytes in F_1 hybrids and 0-3.3% of meiocytes in BC_1F_1 hybrids. Telophase II of F_1 hybrid plants was irregular, with lagging chromosomes found in 25.00-33.33% of meiocytes, while BC_1F_1 plants had 7.14-21.13% of such meiocytes (Tab. 2.).

Completely male sterile plants were found in F_1 hybrid generation and among BC_1F_1 hybrid plants. Pollen viability of male fertile F_1 and BC_1F_1 interspecies hybrids was lower than the parents and it ranged from 27.01-47.98% for F_1, and 37.12-54.30% for BC_1F_1 hybrids (Tab. 2.).

DISCUSSION

Many authors studied meiosis of F_1 interspecies hybrids between *H. tuberosus* and cultivated sunflower (HEISER et al., 1969; GEORGIEVA-TODOROVA, 1990; ATLAGIĆ et al., 1993; ESPINASSE et al., 1995). KOSTOFF (1939, as cited in ATLAGIĆ et al., 1993) was the first to find high percent of bivalents and univalents in meiosis, whilst percent of multivalent configurations was reported to be low.

While analyzing meiosis of F_1 hybrids between *H. tuberosus* and cultivated sunflower, GEORGIEVA-TODOROVA (1990) found 30-31 bivalent in diakinesis and the rest of the chromosomes were uni- or quadrivalents. Fast chromosomes were typical for metaphase I (92% of meiocytes), while 76% of anaphase meiocytes contained lagging chromosomes and chromosome bridges. Cited results that were obtained through analysis of meiosis are most similar to the results of CEDENO et al. (1985) who found that F_1 hybrids (*H. annuus* x *H. tuberosus*) have high percent of meiocytes with bivalents and that only several meiocytes had multivalents besides bivalents.

Large number of authors (WHELAN, 1979; WHELAN and DORRELl, 1980; ATLAGIĆ, 1996; ATLAGIĆ and ŠKORIĆ, 1999; ATLAGIĆ, 2000) emphasizes that BC_1F_1 interspecies hybrids frequently have higher percent of irregularities in meiosis than F_1 interspecies hybrids. They also found aneuploids, plants with different number of chromosomes, and lowered pollen viability in male fertile plants or complete male sterility.

Configurations in diakinesis and metaphase I (16-25 bivalents; 2-18 univalents; 0-1 multivalents) of analyzed BC_1F_1 interspecies hybrids with hexaploid species *H. tuberosus* presented in this paper, are very similar to the ones that were found in BC_1F_1 interspecies hybrids with the species *H. laevigatus* and *H. rigidus* (ATLAGIĆ and ŠKORIĆ, 1999; ATLAGIĆ, 2000). All three studies revealed chromosome configurations that indicate highly irregular chromosome pairing. Bivalents were formed not only by homologue chromosome pairing but also by homologue chromosome pairing and autosyndesis of chromosomes from the genomes of each parent species. Large number of univalents is an indication that parent species differ not only in number, but also in structure of the chromosomes. Univalents are really non-paired chromosomes of the hexaploid species *H. tuberosus*, while multivalents result from the pairing of non-homologue chromosomes of the crossed species. After backcrossing the tetraploid (2n=4x=68) F_1 interspecies hybrid generation with diploid (2n=2x=34) *H. annuus*, triplod plants are expected (2n=3x=51) in BC_1F_1 generation and the results show large number of aneuploid plants.

GEORGIEVA-TODOROVA (1984) also found large number of aneuploid plants in BC_2F_1 interspecies cross between *H. annuus* (2n=34) and *H. decapetalus* (2n=68). The number of chromosomes varied from 35 to 68.

CONCLUSION

- Meiosis was regular in the lines of cultivated sunflower that were used for interspecies crosses. Pollen viability was high and it varied from 96.8 to 98.9%;
- Hexaploid species *H. tuberosus* had regular meiosis (51 bivalent – normal chromosome pairing) and very low percent of meiocytes with non-included chromosomes;
- F_1 hybrid plants obtained through interspecies cross between *H. tuberosus* and the line of cultivated sunflower most often had 34 bivalents in diakinesis (normal chromosome pairing). However, meiocytes with 28 to 32 bivalents and uni- and quadrivalents were also found. ″Non-included″ chromosomes were detected in 24.59-87.20% of meiocytes in metaphase I, 10.53-81.00% in anaphase I and 25.00-33.30% in telophase II. Chromosome bridges were found in 0-9.9% of meiocytes in anaphase I. Pollen viability of F_1 interspecies hybrids was lower than in the parent species and it varied from 27.01 to 47.98%;

- Chromosome pairing was highly irregular in BC_1F_1 hybrids. Number of bivalents per meiocyte varied from 16 to 25. Univalents were very frequent (2-18), while the number of multivalents was 0-1. Although a triploid set of chromosomes (51) was expected, BC_1F_1 hybrids had 45 to 57 chromosomes. Pollen viability was lowered and it varied from 0 to 54.30%.
- The results presented here indicate that the transfer of desirable genes from the hexaploid wild species *H. tuberosus* to the cultivated sunflower and their stabilization is accompanied with many difficulties.

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CITOGENETSKA ISPITIVANJA HEKSAPLOIDNE VRSTE *HELIANTHUS TUBEROSUS* I NJENIH F_1 I BC_1F_1 HIBRIDA SA GAJENIM SUNCOKRETOM, *H. ANNUUS*

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I z v o d

H.tuberosus predstavlja potencijalni izvor otpornosti prema mnogim prouzrokovačima bolesti. U istraživanjima su korišćene tri populacije vrste *H.tuberosus* koje su ukrštane sa gajenim suncokretom. Dobijeno je 6 hibridnih kombinacija F_1 i 2 BC_1F_1 gen. Izvršena je analiza mejoze acetokarmin metodom (Georgieva-Todorova, 1976) i vitalnosti polena bojenom metodom (Alexander, 1969). Mejoza kod gajenog suncokreta proticala je normalno, a vitalnost polena bila visoka (96.8-98.9%). Kod vrste *H.tuberosus* mejoza je proticala uz nizak % nepravilnosti tipa neuključenih hromozoma. Vitalnost polena je bila visoka (97.2-98.7%). Kod F_1 hibrida najčešće je bilo normalno parenje hromozoma (34 bivalenta), a zabeležene su mejocite sa 28-32 bivalenta uz pojavu uni- i kvadrivalenata. Procenat mejocita sa izbeglim hromozomima u metafazi I je bio 24.6-87.2, sa izostalim hromozomima u anafazi I 10.5-81.0, a u telofazi II 25.0-33.3. Hromozomski mostovi su detektovani u 0-9.9% anafaznih mejocita. Vitalnost polena kod F_1 hibrida se kretala od 27.0-47.9%. Kod BC_1F_1 hibrida broj bivalenata je bio od 16-25, univalenata od 2-18, a multivalenata od 0-1. Iako je očekivan triploidan broj hromozoma (51) kod BC_1F_1 hibrida broj hromozoma je bio od 45-57. Vitalnost polena se kretala 0-54.3%.

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