

Recent Information on sunflower

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Imerovski, I. (2014). Use of molecular markers in detection of downy mildew resistance gene *Pl_{arg}*. Ph.D Thesis, University of Belgrade, Faculty of Agriculture, Serbia, pp. 89.

Summary Downy mildew is a common sunflower disease that can cause significant yield reduction. The damage caused by the pathogen can be controlled by growing sunflower varieties resistant to this disease.

The aim of this study was to identify molecular markers closely linked to gene *Pl_{arg}* that will be used in marker assisted selection (MAS). A mapping population of 103 F₂ progeny from the cross RHA 419 x RHA-N-49 was used. Molecular analyses indicated that the gene *Pl_{arg}* is located on LG1 of the genetic map of sunflower. A new co-segregating simple sequence repeat (SSR) marker ORS675 was identified and the co-segregation of markers ORS716 and ORS662 with *Pl_{arg}* gene was confirmed. The markers were validated on twenty two resistant inbred lines (RHA 443, RHA 464 and RH 1-20). Results obtained on lines HA-R4 (*Pl₁₄* i *Pl₁₆*) and HA-R5 (*Pl₁₃*) with markers ORS662, ORS716 i ORS675 showed that *Pl_{arg}* is a resistance locus different from other resistance genes mapped on LG1. Application of the markers ORS675 and ORS716 in marker assisted selection was tested on two different BC₁ populations. Markers were found to be useful both for molecular breeding and genetic purity analyses. The closely linked molecular markers from this study will facilitate transfer of the resistance gene into different sunflower lines.

Čanak, P. (2014): Seed set efficiency of sunflower genotypes in different ecological conditions. PhD Thesis, Faculty of Agriculture, University of Belgrade, Serbia.

Abstract: Analysis of seed set efficiency of sunflower genotypes in different ecological conditions was carried out at Rimski šančevi experimental field. Testing was conducted during a three year period on 30 genotypes developed at the Institute of Field and Vegetable Crops, Novi Sad. Nectar production, visitation of pollinators, pollen viability, self-fertility and seed set efficiency were analyzed. Association between the seed set efficiency and other traits was examined using multivariate analysis. The existence of highly significant differences between genotypes was observed for all of the traits analyzed. Highly significant differences were obtained for nectar production, bumble bee visitation and pollen viability between the years, while for total visitation of pollinators, significant differences were recorded. These results indicate high effect of environmental factors on development of these traits. No significant differences between the years were recorded for bee visitation, self-fertility and seed set efficiency, indicating higher effect of genotype in development of these traits. Multivariate analysis showed positive association between seed set efficiency and pollen viability. Association between seed set efficiency and nectar production varied from strong positive to strong negative. During the study, visitation of pollinators was in weak association with seed set efficiency. Bumble bee visitation had stronger association with seed set efficiency than total visitation and bee visitation. Association between self-fertility and seed set efficiency varied from weak to negative. Additionally, associations

among the seed set efficiency, yield and yield contributing traits were analyzed. Seed set efficiency showed positive association with seed yield, oil yield, head diameter and 1000 seed weight. Oil content and number of flowers per head were negatively associated with seed set efficiency. Results obtained in this study will contribute in better understanding of seed set efficiency in sunflower and factors that affect it.

Dimitrijević, A. (2013) Use of molecular markers and *in vitro* methods for detection of tribenuron-methyl resistance in sunflower. PhD thesis, Faculty of Agriculture, University of Belgrade, Serbia.

Summary Development of an *in vitro* test and molecular markers for detection of tribenuron-methyl (*Ahas1-2* gene) tolerance in sunflower is presented in this study. Since, introduction of tolerance in sunflower by conventional breeding takes years, quick tests and molecular markers that can detect tolerant genotypes will shorten the time for developing tolerant sunflower lines and hybrids. Nine sunflower genotypes: four homozygous tolerant, four heterozygous and one susceptible sulfonylurea genotype were used for development of an *in vitro* test. MS media supplemented with different concentrations of herbicide (in range between 2.0 μM - 4.0 μM) and with pH either 7 or 8 were used for tolerance testing. The effect of medium pH and herbicide concentration on sunflower seedlings was observed in order to identify the optimal conditions and the most useful morphological parameter for tolerance testing. The best pH and herbicide concentration combination for discrimination between homozygous and heterozygous tolerant sunflower genotypes was 3.0 μM at pH 7, while root mass was found to be the best parameter for discrimination between homozygous and heterozygous tolerant genotypes. The test enabled discrimination between tolerant and susceptible genotypes in 5 days, as well as discrimination between homozygous and heterozygous tolerant genotypes in 12 days. Molecular analysis of tribenuron-methyl tolerance included tolerant and susceptible parental lines, F₁ and F₂ progeny. Specific primers for *Ahas1-2* gene were designed and restriction enzymes that have different patterns of restriction between tolerant *Ahas1-2* gene and wild type, *ahas1*, were identified. Some of the combinations of primers and enzymes showed dominant nature, however six markers proved to be co-dominant. Those markers will be used in marker assisted selection and for shortening long period for backcrossing.

Atlagić, J., Terzić, S., (2014): Sunflower Genetic Resources – Interspecific Hybridization and Cytogenetics in Prebreeding In: Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases. Nova Science Publishers. New York. ISBN 978-1-63117-347-9. 95-130.

Abstract

Sunflower (*Helianthus annuus* L.) belongs to the *Helianthus* genus which is composed of 51 species (14 annual and 37 perennial). Lowered genetic variability and sensitivity towards large number of pathogens on cultivated sunflower, point to wild relatives as useful breeding material. Practically from the early breeding efforts in Russia in 1930s, first uses of wild relatives were registered, and it was intensified with introduction of hybrids since 1970s.

Increased usage led to formation of several major collections of *Helianthus* species, starting from the collection in USA. They were since enlarged with new accessions in collecting expeditions

and exchange with other gene banks. The collection at Novi Sad, Serbia was formed in 1980 with 11 annual and 32 perennial species (over 1000 accessions) and considered as one of the largest collections worldwide. Wild species are usually kept as seeds in cold chambers, while perennial species can also be kept as living collections in the field.

Wild species are mostly used in sunflower breeding as a source of desirable genes for resistance to pathogens, to find Cytoplasmic Male Sterility and Restoring fertility genes, specific oil quality, traits for new ideotypes and herbicide resistance. The characterization data significantly increased usability and value of collections by facilitating their use in breeding programs thus justifying the effort of collection and maintenance.

The divergence and heterogeneity of the genus cause considerable difficulties, such as cross-incompatibility, embryo abortiveness, sterility and reduced fertility in interspecific hybrids. All annual species and a large number of perennial species have been crossed with the cultivated sunflower using the conventional hybridization method. Other methods like somatic hybridization, "in vitro" embryo culture and chromosome doubling are less often used but can be helpful for more difficult cross combinations. An example is the interspecific program at IFVCNS which resulted in crosses with 7 annual species (F1 and BC1F1 - BC4F1) and 14 perennial species (F1 - BC2F1), some of which were included in the breeding program. The success of interspecific hybridization is evaluated based on morphological observations, cytogenetic analysis, as well as molecular markers.

Cytogenetic studies are used for determinations of chromosome number and structure and analysis of meiosis-microsporogenesis and pollen viability. Such studies made it possible to establish phylogenetic relations between wild sunflower species and the cultivated sunflower and enabled the use of the former in sunflower breeding.

The experience gathered over such a long period of sunflower prebreeding point to difficulties in wild sunflower collection maintenance, interspecific hybridization and isolation of desirable genes. None the less *Helianthus* genus has become a model genus for studying speciation and evolution while still being a constant source of material for improvement of cultivated sunflower.

Terzić, S., Dedić, B., Atlagić, J., Miladinović, D., Tančić, S., Miklič, V., Jocić, S., (2014): Screening *Helianthus* species for resistance to diseases in field conditions. International Conference on Enhanced Genepool Utilization - Capturing wild relative and landrace diversity for crop improvement, Cambridge, United Kingdom, 16-20 June 2014. Book of Abstracts. Bioversity International, Rome, Italy. pp 90. ISBN: 978929043995-0.

Abstract

Besides collection and conservation, characterisation is essential to promote and enable the use of plant genetic resources by breeders. Evaluation of PGR genetic diversity for economically important traits should be one of the basic activities of each gene bank.

The collection of *Helianthus* species at the Institute of Field and Vegetable Crops in Novi Sad comprises 8 annual and 21 perennial species with 189 accessions of annual and 316 accessions of perennial species. It was founded in 1980 and has since been used in sunflower pre-breeding programs. Main reason for the establishment of the collection was the susceptibility of cultivated sunflower to different pathogens and the possibility of acquiring resistance from wild relatives.

The aim of this research was to determine response of *Helianthus* species to infection in the field, as there are not many references on the reaction of wild sunflowers in ex-situ conservation conditions. Disease incidence and severity was recorded in a naturally infected field during seven years. Total of 29 *Helianthus* species (505 accessions) were included in the survey, while an addition were F1 interspecific hybrids between the cultivated sunflower and perennial species (10 species, 23 genotypes).

Powdery mildew (*Erysiphe chichoracearum*) was the most frequently observed disease. While its development and severity varied due to climatic conditions, it was most abundant in 2011.

Symptoms of other diseases like necrotic spots on leaves (*Alternaria* spp.), stem necrosis (*Phoma macdonaldii*) and head and stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), were observed. One parasitic plant - broomrape (*Orobancha cumana* Wallr.) was also registered in traces.

The obtained results were used to select accessions for inoculation trials on head rot and black stem disease resistance.

Terzić, S., Dedić, B., (2013): Interspecific sunflower progenies selection for tolerance to white rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. EUCARPIA Genetic Resources section meeting, June 10-13 2013. Alnarp, Sweden. pp 132.

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at:

http://www.nordgen.org/ngdoc/nordgen/Eucarpia/Posters/P4_05_Sreten_Terzic.pdf

Abstract

White rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major sunflower disease worldwide. Testing *Sclerotinia* head resistance can be done using artificial infections by spraying heads at R5.1 stage with ascospore suspension using 5000 spores/ml (Becelaere and Miller, 2004). Total of 20 interspecific sunflower progenies were tested and compared with tolerant and susceptible control lines. All tested interspecific progenies were obtained after crosses between cultivated inbred lines and wild annual species, followed by one generation of open pollination due to difficulties in obtaining progenies from controlled backcross pollination. Inflorescences were screened for symptoms of head rot 35 days after inoculation. Disease severity was determined by measuring total infected area of the head. A 1-5 scale was used to describe tolerance where 1 is the most tolerant.

At the end of trial, 8 out of 9 tested *Helianthus annuus* progenies were highly susceptible, where symptoms of white rot covered more than 50% of the head area. Most of the *H. petiolaris* progenies were moderately susceptible, while the most tolerant were two progenies of *H. debilis* originating from accession DEB1810 where less than 12,5% of head rot was found. This study shows the possibility to use open pollinated progenies in sunflower interspecific programs where other hybridization methods are not successful. Careful selection in the following generations of backcrossing and selfing can then lead to the desired resistance trait.

Savić, J., **Terzić, S.,** Miladinović, D., (2013): Intercropping of legumes with *Helianthus* species. First Legume Society Conference (LSC1), May 9 - 11, 2013. Novi Sad, Serbia. pp 269.

Abstract

Intercropping, or growing together more species at the same time in the same place is one of the oldest systems of cultivation in agriculture. Today, intercropping is recommended in sustainable agriculture due to various positive effects. The main advantage of legumes in intercropping is the possibility to increase soil fertility and protein levels with companion plants.

The most common agricultural species of the *Helianthus* genus are sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.). Intercropping of legumes with sunflower has been tested more than with Jerusalem artichoke. Intercropping of Jerusalem artichoke with sweet clover and hairy vetch showed that the level of nitrogen in the soil was not significantly lower than the controls.

Intercropped sunflower showed large variations in yield depending on the variety and location. Species like *Vicia villosa* Roth, *Melilotus officinalis* Lam., *Medicago sativa* L. and *Medicago scutellata* (L.) Mill. have been intercropped with sunflower with the same sowing time and at the V4 and V10 sunflower growth stages. Intercropping in later growth stages had no negative impact on the development of sunflower. Intercropping with beans produced no significant effect on yield, while

alfalfa, soybean, mung and cluster bean significantly reduced sunflower yield. Increased levels of nitrates in the soil have been recorded when sunflower was intercropped with hairy vetch.

Most of the research so far on intercropping of legumes with *Helianthus* species has been done in subtropical area, where the best results were obtained when sunflower was intercropped with bean and hairy vetch.

Terzić, S., Jocić, S., Zorić, M., Marjanović-Jeromela, A., (2012): Influence of pollination method on sunflower seed oil and protein content. International symposium for agriculture and food. December, 12-14th, Skopje. Macedonia. pp 15.

Abstract

Three commercial sunflower hybrids (NSH2023, NSH2026 and RIMI) were used in this work, and their parental lines. They were grown in different pollination conditions: in isolation cages (hand pollinated and with bumblebees) and outside of isolation cages (open pollinated and self pollinated). Seed yield, oil and protein content, as well as pollen viability and nectar content were determined.

Oil content was significantly higher with bumblebee (43,4%) and open pollination (43,8%) than hand (40,8%) and self pollination (40,4%). Protein content was highest in self pollination (23%), while in hand pollination (19,7%) and insect pollination it was significantly lower (18,2%). Oil and protein content are negatively correlated in sunflower, and this trial revealed a correlation of -0,69.

The analysis of variance for seed yield showed significant difference between analyzed genotypes and pollination conditions, as well as their interaction. Oil and protein content also depended on the inflorescence isolation method, so that the determined differences cannot only be assigned to pollination method. None the less, significant differences in oil and protein content have also been found for hand and bumblebee pollination using the same isolation method.

Terzić, S., Atlagić, J., Maksimović, I., Zeremski, T., Zorić, M., Miklič, V., Balalić, I., (2012) Genetic variability for mineral concentration in tubers and leaves of Jerusalem artichoke. *Scientia horticulturae*. 136, 135–144.

Abstract

Data on the concentration of essential elements in the Jerusalem artichoke (*Helianthus tuberosus* L.) as valuable nutritional information is available only for a limited number of accessions and for tubers or leaves analyzed separately. For research or breeding of the Jerusalem artichoke a comprehensive overview of the extent of genetic diversity in existing germplasm is needed. As such, a diverse Jerusalem artichoke collection was analyzed in order to determine concentrations of essential elements separately in tubers and leaves and to assess the potential usability of accessions in breeding programs aimed at improving the quality of a Jerusalem artichoke's vegetative parts for feed and food.

Concentrations of analyzed essential elements in leaves and tubers were significantly different. Of all the analyzed essential elements in leaves (N, P, Ca, Mg, K, Fe, Mn, Zn and Cu) Ca was the most abundant (36 g/kg), while in tubers it was only the fourth of the nine analyzed elements. The Ca/P ratio was approximately the same in tubers of all accessions which corresponds to a desired ratio for cattle feed (1:1–2:1). The leaves contained 18 times more Ca than P. Except for the lack of P in the Jerusalem artichoke herbage, there was an adequate amount of studied mineral elements for use as cattle feed (ruminants).

It was found that tubers contain an adequate amount of macro- and microelements when used as food, and the element content was similar to or greater than in the other root crops. Accessions with the lowest K/(Ca + Mg) ratio were among accessions from

Montenegro with ratio a of 5.8 which is still higher than the prescribed ratio for cattle feed of up to 2.2.

By combining all of the obtained results through multivariate analysis, it was discovered that a group of accessions from Montenegro has potentially the best mineral composition for further work. One possible solution to overcome inappropriate mineral element ratios in leaves and tubers could be the usage of their mixtures for cattle feed, but further studies are required before a conclusion on its success can be made.

Terzić, S., Atlagić, J., Maksimović, I., Zeremski, T., Petrović, S., Dedić, B., (2012): Influence of photoperiod on vegetation phases and tuber development in topinambour (*Helianthus tuberosus* L.). Archives of Biological Sciences. 64 (1), 175-182.

Download at: <http://archonline.bio.bg.ac.rs/VOL64/SVESKA1/21%20-%20Terzic%20.pdf>

Abstract

A topinambour collection was analyzed to determine genetic variability of 141 accessions in reaction to the length of day (light), its influence on vegetative and flowering phases, tuber number and mass. Day length significantly influenced flowering, which started with first shorter days (15.6h) in the third decade of June, while majority of accessions flowered in the third decade of August (13.3h). Differences between accessions were statistically significant for the analyzed phenotype traits. Significantly longer vegetative phase was found in Montenegrin accessions in comparison to the other groups of origin. Duration of vegetative phase was significantly and positively correlated to tuber mass and negatively to their number, while duration of reproductive phase had an opposite effect. For further work on topinambour breeding, it would be important to describe the mechanism of photoperiodic control of flowering initialization. Only by obtaining accessions neutral to photoperiod, topinambour could become a cultivated crop.

Tančić S., Dedić B., Jocić S., Miklič V. (2013): Patogenost *Macrophomina phaseolina* i *Fusarium* spp. na klijance suncokreta. Zbornik referata XII Savetovanja o zaštiti bilja, p. 64, 25-29. Novembar 2013, Zlatibor.

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3. **Tančić S.,** Maslienko Lj. V., Kurilova D.A., Skrobonja J., Marjanović-Jeromela A., Jocić S., Miklič V. (2014): *Trichoderma* spp. Growth Promoting Effect on Oil-crops and Potential Antagonistic Effect on *Sclerotinia sclerotiorum*. Proceedings of the 8th International Conference "Biological Plant Protection as the Basis of Ecosystem Stabilisation. Inovative Technologies for the Application of Biological Plant Protection Agents in Organic Agriculture", 16-18th September, 2014. p. 286-289.

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