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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.

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Laboratory method for detection of tribenuron-methyl resistant sunflower (*Helianthus annuus* L.)

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ABSTRACT

- Sulphonylureas are potent herbicide group that inhibit synthesis of three essential amino acids, valine, leucine and isoleucine. They have proven to be very effective in controlling broad-leaf weeds. Developing herbicide resistant genotypes is of great importance in sunflower breeding. As a part of breeding program of the Institute of Field and Vegetable Crops, tribenuron-methyl resistance gene – *Ahasl1-2* was introduced into commercial sunflower inbred lines. Resistant genotypes have a mutation of a single nucleotide in *ahas1* gene. In order to accelerate breeding process, of great importance is development of fast and reliable tests for detection of resistant genotypes.
- In this study a new, quick *in vitro* test is presented. Three tribenuron-methyl homozygous resistant and one susceptible genotypes were grown *in vitro* on MS media supplemented with three different concentrations of herbicide (2.5 μM, 3.0 μM, 3.5 μM).
- *In vitro* test enabled a clear distinction between resistant and susceptible genotypes using different morphological parameters.
- As opposed to conventional herbicide resistance tests, *in vitro* test is faster and the results obtained by this test are reliable, as they are not affected by the environmental conditions.
- A new *in vitro* test presented in this study will greatly accelerate breeding process and development of herbicide resistant commercial sunflower hybrids and lines.

Key words: *in vitro* testing, resistance, tribenuron-methyl

INTRODUCTION

Weeds compete with sunflower for moisture, nutrients and, depending on species, for light and space (Sala and Bulos, 2011). Blamey and Zollinger (1997) showed that sunflower yield loss in weeded conditions could be between 20 and 53% in comparison to weed-free conditions. Herbicides that inhibit function of acetohydroxyacid synthase (AHAS, EC2.2.1.6, also known as acetolactate synthase ALS; EC 4.1.3.18) proved to be very successful in weed control in a number of crops (e.g. rapeseed, maize, soybean, potato, tomato, etc.). There are several groups of herbicides which inhibit activity of AHAS: sulfonylureas (SUs), imidazolinones (IMIs), triazolopyrimidines (TPs), pyrimidinylthiobenzoates (PTBs) and sulfonylaminocarbonyltriazolinones (SCTs) (Kramer and Schirmer, 2007). From the beginning of the '80s of the last century, SUs are found to be very efficient in controlling broad-leaf weeds. The advantage of use of all AHAS inhibiting herbicides, including SUs, is that they express low toxic effect on mammalian organism and low use rates (Chipman et al., 1998).

Extensive herbicide use lead to appearance of herbicide resistant genotypes in sunflower: IMI resistant common sunflower genotype (Al-Kathib et al., 1998), IMI and SU cross resistant sunflower genotype (White et al., 2002) and IMI resistant sunflower line, CLHA-PLUS, developed by EMS induced mutagenesis (Sala et al., 2008a; b). Recently, new sunflower inbred line, RW-B, cross resistant to IMI and SU herbicides was reported (Sala and Bulos, 2011). First registered SU resistant sunflower genetic stocks were developed in USDA-Fargo, North Dakota: SURES-1 (Reg. no. GS-28, PI 633749) and SURES-2 (Reg. no. GS-29, PI 633750) (Miller and Al-Khatib, 2004). SU resistance gene was transferred these stocks into NS sunflower hybrids Jocić *et al.* (2008; 2011).

The development of AHAS inhibiting herbicide resistant genotypes lead to the need for development of reliable and quick tests which could discriminate between herbicide resistant and susceptible genotypes. Hashem et al. (1998) used seedling test to detect metosulam and triasulfuron resistant wild radish resistant genotypes. In comparison to conventional tests which took 8-10 weeks, this test enabled shortening the time for obtaining results to 5-6 weeks for wild radish. Petri dish-based assay was proven to be effective in detection of tribenuron-methyl resistant *Papaver rhoeas* L. genotypes (Cirujeda et al., 2001). Kuk et.al. (2003) used leaf bioassay for detection of SU resistant *Monochoria vaginalis* genotypes. Richter et al. (1993) reported Petri-dish based method that was based on adding sulfometuron-methyl, triasulfuron or imazapyr to pollen tube growth medium and measuring rigid ryegrass pollen germination after 2 h. Disadvantage of this test was that it could not detect resistant genotypes if resistance mechanism was not expressed in pollen. New rapid pot germination test was recently developed for detection of IMI sunflower resistant genotypes (Breccia et al., 2011).

So far, a quick SU resistance sunflower test was not reported. The aim of our work was to develop quick, reliable and cheap test for screening herbicide resistance during sunflower inbred lines conversion into herbicide resistant form and their further use in breeding. It should enable testing of a large number of plants in controlled conditions, and therefore be used independently from the season of the year. Objective of this work was to find optimal tribenuron-methyl concentration that could most easily discriminate between resistant and susceptible sunflower hybrids.

MATERIALS AND METHODS

Three homozygous tribenuron-methyl resistant and one tribenuron-methyl susceptible sunflower hybrid from Institute of Field and Vegetable Crops, Novi Sad, Serbia, were used in the experiment (Table 1).

Herbicide solution was added in MS medium (Murashige and Skoog, 1962), with pH adjusted to 8, in final concentrations of 2.5 μM, 3.0 μM, 3.5 μM. Before it was added to MS medium, herbicide (Express 50SX, active substance tribenuron-methyl) was dissolved in sterilized dH_2O, and filter sterilized (Rotilabo – syringe filters, Roth).

Seeds of the tested hybrids were sterilized according to Taski-Ajdukovic and Vasic (2005), and were germinated in Petri dishes with filter paper soaked in sterilized water. Seed germination was performed in the dark in 25°C in an incubator for 2 days to obtain healthy seedlings of appropriate size.

Table 1. Sunflower hybrids used for testing

Name	Type	Genotype
HoF	resistant	*Ahasl1-2/Ahasl1-2**
HoT	resistant	*Ahasl1-2/Ahasl1-2*
HoP	resistant	*Ahasl1-2/Ahasl1-2*
Os	susceptible	*ahasl1/ahasl1*

*nomenclature proposed by Sala et al. (2008)

After two days of germination, healthy sunflower seedlings that were around 1.5 cm long were chosen and placed in MS medium supplemented with different concentrations of tribenuron-methyl and the control. Three seedlings were put in each Erlenmeyer flask of 250 ml. Every treatment was set in four repetitions. Erlenmeyer flasks were kept in chamber at 25°C and a photoperiod 16h day and 8h night.

Development of the above-ground part of the seedlings on MS medium was observed for twelve days. On the 12th day of culture, shoots were taken out of the Erlenmeyer flasks and fresh (FAGM) and dry (DAGM) mass of above-ground part was measured. Dry above-ground mass was measured after drying for approximately 48 hours at 105°C.

The bioassay FAGM and DAGM was subjected to analysis of variance. The means were separated by least significant difference (LSD) test (STATISTICA 10).

RESULTS AND DISCUSSION

In this study, preliminary results of an *in vitro* herbicide resistance test were presented. The aim was to create easy, short and reliable test that could be used for detection of tribenuron-methyl sunflower resistant genotypes. First step was to determine optimal herbicide concentration that enables the clearest distinction between tribenuron-methyl resistant and susceptible sunflower hybrids. For that purpose three homozygous tribenuron-methyl resistant sunflower hybrids and one tribenuron-methyl susceptible hybrid were treated with three different herbicide concentrations.

The development of tribenuron-methyl resistant and susceptible plants was observed during the period of 12 days. It took, approximately, two days for cotyledons of all genotypes to turn green and on the 6th day (counting from the day seedlings were placed on MS medium) first leaf pair was formed in all resistant sunflower hybrid plants on all media used, but not in susceptible sunflower plants, except in control. The similar phenomenon was observed in *Papaver rhoeas* L. where susceptible plant stopped growing after cotyledon stage and turned chlorotic after being treated with tribenuron-methyl, while resistant plants continued to developed new leaves in Petri dish based assay (Cirujeda et al., 2001).

Twelve days after being exposed to herbicide treatment, plants were taken out of MS medium and FAGM and DAGM was measured and compared. In susceptible genotype, there was significant decrease of FAGM in all treatments compared to the control (Table 2). In resistant genotypes, LSD analysis of FAGM showed a statistically significant difference between control and treatments at 2.5 μM and 3 μM for HoF and significant difference between control and 3 μM treatment for HoT, i.e. decreace of FAGM compared to the control. There was no significant decrease in FAGM compared to the control in all resistant genotypes when treated with 3.5 μM of tribenuron-methyl. These results indicate that concentration of 3.5 μM could be marked as the most suitable concentration for herbicide resistance testing of sunflower genotypes. Cirujeda et al. (2001) were able to differentiate between resistant and susceptible *Papaver rhoeas* L. populations with concentrations that were in between concentrations used in this work. The lowest concentration that could discriminate between resistant and susceptible genotypes was 0.24 μM tribenuron-methyl (when no gibberellin was added to Petri dish). The same effect was achieved with 7.68 μM (when 0.2 g gibberellin/L was added to Petri dish) and 61.44 μM tribenuron-methyl (when 0.5 g gibberellin/L was added to Petri dish).

Table 2. LSD test for tested values obtained by measurement of fresh above-ground mass of tribenuron-methyl resistant and susceptible sunflower hybrids genotypes

Treatment / Genotype	Control	2.5 μM	3 μM	3.5 μM
HoF	1.2333 de	0.8946 f	1.0136 f	1.0763 ef
HoT	1.5916 ab	1.392 abcd	1.3842 cd	1.5939 a
HoP	1.4524 abc	1.374 cd	1.3777 cd	1.3869 bcd
Os	1.2425 de	0.398 g	0.4014 g	0.3599 g

Values (presented in grams) within the table marked with different letter differ significantly at $\alpha_{0.05}$

In susceptible genotype, LSD test for DAGM showed that all treated plants differed significantly from DAGM values of plants in control (Table 3). Analysis of DAGW showed no statistically significant difference between control and all treatments for HoF, HoT and HoP.

Regarding its duration, resistance test presented in this paper belongs to quick resistance tests (in comparison to conventional testing). Results of tribenuron-methyl resistance Petri-dish test of *Papaver rhoeas* L. were obtained after 14 to 17 days after sowing (Cirujeda et al., 2011). New pot germination sunflower IMI resistance test lasted between 8 and 15 days (Breccia et al., 2011).

Table 3. LSD test for tested values obtained by measurement of dry above-ground mass of tribenuron-methyl resistant and susceptible sunflower hybrids genotypes

Treatment / Genotype	Control	2.5 μM	3 μM	3.5 μM
HoF	0.0798 efg	0.0684 gh	0.0725 fgh	0.0801 efg
HoT	0.1180 a	0.122 a	0.1140 ab	0.1154 ab
HoP	0.0957 cd	0.0916de	0.0862 def	0.0986 cd
Os	0.1020 bc	0.0612 h	0.0607 h	0.0645 h

Values (presented in grams) within the table marked with different letter differ significantly at $\alpha_{0.05}$

All parameters examined in *in vitro* tribenuron-methyl test discriminated between SU resistant and susceptible genotypes. Dry above-ground mass was found to be better resistance parameter since there was no statistically significant difference between control and treatments for all tested resistant genotypes. If fresh above-ground mass was used as resistance parameter, analysis should be performed at 3.5 μM treatment since there was not a statistically significant difference between treatment at that concentration of herbicide and control for all tested resistant genotypes. Advantage of the test presented in this work is that it gives reliable results in a short period of time. The results are environmentally independent and pollution of environment is minimized since the remaining of MS medium with herbicide is autoclaved after testing and thus the herbicide decomposes in such high temperature conditions.

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REFERENCES

Al-Khatib, K., Baumgartner, J.R., Peterson, D.E., and R.S. Currie. 1998. Imazethapyr resistance in common sunflower (*Helianthus annuus*). Weed Sci. 46:403-407.

Blamey, F. P., and R. K. Zollinger. 1997. Sunflower production and culture. P. 596-670. In: A.A. Schneiter (ed.), Sunflower Technology and Production. American Society of Agronomy Inc., Crop Science Society of America Inc., Soil Science Society of America Inc., Madison, WI, USA.

Breccia, G., Vega, T., Nestares, G., Mayor, M.L., Zorzoli, R., and L. Picardi, L. 2011. Rapid test for detection of imidazolinone resistance in sunflower (*Helianthus annuus* L.). Plant Breeding 130:109—113. doi:10.1111/j.1439-0523.2009.01756.x

Chipman, D., Z. Barak, and J.V. Schloss. 1998. Biosynthesis of 2-aceto- 2 hydroxy acids: acetolactate synthases and acetohydroxyacid synthases. Biochim. Biophys. Acta. 1385:401-419.
Cirujeda A, Recasens J and A. Taberner. 2001. A qualitative quick-test for detection of herbicide resistance to tribenuron-methyl in *Papaver rhoeas*. Weed Res. 41:523–534.
Hashem A, Hallem S, Bowran D and M. Allan. 1998. Herbicide resistance in wild radish (*Raphanus raphanistrμM*): new seed soaking method for resistance testing. In: 9th Australian Agronomy Conference. Poster Presentation. [Online]. Available: http://www.regional.org.au/au/asa/1998/6/290hashem.htm [html 23.11.11.]
Jocic, S., Miklic, V., Malidza, G., Hladni, N., and S. Gvozdenovic. 2008. New sunflower hybrids tolerant of tribenuron-methyl. p. 505-508. In: Proc. 17th Int. Sunfl. Conf., Cordoba, Spain.
Jocic, S., Malidza, G., Cvejic, S., Hladni, N., Miklic, V., and D. Skoric. 2011. Development of sunflower hybrids tolerant to tribenuron methyl. Genetika. DOI: 10.2298/GENSR1101175J.
Koley, N. K., Sahoo, B. C., and A.K. Das. 1993. Dissipation and effect of chlorophenoxy herbicide on plant pigments and carbohydrates of sunflower (*Helianthus annuus* L.). Toxicological & Environmental Chemistry. 39:97-102.
Kramer, W and U. Schirmer. 2007. Modern Crops Protection Compounds, Vol.3. WILEY-VCH Verlag GmbH & Co, KGaA, Weinheim.
Kuk, Y.I., Jung, H.I., Kwon, O.D., Lee, D.J.,Burgos, N.R., and J.O. Guh. 2003. Rapid diagnosis of resistance to sulfonylurea herbicides in monochoria (*Monochoria vaginalis*). Weed Sci. 51:305-311.
Lovell, ST, Wax, L.M., Simpson, D.M., and M. McGlamery. 1996. Using the in vivo acetolactate synthase (ALS) Assay for identifying herbicide-resistant weeds. Weed Sci. 10:936-942.
Miller, J.F., and K. Al-Khatib. 2004. Registration of two oilseed sunflower genetic stocks, SURES-1 and SURES-2, resistat to tribenuron herbicide. Crop Sci. 44:1037–1038.
Murashige, T., and F. Skoog. 1962. A revides medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.
Orcaray, L., Igal, M., Zabalza, A., and M. Royuela. 2011. Role of Exogenously Supplied Ferulic and p-Coumaric Acids in Mimicking the Mode of Action of Acetolactate Synthase Inhibiting Herbicides. J. Agric. Food Chem. 59:10162–10168
Richter, J., and S.B. Powles. 1993. Pollen expression of herbicide target resistance genes in annual ryegrass (*Lolium rigidum*). Plant Physiol. 102:1037–1041.
Sala, C.A, Bulos, M, and A.M. Echarte. 2008a. Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. Crop Sci. 48:1817–1822.
Sala, C.A., Bulos M., Echarte, A.M., Whitt, S.R., and R. Ascenzi. 2008b. Molecular and biochemical characterization of an induced mutation conferring imidazolinone resistance in sunflower. Theor. Appl. Genet. 108:105–112.
Sala, C.A., and M. Bulos. 2011. Inheritance and molecular characterization of broad range tolerance to herbicides targeting acetohydroxyacid synthase in sunflower. Theor. Appl. Genet. DOI 10.1007/s00122-011-1710-9.
Taski-Ajdukovic, K.J. and D.M. Vasic. 2005. Different sterilization methods for overcoming internal bacterial infection in sunflower seeds. Proc. Nat. Sci. Matica Srpska, Novi Sad. 109:59-64.
White, A.D., Owen M.D., Hartzler, RG, and J. Cardina. 2002. Common sunflower resistance to acetolactate-inhibiting herbicides. Weed Sci. 50:432-437.