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NEW SUNFLOWER (*H. ANNUUS* L.) LINES AS RESULTS OF INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION AND APPLICATION OF METHOD OF DIRECT ORGANOGENESIS IN F1 IMMATURE EMBRYO

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Abstract

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The direct organogenesis method in immature F1 embryos from sunflower was successfully applied for production of new forms from the interspecific crosses *H. annuus* (cv. Albena) x *H. tuberosus*, *H. annuus* (cv. Albena) x *H. salicifolius* and intergeneric cross *Helianthus annuus* (cv. Albena) x *Verbisina helianthoides* (genera *Verbisina*). After repeated selfing and continuous selection, a great diversity of new sunflower lines were developed. The available literature does not provide data on the obtaining of these hybrid combination by direct organogenesis method. The applied molecular method (RAPD) confirmed the hybrid nature of the obtained breeding material and indicated an introgression of *H. tuberosus* and *V. helianthoides* DNA into some of the hybrid progenies produced. Our results are also the first attempt to investigate the combining ability of the new restorer lines produced through interspecific and intergeneric hybridization. Some of the new lines possess resistance to important diseases and parasite broomrape and very good combining ability. The new hybrids developed showed an increase of the indexes seed yield and oil yield in comparison to the mean standard (commercial hybrids Albena and Super start).

Key words: Helianthus annuus, *H. tuberosus*, *H. salicifolius*, *Verbisina helianthoides*, organogenesis, new breeding material, RAPD analysis, resistance, *Phomopsis helianthi*, *Phoma macdonaldii*, *Alternaria helianthi*, *Plasmopara helianthi*, *Orobanche cumana*

Introduction

The changed climatic conditions consisted development of new genetic variability suitable to cover increased necessities of sunflower growers. Because of limited genetic base of this crop, new approaches should be applied in sunflower breeding programme.

Investigation on the productivity of cultivated sunflower showed that the diseases are the most important and limiting factor in the majority of sunflower growing countries. The widely spread diseases such as *Phomopsis helianthi*, *Phoma macdonaldii*, *Alternaria helianthi* and *Plasmopara helianthi* and parasite *Orobanche cumana* drastically decrease sunflower yield especially in central and southern Europe. The wild *Helianthus* species are potential source of genes for resistance to diseases (Georgieva-Todorova, 1993, 1997; Sackston, 1992; Seiler, 1992; Skoric and Rajcan, 1992; Skoric, 1994). Some accessions of *H. eggertii*, *H. strumosus*, *H. laevigatus, H. hirsutus* and *H. mollis* showed a high degree of resistance to *Sclerotinia*. (Christov, 1987 by Georgieva-Todorova, 1990). By using the embryo cultural method, hybrid plants resistant to *Phomopsis helianthi* (Dozel et al., 1996) and partial resistance to *Sclerotinia* (Köhler et al., 1999) were produced as a result from the crossing to the wild species *Helianthus tuberosus*.

Biotechnological methods have been developed which aim to increase the available genetic variability of the initial breeding material or to intensify the breeding process by applying appropriate methods for evaluation and selection of desirable genotypes.

The *embryo rescue* technique is most commonly used for overcoming the incompatibility between *H. annuus* L. and dif-

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ferent alien species; it however does not always contribute to the production of hybrid plants (Chandler and Beard, 1983; Krauter et al., 1991; Korell et al., 1996a; Bohorova et al.; 1991; Friedt, 1992). This allowed us to investigate the possibilities of the direct organogenesis method, which has not been applied up to now as an approach for overcoming the interspecific and intergeneric incompatibility in sunflower hybridization (Encheva et al., 1992). In comparison to the authors mention above our study presented data on the hybrid progenies at advanced generation, as well as data on their combining ability.

The aim of this study was to: a/ follow the reaction of the hybrid progenies in generation F10 from the interspecific cross *H. annuus* (cv. Albena) x *H. tuberosus*, *H. annuus* (cv. Albena) x *H. salicifolius* and intergeneric cross *H. annuus* (cv. Albena) x *Verbisina helianthoides* (genera *Verbisina*) produced through the direct organogenesis method, to the diseases *Phomopsis helianthi*, *Phoma macdonaldii*, *Alternaria helianthi* and *Plasmopara helianthi* and local population of parasite *Orobanche cumana;* and b/ to investigate the combining ability of the new fertility restorer lines.

Materials and Methods

Cultural sunflower (hybrid Albena – 2n = 34) and the wild species *H. tuberosus* (2n = 64), *H. salicifolius* (2n = 34), *Verbisina helianthoides* (2n = 34) – genera *Verbisina* were grown under field conditions at DAI-General Toshevo.

After sterilizing the pollen of the female parent (hybrid Albena) with GA_3, hand pollination with pollen from the male parent *H. tuberosus*, *H. salicifolius*, and *Verbisina helianthoides* was made. Direct somatic buds and plants were induced after plating of hybrid immature zygotic 13-15 days embryos on nutrition media I, II and III (Encheva et al., 1992). As a result from repeated selfing and continuous individual selection in the hybrid materials, fertility restorer lines were produced in F_{10} generation. All hybrid materials possessed a CMS source of *H. petiolaris* from Leclercq (1969). In the crosses made, the mother form had sterile cytoplasm of *H. petiolaris* and therefore only the fertile forms were considered in this research work, i.e. the ones possessing a genes for restoration. The origin of this gene has not been proved by us. It may originate both from the mother form hybrid Albena, and from the wild father parents; there is evidence that they carry genes for restoration of this cytoplasm (Christov and Vassilevska, 1999).

The phytopathological evaluation of the parental forms and the obtained stabilized hybrid progenies in F10

This was performed with regard to the local broomrape population (*Orobanche cumana* Wallr.), phomopsis (*Phomopsis helianthi*, Munt.-Cvet. et al.), phoma (*Phoma macdonaldii* Boerema/*Phoma oleracea* var. *helianthi-tuberosi* Sacc.), alternaria (*Alternaria helianthi*) and downy mildew (*Plasmopara helianthi* Novot.-race 700) at the Sunflower Phytopathology Laboratory and infection fields of DAI, General Toshevo, during the period 1997-1999.

Broomrape resistance was evaluated under greenhouse conditions according to Panchenko (1975), slightly modified to local conditions. A mixture of soil and sand (2:1) was prepared, and 0,2 mg broomrape seeds were added to each kilogram of the mixture. Sunflower was grown in this substrate in the following order: 50 plants + 10 plants (standard-AD-66) in every container. They were placed in a greenhouse under controlled conditions with irrigation. Forty-five days after planting, the roots of all sunflower plants were cleaned and checked for the existence of the parasite. Broomrape resistance was calculated as percentage of non-infected plants. The reaction of 50 plants from each genotype was recorded using the following scale: 0% = S (sensitive); 100% = R (resistant).

According Peres and Regnault (1986) the natural infection of grey and black spots was estimated at the middle of August. The plant residues (stems) showing symptoms were collected from the field. At stage 5-6th leaf of sunflower 3-4 stems per m^2 were scattered between the rows. Each week the plants were irrigated from the beginning of buttoning till mass flowering. The number of sprinklings was determined by the meteorological conditions and varied from 2 to 3 per week. The reaction of the lines was registered at full flowering according to the following scale for *phomopsis:* 0 - no symptoms; 1 - small single spot around the leaf petiole; 2 - spots up to 5 cm long; 3 - spots covering two or more internodules; 4 - stem breaking at the place of damage.

To estimate *phoma* ware use four level scale: 0 - no symptoms; 1 - necrotic spot around the leaf petiole; 2 - several merge necrotic spots; 3 - all stem is cover with necrotic spots. Infected plant residues (steams) with symptoms of the diseases were annually introduced in the testing plots. The stems were collected the previous year and left in the field during the winter. They were spread in zig-zag between the rows after emergence of the sunflower plants. The type and degree was estimated in phase milk ripeness on the following scales type of attack on brown spots: 0 - no symptoms; 1 - necrotic leaf spot localized around handle; 2 - merged several necrotic spots on the stem; 3 - whole stem covered with necrotic spots. Degree of attack - what part of the stem of the plant is covered with patches of the pathogen (1/3, 2/3, 3/3). In parentheses are shown number of spots.

With a view to characterizing the resistance to downy mildew were used the method suggesting by Gulya et al. (1991). The evaluation of 50 plants from each line was carried out using standard methodologies: 0% = S (sensitive); 100% = R (resistant).

Biometric evaluation of the new lines

As a result from long-term selfing and individual selection, new sunflower lines were produced in F10 generation. The lines were investigated with regard to some main characteristics concerning breeding in sunflower, also. In each generation biometric studies of plants were carried out.

The biometric evaluation of the control genotype and the newly developed lines was made on 10 plants for each individual year, and included 17 main agronomic traits as oil content in seed, 1000 seed weight, plant height, leaf width, leaf length, number of leaves, leaf petiole length, internode length, head diameter, number of branches, length of branches, diameter of branch head and stem diameter, number of ray flowers, seed length, seed width and seed thickness.

1000 seed weight (g) was determined on three samples of 50 seeds per head each.

The biometric evaluation and biochemical analysis of the new developed hybrids

The biometric evaluation and biochemical analysis was made on 30 plants in tree replications for each individual year, and included the main agronomic characters: seed yield and oil yield. Nuclear-magnetic resonance (Newport Instruments Ltd., 1972) was used to determine oil content of air dry seeds from the developed hybrids.

Hybridization

To determine the combining ability of the new developed sunflower restorer lines the sterile analogue of the Bulgarian selfed line 2607 was used. The standards for comparing the new hybrids № 15, 16, 18, 40, 46, 47, 52, 56, 64, 71 and 72 were commercial hybrids Albena and Super Start. The obtained hybrid combinations were tested during the period 1997-2000 at the breeding fields of DAI according to the block-design method, in three replications, the area of each replication being 10 m^2 (Barov and Shanin, 1965).

Results and Discussion

For the first time the method of direct organogenesis (Figure 1) has been successfully used for overcoming the inability for crossing between *H. annuus* x *H. tuberosus*, *H. annuus* x *H. salicifolius* and *H. annuus* x *Verbisina helianthoides*.

New restorer lines 101 R and 104 R with valuable agronomic characteristics were produced from the cross *H. annuus* x *H. tuberosus* (Encheva at al., 2003); 107 R, 114 R and 120 R from the cross *H. annuus* x *H. salicifolius* (Encheva and Christov, 2006a) and 131 R, 138 R, 140 R, 143 R, 144 R, 146 R from the cross *H. annuus* x *Verbesina helianthoides* (Encheva and Christov, 2005a).

Through the direct organogenesis method new lines were produced from the interspecific cross *Helianthus annuus* L. (hybrid Albena) *x H. tuberosus*. Lines 101 R и 104 R showed 64.7% intermediate phenotype in comparison to the parental lines. The positive transgressive forms were for the character number of branches, length of branches, and number of ray flowers and diameter of branched head. The negative transgressive forms were found at line 101 R and line 104 R for the indexes plant height (Figure 2). Lines 101 R and 104 R showed 122 cm and 143 cm, respectively plant height in comparison to 177.2 cm at parent hybrid Albena (with the lowest value).

Some promising R lines from the cross *H. annuus x H. tuberosus* were tested for resistance to diseases and parasite broomrape. Tolerance to *Phoma macdonaldii* Boerema demonstrated lines 101 R and 104 R. The species *H. tuberosus* showed a complete resistance to *Phoma macdonaldii* Boerema at field and 75% at artificial condition (Christov, 1996).

Evaluation for resistance to phomopsis and parasite broomrape for species *H. tuberosus* is according Scoric (1987), Seiler (1992) and Christov (1990), and to downy mildew according Scoric (1987) и Seiler (1992).

The resistance of line 104 R to the local broomrape population (up to 70%) came from the species *H. tuberosus*, which, according to Pustovoit (1978) and Christoiv (1996) showed complete resistance to the parasite under field and greenhouse conditions. Line 104 Rf was characterized with 100% resistance to downy mildew (race 700) also.

Fig. 1. Direct organogenesis method at cross *H. annuus* x *Verbisina helianthoides* (genera *Verbisina*)

The direct organogenesis method in immature F1 hybrid embryos from sunflower was successfully applied for production of new forms from the interspecific cross *Helianthus annuus* L. (hybrid Albena) *x H. salicifolius.* A considerable number of new sunflower lines were produced after self-pollination and individual selection. The lines 107 R, 114 R and 120 R show 76.5% intermediate phenotype in comparison to the two parental forms. The positive transgression was established for lines 114 R and 107 R for number of branches, length of branches and diameter of branch head. The negative transgressive forms were found at lines 107 R, 114 R and 120 R for the indexes plant height (Figure 3), leaf length and petiole length.

The phytopathological evaluation of the parental form Albena and the obtained hybrid progenies was performed with regard to the local broomrape population and the diseases phomopsis, phoma, alternaria and downy mildew. Complete resistance to phomopsis and 100% resistance to downy mildew, as well as tolerance to phoma of line R 114 for the period 1997-1999 was registered. Resistance to altenaria and tolerance to phoma was observed in line R 120. The resistance of lines R 114 and R 120 to some of the economically important diseases probably comes from the wild species *H. salicifolius* which according to the investigations of Christov (1990), Christov et al. (1996) has shown complete resistance to downy mildew, phomopsis and phoma. Among the sources of resistance to phomopsis and alternaria, Scoric, 1987, pointed out *H. salicifolius*. The mother form (hybrid Albena) is, on its part, susceptible to the diseases investigated above, with the exception of downy mildew.

Broomrape is a major diseases in parts of Europe, Spain, the Near East and China (Skoric, 1994). Therefore development of new lines resistant to this parasite is essential for sunflower breeding. As a result from interspecific hybridization line R 107 was developed, which showed 100% resistance to broomrape against artificial infection background. The resistance of line R107 comes from the wild species *H. salicifolius*, which, according to Christov et al. (1996), has complete resistance to the parasite under both field and laboratory conditions.

For the first time the method of direct organogenesis has been successfully used for overcoming the inability for crossing between *H. annuus* (h-d Albena) x *Verbisina helianthoides* (genera *Verbisina*). Larger part of lines R 131, R 140, R 143 и R 144, produced from the cross *H. annuus* x *Verbesina helianthoides* possess intermediate value of the characteristics with exception of oil in the seeds % at the two lines 138 R and 140 R (Figure 4). These lines showed positive transgression of oil in seed from 48.2% to 50.6% in comparison to 47.7% at parent hybrid Albena (with the highest value). Positive transgression was registered for indexes plant height, number of branches, length of branches, diameter of branch head and length of seed, also.

Lines 131 R, 135 R, 140 R и 144 R posses resistance to *phomopsis* and *alternaria*. Lines 140 R, 143 R, 144 R and 146 R were resistant to orobanche from 53% to 78%. The resistance to *phomopsis*, *alternaria* and *broomrape* comes from species *Verbisina helianthoides.* Mother form (hybrid Albena) is susceptible to the diseases and parasite mention above.

Molecular investigation in progeny of interspecific cross H. annuus x H. tuberosus and intergeneric cross H. annuus x V. helianthoides (genera Verbisisna)

The molecular characterization of the novel sunflower germplasm derived from interspecific and intergeneric cross was carried out by RAPD analysis. The primer OPAE-03 produced some fingerprints (400 and 1400 bp) in the hybrid progenies which were detected in the wild species *H. tuberosus*, but not

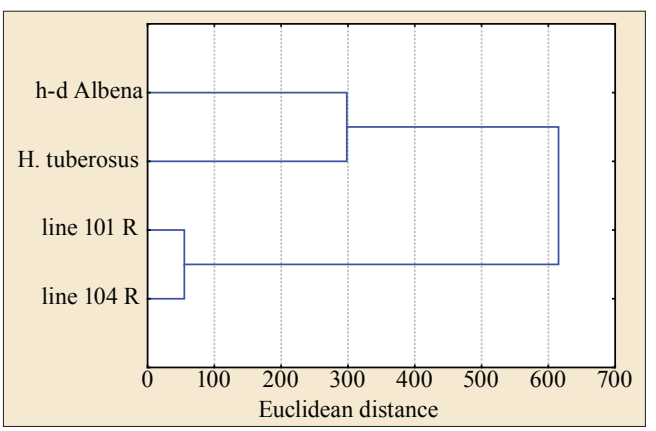

Fig. 2. Degree of similarity between lines (F10) of cross *H.annuus* (hybrid Albena) *x H. tuberosus* and parental forms, based on plant height

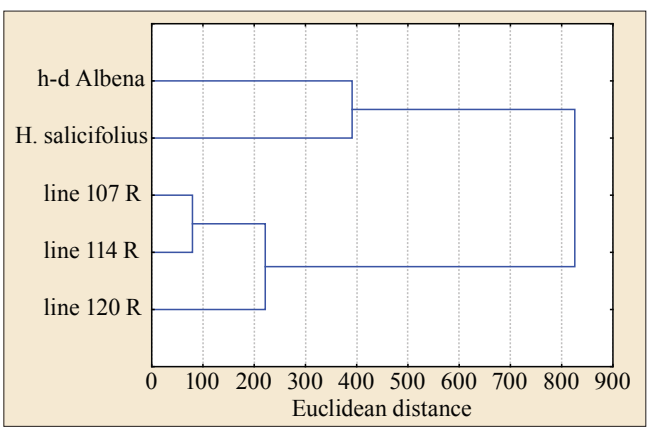

Fig. 3. Degree of similarity between lines (F10) of cross *H.annuus* (hybrid Albena) *x H.salicifolius* and parental forms, based on plant height

in the cultivated Albena (Encheva at al., 2004a). This DNA fingerprints (400 and 1400 bp) indicated an introgression of *H. tuberosus* into the lines 101 R and 104 R, respectively.

- Primer OPAJ-19 produced some fingerprint (300 bp) in the hybrid intergeneric progenies which had been detected in the wild species *V. helianthoids* (genera *Verbisina*). This DNA fingerprint indicates the introgression of *V. helianthoides* DNA into some of the hybrid progenies produced. Band 300 bp was specific for line 140 R, 144 R and 143 R. This is a demonstration that DNA polymorphism could be detected between the amplified products of the 6 inbred lines (Encheva at al., 2005b).

Combining ability of the new restorer lines produced from the cross H. annuus x H. tuberosus

- The tree years testing of lines 101 R and 104 R demonstrated 100% restoration and very good combining ability. (Encheva et al, 2004b). The sterile analogue of the Bulgarian self-pollinated line 2607 B was used as a tester. Seed yield of the produced hybrids № 40 and № 46 showed an increase of index "seed yield" with comparison to mean standard (commercial hybrids Albena and Super Start) with 13.2% and 7.2%, respectively.
- The hybrids were also, characterized with higher oil yield. The increase according to the mean standard was with 13.2 and 5.6%, respectively.

Combining ability of the new restorer lines produced from the cross H. annuus x H. salicifolius

- The tree years testing of lines 107 R, 114 R and 120 R demonstrated 100% restoration and very good combining ability (Encheva et al, 2006b). As a tester was used sterile analogue of the Bulgarian self-pollinated line 2607 B.
- Seed yield of the produced hybrids № 47, № 52, and № 56 showed an increase of the indices seed yield to 114.0% and oil yield to 117.8% in comparison to the mean standard (commercial hybrids Albena and Super start).

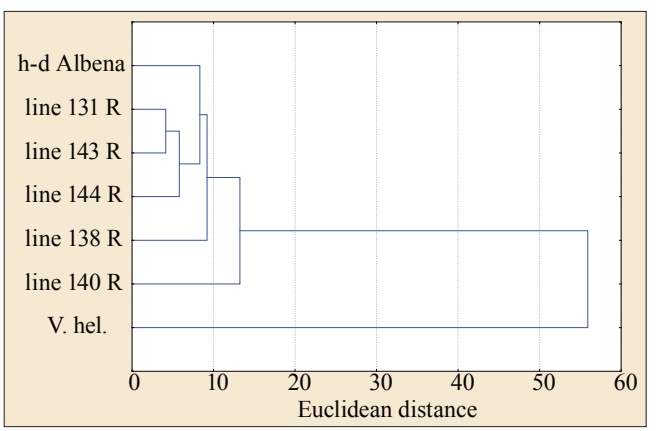

Fig. 4. Degree of similarity between lines (F10) of crosses *H.annuus* (hybrid Albena) *x Verbisina helianthoides* (genera *Verbisina*) and parental forms, based on oil in the seed, %

Combining ability of the new restorer lines produced from the cross H. annuus x V. helianthoides

- The two years testing of lines 131 R, 138 R, 140 R, 143 R, 144 R and 146 R demonstrated 100% restoration and very good combining ability. (Encheva et al., 2004b). The sterile analogue of the Bulgarian self-pollinated line 2607 B was used as a tester. The produced hybrids showed an increase of the index "seed yield" with comparison to the mean standard (commercial hybrids Albena and Super Start) from 5.2% to 20.5%.
- The hybrids were also, characterized with higher oil yield. The increase according to the mean standard was with 6.1% to 33.0%.

Conclusions

The interspecific crosses *H. annuus* (cv. Albena) x *H. tuberosus*, *H. annuus* (cv. Albena) x *H. salicifolius* and intergeneric cross *H. annuus* (cv. Albena) x *Verbisina helianthoides* (genera *Verbisina*) is obtained by using to the method of direct organogenesis of immature zygotic embryos. In our study through this method 3 to 8 hybrid plants were produced from a single embryo. This is a valuable method because it allows to obtain more than one plant from a hybrid embryo, which is not possible with the commonly used *embryo rescue* technique.The available literature does not provide data on the obtaining of intergeneric cross *H. annuus* (cv. Albena) x *Verbisina helianthoides* (genus *Verbisina*) by this method. Our results are also the first attempt to investigate the combining ability of the restorer lines produced. On the basis of the test carried out on the restorer ability, a conclusion was drawn that all lines restored at 100%.

RAPD analysis confirmed the hybrid nature of the obtained breeding material and indicated the introgression of *H. tuberosus* and *V. helianthoides* DNA into some of the hybrid progenies produced.

Lines 101 R, 104 R, 107 R, 114 R, 120 R, 131 R, 138 R, 140 R, 143 R, 144 R, 146 R were successfully included in heterosis breeding of sunflower. The new hybrids developed showed an increase of the indexes seed yield to 20.5% and oil yield to 33.0% in comparison to the mean standard (commercial hybrids Albena and Super start).

Lines 104 R, 107 R, 140 R, 143 R, 144 R, 146 R produced instead very good combining ability possess resistance to im-

portant decease as phoma, phomopsis, alternaria and downy mildew and the local population of parasite broomrape.

The good economic indices of the hybrids developed with participation of the new restorer lines, their resistance to some economically important diseases and the parasite broomrape reveal the high importance of interspecific and intergeneric hybridization for improvement of cultural sunflower.

References

Barov, V. and J. Shanin, 1965. Methodics of field experiment. *Zemizdat*, Sofia, 127 pp. (Bg).

Bohorova, N. E., 1991. Report on cytogenetic problems in wild sunflower species and interspecific hybrids (1988-1990), application of tissue culture methods and plan for 1991-1993 (Consultation Meeting of the FAO-Sunflower Network, Pisa 1991).

Dozel, B., J. Atlagic and D. Vasic, 1996. Transferring stem canker resistance from *Helianthus tuberosus* L. into inbred line of sunflower by embryo rescue technique. *Helia,* **19**: 87-94.

Chandler, J. M. and B. H. Beard, 1983. Embryo culture of *Helianthus* hybrids. *Crop. Sci*., **23**: 1004-1007.

Christov, M., 1990. Study on the wild species from genus *Helianthus* with a view of their use in sunflower breeding. *Ph.D. Thesis*, Sofia, pp. 208 (Bg).

Christov, M., 1996. Characterization of wild *Helianthus* species as sources of new features for sunflower breeding. In: P. D. S. Caligari and D. J. N. Hind (Eds.), Compositae. Biology & Utilization. (Proceedings of the International Compositae Conference, Kew), Royal Botanic Gardens, Kew, 1994, **2**: 547-570.

Christov, M. and R. Vassilevska-Ivanova, 1999. Intergeneric hybrids in *Composite* (*Asteraceae*), I. Hybridization between cultivated sunflower (*H. annuus* L.) and *Composite* genera. *Helia*, **22** (31): 13-22.

Encheva, J., M. Christov and P. Ivanov, 1992. Use of direct organogenesis *in vitro* from immature embryos of interspecific and intergeneric hybrids of *Helianthus annuus* L. (Proceedings of the 13th International Sunflower Conference, Pisa, Italy, 7-11 September), **2**: 1455-1460.

Encheva, J., M. Christov and P. Ivanov, 2003. Characterization of interspecific hybrids between cultivated sunflower *H. annuus* L. (cv. Albena) and wild species *Helianthus tuberosus*. *Helia*, **26** (39): 43-50.

Encheva, J., M. Christov, H. Köhler, P. Shindrova, V. Encheva and W. Friedt, 2004a. Interspesific hybrids between *Helianthus annuus* and *H. tuberosus*: RAPD analysis, disease resistance, combining ability. *Bulgarian Jjournal of Agricultural Science*, **10** (2): 169-175.

Encheva, J., M. Christov, M. Todorova, P. Shindrova and V. Encheva, 2004b. New sunflower restorer lines, developed by direct organogenesis method from intergeneric cross *Helianthus annuus* (cv. Albena) x *Verbesina helianthoides* (genus *Verbesina*)-disease resistance, combaining ability. *Bulgarian Journal of Agricultural Science,* **10**: 169-175.

Encheva J. and M. Christov, 2005a. Intergeneric hybrids between cultivated sunflower (*Helianthus annuus* L.) and *Verbesina helianthoides* (genus *Verbesina*)- Morphological and Biochemical aspects. *Helia*, **28** (42): 27-36.

Encheva, J., H. Köhler, M. Christov and W. Friedt, 2005b. Intergeneric hybrids between cultivated sunflower (*Helianthus annuus* L.) and *Verbesina helianthoides* (genus *Verbesina*)-RAPD analysis. *Helia*, **28** (42): 37-44.

Encheva, J. and M. Christov, 2006a. Interspecific hybridization between cultural sunflower *Helianthus annuus* L. and *Helianthus salicifolius* L. morphological and biochemical characterization. *Helia*, **29** (45): 107-116.

Encheva, J., M. Christov, P. Shindrova, M. Drumeva and V. Encheva, 2006b. New sunflower restorer lines, developed by direct organogenesis method from Interspecific cross *Helianthus annuus* L. (cv. Albena) x *Helianthus salicifolius*-Decease resistance, combining ability. *Helia,* **29** (45): 95-106.

Friedt, W., 1992. Present state and future prospects of biotechnology in sunflower breeding. *Field Crops Research,* **30**: 425-442.

Georgieva-Todorova, J., 1990. Genetic and cytogenetic investigation of genus *Helianthus* L., pp. 131.

Georgieva-Todorova, J., 1993. Interspecific hybridization and its application in sun-flower breeding. *Biotechnology and Biotechnology Equipment,* **7** (4): 153-157.

Georgieva-Todorova, J., 1997. Interspecific relationship in the genus *Helianthus*. *Bulgarian Journal of Agricultural Science,* **2:** 57–62.

Gulya, T. J., J. F. Miler, F. Firanyi and W. E. Sackston, 1991. Proposed internationally standardized method for race identification of *Plasmopara halstedii*. *Helia,* **14**: 11-20.

Krauter, R., A. Steinmetz and W. Friedt, 1991. Efficient interspecific hybridization in the genus *Helianthus* via 'embryo-rescue" and characterization of the hybrids. *Theor. Appl. Genet*., **82**: 521-525.

Köhler, H. and W. Friedt, 1999. Genetic variability as identified by AP-PCR and reaction to mid stem infection of *Sclerotinia sclerotiorum* among interspecific sunflower (*Helianthus annuus* L.) hybrid progenies. *Crop Sci.,* **39**: 1456-1463.

Korell, M., L. Brahm, R. Horn and W. Friedt, 1996a. Interspecific and intergeneric hybridization in sunflower breeding, I: General breeding aspects. *Plant Breeding Abstracts*, **66**: 925-931.

Leclercq, P., 1969. Use sterile male cytoplasmique chez le turnesol. *Ann. Amelior. Plant*, **19**: 99-106.

Newport Instruments Ltd., 1972. Use of the Newport quantity analyzer as a replacement for solvent extraction for measuring the oil and fat content of oil seeds, chocolate, meat and other materials. *Newport Pagnell*, England.

Panchenko, A. N., 1975. An early diagnostic method for resistance to *Orobanche cumana* Wallr. *Agricultural Newspaper*, **2**: 225-228 (Ru).

Peres A. and Y. Regnault, 1986. *Phomopsis helianthi*: production d'inoculum et mise au point d'une methode de contamination artificielle. *Inf. Tech. CETIOM* 95, **24**.

Pustovoit, G. V., 1978. Main results of sunflower breeding for group resistance in VNIMK. Zbornik-VNIMK. Krasnodar. *Pest and Disease of Oil Crops*, pp. 32-40 (Ru).

Sackston, W. E., 1992. On a treadmill: Breeding sunflower for resistance to diseases. *Ann. Rev. Phytopatology*, **30**: 529-551.

Seiler, G. J., J. S. Pomeroy and R. Marinkovic, 1992. Valuable sunflower germplasm collected from the Northwestern United States. *Helia,* **15** (17): 1-6.

Skoric, D., 1987. Progress report 1984-1986. FAO Subnetwork. In: D. Skoric (Ed.) Genetic evaluation and use of *Helianthus* wild species and their use in breeding programs. *FAO*, Rome, Italy, **1**: 17.

Skoric, D. and I. Rajcan, 1992. Breeding of *Sclerotinia* tolerance in Sunflower. (Proceedings of the 13th International Sunflower Conference, Pisa, Italy, 07-11 Sept., 1992), **1**: 1257-1262.

Scoric, D., 1994. Sunflower breeding for resistance to dominant diseases. In: EUCARPIA – "Section of disease and pests resistance session". (Albena, Bulgaria, 22-24 October), pp. 30–48.

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