Molecular mapping of a sunflower rust resistance gene from HAR6

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Sunflower, caused by *Puccinia helianthi* Schw., can result in significant yield losses in cultivated sunflower (*Helianthus annuus* L. var. *macrocarpus* Ckll.). HAR6 is a germplasm population resistant to most predominant rust races. The objectives of this study were to map the resistance factor present in HAR6 (*R_HAR6*), and to provide and validate molecular tools for the identification of this gene for marker assisted selection purposes. Virulence reaction of seedlings for the F1 population and F2:3 families suggested that a single dominant gene confers rust resistance in HAR6-1, a selected rust resistance line from the original population. Genetic mapping with eight markers covered 97.4 cM of genetic distance on linkage group 13 of the sunflower consensus map. A co-dominant marker ZVG61 is the closest marker distal to *R_HAR6* at a genetic distance of 1.5 cM. Validation of these markers was assessed by converting a susceptible line into a rust resistant isoline by means of marker assisted backcrossing. The application of these results to assist the breeding process and to design new strategies for rust control in sunflower is discussed.

Key Words: sunflower rust, *Puccinia helianthi*, molecular breeding, disease resistance, marker assisted backcrossing.

Introduction

Sunflower (*Helianthus annuus* L. var. *macrocarpus* Ckll.) is grown all over the world for three main purposes: beauty (ornamental sunflower), direct consumption of the seeds (confectionary sunflower) and oil production (oilseed sunflower). By far, the last of them is the most important objective in terms of acreage and production (Fernández-Martínez et al. 2009). Sunflower oil has been traditionally viewed as a healthy vegetable oil and it is considered premium oil for salad, cooking and margarine production and is also being evaluated as a source of biodiesel. With a cultivated acreage of over 22 million ha and an annual production of around 9 million ton, sunflower is grown on every continent, but its production is mainly concentrated in the Russian Federation, Ukraine, India and Argentina. Sunflower oil is the fourth most important vegetable oil in world trade after soy, palm and colza oils. Sunflower is primarily an oil crop, with high protein meal being a by-product. The world production of sunflower pellets is also important, as it is the principal grinding subproduct. Argentina is the leading exporter and the European Union is the greatest importing block (Sala et al. 2012).

Sunflower rust, caused by *Puccinia helianthi* Schw., is a serious fungal disease that can cause significant losses in both yield and seed quality of cultivated sunflower especially in Australia, Argentina, South Africa and USA (Sendall et al. 2006, Yang et al. 1986). *P. helianthi* is autoecious and macrocyclic rust that occurs on wild perennial, wild annual and cultivated *Helianthus* species (Markell et al. 2009).

Deployment of resistant cultivars provides an effective approach for disease control, eliminates the use of fungicides and minimizes crop losses. Several genes conferring resistance to different physiological races of rust have been identified in sunflower including: *R1*, *R2*, *R5*, *R6*, *Ptu*, *R_ab*, *R_1* and *R_5* (Gong et al. 2012, Lawson et al. 1998, Miah and Sackston 1970, Miller et al. 1988, Yang et al. 1989). In addition to the already named rust resistance genes, several inbred lines and interspecific germplasm lines were reported to have resistance to different rust races of sunflower (Bulos et al. 2012, Quresh et al. 1993). Many of these sources have been discovered in Australia, Argentina and North America, and most of them are traced back to wild *H. annuus* (Sendall et al. 2006).

HAR6 (PI607509) is a confectionary sunflower germplasm population developed and released by the USDA-ARS and the North Dakota Experimental Station (USA) in 2001 (Miller and Gulya 2001). It was developed from a French sunflower accession named 6 SC U6 L6 (PI650362). HAR6 is resistant to rust physiological races 7771 (Miller and Gulya 2001) and 336 (Qi et al. 2011a) but shows

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1 Nomenclature of sunflower rust races according to Gulya and Masirevic (1988, 1996). This internationally accepted system uses a triplet code to produce a virulence formula using a set of nine differential lines.
susceptibility when inoculated with some isolates belonging to the physiological group race 700 (Moreno et al. 2012). Recently, the inheritance of rust resistance in HAR6 was reported to be controlled by one dominant gene located on linkage group (LG) 13 (Bulos et al. 2012).

A significant problem is that sunflower rust resistance genes are frequently overcome by virulent races within a short time period after introduction to agriculture. Consequently, it is necessary to search for new rust resistance genes/alleles and to pyramid several resistance genes in a single cultivar to achieve effective resistance. In this sense the objectives of this work were to map the resistance factor present in HAR6 and also to provide and validate molecular tools for the identification of this gene for marker assisted selection purposes.

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). Urediniospores were increased by inoculating an aqueous spore suspension onto 21-day-old seedlings of susceptible oilseed line HA89 with a paintbrush in the first multiplication cycle. After collecting the urediniospores from these plants, a second multiplication cycle was carried out inoculating the freshly collected urediniospores with a vacuum-pump powered atomizer onto 21-day-old seedlings of HA89 previously sprayed with distilled water.

Plant materials and mapping population

The HAR6 sunflower germplasm population was obtained from the U.S. National Plant Germplasm System (NPGS, USA). Seeds of this population were sown under greenhouse conditions and inoculated as above with rust isolate B.A. & S. 2009. The original population was heterogeneous for its reaction to this isolate since susceptible and resistant plants were observed. One resistant plant was selfed and its progeny was inoculated again with the same isolate approximately 2400 F2 individuals. Twenty seeds of the lines HA89, HAR6-1 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 F1 plants, parental lines and susceptible control (HA89) were inoculated with P. helianthi spores of race 760 at the V2 developmental stage (Schneider and Miller 1981), using the procedure described by Gulya and Masirevic (1996). 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. Evaluation of symptoms was conducted twelve days after inoculation, using a 0–4 rating that classifies 0, 1 and 2 as resistant plants and 3 and 4 as susceptible (Yang et al. 1986). In this way, 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 DNaseasy 96 Plant Kit (Qiagen Inc., USA). DNA quality and quantity was determined using agarose gel electrophoresis.

Fifteen microsatellite (SSR) markers located on LG13 of the public sunflower genome map (Tang et al. 2002, Yu et al. 2003) and two other SSRs from INTA Castelar located on the same LG (Paniego et al. 2007) were screened for polymorphism in the parental lines. Additionally, three SSR markers were developed from the BAC clone P408L01 located on LG13 (Genbank accession HQ222262, Bachlava et al. 2011), using the SSR Hunter version 1.3 software (http://www.biosoft.net/dna/SSRHunter.htm) utilizing parameters set to more than seven repetitions for di-nucleotide repeats, five for tri-, four for tetra- and three for penta- and hexa-nucleotide repeats (Li and Wan 2005). Sequences of the primers of these SSR markers are provided in Table 1. The SCAR marker HRG01 (Horn et al. 2003) located on LG13 was also used.

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), 200 nM of each primer and 25 ng of genomic DNA. PCR products were separated in 2% agarose gel and visualized in ultraviolet light. Results were recorded as presence (1) or absence (0) of the bands for the parental lines and F2 plants.

Table 1. Primer sequence information of three SSR markers developed from the sequence of the BAC clone P408L01

| Marker name | Forward | Reverse | Motif |
|-------------|---------|---------|-------|
| NidGi1      | AGTGAAGTTAGATGGTGGTGAATC | TTTATGATGTTGATC | at |
| NidGi2      | CAGAACTGGAACGTGTTTATTCT | CCGTGAATACGAGTC | tcc |
| NidGi3      | GAGGGTTAGCCCTCAAAG | GACCTGGTGACGGTC | mix |
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% Metaphor agarose stained with SybrSafe (Invitrogen Life 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 1944). Goodness of fit to a 1:2:1 segregation ratio of F1 progeny was tested using the previously described procedure was selfed to obtain a BC2 population that was homozygous resistant, 48 heterozygous and 22 susceptible plants. A Chi-square test indicated that this fits a theoretical 1RR : 2Rr:1rr segregation ratio ($\chi^2 = 0.333, df = 2, p = 0.846$) which would be expected for a single segregating dominant gene. The combined F2 and F2.5 family data indicated that resistance in HAR6-1 was conferred by a single dominant gene, which was temporarily designated as $R_{HARS6}$. The combined F2 and F2.5 family data indicated that resistance in HAR6-1 was conferred by a single dominant gene, which was temporarily designated as $R_{HARS6}$.

**Identification of SSR markers linked to the resistance gene**

A set of 21 SSR selected from LG13 was screened to identify polymorphisms among resistant and susceptible parental lines. Only eight of them showed polymorphism between parental lines and were used for F2 genotypic analysis. Genotyping of these markers in the entire F2 population showed that markers ORS224, NidGi1, HRG01, ORS581, ZVG61, HA3330, HA4011 and ORS316 were mapped close to the $R_{HARS6}$ locus. ORS581 and ZVG61 were each found to be linked in coupling to the resistant gene at 1.5 and 0.7 cM, and exhibited dominant and co-dominant inheritance patterns respectively (Fig. 1).

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

The BC1F1 population was screened with the molecular marker ZVG61, tightly linked to $R_{HARS6}$ and 286 plants were selected. These plants were screened with 34 additional SSR polymorphic markers well distributed in the sunflower genome to select a plant highly similar to the susceptible parent. BC2F1 plants were screened in the same way and a single plant was selfed to obtain a BC2F2 family. Fifty seven plants of this backcross generation were screened for homozygosity by the marker ZVG61 in order to detect homozygous plants for the resistant gene $R_{HARS6}$. Thirteen homozygous plants were detected and they were screened for two remaining SSR markers to select for genetic background. One plant which was highly similar to R702CLPlus and homozygous for ZVG61 was selected and selfed. Twenty selfed individuals from this plant showed completely resistance to rust isolate B.A. & S 2009 indicating that a marker assisted backcrossing procedure for introgressing $R_{HARS6}$ into a susceptible oilseed background is feasible.

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**Results**

**Inheritance of rust resistance from HAR6**

The inbred lines HA89 and R702CLPlus were highly susceptible to rust isolate B.A. & S 2009 showing big pustules (reaction type 4), whereas the selected inbred line HAR6-1 was resistant, with only localized necrosis at infection sites (reaction type 1). F1 plants were scored as resistant, indicating a dominant effect of the resistance gene (Table 2). The 96 F2 individuals segregated at a ratio of 74 R : 22 S which did not differ significantly from the expected 3 : 1 ratio ($\chi^2 = 0.222, df = 1, p = 0.637$). Rust phenotyping of 96 F2 plants showed that the F2 population had 26 homozygous resistant, 48 heterozygous and 22 susceptible plants. A Chi-square test indicated that this fits a theoretical 1RR : 2Rr:1rr segregation ratio ($\chi^2 = 0.333, df = 2, p = 0.846$) which would be expected for a single segregating dominant gene. The combined F2 and F2.5 family data indicated that resistance in HAR6-1 was conferred by a single dominant gene, which was temporarily designated as $R_{HARS6}$.

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**Table 2.** Inheritance of rust resistance in HAR6. Response of sunflower plants [Resistant (R), Susceptible (S)] to rust in F1, F2 and BC1F1 populations resulting from crosses between resistant line HAR6 and R702CLPlus and Chi-square tests of single locus model for control of resistance

| Rust Reaction | F1 | F2 | Ratio | X2 | F2.5 | Ratio | X2 |
|---------------|----|----|-------|----|------|-------|----|
|               | R  | S  |       |    |      |       |    |
| Number of plants | 20 | 0  | 74    | 22 | 3 : 1 | 0.637 | 26 |
| Genotypes     | R  | H  | S     |    |      |       |    |
| p-value       |    |    | 0.846 |    | 1 : 2 : 1 |
Rust resistance in the line HAR6 was shown to be controlled by a single dominant gene by means of classical genetic analysis of F$_2$ population derived from the cross of the susceptible line R702CLPlus with HAR6-1 and later confirmed in the analysis of the F$_2$$_3$ families. This genetic factor, tentatively named \( R_{HAR6} \), was mapped to LG13 between molecular markers ZVG61 and ORS581, at 0.7 and 1.5 cM, respectively. The marker ZVG61 proved to be efficient for selection purposes to convert a susceptible line to a rust resistant isoline by means of marker assisted backcross selection.

Other rust resistance genes were also mapped on this LG. The rust resistant locus \( R_c \), present in sunflower inbred lines HA-R1, HA-R3, HA-R4 and HA-R5, was mapped to the same genomic region flanked by markers ZVG61 and ORS581 (Qi et al. 2011b). Likewise, two other genes coming from different sources and named \( R_{ab} \) have been mapped to LG13. One of them traces back to the proprietary line P2 (Lawson et al. 1998) and maps near ORS191 (Lawson et al. 1998, Yu et al. 2003). The other resistant gene traces back to the inbred line RHA340 and maps 0.2 cM away from RGC260 and 3 cM away from ORS316 (Bachlava et al. 2011, Radwan et al. 2008). Additionally, the rust resistant factor present in the inbred line RHA397 which traces back to South African germplasm is allelic to the gene \( R_{HAR6} \) (Gong et al. 2012). Finally, \( R_{11} \), a gene derived from a wild population of \( H. annuus \) also is located at the lower end of LG 13 (Qi et al. 2012). In summary, 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. Clusters of genes conferring resistance to plant diseases in the host chromosomes have been identified in diverse plant species (Jones and Dangl 2006 and references herein). Genes within a cluster can be allelic variants of the same gene or closely linked genes. Interestingly, this region of the sunflower genome was previously described as a region populated of resistant genes analogs of CC-NBS-LRR subfamily (Radwan et al. 2008, Radwan 2010).

Three out of the four inbred lines carrying \( R_c \) (HA-R1, HA-R3 and HA-R4) were derived from an Argentinian interspecific pool obtained from crosses among Russian open pollinated varieties and the wild sunflower species \( H. annuus \), \( H. argophyllus \) and \( H. petiolaris \). The fourth line carrying \( R_c \), HA-R5, was derived from a selection of the Argentine open pollinated cultivar Guayacan INTA (Gulya 1985). \( R_{ab} \) from RHA340 traces back to the wild species \( H. argophyllus \) (Miller and Gulya 1988). HAR6, in turns, derived from a French introduction (Miller and Gulya 2001) and \( R_{11} \) from a wild population of \( H. annuus \) (Seiler and Jan 1997). It is clear that the rust resistant sources located in this region of LG13 are very diverse from a genealogical point of view.

Furthermore, these sources are also very different from a pathological point of view. In fact, HA-R1, HA-R3, HA-R4 and HA-R5 are members of the international set of rust differentials (Gulya and Masirevic 1988) and for this reason,
they can be easily recognized by their pattern of resistance/susceptibility to different rust races. In addition, all of them are susceptible to the North American race 777. HAR6, on the other hand, is resistant to this physiological race (Qi et al. 2011a) but shows susceptibility to certain isolates belonging to race 700 (Moreno et al. 2012). _R_aab_ is resistant to race 700 (NA race 4, Miller and Gulya 1988), but is not effective against the new virulent races of 336 and 777 (Qi et al. 2011a). Tests with race 777 distinguished _R_f_ and _R_ig, the first being susceptible, the second resistant, but both these genes are resistant to race 336 (Qi et al. 2011a).

All these rust resistant sources seem to encode different rust resistance specificities and appear to be different from each other taking into account the genealogical and pathological information available. Nevertheless, it is imperative to carry out allelism tests and to saturate this region with different types of molecular markers in order to determine the organization of this particular genomic region.

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). As was pointed out by Lawson et al. (2011) for sunflower rust, precognition and thus strategic planning, these occurrences require 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. In this sense, molecular markers tightly linked to the resistance genes permit to pyramidize different genes conferring resistance in a single line. Furthermore, _R_Hlab_ belongs to a cluster of rust resistance genes which are located at the end of LG13, as was the case for many other clusters of resistance gene analogs in the sunflower genome (Radwan et al. 2008). A fine elucidation of this particular architecture will have an impact on molecular breeding, not only by the design of molecular markers targeting these regions, 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).

**Literature Cited**

Bachlava, E., O.E. Radwan, G. Abrati, S. Tang, W. Gao, A.F. Heesacker, M.E. Bazzalo, A.Zambelli, A.J. Leon and S.J. Knapp (2011) Downy mildew ( _Pl_ and _Pl_2) and rust ( _R_aab_ ) resistance genes reside in close proximity to tandemly duplicated clusters of non-TIR-like NBS-LRR-encoding genes on sunflower chromosomes 1 and 13. Theor. Appl. Genet. 122: 1211–1221.

Bulos, M., E. Altieri, M.L. Ramos, P. Vergani and C.A. Sala (2012) Genome localization of rust resistance genes. In: Proceedings 18th International Sunflower Conference, Mar del Plata, Argentina, pp. 980–985.

Fernández-Martinez, J., B. Pérez-Vinch and L. Velazco (2009) Sunflower. Chapter 6. In: Vollmann, J. and I. Rajcan (eds.) Oilcrops. Handbook of Plant Breeding, 4, pp. 155–232.

Gong, L., B.S. Hulke, T.J. Gulya, S.G. Markell and L.L. Qi (2012) Molecular tagging of a novel rust resistance gene _R_ig in sunflower ( _Helianthus annuus_ ). Theor. Appl. Genet. 126: 93–99.

Gulya, T.J. (1985) Registration of five disease-resistant sunflower germplasms. Crop Sci. 25: 719–720.

Gulya, T.J. and S. Masirevic (1988) International standardization of techniques and nomenclature for sunflower rust. In: Proc. 10th Sunflower Research Workshop, Fargo, ND. National Sunflower Assoc., Bismarck, ND, USA, p. 10.

Gulya, T.J. and S. Masirevic (1996) Inoculation and evaluation methods for sunflower rust. In: Proc. 18th Sunflower Research Workshop, Fargo, ND. National Sunflower Assoc., Bismarck, ND, USA, pp. 31–38.

Horn, R., B. Kasterer, E. Lazarescu, M. Prufé and W. Friedt (2003) Molecular mapping of the _R_f_ gene restoring pollen fertility in _PET1_ based _F_ hybrids in sunflower ( _Helianthus annuus_ ). Theor. Appl. Genet. 106: 599–606.

Jones, J.D.G. and J.L. Dangl (2006) The plant immune system. Nature 444: 323–329.

Kelly, J.D. and P.N. Miklas (1998) The role of RAPD markers in breeding for disease resistance in common bean. Mol. Breed. 4: 1–11.

Kosambi, D.D. (1944) The estimation of map distances from recombinant values. Ann. Eugen. 12: 172–175.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newburg (1987) MAPMAKER: an interactive computer package for constructing primary genetic maps of experimental and natural population. Genomics 1: 174–181.

Lawson, W.R., K.C. Goulter, R.J. Henry, G.A. Kong and J.K. Kochman (1998) Marker assisted selection for two rust resistance genes in sunflower. Mol. Breed. 4: 227–234.

Lawson, W.R., C.C. Jan, T. Shatte, L. Smith, G.A. Kong and J.K. Kochman (2011) DNA markers linked to the _R_2 resistance gene in sunflower ( _Helianthus annuus_ ) facilitate anticipatory breeding for this disease variant. Mol. Breed. 28: 569–576.

Li, Q. and J.M. Wan (2005) SSR Hunter: Development of a local searching software for SSR sites. Hereditas 27: 808–810.

Markell, S., T. Gulya, K. McKay, M. Hutter, C. Hollingsworth, V. Ulstad, R. Koch and A. Knudsvig (2009) Widespread occurrence of the aecial stage of sunflower rust caused by _Puccinia helianthi_ in North Dakota and Minnesota in 2008. Plant Disease 93: 668–668.

McIntosh, R.A. and G.N. Brown (1997) Anticipatory breeding for resistance to rust diseases in wheat. Annu. Rev. Phytopathol. 35: 311–326.

Miah, M.A.J. and W.E. Stackton (1970) Genetics of host-pathogen interaction in sunflower. Phytoprotection 51: 1–16.

Miller, J.F. and T.J. Gulya (1988) Registration of six downy mildew resistant sunflower germplasm lines. Crop Sci. 28: 1040–1041.

Miller, J.F., R.H. Rodriguez and T.J. Gulya (1988) Evaluation of genetic materials for inheritance of resistance to Race 4 rust in sunflower. In: Proceedings 12th International Sunflower Conference, Novi Sad, Yugoslavia International Sunflower Association, Paris, pp. 361–365.

Miller, J.F. and T.J. Gulya (2001) Registration of three rust resistant sunflower germplasm populations. Crop Sci. 41: 601.

Moreno, P.S., A. Bertero de Romano, M.C. Romano, P. Vergani, M. Sposaro, M. Bulos, E. Altieri, M.L. Ramos and C.A. Sala (2012) A survey of physiological races of _Puccinia helianthi_ in Argentina. In: Proceedings 18th International Sunflower Conference, Mar del Plata, Argentina, pp. 278–283.

Paniego, N., R. Heinz, F. Fernandez, P. Talia, V. Nishinakamasa and H.E. Hopp (2007) Sunflower. In: Kole, C. (ed.) Genome Mapping
and Molecular Breeding in Plants, Volume 2 Oilseeds, Springer-Verlag Berlin, Heidelberg, pp. 153–177.

Paniego, N., M.E. Bazzalo, M. Bulos, V. Lia, C. Fusari, D. Alvarez, E. Altieri, M.L. Ramos, M.T. Galella, M. Kaspar et al. (2012) Genomics, mapping and marker assisted selection strategies for disease resistance. In: Proceedings 18th International Sunflower Conference, Mar del Plata, Argentina, pp. 44–50.

Qi, L.L., T.L. Gulya, G.J. Seiler, B.S. Hulke, and B.A. Vick (2011a) Identification of resistance to new virulent races of rust in sunflowers and validation of DNA markers in the gene pool. Phytopathology 101: 241–249.

Qi, L.L., B.S. Hulke, B.A. Vick, and T.J. Gulya (2011b) Molecular mapping of the rust resistance gene \( R_{4} \) to a large NBS-LRR cluster on linkage group 13 of sunflower. Theor. Appl. Genet. 123: 351–358.

Qi, L.L., G.J. Seiler, B.A. Vick, and T.J. Gulya (2012) Genetics and mapping of the \( R_{11} \) gene conferring resistance to recently emerged rust races, tightly linked to male fertility restoration, in sunflower (Helianthus annuus L.). Theor. Appl. Genet. 125: 921–932.

Quresh, Z., C.C. Jan, and T.J. Gulya (1993) Resistance to sunflower rust and its inheritance in wild sunflower species. Plant Breed. 110: 297–306.

Radwan, O., S. Gandhi, A. Heesacker, B. Whitaker, C. Taylor, A. Plocik, R. Kesseli, A. Kozik, R.W. Michelmore, and S.J. Knapp (2008) Genetic diversity and genomic distribution of homologs encoding NBS-LRR disease resistance proteins in sunflower. Mol. Genet. Genomics 280: 111–125.

Radwan, O. (2010) Isolation and Expression of an NBS-LRR Protein-encoding resistance gene candidate that segregates with a rust resistance gene in sunflower. Journal of Phytopathology 158: 433–443.

Sala, C.A., M. Bulos, and A.M. Echarte (2008) Genetic analysis of an induced mutation conferring imidazolinone resistance in sunflower. Crop Sci. 48: 1817–1822.

Sala, C.A., M. Bulos, E. Altieri, and M.L. Ramos (2012) Sunflower: improving crop productivity and abiotic stress tolerance. In: Tuteja, N., S. Gill, A.F. Tubercio, R. Tuteja (eds.) Improving Crop Resistance to Abiotic Stress, Wiley-Blackwell Wiley-VCH Verlag GmbH & Co., Germany, pp. 1205–1249.

Schneiter, A.A. and J.F. Miller (1981) Description of sunflower growth stages. Crop Sci. 21: 901–903.

Seiler, G.J. and C.C. Jan (1997) Registration of 10 interspecific germplasm fertility restoration populations for sunflower PET1 male-sterile cytoplasm. Crop Sci. 37: 1989–1991.

Sendall, B.C., K.C. Goulter, E.A.B. Aitken, K.C. Goulter, S.M. Thompson, J.H.M. Mitchell, J.K. Kochman, W. Walters, T. Shuttle and T.J. Gulya (2006) Review of research on the sunflower: Puccinia helianthi pathosystem in Australia. Australasian Plant Pathol. 35: 657–670.

Tang, S., J.K. Yu, M.B. Slabaugh, D.K. Shintani, and S.J. Knapp (2002) Simple sequence repeat map of the sunflower genome. Theor. Appl. Genet. 105: 1124–1136.

Yang, S.M., E.F. Antonelli, A. Luciano, and N.D. Luciani (1986) Reactions of Argentine and Australian sunflower rust differentials to four North American cultures of Puccinia helianthi from North Dakota. Plant Disease 70: 883–886.

Yang, S.M., W.M. Dowler, and A. Luciano (1989) Gene \( Pu_{6} \): a new gene in sunflower for resistance to Puccinia helianthi. Phytopathology 79: 474–477.

Yu, J.K., S. Tang, M.B. Slabaugh, A. Heesacker, G. Cole, M. Herring, J. Soper, F. Han, W.C. Chu, D.M. Webb et al. (2003) Towards a saturated molecular genetic linkage map for cultivated sunflower. Crop Sci. 43: 367–387.