

Sequential evaluation for resistance to three characters in sunflower seedlings.

Silvana Maria Piubello¹

Santiago Renteria²

¹Conversion Service and Generations Advance in Greenhouse. Primera Junta 1235 - Junín (6000), Buenos Aires, Argentina. silvana.piubello@gmail.com.

² Pannar Seeds SRL. Mitre 745, Venado Tuerto (2600) Santa Fe, Argentina. srenteria@pannar.com.ar

Abstract

- At the moment the market demands from the Seed Companies more competitive sunflower hybrids, not only in yield but also with the aggregate of the new events and disease resistance characters. With the discovery of the resistance gene to the imidazolinones herbicides family *Ahas11-1* (*Imisun*) and recently *Ahas11-3* (*CLHA Plus*) along with other major *Pl* genes for Downy Mildew (MR) resistance, caused by *Plasmopara halstedii* and, *Ph* and *PU6* genes for resistance to Rust (PR), caused by *Puccinia helianthi*, moved seed companies to speed up the conversion process of the key elite lines. The conversion work in sunflower is a relatively long process if it is compared with these tasks.
- In addition to that, in each new gene introgression is a real risk to lose other important traits present in elite lines or directly not present in homozygous. To shorten the times to obtain the new genotypes a common practice already implemented by the industry is to increase the number of generations per year with the integrated use of the growth station, winter nursery and/or greenhouse. The sunflower culture in greenhouse demands efficiency in many aspects, especially thermal-luminance, nutritional and sanitary requirements in order to obtain a suitable production of seeds, being the last one the main limiting factor of success in the advance of generation processes and in the increase of the number of generations per year.
- The object of this work was to make more efficient the use of seeds and the winter generation for the evaluation of a group of characters in a same seedlings group. The genes whose presence was evaluated were *Ahas11-3* (CL+), object of the conversion of the elite lines, and *Pl6* (MR) and *Pu6* (PR) genes already present in the families.
- The sequence of the treatments was designed on the basis of the different development stages required in each protocol to obtain the resistance or susceptibility manifestation present in each individual. For this reason, first the seedlings when germinated were inoculated with *Plasmopara halstedii* spores and then cultivated in a chamber. After the evaluation the resistant plants were taken to greenhouse. In V4 they were sprayed with CL+ herbicide (Imazamox + Imazapir). The plants selected as resistant were sprayed with a solution of spores of *Puccinia helianthi*. After 45/50 days germination was initiated, the resistant plants selection to the 3 characters was finalized and then cultivated until completing their cycle with production of seeds.
- The sequential evaluation of the resistance to MR, CL+ and PR on a same group of seedlings is an efficient technique to apply in a conversion process of elite lines in sunflower, obtaining the selection of the individuals resistant to the three characters in 45/50 days, using a low number of seeds by family and reducing the number of days required to complete each one of the generations.

Key words: sequential evaluation, resistance, gene *Ahas11-3*, *Plasmopara halstedii*, *Puccinia helianthi*, sunflower.

INTRODUCTION

At the moment the market demands more competitive hybrids from the plant breeding companies, not only in yield but also with the aggregate of the new events and disease resistance characters. The yield of a culture can fail by numerous factors. Within the factors that can affect the stability of the sunflower yields, the diseases occupy a preponderant place. Ferenc Virányi (2008) in 17^o International Sunflower Conference evaluated the relative importance of different diseases. Downy Mildew (MR) produced by *Plasmopara hastedii* can be considered the most important pathogen and in fact the most studied. For growers, the genetic resistance is more important than fungicide seed treated because the fungicide seed treated protect against the primary infection but not against the secondary one. The genes *Pl6* and *Pl8* are those that are conferring resistance to the present races in recent years.

Anyway, in the last years the great challenge for the companies is to obtain hybrids with a durable resistance to the main diseases, necessity already indicated by Felicity Vear in 16^o International Sunflower Conference (2004), since the times of appearance between races are shorter, added that some races showed resistance to metalaxil fungicide (Gulya, 2000). Another important disease is rust (PR) produced by the fungus *Puccinia helianthi* which, according to the year and Argentina regions, can severely affect the yields. The genes *Pu6* and *Ph* confer resistance to this disease (Moreno, 2006).

In general terms, the sunflower always was a relegated culture with respect to the technology incorporation, especially in weed control. But the appearance in 1996 of resistant wild sunflowers to the imidazolinonas herbicide family was the most important step in this subject (Al-Katib et al, 1998). This gene was identified with the *Ahas1-1* name (*Imisun*) (Al-Katib and Miller, 2000) and was immediately incorporated to the hybrids, although in the last years it was surpassed by the *Ahas1-3* CLHA (Plus) gene (Sala, 2010).

It is an important challenge for the plant breeding companies to incorporate in the shorter possible time the greater amount of genes that improve the behavior of the genotypes against different environmental adversities, and to allow the hybrid to express its productive potential. With the incorporation of each new gene the risk is to lose other important traits present in elite lines or directly not present in homozygous. Therefore, it is necessary to control the presence of those characters already present in the parental lines. The time necessary for this work is always long, compared with the market competitive exigencies. Thus, it becomes necessary to work in winter nursery and/or greenhouse, not only for the generations advance in the conversion process of parental lines to the new characters, but also to check the traits that should be present in the genotypes.

The challenge of this work is to be efficient, especially in the use of seeds, time and space that are translated into smaller costs. Perhaps the seeds number is the more important item in this stage of the improvement, where the selection is done at an individual plant level.

In the necessity to evaluate the presence of several genes in a sister family group coming from individual plants selected by their agronomics characteristics to field, therefore with a low seeds number to carry out all the necessary evaluations, it became necessary to make the test on a same group of seedlings.

The object of this work was to obtain more efficiency in the use of seeds and winter generation for a group of traits evaluation on the same seedling group. The genes whose presence was required to state were *Ahas1-3* (CL+) object of the conversion of the elite lines, and the genes *Pl6* (MR) and *Pu6* (PR) already present in the families.

MATERIALS AND METHODS

The work was carried out in “Cultivos Don Juan”, an establishment located in Junín, Buenos Aires province.

The genetic material used, property of the company Pannar Seeds, consisted of 8 sister families derived from the elite restoring line denominated P34, with the genes *Pl6* (MR), *Pu6* (PR) and *Ahas1-3* (CL+) incorporated.

These genes were incorporated to this line through backcrosses of 2 isolines with the same genetic background, one with the genes *Pl6* (MR), *Pu6* (PR) and another one with the gene *Ahas1-3* (CL+) and *Pu6* (PR) combining sowings of station along with greenhouse, obtaining an advance of 3 generations per year. The 8 family seeds were in backcross 4 filial 5 (BC4F5) and 50 seeds of each family were used for the sequential evaluation of the resistance to MR, PR and CL+.

The sequence of the treatments was designed on the basis of the different development stages required in each protocol to obtain the resistance or susceptibility manifestation present in each individual.

Therefore the order was:

1°) MR Test: since the moment of the inoculation is when the seedlings have the root length of 0.5-2.5 cm (Molinero and col, 2000). It was use 770 race (Tourvieille and col., 2000)

2°) CL +Test: since the state of V4 is the development state recommended to obtain a good effectiveness of the product. The product that was used was precommercial herbicide BAS723 00 H whose active principles are Imazamox + Imazapir, and correspond to the commercial product Clearsol Plus (BASF, 2010).

3°) PR Test: where susceptibility to disease can be given in all the development stages, although in the protocol the first pair of leaves for the inoculation is recommended (Maih and Sackston, 1967), in this case took place in V6-V8. In all the cases a susceptibility control was used to confirm the treatment quality and to determine the moment of evaluation of the CL + and PR tests. It is necessary to emphasize the importance of the susceptibility control in these tests where one hoped that the materials were 100% resistant for the 3 characters.

The susceptible control for the test MR and CL+ was hybrid Pan7034 and for PR was hybrid Paraíso 21.

The tests were begun in April 20th of 2011, in order to complete the selection of the families before the following campaign 2011/2012.

The MR test begins with the seeds germination on paper. After 48 hours, when roots length are between 0,5 and 2,5 cm, the seedlings are submerged in an esporangious solution (2×10^4 /ml) during 3 hs., in a temperature of 15°C. Next they are planted in plugs of 128 cells with substrate. The substrate is made up of soil (30%), perlite (50%), earthworm-compost (10%), peat (10%) and basic fertilizer (0.5g/lit of substrate). The culture conditions are 18/22°C (night/day) of temperature and a photoperiod of 12 hs. With respect to the irrigation, it is necessary to maintain the substrate permanently humid. After 10/12 days, when seedlings are expanding the first leaves, a humid chamber of plugs is made during 48 hs to favor the fungus sporulation. The evaluation is done and the resistant seedlings are taken to greenhouse and transplanted to 0.5 lt vases with the same substrate that is used in plugs. The culture conditions are 18/25°C of temperature, 16hs of light and sanitary control for a good plant growth. When they reach the V4 state, they are sprinkled with the herbicide solution, at 1 lt of commercial product/ha (1X). After 7 days, when the susceptibility control indicates the herbicide action, the evaluation is done. The resistant plants are pulverized with an industrial talc and *Puccinia helianthi* spore mixture. Once innoculated, they are put in a humid chamber during 72 hs to favor the spore germination. From 12 to 15 days after the inoculation, when in the susceptibility control plants the pustules appear, the evaluation should be done. Of each family 4 resistant plants are chosen and they are transplanted to 6lts vases with the substrate used previously to complete its cycle until the seeds production. During the rest of the cycle the plants receive complementary fertilization with irrigation water (18-18-18) in a concentration of 0.5gr/lit of water.

RESULTS

The accomplishment of the work took 48 days from the beginning (sowing of the seeds) to the end of the last evaluation (PR reading).

Picture N° 1: Test results (in %) to verify the presence of the genes of resistance *Pl6* (MR), *Ah1-3* (CL+) and *Pu6* (PR).

Genotypes	Resistant plants MR (%)	Susceptible plants MR (%)	Resistant plants CL+ (%)	Susceptible plants CL+ (%)	Resistant plants PR (%)	Susceptible plants PR (%)
P34CL+ 55	100	0	100	0	100	0
P34CL+ 57	100	0	100	0	100	0
P34CL+ 58	100	0	100	0	100	0
P34CL+ 59	100	0	100	0	100	0
P34CL+ 60	100	0	100	0	100	0
P34CL+ 62	100	0	100	0	100	0
P34CL+ 64	100	0	100	0	100	0
P34CL+ 65	100	0	100	0	100	0
P35CL+ 75	100	0	100	0	100	0

Susceptible control	0	100	0	100	0	100
---------------------	---	-----	---	-----	---	-----

The picture n° 1 shows the percentage of resistant and susceptible plants for the 3 evaluated characters.

In the first place we can confirm that the three evaluations are done on the same group of plants. One can see that all the evaluated families are resistant to MR, PR and CL+. In addition, the susceptible controls behaved as such, which does it means that the treatments are effective.

DISCUSSION

The importance of this work is based on the development of a technique combining separate protocols of routinary use in order to make more efficient the use of winter nursery and greenhouse in the conversion process of sunflower elite lines. The total resistance results obtained of the three characters evaluated in BC4F5 for all families show that the same had the three genes in homozygous state not finding variation within families.

Although the counterpart of the 3 tests in 3 groups of seedlings was not accomplished (the number of seeds being an important obstacle) , it is possible to assure that, for example, the space in the greenhouse should have tripled, as well as all the inputs for the growth of the plants until the moment of the test evaluation, especially manpower for the care of those plants; that the plants fulfilled their cycle in the normal time; and that enough seeds could be harvested to sow in the following campaign.

CONCLUSION: - The sequential evaluation of the joint resistance to MR, CL+ and PR on a same seedling group is a possible and efficient technique to apply in the conversion processes of sunflower elite lines, obtaining the selection of the resistant individuals to the three characters in 45/50 days, along with a low number of seeds to be used by family and a smaller number of days required to complete each one of the generations. The described protocol would allow accomplishing the selection work sequentially in early generations of backcrosses towards a same genetic background. The fast and efficient incorporation of qualitative characters within complex genotypes allows to diminish the time of arrival of the commercial hybrids to the market and, therefore, the yield penalty that is usually associated to the introgression of new characters in the crop..

REFERENCIAS

- Al-Katib L, Baurgartner, J., Peterseon, D. and Currie R. 1998. Imazethapir resistance in common sunflower (*Helianthus annuus*)- Crop Sci. 40: 403-407.
- Al-Katib K. and Miller J. 2000. Registration of four genetic stocks of sunflower resistant imidazolinone herbicides. Weed Sci. 40:867-870.
- Basf Corporation (2010). Marbete de Clearsol Plus. http://agro.basf.com.ar/productos/Clearsol_Plus.pdf.
- Gulya, T. (2000). Metalaxyl resistance in sunflower downy mildew and control through genetics and alternative fungicides. Proceedings del 15° Conferencia Internacional de Girasol, Vol II: 179-184.
- Maih, M.A. and sackston W.E. 1967. A simple method for inoculating individual leaves of sunflower and wheat with several races of rust. Phytopathology, 57: 1396-1397.
- Molinero-Demilly V., Guénard M., Giroult C., Herbert O., Tourville D., Jacqueline P., Peanud A., Tardin M.C., Argeles G., Costes M., Mezzarobba Al., Cellier V., Grégoire S. 2004. Improving the sunflower downy mildew resistant test. Proc. 16th International Sunflower conference, Fargo, ND USA, Vol I: 99-110.
- Moreno, P.2006. Determinación de razas fisiológicas en poblaciones de “Roya Negra (*Puccinia helianthi* Schw.) de Argentina.
- Sala C. 2008. Nuevas tecnologías en tolerancia a herbicidas. <http://www.asagir.org.ar>. 5° Congreso Argentino de Girasol. Modulo 4: I129-I139.
- Tourvieille D., Gulya T, Masirevic S., Penaud A., Rashid K. y Viranyi F. 2000. New nomenclature of Races of *Plasmopara halstedii* (Sunflower Downy Mildew). Proceedings de la 15° Conferencia Internacional de Girasol, Vol II: I61-I65.

Vear, F. 2004. Breeding for durable resistance to the main diseases of sunflower. Proc. 16th International Sunflower conference, Fargo, ND USA, Vol I: 15-28.

Virányi F. 2008. Research progress in sunflower diseases and their management. Proc. 17th International Sunflower conference, Córdoba, Spain, Vol 1: 1-12.