INTERNATIONAL
SUNFLOWER ASSOCIATION
ISA

Proceedings

18th International Sunflower Conference

MAR DEL PLATA & BALCARCE - ARGENTINA

February 27 - March 1 / 2012

ASAGIR
ASOCIACION ARGENTINA DE GIRASOL

ISA Board

Carlos FEOLI, Argentina - President

André POUZET, France - CEO

Nikolai BOCHKARYOV, Russia

Maria JOITA-PACUREANU, Romania

Johan POTGIETER, South Africa

Gerald SEILER, USA

Dragan SKORIC, Serbia

Gian Paolo VANNOZZI, Italy

Felicity VEAR, France

Leonardo VELASCO, Spain

Yalcin KAYA, Turkey

ASAGIR Board

PRESIDENT
Ricardo Negri, Capelle Hnos

VICE PRESIDENT
Orlando Vellaz, Advanta Semillas

SECOND VICE PRESIDENT
Guillermo Pozzi

SECRETARY
Juan Carlos Arana, OMHSA

TREASURE
Ramiro Costa, Bolsa de Cereales

PRO SECRETARY
Carlos Sosa, Pannar SA

PRO TREASURE
Fernando Cozzi, Cargill SA

Norma Huguet
Luis Arias, Cazenave y Asociados
Alicia Rupel, Bolsa de Cereales de Bahía Blanca
José María Dodds, Nidera Semillas
Marcelo Morini, ArgenSun
Armando Casalins, Federación de Acopiadores
Antonio Hall
Raúl Tomá, SRA
Santiago Sanchez, AGD
Horacio Urpi
Hernán Busch, Banco de Galicia
Jorge Harguindeguy, AACREA

Local Organizing Committee

Antonio Hall (President) *

Carlos Feoli (ISA President) *

Ezequiel Fonseca *

Archibaldo Salvador *

José Dodds *

Pablo Paoloni

Norma Huguet

Florencia Bedacarratz

Ramiro Costa

Carlos Sosa

Facundo Quiroz

Javier Mallo

Daniel Alvarez

Daniel Funaro

Mariano Martín Sposaro

Jorge Moutous

Marcelo Morini

Arnaldo Vázquez

Marisa Della Maddalena

Maria Carolina Alegre

* Executive Committee

International Steering Committee

Dr. Antonio Hall (Argentina)

Dr. Ruth Heinz (Argentina)

Dr. Luis Aguirrezabal (Argentina)

Dr. Loren Rieseberg (Canada)

Dr. Leonardo Velasco (Spain)

Dr. Felicity Vear (France)

Program Committee

Dr. Luis Aguirrezábal (co-chair)

Dr. Miguel Cantamutto (co-chair)

Dr. Antonio Hall

Dr. Ruth Heinz

Dr. Carlos Sala

Dr. Abelardo De La Vega

Ms. Amelia Romano

Dr. Mónica Poverene

Mr. Guillermo Pozzi

Dr. Pablo Calviño

Dr. Alberto Escande

Dr. María Eugenia Bazzalo

Dr. Andrés Zambelli

Dr. Sergio Alemano

Foreword

The International Sunflower Association (ISA) and the Argentine Sunflower Association (ASAGIR) are pleased to present this guide to the 18th International Sunflower Conference.

At the time the main objectives for the meeting were defined, organizers aimed to provide a forum for the international sunflower research community with interest in any aspect of science and technology relating to the crop (in its oil-seed and confectionery variants) that would allow all involved to:

- Update knowledge in all fields of sunflower research since the previous conference held at Córdoba, Spain, June 2008;
- Review recent technological advances in sunflower production and identify knowledge gaps that require attention;
- Analyze the status and expectations for current and prospective demands for sunflower products;
- Provide a venue for workshops and special-interest meetings focusing on unresolved research, market, and production issues;
- Provide new generations with an opportunity to interact with global leaders in sunflower research.

The local Program Committee, with the help of the International Steering Committee, has developed a program covering the whole spectrum of relevant topics from genes and genomics through to field agronomy, crop protection, and industry and market issues. The program comprises 14 plenary and 13 invited presentations, 14 short oral presentations, an exhibition of 160 posters that can be visited during each of the first three days of the meeting. In addition, there will be three associated workshops (Bird Damage, Breeding, International Sunflower Genome Initiative), a special-interest presentation of the Global Crop Diversity Trust, and facilities will be available on request for small groups who wish to discuss business or scientific topics.

On the last day of the meeting, the Conference Field Day will be held at the joint INTA-Universidad de Mar del Plata facility in Balcarce. This time the traditional Conference demonstration plots of hybrids from International Sunflower Association member countries and from the host country will be complemented by a broad range of demonstrations of production and management techniques, as well as demonstrations of research techniques in current use by Argentine sunflower research teams.

This Conference has been made possible by the work of many people, by the support of sponsors from both the public and the private sector (sponsors are recognized on the back covers of this guide) and last, but certainly by no means least, those responsible for the lectures, short oral presentations, posters, associated workshops and special interest meetings, and field and laboratory demonstrations that make up the rich and varied bill of fare for this Conference, as reflected in this guide. The Organizing Committee extends their heartfelt thanks to all these individuals and organizations.

ISA and ASAGIR trust that this guide will enable all attendees to have an interesting and fruitful 18th International Sunflower Conference.

Welcome

It has been 27 years since the 11th International Sunflower Conference was held in Mar del Plata, Argentina, March 10-13, 1985. Since then, very many things have changed in the world of sunflower science, technology, and crop production and management. As the global sunflower community reconvenes once again in the same city, its members will have the opportunity to review progress in the last four years, which has been substantial in many areas.

Mar del Plata, a vibrant city located by the sea, with a fishing port, good restaurants, an unusually good choice of golf courses, and kilometers of sandy beaches, together with Balcarce, provide excellent venues for the Conference lectures and Field Day, and will allow attendees to appreciate a unique combination of seas, hills and Pampas. It is a great pleasure for the Organizing Committee to be able to host attendees to this meeting, which we hope will be both enjoyable and fruitful.

Welcome to Argentina, to Mar del Plata and Balcarce, and to the 18th International Sunflower Conference.

| 10-VC-4 |

Towards *Orobanche* resistance in sunflower - SSR analysis of a novel resistance source

Ivana Imerovski,
Aleksandra Dimitrijevic, Dragana Miladinovic*, Bosko Dedic, Sinisa Jocic, Vladimir Miklic
Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21 000 Novi Sad, Serbia
*Corresponding author: dragana.miladinovic@ifvcns.ns.ac.rs

ABSTRACT:

- Broomrape is an aggressive parasite that penetrates through the roots of the host, absorbs nutrients directly from the vascular system and causes significant reduction in sunflower yield. Genetic resistance proved to be the most efficient method for suppressing severe broomrape attacks in the field; however selection pressure resulted in occurrence of new and more aggressive races of the parasite. Hence, finding new sources of resistance is immensely significant. One of the major focuses of the breeding programs of the Institute for Field and Vegetable Crops, Novi Sad, is finding a source that would provide durable resistance, and introducing this resistance into commercial sunflower hybrids. It was recently observed that line AB–VL-8 was not affected by broomrape attacks in the areas where races F and G were abundant. After repeated field tests it was concluded that the AB–VL-8 is resistant to broomrape races higher than F, and could potentially be used as a new source of resistance. The objective of the present study was to screen and compare the genome of resistant line AB–VL-8 and a susceptible line L-OS-1. The goal was to narrow down the region where the resistance locus is placed, and to get a preliminary idea if the trait is qualitative or quantitative.
- Markers were chosen from each linkage group. A total of 85 SSRs strategically positioned throughout the genome were used with an aim of finding differences on molecular level between the resistant and the susceptible line. Molecular profiles were compared, and genome region that presented as polymorphic between the tested genotypes was closer examined with an additional set of SSR markers.
- SSR analyses revealed differences between the examined genotypes. The polymorphic markers from the same linkage group indicated the potential placement of the resistance locus.
- Preliminary position of a new resistance locus was presented. Based on observed clustering pattern of polymorphic markers between the resistant and susceptible genotype, a hypothesis about the way the trait is inherited was proposed. The results will be compared with the observations from the field tests, and SSRs that showed polymorphism between the lines will be used for bulk segregant analyses (BSA).
- New source of resistance could significantly increase the ability of sunflower to withstand attack of highly virulent broomrape races. The final goal is to develop molecular tools that could facilitate detection of the new resistance gene(s). To achieve that, a detail genetic analysis of the targeted genome region and map construction will be performed, thus the results presented herein will be used as a starting point.

Key words: broomrape - resistance genes – SSR - sunflower

INTRODUCTION

Broomrape is a parasitic plant that penetrates through the roots, absorbs nutrients directly from the vascular system of sunflower and causes significant reduction in yield. Several methods are available for suppressing the parasite, however genetic resistance proved to be the most efficient one. The resistance of sunflower to races A-E is conferred by five dominant genes: Or_1, Or_2, Or_3, Or_4 and Or_5 (Vranceanu et al., 1980), whereas for Or_6 gene different inheritance patterns have been reported by several authors (Pacureanu et al., 2008; Fernandez-Martinez et al., 2008). *Or* genes are considered to be allelic or closely linked. Similar to downy mildew resistance genes, it is often the case that new *Or* genes ensure resistance to earlier races of the parasite as well (Tang et al., 2003). As the *Or* genes are race-specific, new broomrape races overcome the existing genetic resistance. In the past decade, new aggressive *Orobanche* races have been reported in Spain (Alonso et al., 1996; Fernandez-Martinez et al., 2000), Romania (Pacureanu-Joita et al., 2008; Skoric and Pacureanu-Joita, 2010), Turkey (Kaya et al., 2004) and other countries. Hence, finding new sources of resistance and developing molecular markers for detecting the resistance genes comes in focus of sunflower breeders.

Breeding programs of the Institute for Field and Vegetable Crops, Novi Sad, aim to develop hybrids that, apart from having high yield, are resistant to the most threatening diseases. Since broomrape is an important limiting factor in crop production, finding a source that would provide durable resistance, and introducing this resistance into commercial sunflower hybrids is one of the biggest concerns. In this process, a reliable evaluation of resistance of the sunflower genotypes is crucial. Beside evaluation in the field, resistance can be checked with use of molecular methods. Molecular markers, when located in vicinity to the resistance gene, can detect the gene of interest, and give more reliable information about plant's resistance. This study was conducted as a first step in finding molecular marker for potentially new source of resistance. The aim was to closer investigate the nature of the resistance in the line AB–VL-8, and mark the region of the genome which could potentially carry the resistance locus.

MATERIALS AND METHODS

Inbred lines AB-VL-8 (resistant) and L-OS-1 (susceptible) were developed at the Institute of Field and Vegetable Crops, Novi Sad. AB-VL-8 was developed by interspecific hybridization of cultivated sunflower with *Helianthus divarticatus*. Trials that were conducted during the period of 2007-2010 showed that this line was repeatedly resistant in all of the experimental fields, including the ones located in Seville region (Spain) and Tulcea (Romania), where differentials carrying *Or5* and Or_6 were heavily attacked. These results indicate that AB-VL-8 is resistant to broomrape races higher than F, and is therefore potentially a novel source of resistance (Cvejic et al., unpublished data). Line L-OS-1 was chosen from the genetic bank of the Institute as a susceptible line that in consecutive greenhouse testing and trials in the field where race E was abundant showed a high susceptibility to broomrape. It could therefore be assumed with high certainty that this line does not have Or_5, if any resistance genes. DNA was extracted from young leaf tissue of the examined lines using a modified CTAB method described by Permingeat et al. (1998). In order to thoroughly screen the genome, approximately 5 markers were taken from each linkage group of the genetic map developed by Yu et al. (2003). After analyses performed with 85 SSRs, additional markers were selected from the LG3, bringing to a total of 93 primer pairs. PCR was performed in Mastercycler gradient Eppendorf machine by using 15 µl of reaction mixture containing 1xPCR buffer, 3 mM $MgCl_2$, 0.2 mM of dNTPs, 0.3 µM each of 3'- and 5'-end primers, 1 U of DNA polymerase, 25 µg BSA and 40 ng of genomic DNA. To reduce non-specific amplification touchdown program was used, with an initial denaturation at 95°C for 5 min, followed by one cycle at 95°C for 1 min, 1 min at 64°C, and then 1 min at 72°C. The annealing temperature decreased 1°C per cycles during each of 10 cycles, after which came 29 cycles at 95°C for 1 min, then 1 min at 55°C and 2 min at 72°C. The final extension was carried out for 10 min at 72°C. All of the products were evaluated by electrophoresis on 2% metaphor agarose gels using 1xTBE buffer. The gels were stained with ethidium bromide 10 mg/ml and visualized with the BIO-Print system (Vilber Lourmat, Marne la Velee, France). Fragment size was evaluated with BIO-CAPT V.97 program (Vilber Lourmat).

RESULTS AND DISCUSSION

Among 93 SSR markers, 31 were polymorphic. More than 2 polymorphic markers per group were detected on LG 8 (ORS 166, ORS 1043, ORS 762, ORS 1013) and LG3 (ORS 1036, ORS 1040, ORS 1112, ORS 665, ORS 1021, ORS 718).

The differences between L-OS-1and AB–VL-8 obtained with markers from LG8 were expected, since the line L-OS-1 has Pl_6 whereas AB–VL-8 lacks all downy mildew resistance genes. Slabaugh et al.

(2003) found that LG8 is highly complex and diverse region of the sunflower genome, and mapped two SSR markers (ORS 166 and ORS 1043) in this region within the HaRGC1 cluster. Pankovic et al. (2007) confirmed that the two SSR markers co-segregate with Pl_6 and could therefore successfully be used for marker assisted selection. Molecular profile of the line L-OS-1 matches the expected one (data not shown), so the presence of the gene Pl_6 was confirmed in this manner.

High level of polymorphism detected on LG3 was particularly interesting, since in this part of the genome resides Or_5 (Tang et al., 2003). In order to examine it in more detail, LG3 was screened with the use of 18 SSR markers in total, three of which (ORS 820, ORS 555, ORS 545) were later excluded due to a high amount of unspecific bands. Markers located on the upper part of the LG3 (ORS 1036, ORS 1040, ORS 1112, ORS 665, ORS 1021 and ORS 718) were polymorphic between the resistant and susceptible genotype, whereas markers residing on the lower part of the same linkage group (ORS 1222, ORS 976, ORS 898, ORS 485, ORS 529, ORS488 and ORS 1114) were monomorphic.

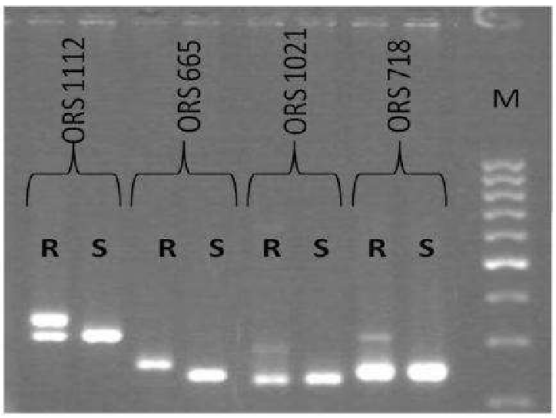

Figure 1. Molecular profiles of the resistant (R) and susceptible (S) line with the selected polymorphic SSR markers from LG3; M=100 bp DNA ladder;

ORS 665 amplified a single polymorphic locus with two alleles (280 and 275 bp in length), while the remaining eight SSR markers amplified two or three polymorphic loci each. ORS 1112, which has a known duplicated locus on LG 10, amplified 3 alleles, one of which being null. Similar molecular patterns were obtained with ORS 1021 and ORS 718 (Figure 1).

ORS 1036 and ORS 1040 each amplified one locus in the resistant, and two loci in the susceptible genotype. With ORS 1036, fragment of 255 bp was obtained in the resistant line, whereas susceptible line had 255 bp and 280 bp long fragments. Even though these results do not match the findings of Tang et al. (2003), who reported a single polymorphic locus of 255 bp long allele in the susceptible line and 245 bp allele in the line carrying Or_5, we were eager to find out if the line that was subject of our investigation has Or_5. Line AB-VL-8 was resistant to broomrape races that overcome race F in the field, hence the gene (or genes) providing resistance in this genotype should be other that Or_5 or Or_6. However, we cannot exclude the possibility that it has Or_5 and Or_6 in addition to some other gene(s). In order to further explore this possibility, molecular profiles of AB–VL-8 and L-OS-1 were compared with profiles of the differential lines for Or_5 (LC 1003) and Or_6 (LC 10093) (Figure 2).

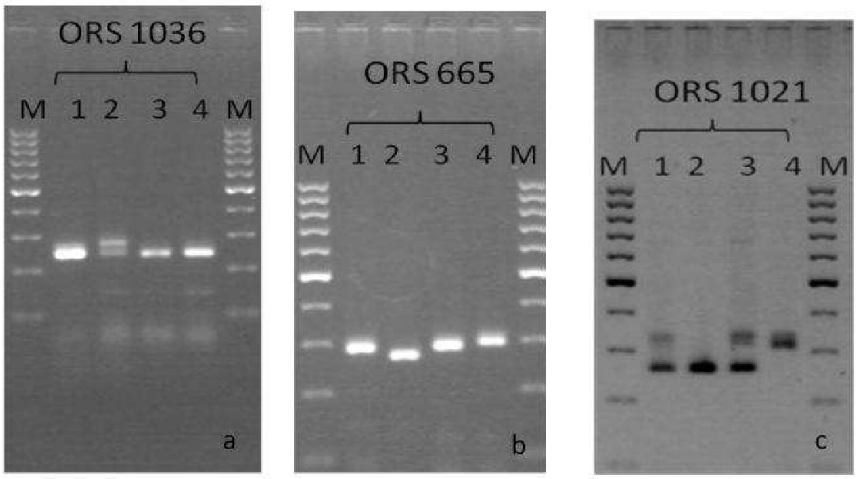

Figure 2. Molecular profiles obtained ORS 1036 (a), ORS 665 (b) and ORS 1021 (c). Lanes 1 to 4 represent the DNA profiles of the lines AB-VL-8 (R), L-OS-1 (S), LC 1003(*Or*) and LC 10093 (Or_6); M=100 bp DNA ladder;

DNA patterns obtained with markers ORS 1036 (Figure 2a) and ORS 1021 (Figure 2c) were clearly different. Each of the lines tested had a unique profile, however since the difference in the length of the amplified fragments was less than 5bp, further research should be done on a gel of higher resolution, or with the use of capillary electrophoresis so the exact size could be determined. Previous investigation on various lines of different origin and with different genetic constitution showed that ORS 1036 was not able to undoubtedly single out lines possessing Or_5 gene from the susceptible lines and lines with genes Or_1 to Or_4 (Imerovski, 2010). The effect of the genetic background on the molecular profiles of the genotypes that were subject of this research remains to be investigated.

In contrast to Tang et al. (2003), who found ORS 665 to be monomorphic between the resistant and susceptible bulk, we found four different alleles: 280 bp, 275 bp, 295bp and 300 bp long bands were detected in AB-VL-8 (R), L-OS-1 (S), LC 1003(Or_5) and LC 10093 (Or_6), respectively (Figure 2b). The amplified bands were clearly separated even on agarose gel.

In conclusion, having all of the observed differences in mind, it could be stated that the source of resistance in AB-BL-8 is not Or_5 or Or_6, but rather a new gen that provides resistance to broomrape races higher than F, and could be vary valuable to the breeders as such. Since *Or* genes were introgressed from wild *Helianthus* species, flanking DNA sequences are expected to be extraordinarily polymorphic (Tang and Knapp, 2003) as it was the case in the work here presented. It is very likely that clustering of different resistance genes occurred on LG3. This phenomenon was already reported in sunflower, i.e. in the case of the downy mildew resistance genes *Pl1, Pl2* and *Pl6,* which were found to be linked to the same set of RFLP markers and formed a cluster on the linkage group 1 (Vear et al. 1997). It is possible that *Or* genes show a similar pattern of forming resistance gene clusters. In order to fully understand the nature of the newly discovered resistance, and to confirm if the polymorphisms presented herein are correlated to the trait of interest, further investigation has to be done on an appropriate mapping population.

High number of polymorphic markers located on one linkage group could mean that the trait is rather qualitative than quantitative, and that the resistance locus could be placed on LG3. The segregation ratio of the progeny will reveal if the resistance is in fact controlled by a single gene or not. With the use of the bulk segregant analyses, molecular differences that are in correlation with resistance will become more notable. Further research should include close examination of LG3 with additional SSR and SNP markers, as well as the development of sequence specific molecular markers for resistance gene clusters (RGCs) that are located in the vicinity of the detected polymorphic markers. In the light of the work that is to be done, the results presented herein should be regarded as the initial step of the research that aims at finding a molecular marker useful for marker assisted selection.

ACKNOWLEDGEMENTS

This work was supported by Ministry of Education and Science, Republic of Serbia, project TR 31025.

REFERENCES

Alonso, L.C., Fernandez-Escobar, J., Lopez, G., Rodriguez-Ojeda, M., Sallago, F., 1996. New highly virulent sunflower broomrape (*Orobanche cernua* Loefl.) pathotype in Spain. In: Moreno, M., Cubero, J., Berner, D., Joel, D., Musselman, L., Parker, C., (eds.) Advances in Parasitic Plant Research. Proc. 6th Int. Symp. Parasitic Weeds. Cordoba, Spain, 639-644.

Fernández-Martínez, J., Melero-Vara, J., Munoz-Ruz, J., Ruso, J., Domínguez, J., 2000. Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance of the *Or5* gene. Crop Sci 40: 550-555.

Fernández-Martínez, J.M., Domínguez, J., Pérez-Vich, B., Velasco, L., 2008. Update on breeding for resistance to sunflower broomrape. Helia 31: 73-84.

Imerovski, I. 2010. The use of SSR markers in detection sunflower resistance to broomrape. Master thesis. Faculty of natural sciences, University of Novi Sad (in Serbian)

Kaya, Y., Evci, G., Pekcan, V., Gucer, T., 2004. Determining new broomrape infested areas, resistant lines and hybrids in Trakya region of Turkey. Helia 27: 211-218.

Pacureanu-Joita, M., Raranciuc, S., Stanciu, D., Sava, E., Nastase, D., 2008. Virulence and aggressiveness of sunflower broomrape (*Orobanche cumana* Walr.) populations, in Romania. Rom Agric Res 25: 47-51.

Pankovic, D., Radovanovic, N., Jocic, S., Satovic, Z., Skoric, D. 2007. Development of co-dominant amplified polymorphic sequence markers for resistance of sunflower to downy mildew race 730. Plant Breeding 126: 440–444.

Skoric, D., and Pacureanu, M., 2010. Sunflower breeding for resistance to broomrape *(Orobanche cumana* Wallr).Proc. Int. Symp. "Sunflower Breeding for Resistance to Disease", Krasnodar, 19-29.

Slabaugh M B, Yu J K, Tang S, Heesacker A, Hu X, Lu G, Han F, Bidney D, Knapp S J (2003): Haplotyping and mapping a large cluster of resistance gene candidates in sunflower using multilocus intron fragment length polymorphisms. Plant Biotech J 1: 167-185.

Tang, S., and S.J. Knapp. 2003. Microsatellites uncover extraordinary molecular genetic diversity in Native American land races and wild populations of cultivated sunflower. Theor Appl.Genet 106: 990-1003.

Tang, S., Heesacker, A., Kishore, V.K., Fernandez, A., Sadik, E.S., Cole, G., Knapp, S.J., 2003. Genetic mapping of *Or5* gene for resistance to Orobanche race E in sunflower. Crop Sci 43: 1021–1028.

Vear, F., L. Gentzbittel, J. Philippon, S. Mouzeyar, E. Mestries, P. Roeckel-Drevet, D. Tourvielle de Labrouhe, and P. Nicolas. 1997. The genetics of resistance to five races of downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 95: 584–589.

Vranceanu, A.V., Tudor, V.A., Stoenescu, F.M., Pirvu, N., 1980. Virulence groups of *Orobanche cumana*Wallr., different hosts and resistance sources and genes in sunflower. In: Proc. 9th Int. Sunflower Conf., Torremolinos, Spain, 74–82.