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# CONTENTS

## BIODIVERSITY

- The effects of human disturbance on the diversity of aquatic insects in a tropical headwater stream in southern Thailand** .....1-5  
Parnrong Davison Supatra, Khwansut Sanchai
- Evaluation and utilization of cowpea (*Vigna unguiculata* (L.) walp.) germplasm for varietal improvement of resistance to cowpea aphid (*Aphis craccivora* Koch.) in Thailand** .....6-18  
Nualsri Charassri, Potarot Somsak, Chansod Jitra, Milošević Dušan,  
Wuttiwong Kanok-on, Benchasri Sorapong, Maneelert Veera
- Improvement of pre-harvest sprouting tolerance in wheat: challenges and successes**..... 19-24  
Hristov Nikola, Mladenov Novica, Kondić-Špika Ankica, Đurić Veselinka, Jocković Bojan
- Collection of minor oil crops and how to utilize it** ..... 25-29  
Marjanović-Jeromela Ana, Terzić Sreten, Marinković Radovan, Atlagić Jovanka,  
Milovac Željko, Jocković Milan, Mikić Aleksandar
- Genetic variability of *Gibberella fujikuroi* (Sawada) Ito. in Serbia** ..... 30-33  
Tančić Sonja, Stanković Slavica, Lević Jelena, Krnjaja Vesna
- Resistance of wild sunflower species to *Macrophomina phaseolina*** .....34-37  
Tančić Sonja, Terzić Sreten, Dedić Boško, Atlagić Jovanka, Jocić Siniša, Miklič Vladimir
- Biodiversity of rapeseed insects** ..... 38-41  
Milovac Željko, Kereši Tatjana, Pešić Snežana, Marjanović Jeromela Ana,  
Marinković Radovan, Mitrović Petar
- Composition of weed flora in organic farming** ..... 42-46  
Nikolić Ljiljana, Ljevnaić-Mašić Branka, Džigurski Dejana
- Analysis of agronomic traits and their influence on the preservation and enhancement of the agro-biodiversity in wheat** ..... 47-54  
Mladenov Velimir, Banjac Borislav, Milošević Mirjana, Krishna Avineerappa
- The effect of osmotic stress on physiological parameters in different maize genotypes** .... 55-59  
Kravić Natalija, Vuletić Mirjana, Nikolić Ana, Babić Vojka, Ristić Danijela,  
Perić Vesna, Anđelković Violeta
- Classification of local and introduced maize landraces for grain quality** ..... 60-63  
Grčić Nikola, Pavlov Jovan, Vančetović Jelena, Ignjatović-Mićić Dragana,  
Marković Ksenija, Anđelković Violeta
- Characterization of soybean varieties based on protein and oil content** ..... 64-67  
Perić Vesna, Nikolić Ana, Mladenović Drinić Snežana, Kravić Natalija, Kovačević Dragan

<b>The effect of foliar application of boron on alfalfa seed yield</b> .....	<b>68-71</b>
Terzić Dragan, Stanisavljević Rade, Dinić Bora, Vučković Savo, Đokić Dragoslav, Marković Jordan	
<b><i>Phthorimaea operculella</i> (Zeller, 1983), in the tobacco agro-ecosystem.</b> .....	<b>72-77</b>
Krsteska Vesna, Stojanoski Petre	
<b>Detection of oxytetracycline residues in milk of cows in rural area in Tetovo, Macedonia</b> ...	<b>78-81</b>
Kamberi Mensur, Sulaj Kapllan	
<b>Chemical activations of systemic acquired resistance in pea to rust (<i>Uromyces pisi</i>).</b> .....	<b>82-86</b>
Barilli Eleonora, Prats Elena, Castillejo M. Angeles, Moral Ana, Rubiales Diego	
<b>Protein nitration in barley during the defense response against</b> <b><i>Blumeria graminis</i> f.sp. <i>hordei</i></b> .....	<b>87-90</b>
Caballero E, Castillejo M. Angeles, Espejo B, Rubiales Diego, Laj Mur, Prats E.	
<b>Use of physiological parameters as tools to discriminate water stress tolerance in pea.</b> ....	<b>91-94</b>
Iglesias-García Rebeca, Prats Eleonora, Rubiales Diego	
<b>Horse chestnut (<i>Aesculus hippocastanum</i> L.) seeds storing</b> .....	<b>95-98</b>
Čukanović Jelena, Ninić-Todorović Jelena	
<b>Effect of seed storage of meadow fescue (<i>Festuca pratensis</i> huds.)</b> <b>on changes in dormancy, germination, and longevity of seeds</b> .....	<b>99-103</b>
Stanisavljević Rade, Đokić Dragoslav, Terzić Dragan, Beković Dragoljub, Đukanović Lana, Milenković Jasmina, Marković Jordan	

## BIOTECHNOLOGY

<b>Influence of medium on growth and development of wild rose in vitro</b> .....	<b>104-108</b>
Tkalec Monika, Parađiković Nada, Zeljković Svjetlana, Vinković Tomislav	
<b>Findings of specific antibodies against maedi-visna virus in sheep</b> <b>population in the region of Vojvodina.</b> .....	<b>109-114</b>
Savić Sara, Vidić Branka, Bugarski Dejan, Grgić Živoslav	
<b>Screening test in determination of colorado potato beetle</b> <b>(<i>Leptinotarsa decemlineata</i> Say.) sensitivity to insecticides</b> .....	<b>115-123</b>
Indić Dušanka, Vuković Slavica, Vukša Petar, Grahovac Mila, Gvozdenac Sonja, Janković Dragica, Forgić Gordana, Mrdak Gordana	
<b>Chemical properties of raw milk from conversion period in organic production</b> .....	<b>124-129</b>
Popović-Vranješ Anka, Pejanović Radovan, Krajinović Milan, Kasalica Anka, Cvetanović David, Kralj Aleksandar	
<b>Directed microbiological processes as a possibility of safe management</b> <b>by - products of animal origin</b> .....	<b>130-133</b>
Košarčić Slavica, Plavša Nada, Kovačević Mira, Babić Jelena	

---

<b>Development, status and possible improvement of yield and seed quality of forage grasses in Serbia</b> . . . . .	134-140
Stanisavljević Rade, Đokić Dragoslav, Milenković Jasmina, Terzić Dragan, Đukanović Lana	
<b>Molecular determination of kti in F2 soybean population.</b> . . . . .	141-144
Kovačević Dragan, Srebrić Mirjana, Perić Vesna, Mladenović Drinić Snežana	
<b>Screening of antibiotics production by <i>Streptomyces</i> spp. strains isolated from the environment</b> . . . . .	145-149
Dodić Jelena, Grahovac Jovana, Tadijan Ivana, Dodić Siniša, Vučurović Damjan, Popov Stevan	
<b>Relationship between grain yield and some chemical traits of maize inbred line seed</b> . . .	150-154
Milenković Jasmina, Stanisavljević Rade, Marković Jordan, Đokić Dragoslav, Anđelković Snežana, Đukanović Lana, Vasić Tanja	
<b>Fluctuation of abundance of microorganisms in the rhizosphere of alfalfa during the growing season</b> . . . . .	155-159
Anđelković Snežana, Jarak Mirjana, Vasić Tanja, Radović Jasmina, Filipović-Trajković Radmila, Sokolović Dejan, Dinić Bora	
<b>Yield and yield components of alfalfa seed as affected by sowing system</b> . . . . .	160-163
Beković Dragoljub, Stanisavljević Rade, Stevović Vladeta, Biberdžić Milan, Stojković Slaviša, Knežević Jasmina	
<b>Fatty acid composition of tobacco seed oil and its potential as source of linoleic acid</b> . . . . .	164-167
Srbinska Marija, Filipovski Kiril, Rafajlovska Vesna	
<b>The effects of thermal treatment on the quality of oriental tobacco</b> . . . . .	168-171
Zdraveska Natasha, Srbinska Marija, Kirkova Stefka	
<b>Genetic diversity assessment of maize inbred lines with microsatellite markers</b> . . . . .	172-175
Treskić Sanja, Brbaklić Ljiljana, Kondić-Špika Ankica, Trkulja Dragana, Stanisavljević Dušan, Mitrović Bojan, Nastasić Aleksandra	
<b>Wheat microsatellite markers in association analysis.</b> . . . . .	176-179
Brbaklić Ljiljana, Kondić-Špika Ankica, Trkulja Dragana, Treskić Sanja, Hristov Nikola, Mladenov Novica, Kobiljski Borislav	
<b>Conventional and molecular breeding for broomrape resistance in sunflower</b> . . . . .	180-183
Miladinović Dragana, Imerovski Ivana, Dimitrijević Aleksandra, Jocić Siniša, Cvejić Sandra, Dedić Boško, Miklič Vladimir	
<b>Identification of the causal agent of bacterial blight of bean (<i>Phaseolus vulgaris</i> L.)</b> . . . .	184-187
Ignjatov Maja, Nikolić Zorica, Zdjelar Gordana, Vasić Mirjana, Gvozdanović-Varga Jelica, Milošević Dragana, Jovičić Dušica	
<b>Identification of the causal agent of soybean bacterial blight (<i>Pseudomonas savanstanovi</i> pv. <i>glycinea</i>)</b> . . . . .	188-191
Ignjatov Maja, Vidić Miloš	

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---

<b>Outbreak of clinical <i>Prototheca zopfii</i> mastitis in a herd of dairy cows</b> . . . . .	<b>192-196</b>
Milanov S. Dubravka, Stojanović M. Dragica, Košarčić P. Slavica, Bugarski D. Dejan	
<b>Application of the process of extrusion and micronisation and their influence on nutritive value of feedstuffs</b> . . . . .	<b>197-202</b>
Puvača Nikola, Stanačev Vidica, Glamočić Dragan, Lević Jovanka, Stanačev Vladislav, Milić Dragan, Vukelić Nataša, Ljubojević Dragana	
<b>Extruded rapeseed and rapeseed meal in nutrition of broilers</b> . . . . .	<b>203-209</b>
Stanačev Vidica, Stanačev Vladislav, Milošević Niko, Puvača Nikola, Plavša Nada, Milić Dragan, Beuković Dejan, Džinić Natalija	
<b>Geldanamycin produced by a newly found actinomycete, <i>Streptomyces</i> sp. gdn8-88</b> . . . .	<b>210-214</b>
Thawai Chitti	
<b>Isolation and characterization of antibiotic-producing actinomycetes from hot spring sediment of Thailand</b> . . . . .	<b>215-219</b>
Thawai Chitti	

## **ECONOMY**

<b>Some economic indicators of the organic plant production</b> . . . . .	<b>220-225</b>
Pejanović Radovan, Tomaš Mirela, Popović-Vranješ Anka, Glavaš-Trbić Danica	

## **INGENERING**

<b>Electrical palm fruit cutting machine</b> . . . . .	<b>226-229</b>
Mueangdee Nongyao	

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# Conventional and molecular breeding for broomrape resistance in sunflower

Dragana Miladinović, Ivana Imerovski, Aleksandra Dimitrijević, Siniša Jocić,  
Sandra Cvejić, Boško Dedić, Vladimir Miklič  
Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia,  
e-mail: dragana.miladinovic@ifvcns.ns.ac.rs

## Abstract

Broomrape (*Orobanche cumana* Wallr.) is a flowering plant that significantly reduces the yield of sunflower and causes great economic losses. Genetic resistance, obtained by crossing cultivated sunflower with wild relatives, proved to be the most effective way to fight it, but because of the appearance of new races of the pathogen, it is necessary to find new sources of the resistance. Intensive research on the molecular level has focused on the development of specific DNA markers, in order to facilitate and accelerate the introduction of resistance into cultivated sunflower.

**Key words:** sunflower, broomrape, resistance, molecular markers

## Introduction

Broomrape (*Orobanche cumana* Wallr) is the flowering plant that parasitizes in sunflower (*Helianthus annuus* L.) and can reduce yields by more than 50%. It has a high potential for breeding and fine seeds, which allows it to easily expand its distribution area. Therefore, broomrape needs to be controlled by using available strategies, although the use of resistant sunflower cultivars is the most reliable way to control the parasite (Dedić et al. 2009, Miladinović et al. 2011).

Achieving sustainable sunflower resistance to broomrape is one of the most important goals in sunflower breeding (Pacureanu et al. 2009, Fernandez-Martinez et al. 2010). The introduction of resistance genes into cultivated sunflower began in the early 20th century. These genes are mostly found in wild relatives of cultivated sunflower, especially in species *Helianthus tuberosus* L., *Helianthus maximiliani* Schrad., and *Helianthus debilis* Nutt (Fernandez-Martinez et al. 2008). Vranceanu et al. (1980) defined the five broomrape races (A, B, C, D and E), and five dominant genes ( $Or_1$ ,  $Or_2$ ,  $Or_3$ ,  $Or_4$  and  $Or_5$ ) that provide resistance to these races. In order to facilitate identification of broomrape races, Vranceanu et al. (1980) formed a set of differentials for  $Or_1$ ,  $Or_2$ ,  $Or_3$ ,  $Or_4$  and  $Or_5$ , which included lines Kruglik A-41, Jdanovsky 8281, Record, S-1358 and P-1380. The set was later complemented with the line LC1093 carrying  $Or_6$  (Pacureanu et al. 1998).

The widespread use of resistant genotypes of sunflower increased the selection pressure and lead to the emergence of new races of broomrape. Pacureanu et al. (2004) found that there are differences between new races that infect fields in Turkey in comparison to those present in Romania, and that there are differences in the populations present in Romania and Serbia in comparison to those of Spain. While testing races E and F from different geographic areas, Pacureanu et al. (2003) came to the conclusion that the same race from different countries differ in virulence. The authors have found that the most virulent races were from Turkey, followed by the ones from Romania, Spain, and Serbia.

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## ***Conventional Breeding - Wild Species as Resistance Genes Sources***

According to Fernandez-Martinez et al. (2007), wild sunflowers are a source of resistance genes that can provide protection not only from existing, but also from new, more aggressive races of broomrape. Resistance genes found in wild species of the genus *Helianthus* and have been incorporated into cultivated sunflower genotypes by interspecific hybridization (Škorić et al. 2010).

Resistance to race F was found in germplasm of both cultivated and wild sunflower (Fernandez-Martinez et al 2000, Pacureanu et al. 1998). Jan and Fernandez-Martinez (2002) crossed the wild sunflower species *H. maximiliani*, *H. grosseserratus* Mart., and *H. divarticatus* L. with cultivated sunflower and developed four populations (BR1-BR4) resistant to race F in Spain, while genotypes resistant to races E and F were developed through interspecific hybridisation with *H. tuberosus* (Škorić et al. 2010). Cvejić et al. (2012) found the source of resistance to race G and more virulent races in an inbred line derived from interspecific hybridization with *Helianthus divarticatus*. The inbred lines possibly resistant to race G were developed from crosses with *H. tuberosus*.

## ***Molecular Breeding - Development of Molecular Markers***

To create a resistant line it is necessary to have reliable evaluation of resistance breeding material. Phenotypic evaluation of resistance is laborious, time-consuming, highly dependent on the environmental conditions and therefore prone to errors. Resistance evaluation using DNA markers is much more reliable and faster method.

Previous molecular studies were mostly related to the mapping of the gene  $Or_5$ , that confers resistance to race E of the broomrape. Lu et al. (2000) identified five RAPD and SCAR markers significantly linked to the  $Or_5$  gene. Markers RTS05, RTS28, RTS40, RTS29 and RTS41 were mapped at 5.6, 13.6, 14.1, 21.4 and 39.4 cM, and the RAPD marker UBC 120\_660 was located distally at a distance of 22.5 cm from the  $Or_5$  locus. Lu et al. (1999) linked the marker RTS05 with RFLP markers S009 and S010, and thereby position the gene on LG17 GIE Cartisol RFLP map. Tang et al. (2003) found that the  $Or_5$  is located on LG3 on the map of Yu et al. (2003), and identified ORS1036 as a closely linked marker.

In addition to studies on monogenic resistance, Perez-Vich et al. (2004) investigated the quantitative nature of broomrape resistance of sunflower and identified five loci important in the defence to race E, and six loci involved in the defence to race F of broomrape. Letousey et al. (2007) found that the defence mechanism is different in two resistant genotypes, but that in both it involves an increased expression of the gene for methionine synthase, glutathione S-transferase and quinon-oxidoreductase, and callose synthetase.

As the broomrape resistance is introduced into cultivated sunflower from various sources, the origin of the material is an important factor when it comes to mode of inheritance and the position of resistance genes. Pacureanu et al. (2009) found that the resistance to the race F could be controlled either with two recessive genes or one dominant gene, in inbred lines depending on the origin of the inbred lines. These differences are apparent at the molecular level, and it is often the case that the marker is specific to the material on which it has been developed (Imerovski et al. 2011). For this reason, the work on molecular marker development in Institute of Field and Vegetable Crops is directed to the development of markers on NS breeding material. Besides working on the development of markers for  $Or_5$ , current research is aimed to the mapping of a new gene that provides resistance to broomrape races higher than F (Cvejić et al. 2012). By comparing the molecular profile of resistant and susceptible genotype, a polymorphism was observed on LG3 of SSR map. Accordingly, it can be assumed that the new gene is located on this linkage group (Imerovski et al. 2012). Further tests on the mapping population will determine the exact position of the gene, and enable the development of specific molecular marker that will accelerate the introduction of resistance to new races of broomrape into the commercial sunflower lines.



## Conclusions

Broomrape is serious constrain in sunflower production. Cultivated sunflower has limited genetic variability and it lacks genes for broomrape resistance, but wild species constitute valuable source of resistance genes. Marker assisted selection has proved to be a valuable tool in sunflower breeding programs for broomrape resistance gene identification, accelerating and facilitating broomrape resistance breeding.

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