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Original article

Herbicide tolerance in sunflower and the contribution of Argentinian science to its development

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Abstract

Sunflower is grown in Argentina across a large and heterogeneous target population of environments. Weed control is one important challenge for this crop to keep it competitiveness with other production schemes. The role of herbicide tolerance research in the public and private sector in Argentina was critical for the development of new technologies across the last two decades, which had impact not only at the regional but also at the global level. The activities include development of initial elite germplasm carrying mutations in Ahal1, but also the discovery and creation of new mutations on the same locus to keep this technology evolving. To create a more efficient way for the deployment of these technologies the development of molecular markers is crucial, and this was attended by local teams too. Finally, the impact of this new technologies on the environment and the analysis of the gene flow from herbicide tolerant materials to sunflower wild relatives was conducted by public research teams in the country. These analyses must be done to create a safe way to translate this genetic modification into technologies than can be applied in every sunflower production area. The aim of this article was to review and highlight some of the milestones obtained by Argentinian researchers in the sunflower herbicide tolerance developments through the last decades.

Keywords: Herbicide Tolerance, Sunflower, Science and Development.

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INTRODUCTION

Sunflowers (*Helianthus annuus* L.) are members of the Asteraceae family (=Compositae), *Asteriodae* subfamily, *Helianthae* tribe, *Helianthinae* subtribe (Seiler and Rieseberg 1997). Asteraceae family is one of the most important considering flowering plants, containing 10% of the all the angiosperms species. Composites are recognized because of their reproductive structure, the capitulum, where the flowers are disposed in a sesil pattern over a broader receptacle (Katinas et al. 2007).

Helianthus genus comprises 36 perennial species and 12 annual herbs (Schilling and Heiser, 1981), among the ones diploids, tetraploids and hexaploids plants are included, all of them having a basic chromosome number of x=17 (Heiser and Smith, 1955).

H. annuus is composed by three subspecies: *H. annuus* ssp. *macrocarpus* (sunflower crop), *H. annuus* ssp. *lenticularis* y *H. annuus* ssp. *annuus*, both as wild relatives of the sunflower crop. Sunflower is a diploid specie (2n = 2x = 34), having a genome of 3500 Mbp as an average size (Baack et al. 2005) and an average DNA content (2C) of 7,3 pg (Jan and Seiler, 2007).

Sunflower production during 2021-22 was estimated at 57.20 million (M) metric tons, according to USDA. Increased production in Russia and Ukraine on more acres and a higher output than last year’s drought-reduced production are the primary drivers for the increase from 2020 (USDA, 2021).

In Argentina sunflower is produced between latitudes 26ºS (Chaco) and 39ºS (southern Buenos Aires) across a large and heterogeneous target population of environments (TPE). These include subtropical (Northern) and temperate (Central and Southern) climates and a wide range of soil types, water regimes, pest and disease conditions, and management practices. During 2023, expected production is close to 3,5 M Tons being produced in 2,2 M ha.

The problem of weeds and associated control measures before selective herbicides

Weeds continue to pose a huge challenge for the sustainable production of sunflower despite decades of implementation of contemporary methods in order of their control. Weeds compete with sunflower for moisture, nutrients, and depending on species for light and space. Weed competition can cause substantial yield losses in sunflower, with reports ranging from 20 to 70% (Blamey et al. 1997). The development of weeds tolerant to herbicides and weed shifts indicate the inefficient of modern agro-technical measures. Integrated Weed Management (IWM) is a sustainable approach to the management of weeds by combining all available weed control techniques, including preventative measures, monitoring, crop rotations, tillage, crop competition, mechanical and physical control, herbicide rotation, herbicide mixtures, biological control, nutrition, irrigation, flaming, etc. in a way that minimizes economic, health, and environmental risks (Swanton et al. 2008).

Producers have fewer herbicide options for broadleaf weed control in sunflower compared to most other row crops. They traditionally relied on pre-emergence herbicides, which require timely rainfall for activation (Kerr et al. 2004). On the other hand, the agricultural chemical industry has not brought any new herbicides with novel sites of action to market in last 30 years. Before commercialization of imidazolinone- and tribenuron-tolerant sunflower hybrids, soil-applied herbicides were especially important because there were no post-emergent herbicides for control of broadleaf weeds that could be applied.

Common pre-emergence herbicides used in sunflower cultivation included ~~atrazine~~, some triazines, metolachlor, and pendimethalin. These herbicides provided residual control, reducing weed competition during the early stages of sunflower growth. Pre-emergence herbicides were particularly useful in minimizing weed pressure during the critical period when sunflower plants were establishing themselves and vulnerable to competition. Soil-active preemergence herbicides are expensive and require timely rainfall or irrigation for activation. Also, some are marginally effective because of the narrow spectrum of weeds controlled (Miller and Alford, 2000).

It's worth noting that while non-selective herbicides and pre-emergence treatments were effective in controlling weeds, they have some limitations. Non-selective herbicides kill all plants, including sunflowers. Pre-emergence herbicides have limited effectiveness against perennial weeds or those with deep root systems. Additionally, the development of herbicide tolerance in weed populations has been a challenge, emphasizing the importance of integrated weed management practices and rotation of herbicides with different modes of action (Malidza et al. 2016)

Because of the importance of the crop in Argentina, the existing weed pressure in its main cultivation regions and the long history in sunflower research in the public and private sector, a big portion of the herbicide tolerance developments were direct or indirect connected with the work of Argentinian scientists.

The development of herbicide tolerant sunflower materials

Given their high effectiveness and low-toxicity, imidazolinone (IMI) and sulfonylurea (SU) herbicides are favored for agricultural use. However, the ability to use both types of herbicides in a sunflower production system depends upon the availability of IMI- and SU-tolerant hybrid cultivars. To produce such tolerant cultivars, it is imperative to develop IMI- or SU-tolerant plants with altered AHAS genes and enzymes. These plants have been discovered in sunflower, which permitted the development and commercialization of several herbicide-tolerant traits. Tolerance in these traits is due to a form of the AHAS large subunit enzyme (AHASL) that is less sensitive to herbicide inhibition and is conferred by a single, partially dominant nuclear gene. The introduction of selective herbicides has provided more targeted and efficient weed control in sunflower fields, as they selectively target specific weed species while minimizing damage to sunflowers.

Imidazolinone tolerance were discovered firstly in wild types in US then this tolerance gene was transferred to cultural ones (Al Khatib et al. 1998; Miller and Al Khatib 2002). IMI herbicides imazamox + imazapyr were firstly registered in Turkey in IMI sunflower, both to control weeds and broomrape. With IMI tolerant sunflowers and herbicides (called as Clearfield (CL)* system from BASF company), farmers had been able to utilize much more effective post-emergence herbicides controlling both major broadleaf weeds and broomrape (Demirci and Kaya 2009). CL technology has been used successfully for ten years in Eastern Europe and has one third of market in Hungary and Turkey and increase rapidly in Bulgaria, Hungary, Romania, Russia and Ukraine (Elezovic et al. 2012; Evci et al. 2011).

Jose Maria Bruniard, and Argentinian researcher from INTA, and Miller (2001) studied the inheritance of herbicide tolerance in the Imisun HA425 inbred line. These authors demonstrated that this trait is controlled by 1 semidominant gene, named *Imr1*, and a second modifier gene, named *Imr2*. Kolkman, together with Bruniard and a research team from Advanta in Argentina, showed that *Imr1* is an allelic variant of the *Ahasl1* locus coding for acetohydroxyacid synthase (AHAS) catalytic subunit (Kolkman et al. 2004) harboring a C-to-T mutation in codon 205 (*Arabidopsis thaliana* nomenclature). However, the nature of the tolerance endowed by *Imr2* was not determined. The fact that AHAS target-site modification does not confer 100% of the Imisun tolerant phenotype, and that the background genotype must be conducive to tolerance was first demonstrated by Sala et al. (2012), who leads the Nidera Seeds biotech department in Argentina, also located in Argentina.

Bertero and Vasquez, Nidera's sunflower breeders, developed the first high yielding CL sunflower hybrid (Paraiso 101) adapted to Argentina, and later developed many different materials for this region and for Europe. Clearfield hybrids were widely adopted in Argentina by farmers who use direct sowing, reaching in less than 20 years, more than 70% of the market share in this country, but also having a big impact in many others.

The team lead by Sala, continue with the discovery and development of sunflower herbicide tolerance. They discover a second IMI tolerance trait in sunflowers, known now as CLPlus, that is controlled by the expression of the partially dominant nuclear allele *Ahasl1-3*, and it confers high levels of IMI-tolerance (Sala et al. 2008c). *Ahasl1-3* presents a G-to-A mutation in codon 122. This trait was developed by seed mutagenesis and selection with imazapyr (Sala et al. 2008b). Based on a vast array of environmental conditions and in biochemical studies, it was determined that the CLPlus trait provides superior herbicide tolerance to IMI when compared with Imisun trait (Sala et al. 2008a, c; 2012d; Weston et al. 2012). In fact, CLPlus trait displays the lowest level of inhibition of the AHAS enzyme extracts by IMI, which results in a higher level of accumulation of biomass after IMI application at the above-ground (Sala et al. 2012a) and root levels (Sala et al. 2012e). Due to the high levels of tolerance, only one homozygous component, namely *Ahasl1-3*, or the combination of both *Ahasl1-1* and *Ahasl1-3* alleles in the final hybrid variety, are required to achieve commercial tolerance levels (Sala et al. 2008a; 2012a; Sala and Weston 2010). High level of tolerance of this trait over Imisun sunflowers also permits to develop new herbicide formulations providing more flexible and reliable weed control (Pfenning et al. 2012).

A few years later, a new technology, known as AIR was launched by Syngenta. The initial discover, and initial development was conducted by the same Argentinian team and is based on another *Ahasl1* allele, named as *Ahal1-4*. *Ahasl1-4* presents a completely new pattern of cross-tolerance for sunflower, since it shows a broad range level of tolerance to different AHAS-inhibiting herbicides. Furthermore, this allele also presents a higher level of tolerance to IMI and SU than lines carrying the Imisun and the Sures traits, respectively (Sala and Bulos 2012a). *Ahasl1-4* harbors a G-to-T mutation in codon 574, which endows broad range tolerance to four families of herbicides targeting AHAS (Sala and Bulos 2012a).

Nestares and her team, from the National University of Rosario, in Argentina, conducted several studies in order to obtain a deep characterization of the target and non-target sites associated tolerances. A simple and rapid screening assays was described that was useful in discriminating imidazolinone tolerance at the seedling stage. This bioassay could be a potential tool for early screening of imidazolinone tolerance genes from large sunflower populations (Vega et al. 2009). Another study from this team contributed to the understanding of AHAS expression in sunflower genotypes and its response to herbicide treatments. The aim of the work was to evaluate *Ahas* gene expression and AHAS activity in different tissues of sunflower plantlets (Breccia et al. 2013). Finally, the team contributed with significant research associated with non-target site tolerance (NTS), and suggest that a NTS related mechanism mediated by P450 isozymes, contributes to imazethapyr tolerance in Imisun sunflowers. In addition, stress-related proteins were associated with the response to the imazethapyr in both tolerant and susceptible genotypes, whereas cytochrome P450 proteins were upregulated only in tolerant lines. These results strengthen the hypothesis that NTS related mechanisms are involved in IMI tolerance in Imisun sunflower and also reveal the contribution of biotic and abiotic stress-related proteins to this trait (Vega et al. 2020). It was also found that this non-target-site tolerance mechanisms contributing to herbicide tolerance in Imisun sunflower could be related to the modifier gene *Imr2*. Gil et al. (2018), using cDNA-AFLP, were able to detect candidate detoxification-related genes potentially involved in imidazolinone tolerance in sunflower.

Further work was conducted in Argentina by Kaspar et al. (2011) to help to manage herbicide tolerant weeds by allowing additional herbicides to be used in this oilseed crop. A cultivated sunflower line with multiple herbicide tolerance was selected and characterized in the field and in the greenhouse to evaluate its response to the herbicides imazamox, prosulfuron, and atrazine at different plant development stages (germination, emergence, and third difoliate) with and without malathion. For all herbicides and all development stages analyzed, a line having significantly higher tolerance compared with the susceptible line was found.

Molecular markers for herbicide tolerance management

Introgression of the genes responsible of herbicide tolerance into high yielding sunflower germplasm should be complemented using molecular markers to unambiguously identify the different alleles involved in tolerance. Bulos et al. (2013) developed a set of allele specific molecular markers for the different herbicide tolerant traits of sunflower, which can be used with various technological platforms, and applied to different objectives in a breeding program. The *Ahasl1* gene sequences from lines carrying different alleles for susceptibility or tolerance showed single nucleotide polymorphisms and length variations for a simple sequence repeat. These differences were utilized to develop three types of PCR markers (SSRs, CAPS and SNPs) which allow the precise identification of each allele at the *Ahasl1* locus. Usefulness of these markers with respect to the application of phenotypic selection for herbicide tolerance, and the strategic implementation of these techniques for developing inbred lines with herbicide tolerance was also fully disclosed.

Gene flow between cultivated sunflower and its wild relatives

A research team located in the southern region of Buenos Aires province of Argentina, provided very important insights related to the estimation of the occurrence of crop-to-wild and wild-to-crop gene flow, the characterization of natural hybrids, and about the detection of the presence of crop alleles in wild populations. Morphological traits and molecular markers were used to attempt hybrid characterization (Gutierrez et al. 2010). The likely gene transfer from sunflower crop to relatives like *H. petiolaris* populations is of concern regarding the commercialization of new herbicide-tolerant hybrid cultivars. Herbicide tolerance was transferred with high frequency (79%) to wild plants (Massinga et al. 2003) and this team postulated that a similar situation could take place if genetically modified (GM) sunflower varieties were released, like glyphosate tolerant ones. In Argentina, about 77 GM sunflower petitions have been authorized for field trials, but no varieties have been commercially released up to date (Cantamutto and Poverene 2007).

CONCLUSION

Argentina had been a country close related to sunflower research during the last 100 years. The development of sunflower herbicide tolerance technologies was not the exception. Argentinian researchers who belong to public and private institutions have been heavily involved in the discovery, development, and deployment of these technologies in their own country and in the rest of the world. Additionally, associated tools like molecular markers were establish and shared during the last decades. Numerous examples have been cited in this work to illustrate the global impact being generated by the research conducted in this country and to highlight the importance of maintaining the investments and efforts in this critical field.

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