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Chapter 4

GENETICS AND GENOMICS APPLIED TO SUNFLOWER BREEDING

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ABSTRACT

Since sunflower domestication by pre-hispanic American cultures at least 3000 BC, the use of empiric and scientifically based genetics led to an amazing genetic diversification of the crop going from sophisticated nutraceutical applications up to ornamental purposes, including the traditional confectionary and oilseed production. Commercial sunflower breeding based on genetics started in the first half of the twentieth century and genomics at its endings, with breeding efforts being directed towards the most economically important traits such as increasing seed and oil yield, improving quality traits and conferring resistance or tolerance to biotic and abiotic stresses. In the last few years, advancements in genotyping and sequencing technologies allowed the development of increasingly dense genetic and physical maps, enabling the development of new breeding strategies based on molecular markers, like QTL mapping, association mapping and genomic selection. The need to increase efficiency and precision has motivated the application of marker assisted selection (MAS) in sunflower breeding programs. This chapter will review the different genomic breeding approaches that are currently used to improve sunflower tolerance to biotic and abiotic stresses, increase oil quality and enhance agronomic yield associated traits in order to reduce the gap between potential and actual sunflower production in the present cultivated sunflower area and

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under global weather changing conditions that negatively impact on it. An overview of the state of the art on sunflower genomics is presented and the potential of high throughput sequencing and genotyping technologies for crop breeding is discussed.

Keywords: Sunflower, marker assisted selection, QTL mapping, association mapping, cytogenetyc mapping, linkage mapping

INTRODUCTION

The Compositae (*Asteraceae*) is the largest plant family on earth, with over 24000 described species, representing almost 10% of all flowering plant species (Stevens 2010). Compositae species include economically important crops, rare and beautiful wildflowers, invasive weeds and several species harboring common allergens and valuable medical molecules (Dempewolf et al., 2008). They are referred to as Composites because what looks like a single large flower is actually a composite of many, maybe thousands tiny flowers. It includes sunflowers, lettuce, artichokes, dandelions, thistles, daisies, ragweed, goldenrod and chicory (Kane et al., 2011).

The genus *Helianthus* contains about 50 species of annual and perennial herbs (Heiser and Schilling 1981) native to America. It includes diploids, tetraploids and hexaploids, all with the basic chromosome number of 17 (Rieseberg 1991).

Asteraceae and its related families *Goodeniaceae* and *Calyceraceae* do not have an extended fossil record. In 2010, Barreda et al. described a fossil capitulum and pollen grains from the Eocene that were found in Patagonia, Southern Argentina. This finding evidenced that the family evolved from an ancestor originated putatively in South America about 50 million years ago (Barreda et al., 2010).

Sunflower *(Helianthus annuus* L. var. macrocarpus) was originally domesticated in the east-central part of North America circa 3000-4000 years ago (Crites 1993; Harter et al., 2004; Smith and Yarnell 2009; Bowers et al., 2012) by pre-Columbian civilizations that used it as a source of edible seeds and for other applications (as a source of natural dyes and for ceremonial purposes) (Mandel et al., 2013). Since then, sunflower has been grown with many purposes: as oil crop (its main use), for beauty (ornamental sunflower) and for direct consumption of the seeds (confectionary sunflower). Furthermore, there is an increasing interest in the use of sunflower proteins in human nutrition. The content of protein remaining in cakes and extraction residues after seed oil extraction, accounts for 30 to 50% (Dorrell and Vick 1997). The properties of sunflower proteins are comparable with those of soy and other leguminous proteins (González-Pérez et al., 2005; Zilic et al., 2010). Regarding its main use, sunflower is currently the world's fourth largest source of vegetable oil (http://www.fas.usda.gov/). Sunflower oil is considered premium due to its high unsaturated fatty acid composition and low content of linolenic acid. Nowadays, it is cultivated on over 23 million hectares worldwide (http://www.fao.org/), with an annual production of 32 million metric tons, mainly concentrated in the Russian Federation, Ukraine, India, and Argentina (Bowers et al., 2012).

Conventional breeding has been successful in raising sunflower yield potential and its stability, as well as in controlling resistance to some fungal diseases, pests and parasitic

weeds (Sala et al., 2012). The advent and development of molecular markers and genetic maps have facilitated understanding the genetic basis of different agronomic traits.

In this chapter, we present and discuss the different genetic and genomic breeding approaches that are currently used to improve sunflower yield and its tolerance to diseases, focusing on the study of pathogen resistance responses and reviewing the state of the art of sunflower genomics.

1. SUNFLOWER BREEDING

Commercial sunflower breeding started in most of the producing countries (Eastern and Western Europe, North and South America) between 1920 and 1950 by phenotypic selection, a method of selecting desirable plants from a population on the basis of phenotypic traits.

The introduction of heterosis, first described in 1966 (Leclercq 1966), the incorporation of cytoplasmic male sterility after interspecific crossing with *H. petiolaris* Nutt (Leclercq 1969), and the development of fertility restorer lines by Kinman in 1970 (Miller and Fick 1997), allowed the development of sunflower hybrids. This process is based on a single cytoplasmic male sterility (CMS) source, the PET1. The CMS was associated with the expression of a 16 kDa protein encoded by *orfH522* in the PET1 cytoplasm, which is anther-specific reduced in fertility restored hybrids (Moneger et al., 1994). This protein is co-transcribed with the atpA mitochondrial gene in the male sterile lines.

The first sunflower hybrids were produced in 1972 and reached 80% of production in five years (Miller and Fick 1997), due to their higher yield and quality potential, high homogeneity, maturing time synchronicity and better adaptation to cultural applications.

Sunflower breeding efforts were directed towards the most economically important traits, including: increased seed and oil yield (number of seeds per plant, test weight, 1000-seed weight, low husk content and high oil concentration in the seed), increased harvest index (plant height, head size and shape, angle of the head, leaf area and leaf canopy, early maturation, short stem and uniform height), improved quality traits (oil quality, protein concentration and composition), and resistance to biotic and abiotic stresses (Škorić 1992).

Despite the optimism for continued improvement by conventional breeding, new technologies are needed to significantly increase efficiency and precision, and to save time, resources and efforts. Agriculturally important traits, such as yield, quality and some forms of disease resistance are controlled by many genes and known as quantitative traits. The regions within genomes that contain those genes associated with a particular quantitative trait are known as quantitative trait loci (QTL). Owing to genetic linkage, DNA markers can be used to detect the presence of allelic variation for major genes or QTL underlying the traits of interest.

The first molecular markers described in sunflower were restriction fragment length polymorphism (RFLP) (Berry et al., 1995, 1996, 1997; Gentzbittel et al., 1995, 1999; Jan et al., 1998), random amplified polymorphic DNA (RAPD) (Rieseberg et al., 1993; Rieseberg 1998) and amplified fragment length polymorphisms (AFLPs) (Peerbolte and Paleman 1998; Flores Berrios et al., 2000; Gedil et al., 2001; Al-Chaarani et al., 2004). However, RFLPs are technically laborious for routine use as molecular markers and while RAPD and AFLP markers have many advantages, they are mostly dominant, abundant but often non-specific

and not very useful for comparison of a genome-wide synteny of molecular markers for cross referencing genetic linkage maps (Paniego et al., 2007). Nowadays, the most popular molecular markers are microsatellite markers (also called single sequence repeats, SSR) and single nucleotide polymorphisms (SNP). While SSR markers are multiallelic, SNPs are generally assumed as biallelic markers. However, this disadvantage is compensated by the relative abundance of SNPs (Oraguzie et al., 2007) and emerging high throughput profiling technologies.

Marker-assisted selection (MAS) is a method whereby a phenotype is selected using molecular markers linked to the trait genetic determinants. The advantages of MAS include: time saving by substitution of field trials with molecular tests; selection carried out at seedling stage; the possibility of combining multiple gene selection; avoid the transfer of undesirable genes by background selection; selection of traits with low heritability; selection of single plants (Collard et al., 2005).

One of the most important tools in MAS strategy is having a high-resolution map, composed of polymorphic molecular markers covering all chromosomes, in order to identify markers flanking those QTL that control traits of interest. To avoid losing the selected trait due to recombination events between the marker locus and the QTL, they need to be in strong linkage disequilibrium (at least <5 cM but ideally <1 cM away from the gene). However, not any marker associated with a QTL is directly useful in MAS, and its reliability for predicting phenotypes has to be tested (Collard et al., 2008). Advancements in genotyping technologies, and its direct consequences, lower genotyping costs and denser genome coverage, allowed the development of new breeding strategies like association mapping and genomic selection (GS). Association mapping, or linkage disequilibrium (LD) mapping, has emerged as a tool to resolve complex trait variation, because the historical recombination and natural genetic diversity are exploited for high resolution mapping (Risch and Merikangas 1996; Nordborg et al., 2002; Zhu et al., 2008). Meanwhile, GS uses a genome-wide panel of dense markers as predictors of performance, calculating genomic estimated breeding values for complex traits. Phenotypes and high-density marker scores are analyzed in the population of study, and the breeding values of any line can be predicted with a high level of accuracy (in simulations, the correlation between true and estimated breeding values reached a level of 0.85 (Heffner, Sorrells, and Jannink 2009). This selection method promises to accelerate the breeding times, while changing the role of phenotyping only to corroborate and update prediction models. However, due to the lack of a reference genome, this method cannot be applied in sunflower until the establishment of a much larger number of SNPs markers validated for GS.

2. SUNFLOWER LINKAGE MAP

Over the past decades, several genetic linkage maps differing in length, density and type of molecular markers, were developed for cultivated sunflower (n=17 chromosomes). Gentzbittel et al (1994, 1995) have shown that the RFLP technique can be used to identify sunflower genotypes and that there is sufficient variability to develop an RFLP framework map. Thus, first descriptions of a consensus sunflower map, based on segregation of RFLP markers, were published by Gentzbittel et al., (1995) and Berry et al., (1995). Since then, denser linkage maps were developed, based on RFLP (Berry et al., 1996, 1997; Jan et al.,

1998; Gentzbittel et al., 1999), RAPD (Rieseberg et al., 1993; Rieseberg 1998), AFLPs (Peerbolte and Paleman 1998; Flores Berrios et al., 2000; Gedil et al., 2001; Al-Chaarani et al., 2002), direct amplification of length polymorphism markers (DALPs) (Langar et al., 2003), and target region amplification polymorphisms (TRAPs), that use EST database information to generate polymorphic markers around targeted candidate gene sequences (Hu and Vick 2003).

In sunflower, RAPDs have been used primarily for tagging phenotypic loci (Lawson et al., 1998; Lu et al., 2000), while the RFLP maps have been used as tools for mapping phenotypic and quantitative trait loci (Leon et al., 1995, 2000; Berry et al., 1996; Lu et al., 1999; Bert et al., 2001; Lee et al., 2001; Al-Chaarani et al., 2002; Pérez-Vich et al., 2002). The TRAP technique has been employed to construct a linkage map (Hu and Vick 2003), to define the telomeric regions of sunflower linkage groups through the use of TRAP markers based on *Arabidopsis*-type telomere repeat sequences (Hu 2006), to map some resistance loci (Hu et al., 2004; Yue et al., 2008); and to assess germplasm relationships (Sala et al., 2012).

The development of the first microsatellite markers in sunflower supplied the critical mass of DNA markers needed to create a public reference map and unify independently developed genetic linkage maps. They rapidly became the markers of choice for linkage analysis due to the fact that they are highly polymorphic, usually inherited in a codominant manner and, in most cases, chromosome-specific.

In the last years, development of microsatellites by South American researchers at INTA in Argentina (HAx) together with those of European (CARTISOL; CRS) and North American (ORSx) origin summed up to 2040 markers (Dehmer and Friedt 1998; Paniego et al., 2002; Tang et al., 2002; Yu et al., 2002). These markers were used for developing reference maps using F2 and recombinant inbred line (RIL) populations, which are highly homozygous lines derived from crosses between highly contrasting sunflower inbred lines. The recombinant inbred line parental crosses used were: PAC-2 x RHA266, RHA280 x RHA801 and PHA x PHAB (Flores Berrios et al., 2000; Tang et al., 2002, 2003; Yu et al., 2003; Al-Chaarani et al., 2004). Furthermore, several reference genetic maps that integrate the independently developed linkage maps have been reported (Yu et al., 2003; Paniego et al., 2007). Yu et al., (2003) integrated 120 SSR loci from the public SSR map and 28 RFLP markers from Jan et al., (1998) map with 80 RFLP loci of the genetic map of Berry et al., (1997). Lai et al., (2005a) produced the first SNP map by integrating 243 loci in the RHA280 x·RHA801 reference map (Tang et al., 2002; Yu et al., 2003). Kiani et al (2007b) established a high-density genetic map with a mean density of one marker per 3.7 cM, by integrating 191 SSR marker loci (Paniego et al., 2002; Tang et al., 2003) into a previous map based on 304 AFLP markers (Al-Chaarani et al., 2004). All the maps reported above, plus unpublished linkage maps, can be accessed through the Sunflower CMap Database (http://www.sunflower.uga.edu/cmap/).

These SSR-based maps have been used to identify quantitative trait loci (QTL) underlying numerous traits of agronomic importance (Shunxue Tang et al., 2006; Wills and Burke 2007, Kianni et al 2007, 2008, 2009, Haddadi et al., 2010, Haddadi et al 2011a, 2011b) and to investigate variation in genome structure between sunflower and other *Helianthus* species (Burke et al., 2002; Heesacker et al., 2008).

Nowadays, next-generation sequencing technologies and high-throughput genotyping platforms make it possible to simultaneously interrogate thousands of SNPs throughout the genome (Gupta et al., 2008), enabling the development of increasingly dense genetic maps.

Zubrzycki et al (2012a) reported the construction of an Oligonucleotide Probe Assay (Illumina) including 384 SNPs representing putative candidate genes for stress resistance responses. In parallel, a 10K Illumina Infinium SNP genotyping array was developed (Bachlava et al., 2012) and used in different sunflower mapping populations to produce a consensus linkage map of the sunflower genome, that constitutes the longest map reported in the literature until now (Bowers et al., 2012). In this study, four different sunflower mapping populations were used to produce four separate high-density genetic maps containing 3500–5500 loci each. Although the individual maps were highly colinear, localized variation in recombination rates in several genomic regions and gaps up to 26 cM in length were observed. Because these regions differed by cross between the different populations, the consensus map of 10,080 loci contained no such gaps, demonstrating the value of simultaneously analyzing multiple mapping populations. This high density genetic map has the potential to facilitate the assembly of the forthcoming sunflower genome (Kane et al., 2011), enabling the efficient and detailed genotypic characterization of germplasm collections, and providing an important tool for genome wide association mapping (GWAS) and GS studies.

Bowers et al., (2012) described the genotypic characterization of a subset of 69 lines of the RHA280 x RHA801 mapping population using a custom Affymetrix GeneChip that contains 2389915 features, targeting 86023 unigenes from sunflower and related species. The previously generated genetic map (Bowers et al., 2012), was used to filter out low quality data and place 67486 features, corresponding to 22481 unigenes, on the sunflower genetic map. The resulting map contains a substantial fraction of all sunflower genes, and will facilitate genome assembly and the identification of candidate genes underlying QTL or traits of interest.

3. QTL MAPPING

3.1. Disease Resistance

Biotic stress is considered one of the most important factors affecting sunflower all over the world. Cultivated sunflower is susceptible to several fungal and bacterial diseases, being the infection severity greatly dependent on environmental conditions. Diseases affecting most of the sunflower growing areas include wilt, middle stalk rot, and head rot **(**mainly caused by *Sclerotinia sclerotiorum***)**, downy mildew **(***Plasmopara halstedii***)**, stem canker **(***Phomopsis helianthi***)**, rust **(***Puccinia helianthi***)** and Verticillium wilt **(***Verticillium dahliae***)**. Other diseases such as head rots **(***Rhizopus arrhizus*, *R. stolonifera*, *Botrytis cinerea***)**, phomopsis black stem **(***Phoma macdonaldii***)**, Alternaria leaf and stem spot **(***Alternaria helianthi* or *A. zinniae***)**, Septoria leaf spot **(***Septoria helianthi***)**, charcoal rot **(***Macrophominia phasiolina***)**, bacterial infections **(***Pseudomonas syringae pv. Tagetis***)**, powdery mildew **(***Erysiphe cichoracearum)* have local impacts in some productive areas (Seiler 1992; Pereyra and Escande 1994; Schneiter 1997).

Disease control by applying fungicides is not always possible due to the vast marginal cultivated areas of sunflower crop. Therefore, the most efficient and sustainable strategy to

control the disease and pest impact is to strengthen the crop by the generation of inbred lines with genetic disease tolerance (Sackston 1962).

A relevant contribution to the study of sunflower disease resistance factors is the comprehensive work carried out by Radwan et al., (2008) mapping unique resistance gene candidates, encoding nucleotide binding site-leucine rich repeat sequences **(**NBS-LRR**)**, to 167 loci throughout the 17 sunflower linkage groups. NBS-LRR loci were mainly distributed in cluster or singletons at the upper and lower end of most linkage groups. They described two large clusters on linkage groups 8 and 13, whereas smaller clusters were mapped on linkage groups 1, 4, 9, and 15. Interestingly, most of the resistance loci mapped in sunflower until now, lie in the same linkage groups **(**LG, see below**)**.

A compendium of disease resistance genes mapped in sunflower genome is summarized in Table 1.

Table 1. Disease resistance and oil related genes mapped in sunflower

Trait	Gene	Location	Literature source
Downy Mildew Resistance	Pl1	LG8	Zimmer and Fick, 1974; Gedil et al., 2001; Bertero de Romano et al., 2010
	Pl2	LG8	Zimmer and Fick, 1974; Bertero de Romano et al., 2010
	Pl4	?	Sackston, 1990
	Pl5	LG13	Bert et al., 2001; Radwan et al., 2004
	Pl6	LG8	Miller and Gulya,1991; Bouzidi et al., 2002; Vear et al., 2003; Panković et al., 2007
	Pl7	LG8	Miller and Gulya, 1991; Bert et al., 2001;
	Pl8	LG13	Miller and Gulya,1991; Radwan et al., 2004; Bachlava et al., 2011
	Pl9	?	Sackston, 1990; Molinero-Ruiz et al., 2003
	Pl13	LG1	Mulpuri et al., 2009; Bertero de Romano et al., 2010
	Pl14	LG1	Bachlava et al., 2011
	Pl15	LG8	Bertero de Romano et al., 2010
	PlArg	LG1	Dussle et al., 2004; Wieckhorst et al., 2010; Imerovski et al., 2011
Black Rust Resistance	R1	LG8	Lawson et al., 1996
	Radv	LG13	Lawson et al., 1998; Bachlava et al., 2009
	R4	LG13	Qi et al., 2011; Miller et al., 1988
	R2	LG9	Lawson et al., 2010
	R3	?	Goulter 1990
	R5	LG2	Qi et al., 2011
	Pu6	?	Yang 1989
	R11	LG13	Qi et al., 2012
	R12	?	Gong et al., 2013
Verticillium Wilt	V1	?	Gulya, 2007
Sclerotinia Head Rot Resistance	HaRIC_B	LG11	Fusari et al., 2012
Oil Yield Related	B	LG10	Tang et al., 2006
	Hyp	LG16	Tang et al., 2006
	P	LG17	Tang et al., 2006

Downy Mildew

Genetic analysis of downy mildew resistance includes the characterization and inheritance of both complete and partial resistance (Jocic et al., 2012). Complete, monogenic or qualitative resistance is given by *Pl* genes (Vranceanu et al., 1981; Miller and Gulya 1991). Until today, a total of 18 disease resistance genes have been identified from wild and cultivated sunflower **(***Pl_1* to *Pl_{16}*, *P_{21}*, *Pl_{Arg}***)**. The *Pl_5* gene from *H. tuberosus*, a perennial hexaploid sunflower, protects against race 3 **(**virulence phenotype 700**)** (Pustovoit 1966; Leclercq et al., 1970). Resistance to races 1, 2, 3 and 4 **(**virulence phenotype 730**)** was introgressed into cultivated sunflower from three sunflower species: *H. annuus ssp. annuus* **(***Pl_6***)**, *H. praecox ssp. runyonii* **(***Pl_7***)** and *H. argophyllus* **(***Pl_8***)** (Miller and Gulya 1988, 1991). Also from *H. argophyllus* was isolated *Pl_{Arg}*, a gene which conferred resistance to at least four tested races **(**virulence phenotypes 300, 700, 730, 770**)**. Recently, new sources of resistance were described, one derived from the inbred line HAR5 which was designated as *Pl_{13}* (Mulpuri et al., 2009), while the second was designated as *Pl_{14}* (Bachlava et al., 2011). The effectiveness of the major resistance genes *Pl_6* and *Pl_7* has been overcome by new races in France (Delmotte et al., 2008) and in USA; however, there are no records of races overcoming other broad spectrum **(***Pl_8*, and *Pl_{Arg}***)** genes (Gulya et al., 2010). The gene *Pl_{15}* found in RNID, a restorer inbred line derived from an open pollinated population, shows resistance to the four predominant races of downy mildew in all sunflower producing countries **(**300, 700, 730, and 770 (Gulya 2007), and also to the less prevalent races, including those recently described in USA **(**714 and 734**)** and in France **(**304**)** which overcome *Pl_6* (Vear 2004; Gulya et al., 2010). The *Pl_1* (Mouzeyar et al., 1995), *Pl_2* (Vear et al., 1997), *Pl_6* (Roeckel-drevet et al., 1996), *Pl_7* (Bert et al., 2001), *Pl_{15}* (Bertero de Romano et al., 2010) cluster on LG 8. Finally, *Pl_5* (Bert et al., 2001), *Pl_8* (Radwan et al., 2003) and *Pl_{21}* (Vincourt et al., 2012) were mapped to LG 13.

Partial or quantitative resistance is usually non race-specific and assumed to be polygenic (Jocic et al., 2012). Al-Chaarani et al., (2002) described three major QTL located on LG 1, 9 and 17, and explained 54.9% of the total phenotypic variance. Vear et al., (2008) reported two QTL located on LG 10 and LG 8. Vincourt et al., (2012) confirmed and refined the QTL described by Vear et al (2008) and mapped a third QTL in LG 7. QTL on LG 7 and LG 10 are not located within the major resistance gene clusters **(**LG 8, LG 13, LG 1**)**, suggesting that the genes underlying these QTL may involve other biological processes than those directly involved in plant pathogen interaction. Moreover, QTL mapped in the same region as a gene involved in stomatal opening and root growth, which was suggested as a possible candidate to explain the control of mildew resistance (Vincourt et al., 2012).

The use of partial resistances does not appear to have an effect as strong as the monogenic resistance on pathogen populations. By combining the partial resistance provided by minor genes with specific resistance genes, durable resistance could be achieved (Vear et al., 2008).

Black Rust

Sunflower rust, occasioned by *Puccinia helianthi* can result in significant losses in both yield and seed quality. The rapid changes that occur in its virulence represent a continuous

threat to the effectiveness of existing rust-resistance inbred lines and hybrids. Several genes conferring resistance to rust have been identified in sunflower including R_1, R_2, R_3, R_4, R_5, Pu_6, R_{adv}, R_{11} and R_{12} (Putt and Sackston 1957; Miah and Sackston 1970; Miller and Gulya 1988; Yang 1989; Lawson et al., 1998; Gong et al., 2013), but only a few of them have been genetically characterized, mapped and linked to molecular markers. In addition, several inbred lines and interspecific germplasm lines were reported to have resistance to different rust races (Quresh et al., 1993; Bulos et al., 2012).

Rust resistance R_1 gene was found in the restorer inbred line RHA279 (Lawson et al., 1996) and was mapped on LG 8. Three resistance genes (R_{adv}, from an Australian hybrid (Lawson et al., 1998), R_{adv} from the public line RHA340 (Bachlava et al., 2009) and R_4 from HAR3 (Qi et al., 2011) were located on LG 13. R_1, and both R_{adv} and R_4 are close to the largest clusters of NBS-LRR resistance candidate genes currently described in sunflower genetic maps (Lawson et al., 1998, 2010; Radwan et al., 2008; Qi et al., 2011). R_2 from MC29 was located using SSR markers in LG 9 (Lawson et al., 2010). In each case, it was demonstrated that rust resistance is controlled by a single factor.

Inheritance and mapping of rust resistance genes from different sources were recently reported (Bulos et al., 2012). Bulos and coworkers (2013) identified 8 markers linked to the rust resistance gene from the line HAR6, located between molecular markers ZVG61 and ORS581, at 0.7 and 1.5 cM, respectively. Currently, research is in progress in order to elucidate the allelic relationships among all these sources, and to generate allele specific markers for all of them. A pre-breeding strategy to eliminate linkage drag from the wild donor species is also in progress (Paniego et al., 2012).

Verticillium Wilt

Verticillium dahliae **(**Kleb**)** is a soil-borne pathogen widely distributed which causes the premature death and stem breakage of sunflower. Resistance to this pathogen was described as qualitative or quantitative, dominant or recessive, depending on the host species considered (Pegg and Brady 2002; Fradin and Thomma 2006). In sunflower genetic resistance, based on a single, dominant gene from wild *H. annuus* (V1), was previously identified and incorporated into released inbreds (Gulya 2007). Resistant oilseed hybrids were produced containing this gene. However, a race of *V. dahliae* not controlled by the *V1* gene was previously identified in Argentina (Bertero and Vasquez 1982).

Galella et al., (2004), reported two new races isolated from Argentina which were called Varg1 and Varg2. A new strain of *V. dahliae*, identified in the U.S., as well as these new reported Argentinean races, are able to overcome *V1* resistance gene **(**from HA89 inbred line**)** (Galella et al., 2004; Gulya 2007). A major Varg1 resistance QTL, mapped and incorporated by MAS to hybrids of different backgrounds, gave effective protection under natural infection at four locations, independently of the background of the parental lines (Creus et al., 2007). A second QTL contributing to Varg2 resistance was mapped in a different linkage group. Both QTL were transferred to the same susceptible line conferring resistance to the two most abundant pathogenic races present in Argentina, under controlled independent inoculations. Nevertheless, few individual plants presented typical symptoms under natural infection conditions indicating the presence of minor *Verticillium* races (Galella et al., 2012). These

results indicate that it is possible to combine resistance QTL to different races in order to obtain a more durable resistance to Verticillium wilt.

Alternaria Leaf Blight

Alternaria helianthi is a worldwide distributed pathogen associated to important yield reductions. *A. helianthi* attacks leaves, stems petioles, bracts and heads of sunflower (Kong et al., 1995) causing premature foliar senescence and defoliation due to spot coalescence or petiole necrosis. The nature of resistance is polygenic and limited information about resistance QTL location has been reported (Virányi 2008). The lack of good sources for resistance is a major limiting issue in sunflower breeding (Reddy et al., 2006).

In order to detect resistance sources, 370 lines were evaluated under natural infection in the Northern crop region of Argentina. The lesion density and the percentage of totally necrotic leaves were measured. Three major QTL in LG 4, 10 and 17 showing additive effect were detected (Galella et al., 2010). The QTL were introgressed by backcross from the resistance source to L97 and L03 susceptible lines using MAS. The converted lines and their respective counterparts were evaluated under very severe natural infection conditions. The percentage of total necrotic foliage was reduced from 100 to 50 % in L03 and from 70 to 40 % in L97 backgrounds, respectively.

Sclerotinia Rots

Sunflower production is seriously affected by *Sclerotinia sclerotiorum* infection with very variable attack levels, according to temperature and rainfall at particular periods of crop growth and the presence of other susceptible crops in the rotation or in neighbouring fields. This pathogen is a highly polyphagous and necrotrophic fungus that infects different plant parts, including roots, stem bases **(**stalk rot**)**, leaves, terminal buds and capitula **(**head rot**)** producing great losses. Disease control on *S. sclerotiorum* is difficult, since the fungus persists in soils for long periods and at high inoculum levels (Huang and Dueck 1980; Troglia 2003).

Currently, no complete resistance to *S. sclerotiorum* is available in cultivated sunflower, even if differences in susceptibility exist (Tourvieille de Labrouhe et al., 1996). The nature of resistance has been described as partial quantitative disease resistance **(**QDR**)** and highly dependent on environmental conditions (Castaño et al., 1993) involving several loci with different effects, most of them additives (Vear and Tourvieille de Labrouhe 1988; Gulya et al., 1997).

Conventional breeding programs involve field resistance tests on segregating progenies and inbred lines and field trials on potential hybrid varieties. Regarding this point, Mestries et al., **(**1998**)** identified four QTL for leaf resistance and two for head rot resistance based on a genetic map with RFLP and isozyme markers. One of these zones appears to be involved in resistance to both types of *S. sclerotiorum* attack, while the others appear specific for resistance in one part of the plant. Individual QTL explained between 9% and 48% of the phenotypic variability, confirming the polygenic basis of this trait. Overall, the quantitative trait loci explained 60% of the genetic variation for leaf resistance and 38% for capitulum

resistance to *S. sclerotiorum*. Simultaneously, Gentzbittel et al., (1998) reported several QTL for resistance to penetration or extension of the *S. sclerotiorum* mycelium in different tissues. The latter authors also mapped a serine-threonine protein kinase-like **(**PK**)** marker that co-segregates with a QTL apparently specific to an inbred line, PAC1. It explained 50% of variation in a cross with a very susceptible line and 15% in a cross with a different highly resistant line. However, this QTL has not been found in other populations, even when the resistance source came from recurrent selection of a population from which PAC1 was originated and when the parents showed polymorphism for the PK gene.

Later, Bert et al., (2002) detected 15 QTL for *Sclerotinia* reaction across several linkage groups, whereas Bert et al., (2004) described seven QTL for resistance to *S. sclerotiorum* terminal bud attack, each explaining less than 10% of the phenotypic variance. For capitulum attack, there were 4 QTL, each explaining up to 20% of variation. All QTL found involved six different genetic crosses between cultivated inbred lines, being reported QTL distributed in at least 14 linkage groups.

Regarding *S. sclerotiorum* head rot, relative QTL effect levels vary between different years and locations considered. Thus, QTL effects studied during a specific year and location differ within a range of 23% (Bert et al., 2004) to 44% (Mestries et al., 1998), and also differ from those studied over two or more years in different locations for which the variation ranged from 13% to 16% (Gentzbittel et al., 1998; Bert 2002).

Micic et al., (2004) identified nine QTL for leaf lesion, eight for stem lesion, and six for speed of fungal growth. For stem lesion two genomic regions explained 26.5% of the genotypic variance (Micic et al., 2005b). Three to four putative QTL were detected for stem lesion, leaf lesion and speed of fungal growth explaining between 40.8% and 72.7% of the genotypic variance. Two QTL for stem lesion showed large genetic effects and corroborated earlier findings from the cross NDBLOSsel **(**resistant**)** x CM625 **(**susceptible**)** (Micic et al., 2005a).

Rönicke et al., (2005) identified five QTL for lesion length and head rot explaining 10.6 to 17.1% of the total phenotypic variance. The most frequent QTL being that **(**or those**)** linked to the recessive branching gene b1 on Linkage Group 10.

Yue et al., (2008) identified nine and seven QTL for disease incidence and disease severity, respectively. Each QTL explained 8.4 to 34.5% of phenotypic variance, suggesting the polygenic basis of the resistance to head rot. Five of these QTL were identified in more than one experiment, and each QTL explained more than 12.9% of phenotypic variance. Although a positive correlation existed between the two disease indices, most of the respective QTL were located in different chromosomal regions, suggesting a different genetic basis for the two phenomena indices.

Davar et al., (2010) detected 7 QTL for percentage necrotic area, localized on 7 linkage groups whose effects were small to moderate.

Zubrzycki et al (2012b) identified 20 QTL, accounting for incidence, severity and the area under the disease progress curve **(**AUDPC**)**, located mostly in linkage groups 10, 13 and 14. Each encountered QTL explained less than 10% of the observed phenotypic variance. In the upper end of LG 10, two reproducible QTL associated with lower incidence were found on this population and on the population derived from the cross of HA89 X RHA801 analyzed by Maringolo (2007).

Association studies of transcript expression of candidate genes with disease resistance represent an alternative strategy to find new sources of resistance to *Sclerotinia*. The

development of a large EST public database for sunflower (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) including entries from sequenced differential cDNA libraries with highly represented defense genes (Fernandez et al., 2003, 2012) enables the utilization of the candidate gene approach for searching new QTL determinants.

The QTL studies for *S. sclerotiorum* resistance have shown that the main QTL detected from different crosses appear to be different, thus suggesting that pyramiding QTL for resistance is possible. Furthermore, resistance to this disease is governed by multiple QTL, located on almost all the sunflower linkage groups. However, like any other quantitative trait, it is necessary to confirm the position of the QTL and to carry out fine-scale mapping before MAS can be undertaken.

The possibility of increasing the level of resistance of elite lines by introgression of several QTL with the help of MAS and embryo culture tools, without affecting yield components, constitutes a relative simple way to reduce the impact of this complex disease.

3.2. Mapping Yield-and Quality Related Traits

Sunflower yield is a complex trait regulated by a number of factors that can be studied as component traits. Oil yield per unit area is determined by the product of seed yield per unit area and oil percentage in the seed. Then, one of the most important goals of sunflower breeding programs is to develop high oil yield hybrids considering both components.

Several QTL accounting for yield-related phenotypic variation across environments ranging from 5 to 90.4%, were detected on different LGs (Leon et al., 1995, 2000; Mokrani et al., 2002).

Tang et al., (2006) identified 40 QTL for yield-related traits in a low- x high-oil RIL mapping population (RHA280 x RHA801) segregating for apical branching (B, located on LG 10), phytomelanin pigment (*Hyp*, located on LG 16), and hypodermal pigment (*P*, Located on LG 17) loci. Twenty-four of the QTL were tightly linked to B, P, and Hyp and may have been partly or completely caused by the pleiotropic effects of B, P, and Hyp. Five QTL controlling seed weight were reported explaining 72.8% of the phenotypic variation. However, the contribution of the pleiotropic effect of the apical branching gene B accounted again for the bulk (52.5%) of this variation (Tang et al., 2006). Interestingly, the same relationship between apical branching and seed oil content was observed in other mapping populations (Mestries et al., 1998; Bert et al., 2003). The linked, pleiotropically acting, and epistatically interacting QTL identified for seed traits were presumably targeted by selection in the transition from large-seeded, low-oil to small-seeded, high-oil cultivars in sunflower.

Putative QTL for 1000-grain weight were detected explaining from 5.4% to 37% of total phenotypic variance (Mokrani et al., 2002; Al-Chaarani et al., 2004). Some of the QTL controlling seed weight overlapped with that mentioned above. The QTL on the same LG as the branching gene *b1*, also reported by Mestries et al., (1998) for another cross, was almost certainly linked to capitulum size. The second QTL for seed weight was close to the one for flowering date.

By evaluation of the same mapping population under different conditions it is possible to discriminate constitutive QTL effects from adaptive ones. These studies allow investigating the underlying genetic basis of trait association by looking for colocation of corresponding QTL for yield-related traits on the genetic map under stress and nonstress conditions.

In this context, several studies have conducted QTL mapping for different traits to highlight the existence of adaptive QTL detected only under specific environmental conditions or QTL modifying their expression with the level of an environmental factor. In 2008, Ebrahimi et al (2008) mapped QTL controlling seed quality traits under well-watered and water-stressed conditions. Significant variation among RILs was observed even though that there were no significant differences for oil content in the seed between both parents. Two to 15 QTL explaining from 5% to 31% of phenotypic variation were found depending on trait and growth conditions. Several QTL for oil content overlapped with QTL for palmitic and stearic acid contents in all four conditions. An overlapping region on linkage group 3 was linked to an SSR marker **(**ORS657**)**. Furthermore, the latter author also identified genomic regions controlling other yield-related traits **(**seed protein content, kernel and hull weights, and seed density**)** in the same water-stressed conditions. Several specific and nonspecific QTL were detected for all traits under these conditions. A QTL for protein content accounting for 17% of phenotypic variance under water-stress conditions was detected. Overlapping QTL for protein content and seed density were identified in LG 15. This region probably has a pleiotropic effect on protein content and seed density. QTL for protein content co-localizing with grain weight traits were also identified. Three markers linked to protein content **(**ORS671_2 and HA2714**)** and kernel weight **(**E35M60_4**)** were identified (Ebrahimi et al., 2009). QTL for grain yield per plant **(**GYP**)** under four water treatments were identified on different linkage groups, among which two were specific to a single treatment (Kiani et al., 2009). The identified QTL explained 4% to 40% of phenotypic variance. Three QTL for GYP overlapped with several QTL for drought-adaptive traits detected in a previous study (Kiani et al., 2007a).

In 2010, Haddadi et al., mapped QTL associated with percentage of seed protein, oil and fatty acids content under well-, partial-irrigated and late-sowing conditions. A specific QTL of palmitic acid content on LG 6 was detected under late-sowing conditions. Common chromosomic regions were observed for percentage of seed oil and stearic acid content on linkage group 10 and 15. Seven QTL associated with palmitic, stearic, oleic and linoleic acids content were identified on LG 14. Additionally, overlapping occured for QTL of oleic and linoleic acids content on linkage groups 10, 11 and 16.

Haddadi et al., (2011) mapped QTL associated with agronomic traits such as yield, plant height, days from sowing to flowering, and leaf-related traits. The study was carried out over well- and partial-irrigated conditions. Three to seven QTL, explaining from 4 to 49% of phenotypic variance, were found for each studied trait in both conditions. Three to six QTL were found for each yield-related trait in both conditions. These QTL were located on LG 2, 9, 10, 11, 12, 13 and 14. Likewise, Hadaddi et al (2011) conducted seven experiments in different environments to identify genomic regions underlying the genetic control of tocopherol, a non-enzymatic antioxidant known as lipid-soluble vitamin E, and phytosterol, molecules with interesting properties, which can result in decreased risk of chronic diseases in humans and with several beneficial effects in plants. Five QTL for total tocopherol content accounting for 45% of phenotypic variation were located on LG 1, 8, 10 and 14, whereas four QTL for total phytosterol content on linkage groups 1, 2, 16 and 17 explained 27% of the phenotypic variation. Moreover, four candidate genes co-located with QTL for total phytosterol content **(**GST, PAT2, SFH3 and POD**)** and four candidate genes co-localized with QTL for total tocopherol content **(**VTE4, HPPD, GST and Droug1**)**. Moreover, the candidate gene SMT2 also mapped on linkage group 17 near the QTL of total phytosterol content.

Evidence of co-localization of ESTs and QTL in sunflower had already been provided by Lai el al. (2005a). They reported different candidate genes that map coincident with QTL controlling physiological, morphologic and developmental traits. On the other hand, a high level of linkage disequilibrium has been reported in cultivated sunflower (Kolkman et al., 2007; Fusari et al., 2008)**.** Thus, it has been suggested that association-based approaches will provide a high degree of resolution for the mapping of functional variations in sunflower (Liu and Burke 2006). Interspecific QTL mapping has been reported for the wild annual species *H. annuus* and *H. petiolaris* (Lexer et al., 2005) and for three hybrid sunflower species derived from them: *H. anomalus*, *H. deserticola*, and *H. paradoxus* (Lai et al., 2005b). QTL analysis in these studies indicated that karyotypic differences among species contributed to reproductive isolation and evaluated inter- and intraspecific QTL magnitudes.

Studies of co-localization of developmental and agronomic traits with resistance to pathogens were also reported. A QTL for percentage of plants attacked by *S. sclerotiorum* was detected in a LG close to one for flowering date, with one of the parental alleles that reduced the days to flowering being linked with increases in resistance to this pathogen (Bert et al., 2003). Other co-localization was reported for QTL for resistance to *S. sclerotiorum* and QTL for seed weight and oil content (Mestries et al., 1998; Bert et al., 2003). Furthermore, some morphology-related traits such as branching regulated by the gene *b1* were studied for their association with *S. sclerotiorum* resistance and agronomic characters (Bert et al., 2003).

A review of oil related genes in sunflower is summarized in Table 1.

4. Association Mapping and Genome Selection

Association genetics via Linkage Disequilibrium (LD) mapping is an emerging field of genetic mapping that has the potential for resolution to the level of individual genes (alleles) underlying quantitative traits (Oraguzie et al., 2007). In contrast to classical biparental population mapping, AM is a method that detects relationships between phenotypic variation and gene polymorphisms in existing germplasm, without development of mapping populations. This method incorporates the effects of recombination occurring in many past generations into a single analysis and thus, it is complementary to the classical biparental approach (Jorde 2000; Fusari et al., 2012).

The first association mapping work reported in sunflower focused on the study of *Sclerotinia* head rot resistance using a candidate gene AM based approach on a population of 94 inbred lines belonging to the "Sunflower Breeding Program" of INTA (Fusari et al., 2012). Disease incidence was measured using assisted inoculation with the fungal pathogen during two campaigns in replicated field trials. Given that no biological mechanisms or biochemical pathways have been positively identified for head rot resistance, selection of candidate genes was based on previous transcript profiling studies in sunflower (Giacomelli et al., 2010; Peluffo 2010; Fernandez et al., 2003) and *B. napus* (Zhao et al., 2007) infected with *S. sclerotiorum*. From 43 previously selected genes, 30 candidate genes were amplified in 10 sunflower inbred lines selected as Core Set (CS) for polymorphism discovery, and 16 candidate genes were finally genotyped in the association mapping population. Associations among SHR incidence and haplotype polymorphisms in those 16 candidate genes were tested using Mixed Linear Models (MLM) that account for population structure and kinship

relationships. A significant association between the candidate gene HaRIC_B and SHR incidence was found, accounting for a SHR incidence reduction of about 20 % (Fusari et al., 2012).

A study combining association and linkage mapping was carried out by Cadic et al., (2013) to identify QTL and causative mutations involved in the control of flowering time in cultivated sunflower. Flowering time is a major event in the plant life cycle that is controlled by both genetic and environmental stimuli. A panel of 384 inbred lines and a recombinant inbred line (RIL) population comprising 273 lines were phenotyped, and genotyped with a set of 5,923 SNPs markers. A large similarity between association peaks detected in both the QTL mapped in a RIL population and the linkage disequilibrium mapping approach was shown (Cadic et al., 2013).

In a recent study carried out by Mandel et al (2013), a total of 271 lines capturing nearly 90% of the allelic diversity present within the cultivated sunflower gene pool was genotyped on an Illumina Infinium 10 k SNP array (Bachlava et al., 2012). These data along with phenotypic data gathered from multi-environmental trials, were used to characterize overall patterns of genomic diversity and to characterize associations underlying agronomic and evolutionary important traits (Mandel et al., 2013). Briefly, the number of days to flowering (DTF, calculated from the planting date) and the total number of branches per plant were measured in the field at the r-5 and R-9 reproductive stage of the Schneider & Miller scale, respectively. Four plants per accession were scored for replicates at three locations. Three different association mapping models were run for each trait including a mixed linear model (MLM) accounting for kinship, and two MLMs using kinship and population structure as estimated via either principle component analysis (PCA) or the program STRUCTURE (Pritchard et al., 2000). Significant positive correlations were found for both branching and flowering time across locations, whereas there was an overall significant negative correlation between branching and DTF. The authors described substantial variation in levels of linkage disequilibrium (LD) across the genome, with islands of elevated LD generally corresponding to genomic regions underlying traits that have been targeted by selection, and large region where LD decays relatively rapidly across the genome.

Genomic selection (GS) strategy was implemented for the first time in sunflower breeding research as a method to predict the performance of untested hybrids based phenotypic and genotypic data (Reif et al., 2013). The work was based on the study of 15 female and 8 male elite sunflower lines adapted to Central Europe, genotyped with 572 AFLP markers (Reif et al., 2013). Experimental data from sunflower hybrids and their parental lines were used to examine the accuracy to predict performance for grain yield, oil yield and oil content, based on phenotypic and genomic data. Among the different biometrical approaches to implement GS, in this study ridge regression-best linear unbiased prediction (RR-BLUP; (Whittaker et al., 1999; Meuwissen et al., 2001) was the method of choice, because it appeared to be well suited for regular plant breeding trials (Albrecht et al., 2011). Prediction accuracy of hybrid performance based on general combining ability effects (GCA) was high. In those cases where no information on GCA effects of parental lines was available, hybrid prediction based on GS methods was accurate for groups of related lines. For unrelated lines, a strong decrease in the prediction accuracy was observed, suggesting that prediction of hybrid performance for crosses based on genetically distant parents is still a challenge.

5. PHYSICAL MAPPING

5.1. Molecular Cytogenetic Mapping

As described above, as a consequence of the increasing availability of various molecular markers and high-throughput genotyping assays sunflower linkage maps are becoming more saturated. In spite of that advance, the association between linkage groups (LGs) and chromosomes has not been established yet. Since the 70s, sunflower cytogenetic studies were focused on karyotypic analyses using conventional techniques as Feulgen's staining and C-banding (Raicu et al., 1976; Al-Allaf and Godward 1979; Kulshreshtha and Gupta 1981; Cuellar et al., 1996; Feng et al., 2013). In recent years, development of molecular cytogenetic techniques such as genomic *in situ* hybridization (GISH), fluorescence *in situ* hybridization (FISH) and primed *in situ* labeling (PRINS) provided key tools for association of physical and genetic maps, physical mapping of interesting genomic regions, as well as diversity and evolutionary studies between species of the genus *Helianthus* (Rocco 2002; Rocco et al., 2003). The GISH technique using genomic DNA as probes on cytological preparations has been successfully used in different plant species to evaluate introgression of wild species in cultivated crops (Benabdelmouna et al., 2003; Shigyo et al., 2003; Wei et al., 2003) as well as in evolutionary studies using wild polyploids (Bennett 1995; Poggio et al., 1999; Paniego et al., 2007); FISH has been the method of choice for mapping repetitive sequences, such as rRNA gene, and thus, enabling the characterization of the chromosome complement and the karyotypic analysis in different species (Talia et al., 2011; Minelli, Maggini, and Gelati 2000; Ceccarelli et al., 2007; Cuellar et al., 1996; De Paula et al., 2005); PRINS technique (Koch et al., 1989) represents an alternative to the use of FISH for localization of DNA sequences in plant chromosomes. This strategy was found to be particularly useful for detection of high-copy tandem repeats. However, for the detection of low-copy repeats, a more sensitive variant of PRINS named cycling PRINS (C-PRINS), which involves serial thermal cycles analogous to polymerase chain reaction (Gosden et al., 1991; Kubaláková et al., 2001), has been recommended (Talia et al., 2011). The combination of bacterial artificial chromosome - fluorescence *in situ* hybridization (BAC-FISH) and C-PRINS techniques were evaluated for their use in the integration of physical and genetic maps of sunflower (Talia et al., 2011).

Classical cytogenetic studies have been important for the analysis of interspecific hybridization in the genus. Comparative genetic linkage maps and co-linearity are powerful tools for the study of karyotypic evolution. The evolution of genome structure between two annual sunflower species, *H. annuus* and *H. petiolaris* was examined using a SSR/RAPD genetic linkage map of the *H. petiolaris* genome and an integrated SSR genetic linkage map derived from four independent *H. annuus* mapping populations (Burke et al., 2004). The results of this work indicate the presence of 11 rearrangements (8 translocation and 3 inversions). In spite of that fact, these two species hybridize extensively throughout their shared range, have high rates of complex backcross production (Rieseberg et al., 1999), and have long-term rates of gene flow (Strasburg and Rieseberg 2008; Strasburg et al., 2009). Patterns of genetic diversity and divergence in rearranged versus co-linear regions in *H. annuus and H. petiolaris* were examined using sequence data from 77 loci distributed throughout the genomes of the two species, finding weak evidence for increased genetic divergence near chromosomal break points but not within rearranged regions overall. No

evidence for increased rates of adaptive divergence on rearranged chromosomes was found; suggesting a limited role for chromosomal rearrangements in genetic divergence (Strasburg et al., 2009).

Transposable elements are the major drivers of genome size increases in plants. Thus, understanding the diversity and evolutionary dynamics of transposable elements in sunflower is of considerable importance for understanding the evolutionary history of this species. FISH studies using repetitive sequences derived from retroelements from *H. annuus* allowed the detection of sequences of two families of retroelements dispersed along the length of all chromosomes in all species studied. However, the Ty3 /gypsy-like sequences were localized preferentially at the centromeric regions in most of the studied species, whereas Ty1/ copia-like sequences were less represented or absent around the centromeres and plentiful at the chromosome ends only in *H. annuus* (Santini et al., 2002; Natali et al., 2006; Paniego et al., 2007), suggesting that these two sequence families played a role in *Helianthus* genome evolution and species divergence, evolved independently in the same genomic backgrounds and in annual or perennial species, and acquired different possible functions in the host genomes. The analysis of approximately 25% of the sunflower genome from random sequence reads and assembled bacterial artificial chromosome (BAC) clones, shows that it is composed of over 81% transposable elements, 77% of which are long terminal repeat (LTR) retrotransposons, predominantly chromodomain-containing Gypsy LTR retrotransposons (related to chromoviruses). It was shown that the vast majority of observed LTR retrotransposon insertions have likely occurred since the origin of this species, providing evidence that LTR retrotransposon activity has played a major role in shaping the chromatin and DNA landscape of the sunflower genome (Staton et al., 2012).

The first GISH procedure applied in sunflower was developed for identifying chromosomes or chromosome segments of wild species in the background of cultivated sunflower, by examining interspecific hybrids involving four wild perennial species, *H. californicus, H. angustifolius, H. nuttallii* and *H. maximiliani*. With different blocking/probe ratios and washing stringencies, chromosomes or segments of the four wild species were clearly identified, demonstrating that the procedure is a practical tool for introgression studies in the genus *Helianthus* (Liu et al., 2009).

The use of bacterial artificial chromosomes (BACs) containing large genomic DNA inserts in physical mapping by FISH technology enabled studies of genome diversity, evolutionary pathways as well as chromosomal location of specific genes or genes families in different species (Nagaki et al., 2003; Wei et al., 2003; Ji et al., 2004; Zhang et al., 2004; Paniego et al., 2007)**.** Recently, a bacterial artificial chromosome (BAC) clone containing repetitive sequences with similarity to retrotransposons and a homologous rDNA sequence isolated from the sunflower genome were used for characterize the complete chromosome complement of sunflower. The BAC clone containing highly represented retroelements hybridized with all the chromosome complement in FISH, and used together with the rDNA probe allowed the discrimination of all chromosome pairs of sunflower. The karyotype could be subdivided into 3 clear-cut groups of 12 metacentric pairs, 1 submetacentric pair, and 4 subtelocentric pairs (Talia et al., 2010).

In 2013, Feng et al., tried to assign sunflower linkage groups to individual chromosomes, and integrate the genetic map with the cytogenetic map, by using a previously developed RFLP linkage map with 232 cDNA probes on 20 linkage groups (some small linkage groups were not integrated with the others (Jan et al., 1998) and two BAC and binary bacterial

artificial chromosome (BIBAC) libraries from the cultivated cv. HA89 (Feng et al., 2006). A set of linkage group-specific BAC/BIBAC clones (~100 kb in size) was identified from the libraries using the mapped cDNA derived RFLP markers, and were used as probes to in situ hybridize to HA89 mitotic chromosomes at metaphase using the BAC-FISH technique. All sunflower chromosomes were anchored by one or two BAC/BIBAC clones with specific FISH signals (Feng et al., 2013). As chromosome-specific cytogenetic markers, the selected BAC/BIBAC clones that encompass the 17 linkage groups will be valuable cytogenetic markers for molecular cytogenetic and genomic research in sunflower, helping to improve our knowledge regarding localization of agronomically interesting characters.

6. WHOLE GENOME SEQUENCING

Despite the economic importance of various species of the *Asteraceae*, there is no reference genome sequence available for the family, which impedes research and improvement efforts. Because the sunflower genome is really large (3.5 billion bases long (Baack et al., 2005), and has many repetitive sequences, for several years it has been considered costly and impractical to sequence. However, the advances in DNA sequencing technologies are making it more practical and much less expensive to sequence and assemble (Mardis 2008; Schatz et al., 2010; Kane et al., 2011).

Kane et al (2011) reported a strategy to sequence the whole sunflower genome, combining whole-genome shotgun sequencing using the Solexa and 454 platforms with the generation of high-density genetic and physical maps that serve as scaffolds for the linear assembly of whole-genome shotgun sequences. This hybrid approach combined the benefits of the two technologies: Solexa brings great depth, but cannot bridge regions of low complexity, whereas 454 reads can span longer repeats, but their higher cost makes deep coverage expensive (Dalloul et al., 2010; Schatz et al., 2010). The performance of this approach was enhanced by the construction of a sequence-based physical map that provides unique sequence-based markers every 5–6 kb across the genome.

Preliminary analyses indicated that almost 80% of the sunflower genome consists of repetitive sequences, thus making difficult the ensemble of the reads. However, 76% of contigs longer than 5 kb could be assigned to either the physical or genetic map or to both, suggesting that the approach is likely to deliver a highly accurate and contiguous reference genome for sunflower (Kane et al., 2011).

CONCLUSION

Sunflower is one of the most important oilseed crops, with an annual production estimated at 32 million metric tons. However, yield is often below the maximum potential due to economics, management and many biological factors. Moreover, over the last decade cultivation has been shifting to marginal areas (with lower-fertility soils, drought and drastic temperature changes), so the genetic progress for oil yield may have been masked by the decline in agronomic quality of the growing region. The rapid changes that occur in pathogen

virulence, their high persistence in soil and their distribution all around the sunflower growing areas are also contributing to yield losses.

The challenge now is to breed sunflowers adaptable to these marginal environments, improve resistance to pathogens and, at the same time, increase seed and oil yield.

Significant advances have been made in this area, including the identification of useful germplasm for many agronomic traits, the discovery of a large number of loci affecting yield under stress conditions and several genes and QTL implicated in disease resistance. Furthermore, more integrated and saturated linkage and physical maps are becoming available. Marker-assisted selection could improve selection methods, but to date, the impact on variety development has been minimal, due principally to insufficient linkage between marker and gene/ QTL locus, limited number of markers and polymorphisms among breeding materials, the interaction between QTL and environmental effects and the high cost of marker-assisted selection. The emergence of high-throughput genotyping technologies based on DNA-chips and ultimately on new generation sequencing technologies, along with modern breeding techniques based on population and quantitative genetics, combined with the possibility of obtaining the complete sunflower sequenced genome, should allow MAS to become more widely applicable for sunflower breeding programs. The further step will be to combine the best conventional and modern molecular approaches to improve sunflower germplasm, in order to maintain it as an economically important global crop.

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