Genetic mapping, marker assisted selection and allelic relationships for the Pu6 gene conferring rust resistance in sunflower

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Rust resistance in the sunflower line P386 is controlled by Pu6, a gene which was reported to segregate independently from other rust resistant genes, such as R6. The objectives of this work were to map Pu6, to provide and validate molecular tools for its identification, and to determine the linkage relationship of Pu6 and R6. Genetic mapping of Pu6 with six markers covered 24.8 cM of genetic distance on the lower end of linkage Group 13 of the sunflower consensus map. The marker most closely linked to Pu6 was ORS316 at 2.5 cM in the distal position. ORS316 presented five alleles when was assayed with a representative set of resistant and susceptible lines. Allelism test between Pu6 and R6 indicated that both genes are linked at a genetic distance of 6.25 cM. This is the first confirmation based on an allelism test that at least two members of the Radv/R4/R11/ R13/R15/Pu6 cluster of genes are at different loci. A fine elucidation of the architecture of this complex locus will allow designing and constructing completely new genomic regions combining genes from different resistant sources and the elimination of the linkage drag around each resistant gene.

Key Words: Helianthus annuus, Puccinia helianthi, disease resistance, anticipatory breeding, complex locus, marker assisted selection, molecular breeding.

Introduction

Sunflower rust, incited by Puccinia helianthi Schwein., is one of the most serious fungal disease of sunflower (Helianthus annuus var. macrocarpus) as it can cause significant losses in both yield and seed quality in this crop (Sendall et al. 2006, Yang et al. 1986). The pathogen is a macrocyclic and autoecious basidiomycete capable of infecting commercial and volunteer sunflowers as well as other wild species of Helianthus.

Deployment of resistant cultivars provides an effective approach for disease control, eliminates the use of fungicides, and minimizes crop losses. Sackston (1962) reported the physiological specialization of the sunflower rust, and Putt and Sackston (1963) firstly determined the presence of rust resistance genes in sunflower. Since then, several genes conferring resistance to different physiological races of the rust pathogen have been identified in sunflower including R1, R3, R4, R5, Pu6, Radv, R11, R12 and R11AB6 (Bulos et al. 2013, Gong et al. 2013a, 2013b, Goulter 1990, Lawson et al. 1998, Miah and Sackston 1970, Miller et al. 1988, Qi et al. 2012, Yang et al. 1989). In addition to the already named rust resistance genes, most of them tracing back to wild Helianthus species (Bulos et al. 2012, Sendall et al. 2006), several inbred lines and interspecific germplasm lines were reported to have resistance to different races of P. helianthi (Bulos et al. 2012, Quresh et al. 1993). Sunflower rust resistance genes, however, are frequently overcome by virulent races of the pathogen within a short time period after their use in commercial hybrids. Consequently, it is necessary to search for new rust resistance genes/alleles and to pyramid several resistance genes in a single cultivar to achieve durable resistance. The combination of different strong resistance genes with already overcome ones during hybrid development might be an effective approach to extend the usefulness of new, non utilized, alleles (Kelly and Miklas 1998). The efficient implementation of such strategy, however, requires the use of molecular markers tightly linked to each of the resistant genes. Several sources conferring resistance to sunflower rust have been identified but only a few of them have been genetically characterized, mapped and linked to molecular markers. Up to the present, seven sunflower rust resistance genes were genetically mapped: R7 on LG 8 (Lawson et al. 1998, Yu et al. 2003), R2 on LG9 (Lawson et al. 2011), R6, Radv, R11, R12 and R11AB6 on LG13 (Bulos et al. 2013, Lawson et al. 1998, Qi et al. 2011b, 2012, Yu et al. 2003), R12 on LG11 (Gong et al. 2013a), and R5 on LG2 (Qi et al. 2012), providing an opportunity to combine several rust resistance genes in an inbred line.

P386 is a sunflower inbred line developed and released as a source of rust resistance by Aurelio Luciano and Néstor Luciani in 1986 (Yang et al. 1986). It was selected from the Argentine germplasm population known as Mezcla precoz...
resistente a roya (“Rust resistance early composite”), which was the result of intercrossing several introductions of three wild sunflower species (H. annuus ssp. annuus, H. argophyllus and H. petiolaris) with many lines derived from Russian varieties (Luciano, pers. com.). It is a monochalosaphal restorer line that shows low seed set under self-pollination conditions and low oil content in the seeds. P386 is resistant to the prevalent P. helianthi physiological races found in USA (Friskop et al. 2012, Gulya and Markell 2009), and Australia (Sendall et al. 2006), and to all the races reported for Argentina (Moreno et al. 2012) and Turkey (Tan 2010). It shows susceptibility when inoculated with some isolates belonging to the new virulent race 777 (Gulya and Markell 2009, Qi et al. 2011b). However, it is possible to combine this gene with other resistance genes to attain a wide spectrum of resistance using marker-assisted selection. Since 1996 the line P386 was included in the set of international rust differential lines (Gulya and Masirevic 1996).

The inheritance of rust resistance in P386 was reported to be controlled by one dominant gene named Pu6, which segregates independently from other rust resistant genes, such as R1, R2, R4 and R5 (Yang et al. 1989). It was also shown that it maps on linkage group 13 (LG13) of the sunflower consensus map (Bulos et al. 2012). This last result, however, is somewhat unexpected since R2 also maps on the same LG (Qi et al. 2011b).

In this sense the objectives of this work were (a) to map Pu6 on the LG13 of the sunflower consensus map, (b) to provide and validate molecular tools for the identification of this gene for marker assisted selection purposes, and (c) to determine the linkage relationship of Pu6 and R4.

Materials and Methods

Rust isolate

The rust isolate B.A.&S. 2009, belonging to physiological race 760, is a single-pustule derived isolate obtained by harvesting urediniospores from a single pustule of field growing susceptible plants in summer 2008–2009 at Venado Tuerto, Santa Fe, Argentina (Moreno et al. 2012). Multiplication of urediniospores was described previously (Bulos et al. 2013).

Plant materials and mapping population

Seeds of inbred line P386 and R702CLPlus were sown under greenhouse conditions and the F1 was obtained. R702CLPlus is a restorer, proprietary, rust susceptible, imidazolinone-resistant line (Sala et al. 2008). Hybridity of F1 plants was checked by molecular markers. One selected F1 plant was selfed to obtain F2 seeds which were sown under greenhouse conditions. One hundred and forty F2 plants were selfed and seeds from each plant were harvested separately to obtain F2:3 families. Progeny test of this F2:3 families for rust resistance indicate the genotype of their F2 plants.

In addition, lines HAR1, HAR2, HAR3, HAR4, HAR5, HAR6, HA89 and RHA801 developed and released by the USDA ARS and the North Dakota Experimental Station (USA) were used for DNA polymorphism analysis, while HAR4 was also utilized to carry out the R4/Pu6 allelism test. HAR1, HAR3, HAR4, and HAR5 are rust resistant lines of Argentine origin (Gulya 1985), which carry the resistant gene R4 (Miller et al. 1988). HAR6-1 is a reselection for resistance to race B.A.&S. 2009 (Bulos et al. 2013) conducted over the confectionary, resistant germplasm population HAR6 (Miller and Gulya 2001), which carry the resistant gene R16686. Maintainer line HA89 and restorer line RHA801 are susceptible oilseed type inbred lines used as checks.

Evaluation of rust resistance

Twenty five seeds from each of the 140 F2:3 families were planted in 20 × 20 × 30 cm pots, given a total of approximately 3500 F3 individuals. Twenty seeds of the lines HA89, P386 and R702CLPlus were planted in three replications as controls. Plants were grown in a greenhouse under natural light conditions supplemented with 400 W halide lamps to provide a 16 h day length. Day/night temperatures were 25 and 20°C, respectively.

The F3 plants, parental lines, and susceptible control (HA89) were inoculated with P. helianthi urediniospores of race 760 at the V2–V3 developmental stage (Schneiter and Miller 1981), using the procedure described by Gulya and Masirevic (1996). Race 760 was collected originally from cultivated plants in Venado Tuerto in 2009, and was increased from a single pustule (Moreno et al. 2012). urediniospores were collected from greenhouse grown HA89 plants and used immediately or stored for a few days at 4°C. After inoculation, plants were incubated in sealed chambers at 100% humidity in a dark room for 16 h at 18–20°C. Plants were then returned to the greenhouse and maintained as described previously.

In the susceptible lines rust pustules started to appear in 8–10 days, and evaluations were made at 12 days post inoculation to allow full development of symptoms. Rust evaluations were made using pustule size or infection type (IT). A modified Sackston’s numerical rating system (1962) described by Yang et al. (1986) was used to categorize IT. Infection categories were as follows: 0 = immune, no uredia and no hypersensitive flecks, 1 = highly resistant, presence of hypersensitive flecks or lesions, or pustules smaller than 0.2 mm in diameter with or without chlorotic haloes; 2 = resistant, pustules smaller than 0.4 mm; 3 = susceptible, pustules 0.4–0.6 mm in diameter; 4 = highly susceptible, pustules larger than 0.6 mm. Reactions 0, 1, and 2 were classified as resistant, while reactions 3 and 4 were rated as susceptible. F2:3 families could be unequivocally scored as resistant (R), susceptible (S), or Segregant (Seg).

DNA marker analysis

Genomic DNA was isolated from young leaves of the parental lines and F2 individuals using Qiagen DNeasy 96 Plant Kit (Qiagen Inc., USA). DNA quality and quantity
was determined using agarose gel electrophoresis. DNA was quantified by fluorometry using Qbit® 173 dsDNA BR kit (Cat# Q23850, Qiagen) and diluted to a final concentration of 60 ng/μL.

Fifteen microsatellite (SSR) markers located on LG13 of the public sunflower genome map (Tang et al. 2002, Yu et al. 2003), three SSRs from INTA Castelar (HA3300, HA2598 and HA4011, Paniego et al. 2007), three SSR markers developed from the BAC clone P408L01 (NidGi1, NidGi2 and NidG13, Bulos et al. 2013), and the SCAR marker HRG01 (Horn et al. 2003), all of them located on LG13 were used for polymorphism analysis and mapping purposes.

PCR assays were conducted in 10 μl reaction volume containing 1× PCR buffer, 400 μM dNTPs, 2.5 mM MgCl₂, 0.5 U Taq DNA Polymerase (Biotools, Madrid, Spain), 0.4 μM of each primer and 50 ng of genomic DNA. PCR cycling conditions were as follows: an initial denaturation step at 95°C for 3 min, followed by 38 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 45 s, and a final extension step at 72°C for 10 min. The PCR products were visualized on either 4% or 6% polyacrylamide gels with silver nitrate staining (Silver Sequence; Promega Technologies, Carlsbad, USA) or 6% polyacrylamide gels with silver nitrate staining (Silver Sequence; Promega Biotech, Madison, USA).

**Linkage analysis**

Genetic distances between the rust resistance gene and the molecular markers were calculated using Map Maker v2.0 (Lander et al. 1987) with default parameters of LOD 3.0 and the Kosambi mapping function (Kosambi 1994). Goodness of fit to a 1 : 2 : 1 segregation ratio of F₂ genotypes for rust reaction from the F₂:3 families was tested by means of a chi-squared analysis.

**Identification of allele specific markers for Pu₆**

A DNA polymorphism analyzes was carried out using the closest marker(s) to the locus Pu₆ in order to identify a specific marker that permit to discriminate this gene from the resistant genes present in the rust resistant lines HAR1, HAR3, HAR4, HAR5, HAR6, HA340, and the gene(s) for susceptibility in the lines HA89, RHA801 and R702CLPlus.

**Allelism test between Pu₆ and R₄**

To determine if Pu₆ and R₄ are allelic variants or different genes, the inbred line P386 was crossed with HAR4. HAR4 is a rust resistance line of Argentine origin (Gulya 1985), which carries the resistant gene R₄ (Miller et al. 1988). One F₁ plant was used as male parent in the cross cmsHA89/F₁ (P386/HAR4). Progenies of this cross were evaluated for their rust resistance as previously described.

**Conversion of a susceptible line to its rust resistance isolate**

To confirm the usefulness of the marker linked to the Pu₆ gene in a breeding program, a susceptible line was converted to its rust resistance isolate by using a marker assisted backcross procedure. The susceptible line R702CLPlus was crossed with P386 and the F₁ was backcrossed to the susceptible parent. Two hundred SSR markers developed by Tang et al. (2002) and Yu et al. (2003) were screened for polymorphisms between donor and recipient lines. Forty six well-distributed polymorphic SSR markers (Table 1) were selected for recipient genome background selection. Five hundred and twenty BC₁F₁ individuals were screened by the marker for the rust resistance gene to select rust resistant plants. These plants were also screened for genetic background similarity with the recipient line by using the already selected SSR markers. One selected BC₁F₁ individual was backcrossed again with the susceptible parent to obtain the BC₂F₁ progeny (283 plants). One BC₂F₁ plant selected by using the previously described procedure was selfed to obtain a BC₂F₂ population. This population was screened again by molecular markers and one selected plant was selfed to obtain a BC₃F₂ family. Twenty five plants of this family were screened for their rust resistance as described above.

**Results**

**Inheritance of rust resistance from P386**

The inbred lines HA89 and R702CLPlus were highly susceptible to *P. helianthi* isolate B.A.&S. 2009 showing IT 4, whereas the inbred line P386 was resistant to this rust isolate, showing necrosis at infection sites (IT 1). Heterozygous F₁ plants were scored as resistant, indicating a dominant effect of the resistance gene. Therefore, resistant and susceptible plants could be easily distinguished by their symptoms.

The 140 F₂ individuals segregated at a ratio of 111R : 29S which did not differ significantly from the expected 3 : 1 ratio ($\chi^2 = 1.371$, df = 1, $P = 0.241$). Rust phenotyping of

| Marker | LG |
|-------|----|
| ORS222, ORS509, ORS425 | 1 |
| ORS1073, ORS1282, ORS342 | 2 |
| ORS545, ORS124, ORS529 | 3 |
| ORS963, ORS366, ORS337 | 4 |
| ORS574, ORS852 | 5 |
| ORS1129, ORS650, ORS725 | 6 |
| ORS928, ORS700, ORS966 | 7 |
| ORS830, ORS243 | 8 |
| ORS1001, ORS510, ORS1034 | 9 |
| ORS541, ORS1088, ORS818 | 10 |
| ORS625, ORS934 | 11 |
| ORS671, ORS767, ORS123 | 12 |
| ORS142, ORS317 | 13 |
| ORS578, ORS1180 | 14 |
| ORS703, ORS1141, ORS913 | 15 |
| ORS31, ORS195 | 16 |
| ORS597, ORS634, ORS1241, ORS512 | 17 |
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140 F$_{2:3}$ families showed that the F$_2$ population had 32 homozygous resistant, 79 heterozygous and 29 susceptible plants. A Chi-squared test indicated that this fits a theoretical 1RR : 2Rr : 1rr segregation ratio ($\chi^2 = 2.443$, df = 2, $P = 0.295$) which would be expected for a single segregating dominant gene. The combined F$_2$ and F$_{2:3}$ family data indicated that resistance in P386 is conferred by a single dominant gene.

**Genetic mapping of Pu6**

A set of 22 molecular markers selected from LG13 was screened to identify polymorphisms among resistant and susceptible parental lines. Only six of them showed polymorphism between parental lines and were used for F$_2$ genotypic analysis. The resulting genetic map spans a distance of 24.8 cM, between the SSR markers CRT504 and ORS317. The markers closely linked to Pu6 were ORS 316 at 2.5 cM in the distal position, and ORS 224 at 4.8 cM in the proximal end (Fig. 1).

**Allelism test between Pu6 and R$_4$**

To determine if Pu6 and R$_4$ are allelic variants or different genes, the inbred line P386 was crossed with HAR4 and one F$_1$ plant was used as male parent in the cross cmsHA89/ F$_1$ (P386/HAR4). One hundred and twenty eight plants from this cross were obtained and evaluated for rust resistance together with the three parental lines. The inbred lines HA89 and R702CLPlus were highly susceptible to isolate B.A.&.S. 2009 showing pustules larger than 0.6 mm (IT 4), whereas the inbred line P386 was resistant showing necrosis at infection sites (IT 1). Four susceptible plants were detected among the 128 plants evaluated. These susceptible individuals showed a great number of pustules larger than 0.6 mm, whereas the rest of plants from the same cross only showed necrosis at infection sites. This observation indicates that R$_4$ and Pu6 did not segregate independently, since the observed values of segregation significantly differ from the expected 3 : 1 ratio ($\chi^2 = 32.67$, df = 1, $P = 0.0001$). However, recombination values under the hypothesis of linkage between Pu6 and R$_4$ indicate that they are linked at genetic distance of 6.25 cM.

**Identification of allele specific markers for Pu6**

ORS 316, the closest marker to the gene Pu6, presented 5 alleles when was assayed with the evaluated set of resistant and susceptible lines. Susceptible lines, RHA801 and R702CLPlus, as well as the rust resistant lines HAR1 and HAR5 showed an allele of 206 bp. The inbred lines HAR3 y HAR4, amplified a PCR fragment of 202 bp. HAR6 and RHA340 showed alleles with molecular weights of 204 bp and 198 bp, respectively. Finally, the inbred lines P386 and HAR2 showed an allele of 200 bp (Fig. 2).

**Conversion of a susceptible line to its rust resistance isoline**

Five hundred and twenty plants from the BC$_1$F$_1$ population R702CLPlus //F$_1$ (R702CLPlus /P386) were screened with the molecular marker ORS 316 and 308 plants were selected. Selected plants were screened with 46 additional SSR polymorphic markers well distributed in the sunflower genome to select the most similar plant to the susceptible parent. BC$_2$F$_1$ plants were screened in the same way and a single plant was selfed to obtain a BC$_2$F$_2$ family. Ninety three plants of this backcross generation were screened for homozygosity by the marker ORS366 in order to detect

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**Fig. 1.** Genetic map of the rust resistance gene Pu6 in the lower end portion of Linkage Group 13 of the sunflower consensus map.

**Fig. 2.** PCR fragments for the SSR ORS316 showing the polymorphism obtained for several resistant (HAR1, HAR2, HAR3, HAR4, HAR5, HAR6 and P386) and susceptible (HA89 and R720) inbred lines.
homozygous plants for the resistant gene *Pu₆*. Twenty two homozygous plants were detected and they were screened for three remaining SSR markers to select for genetic background. One plant was highly similar to R702CLPlus, i.e. it was identical to the recipient line for the 46 SSR markers used, was selected and selfed. Twenty five selfed plants from it were assayed for their resistance to rust isolate B.A.&S. 2009 and all of them showed resistance, indicating that a marker assisted backcrossing procedure to introgress *Pu₆* into a susceptible inbred line is feasible.

**Discussion**

Disease resistant loci in plants may be single genes with two or more alleles. Examples of this type are the L locus in flax—with 13 alleles controlling different resistance specificities—(Ellis et al. 1999), and RPM1 in Arabidopsis—with only two alleles—(Grant et al. 1995). More commonly, resistant genes are organized in clusters that show varying levels of recombination between the component genes (see Michelmore and Meyers 1998, Richter and Ronald 2000). The M locus in flax (Ellis et al. 1995), Xa21 in rice (Song et al. 1997), *Prf* in tomato (Salmeron et al. 1996) and *Mia* in barley (Weil et al. 2002) are examples of resistant genes showing this type of organization.

The identification of molecular markers closely linked to many of the major rust resistant genes used in the sunflower differential set (Lawson et al. 1996, 1998) and their localization to specific linkage groups using SSR markers (Tang et al. 2002) led to the observation that LG 13 appeared to carry most of these genetic factors. Based on direct verification using markers and through linkage association with known genes, Sendall et al. (2006) proposed the existence of 21 putative resistant alleles for the *R₄* locus in sunflower. Using a marker completely linked to one of these putative alleles (*R₄*), and a map-based approach, Qi et al. (2011a) demonstrated that *R₄* and *R₄a*, map at a genetic distance of 13.9 cM, which indicate that they are in fact different resistant genes. Other rust resistance genes were also mapped on this LG. The rust resistant *locus R₄₁₄₇₈₆* was mapped to this LG flanked by markers ZVG61 and ORS581 (Bulos et al. 2013). Similar results were reported by Gong et al. (2013b), under the locus name *R₄₁₃₇₈*. Additionally, the rust resistant factor present in the inbred line RHA397 which traces back to South African germplasm is allelic to the gene *R₄₁₃₇₈* (Gong et al. 2012). Finally, the line HAR9 carries the resistant gene *R₂* which derived from a wild population of *H. annuus* (Qi and Seiler 2013) and is also located at the lower end of LG 13 (Qi et al. 2012). However, since an allelism test was not carried out, it can not be excluded the possibility that *R₂* is an allele of *R₄* (Qi et al. 2012) or that *R₂* is an allelic variant of *R₄* (Bulos et al. 2013). Up to the present the genetic factors (genes and/or alleles) carried by nine rust resistant sources are localized in the same genomic region of the LG13 of the sunflower consensus map.

Most of the disease resistance genes in plants cloned to date encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins characterized by nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domains as well as variable amino- and carboxy-terminal domains. As the result of both, segmental and tandem duplications, NBS-LRR-encoding genes are usually clustered in the genome (McHale et al. 2006). This tends to support the contention that the cluster of genes *R₄d/R₄/R₄₁/R₄₁₃b/R₄₁₃b* is a complex locus organized as many other disease resistance genes in plants, since this particular genomic region is populated of NBS-LRR-encoding genes (Bachlava et al. 2011, Radwan 2010).

Mapping results and allelism test reported here indicate that *Pu₆* is also a member of this complex locus. This resistant gene is located at the distal end of LG13 flanked by the markers ORS 224 and ORS 316, in the same genomic region where *R₄* was mapped by Qi et al. (2011a). This result contradicts the reported conclusion that *Pu₆* segregates independently from *R₄* (Yang et al. 1989). Interestingly, segregation ratios from an allelism test between *R₄* from HAR3 and *Pu₆* were interpreted by Yang et al. (1989) as the result of the independent segregation of two loci (*χ² = 3.81, df = 1, *P* = 0.051). However, these observations also can be interpreted as the result of genetic linkage between both genes at approximately 2.5 cM (*χ² = 0.036, df = 1, *P* = 0.849). The result of the allelism test reported here confirmed that both genes, *Pu₆* and *R₄* from HAR4, are genetically linked at a distance of 6.25 cM. This is the first confirmation based on an allelism test that two members of the *R₄d/R₄/R₄₁/R₄₁₃b/Pu₆* cluster of genes are at different loci, since previous contentions on this subject (v.g. Qi et al. 2011a) were based on the comparison of map distances from different segregating populations showing at least one marker in common. Likewise, this indicates that the factors *R₂* and *Pu₆* are not alleles of the same resistant gene, as was postulated by Sendall et al. (2006) under the allelic names *R₄c* and *R₄d*, but members of a complex locus of disease resistant genes.

Anticipatory resistance breeding involves the breeding for disease resistance to virulent pathotypes before they become prevalent and cause significant yield and economic losses (McIntosh and Brown 1997). A detailed understanding of the virulence structure in the pathogen population, the main mechanisms driving pathotype evolution and genetic understanding of the main resistance genes available in cultivars and its genomic organization is essential to develop effective breeding strategies (Lawson et al. 2011). In this sense, a fine elucidation of the architecture of the complex locus *R₄d/R₄/R₄₁/R₄₁₃b/Pu₆*, combining classical and molecular approaches, will have an impact on molecular breeding, not only by the design of molecular markers targeting each resistant factor, but also for the design of completely new regions combining useful disease resistant genes from different sources by recombination and selection with allele specific markers (Paniego et al. 2012). In this context, the PCR fragment of 200 bp for the SSR ORS 316 was demonstrated to be a useful tool for selecting *Pu₆*.
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during its introgression in a susceptible line by means of a marker assisted selection procedure. Also, this marker could be used to analyze individuals derived from populations obtained by crossing P386 with other well known rust resistance sources like HAR1, HAR3, HAR4, HAR5, HAR6 and RHA340. However, ORS 316 cannot be considered as a diagnostic marker for \textit{Pu6} because of the results obtained in HAR2. The availability of high-throughput genotyping technologies for sunflower will permit the development of increasingly dense genetic maps (see Bachlava et al. 2012, Bowers et al. 2012) to accomplish more efficiently these goals.

Markers closely linked to resistance genes have other application perhaps even more important than previously detailed: to assist in the elimination of the linkage drag around the resistant genes. The great majority of resistant genes used in sunflower breeding traced back to wild \textit{Helianthus} species and their incorporation into cultivated germplasm entail also the introduction of a variable amount of foreign DNA carrying genes of negative effect being tightly linked to the trait of interest. For example, the incorporation of \textit{Pl}_{\text{adv}} from \textit{H. argophyllus} involved the introduction of a segment of 31.5 cM from the wild species into the sunflower LG1 which tends to decrease plant fertility (Dußle et al. 2004). Likewise, the incorporation of a gene \textit{Ahasl1-1} for herbicide resistance from a wild \textit{H. annuus} population into cultivated germplasm determined the concomitant introduction of a segment of 40 to 60 cM on LG9 which reduces the seed oil content of the seed (Sala et al. 2012). The amount of introduced linkage drag is variable, the inbred line RHA340 harboured ca. 30 cM of foreign DNA at the distal end of LG13 which includes the resistant genes \textit{R}_{\text{adv}} and \textit{Pl}_{\text{adv}} from \textit{H. argophyllus}, whereas much longer donor DNA segments persist in LG8 of the lines HA336 (ca. 60 cM) and HA337 (ca. 40 cM) which carry the downy mildew resistant gene \textit{Pl}_{\text{adv}} from \textit{H. praecox} ssp. \textit{runyonii} (Slabaugh et al. 2003). The successive incorporation of several useful traits from wild \textit{Helianthus} species can impose a limit to the yield potential of the hybrids carrying them. For this reason, it is advisable to eliminate the linkage drag around the incorporated resistance genes through the selection of recombinants by molecular markers previous to their deployment as commercial traits.

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