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Spinach: better management of downy mildew and white rust through genomics

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Abstract Spinach (*Spinacia oleracea*) has become an increasingly important vegetable crop in many parts of the world. Significant changes in production practices, particularly in the U.S. and E.U., have occurred in the past 10–15 years as a result of increased product demand. These changes likely increased the incidence and severity of downy mildew, caused by *Peronospora farinosa* f. sp. *spinaciae*. Recently, progress has been made to define the genetics of resistance to this pathogen and the closely related white rust pathogen, *Albugo occidentalis*. In this paper, we outline the genetic and genomic resources currently available for spinach, draw parallels between spinach diseases and more thoroughly characterized pathosystems, and describe efforts currently underway to develop new genetic and genomic tools to better understand downy mildew and white rust of spinach. Presently, many crucial tools and resources required to define the molecular underpinnings of disease are unavailable for either spinach or its pathogens. New resources and information for spinach genomics would provide a jumpstart for ongoing efforts to define (and deploy) genetic resistance against downy mildew and white rust.

Keywords Disease resistance · Genomics · *Spinacia oleracea*

Abbreviations

Pfs	*Peronospora farinosa* f. sp. *spinaciae*
Ao	*Albugo occidentalis*
RPF	Resistance to *Peronospora farinosa*
NIL	Near Isogenic Line
MAS	Marker Assisted Selection

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Spinach production

Spinach (*Spinacia oleracea* L.) is an economically important vegetable crop in many parts of the world. FAO statistics indicate that 20 countries produced approximately 14.5 million MT of spinach in 2008 (http://faostat.fao.org/site/339/default.aspx). Based on FAO statistics, the top ten spinach producing countries are China (12.5 million MT), the U.S. (0.353 million MT), Japan (0.293 million MT), Turkey (0.226 million MT), Indonesia (0.152 million MT), France (0.124 million MT), Italy (0.100 million MT), Korea (0.093 million MT), Belgium (0.090 million MT), and Pakistan (0.082 million MT). Globally, approximately 1 million ha of spinach are grown in Asia and approximately 35,000 ha in each of the E.U., the U.S., Japan, and Turkey (Jan de Visser, *personal communication*). In the U.S., spinach is grown on approximately 35,000 ha, with annual production of approximately 350,000 MT tons valued at over $200 million (USDA-NASS 2008). The U.S. spinach industry has undergone dramatic changes in recent years. Demand for spinach in convenient, pre-cleaned, and pre-packaged units has increased significantly throughout the past decade, thus driving substantial increases in production (Lucier and Plummer 2004; USDA-NASS 2008) (Fig. 1). In the U.S., fresh market spinach production increased over 200% from 1987 to 2007 (USDA-NASS 2008). Per capita consumption of fresh market spinach increased from 0.3 kg/person in 1995 to approximately 1.0 kg/person in 2005 (Irish et al. 2007; USDA-NASS 2008), representing one of the fastest growing rates of per capita consumption among vegetables.

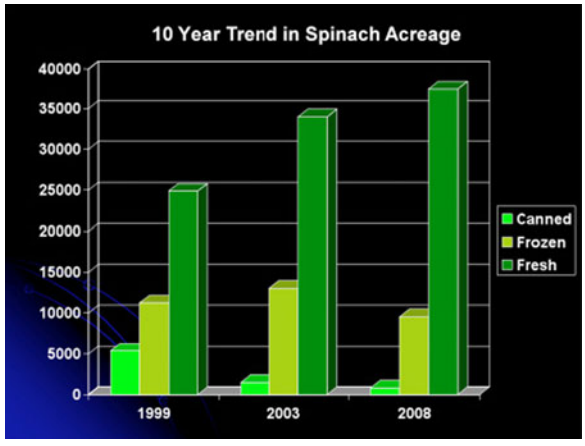

Fig. 1 Spinach production in the U.S. has increased dramatically in the past 10 years

Spinach is a remarkably nutritious vegetable and is increasingly being incorporated into health-conscious diets. Leaves are high in beta carotene and folate, and are also a rich source of vitamin C, calcium, and iron (Ryder 1979; Nonnicke 1989; Dicoteau 2000). Spinach is also an excellent source of antioxidants and has one of the highest ORAC (oxygen radical absorbance capacity) values of any vegetable crop (Prior 2003). In addition, spinach contains high levels of lutein, a carotenoid that prevents age-related macular degeneration (Morelock and Correll 2008).

Seed production

Spinach seed production is limited to areas where environmental conditions include long days to induce flowering and moderate summer temperatures (Metzger and Zeevaart 1985; Morelock and Correll 2008). For U.S. and E.U. spinach production, most seed originates from Denmark (approximately 75% of the world's supply), followed by the Pacific Northwest region of the U.S. (20%) (Daehnfeldt 2007; Foss and Jones 2005). In the Pacific Northwest, approximately 600–1600 ha of spinach seed are grown annually, representing 25%–50% of the U.S. spinach seed supply (Foss and Jones 2005).

Although the genetic basis of sex determination in spinach is complex (Morelock and Correll 2008), spinach is basically dioecious with male and female plants. Hybrid spinach lines were initially developed as a means of combining unique resistances to the downy mildew pathogen (Morelock and Correll 2008). Some open pollinated lines of spinach are still produced, but the vast majority of spinach lines in both Denmark and the U.S. are hybrid cultivars produced by the use of male and female inbred lines each having a different combination of resistances.

Spinach and disease resistance: gaps in our understanding

Several diseases of spinach are economically important, but downy mildew, caused by *Peronospora farinosa* f. sp. *spinaciae* (*Pfs*) (= *P. effusa*) (Brandenberger et al. 1991; Choi et al. 2007), and white rust, caused by

Albugo occidentalis (*Ao*), are of particular concern. Downy mildew is a major production constraint virtually everywhere the crop is grown, whereas white rust is limited to the U.S. (Correll et al. 1994; Morelock and Correll 2008). Although spinach consumption and production are rapidly increasing, spinach has been regarded historically as a minor crop in the U.S and other regions of the world, and the genomic resources available for spinach are limited relative to many other crops. Perhaps not surprisingly, the genetic and molecular basis of resistance to downy mildew and white rust are not well understood in spinach. However, stable genetic resistance to downy mildew and white rust is the lynchpin of effective disease control due to economic and regulatory concerns associated with conventional management strategies (i.e., frequent application of fungicides). To date, durable genetic resistance to either disease has proven to be elusive. Race-specific resistance to *Pfs* has been identified among spinach cultivars and wild relatives, but the underlying genes and signalling pathways are largely undefined. Intriguingly, both pathogens have demonstrated an ability to overcome diverse sources of host resistance, perhaps indicative of diversifying selection and/or intensive reciprocal co-evolution, but the molecular basis for the creation or ascendance of new strains remains a mystery. Despite the lack of information about resistance and virulence at the molecular level, considerable insight can be drawn from the interaction of model species such as *Arabidopsis* with pathogens closely related to *Pfs* and *Ao*. We predict that the steady increase of molecular resources for spinach, *Pfs*, and *Ao* will soon allow long-standing questions about disease interactions to be answered through genomics-based approaches, which in turn will lead to more sustainable management strategies.

In this paper, we outline the genetic and genomic resources currently available for spinach, draw parallels between spinach diseases and more thoroughly characterized pathosystems, and describe efforts currently underway to develop new genetic and genomic tools to better understand downy mildew and white rust of spinach.

Downy mildew and genetics of resistance

Downy mildew, caused by *Peronospora farinosa* f. sp. *spinaciae* (*Pfs*), is perhaps the most significant biotic constraint for spinach production worldwide (Fig. 2) (Correll et al. 1994). Downy mildew occurs everywhere spinach is grown, including areas used exclusively for seed production, and improving resistance is a priority for all breeding programs (Morelock and Correll 2008; Correll et al. 1994). Although the pathogen was first reported in the early 1800's, only three races of the pathogen had been identified before 1990 (Correll et al. 1994). Alarmingly, ten new races of the pathogen were identified between 1990 and 2010, (Fig. 3), and some of the newer races had overcome all known genetic resistance (Irish et al. 2003; 2007). The rapid ascendance of new races is likely a direct result of substantial changes in spinach production during the past decade, including a rapid expansion of planted acreage, 12-month production cycles, high density plantings, reduced usage of rotation crops, and the global movement of the pathogen in seed either as mycelium or as dormant oospores (Inaba and Morinaka 1984; Inaba et al. 1983).

The ability of the pathogen to overcome host resistance is particularly troublesome, as genetic resistance has long been the primary management tool for downy mildew (Morelock and Correll 2008; Correll et al. 1994). Although improving resistance to downy mildew is a critical breeding objective (Morelock and Correll 2008), relatively few studies have been conducted on the genetic control of resistance to the downy mildew pathogen (Irish et al. 2008; Morelock and Correll 2008; Correll et al. 2007).

The emergence of new races of the downy mildew pathogen despite the deployment of new sources of

Fig. 2 Downy mildew on spinach in a controlled greenhouse test

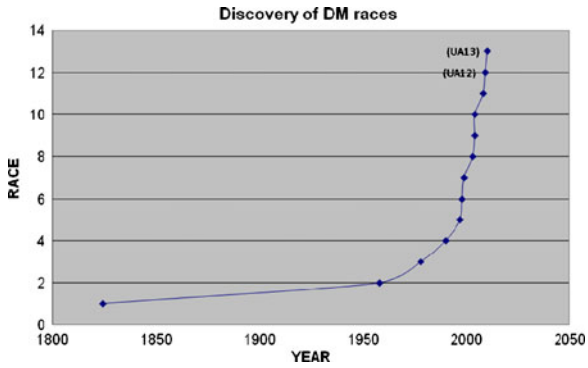

Fig. 3 The observation and documentation of new spinach downy mildew races by year. Note the rapid increase in novel races in the past 10–15 years

resistance is a fascinating case study in diversifying selection. Race 1 was first reported in 1824 (Greville 1824), and over 100 years later, resistance to race 1 was identified in two accessions (PI 140467 and PI 140467) from Iran (Smith 1950). Both sources of resistance manifested as immunity to infection, and the resistance from PI 140467 was conveyed by a single dominant gene (Smith 1950). This source of immunity was incorporated into hybrids 'Califlay' and 'Dixie market,' as well as the parents of 'Early Hybrid 7' and 'Early Hybrid 424' (Ryder 1979). Shortly after race 2 was first described in 1958, resistance was discovered that appeared to be a single dominant gene imparting immunity to race 2 as well as race 1 (Smith et al. 1961; 1962). Later, this resistance was demonstrated to be two closely linked genes rather than a single dominant gene (Eenink 1974, 1976a,b). After race 3 was identified in 1976, resistance was incorporated into hybrids rather quickly. Hybrids 'Mazurka,' 'Polka,' and 'Rhythm' were introduced in 1978, and additional hybrids resistant to race 3 were introduced in subsequent years (Morelock 1999). After race 4 was identified in 1990 (Brandenberger et al. 1991), resistant hybrids 'Bolero' and 'Bossanova' were introduced in 1991 (Morelock 1999). Brandenberger et al. (1992) screened 707 spinach accessions for resistance to race 4 and found nine accessions that were at least partially resistant (9–38% resistance). Accessions CGN09546 (60% of individual plants were resistant) and SP1 82/87 (80% of individual plants were resistant) exhibited the highest levels of resistance to race 4 (Brandenberger et al. 1992). Although inheritance studies were not conducted, seed from the two accessions was increased and distributed to breeders in the private sector. The rapid emergence of races 5, 6, 7, 8, 9, 10, 11, and two additional deviating strains UA12, and UA13 (Correll et al. 1990; 2001; 2003; Correll 1998; Handke et al. 2000; Irish 2004; Irish et al. 2003; 2004; 2007) ignited efforts to broaden host resistance and develop a more thorough understanding of the genetics of resistance to *Pfs*.

Currently, spinach breeding programs rely on pathogenicity tests to identify resistance to downy mildew (Table 1) (Morelock and Correll 2008). However, pathogenicity tests are time consuming, labour intensive, subject to specific environmental conditions, and require the maintenance of reference isolates, which is not trivial for an obligate pathogen. Thus, molecular markers linked to downy mildew resistance loci are urgently needed to provide a more robust means to introgress resistance into advanced spinach breeding material (Michelmore 2003).

More recently, a systematic effort to define downy mildew resistance in spinach led to the identification of a resistance locus (*Pfs1*) and a closely linked molecular marker (Irish et al. 2007; Correll et al. 2007. This locus was identified by developing a near isogenic line (NIL1) using the hybrid Lion as the resistant parent and Viroflay as the susceptible recurrent backcross parent (Irish et al. 2008). The line went through four backcross generations, selection of plants resistant to race 6, and three subsequent selfings, before producing seed for evaluation. The NIL1 line was resistant to multiple races (races 1–7,9,11 and UA13) as anticipated and resistance segregates as a single dominant locus (Irish et al. 2007, Table 2). A second NIL line, NIL2, has resistance to races 1–10. This line is in the advanced stages of development (Correll and Feng *unpublished*).

A total of 6 loci has been hypothesized to control resistance to the 11 known races and 2 additional deviating strains (UA12 and UA13) of the spinach downy mildew pathogen (Correll et al. 2007; Irish et al. 2008) (Table 3). Although the first *P. farinosa* f. sp. *spinaciae* resistance locus described was designated *Pfs-1* (Irish et al. 2008) and other hypothesized loci have been given a "*Pfs*" acronym, this has caused confusion in the spinach community, as the races of the downy mildew pathogen are often designated *Pf1*, or *Pfs1*. Thus, we have adopted the convention used with the Arabidopsis downy mildew pathogen system and have renamed the loci involved in downy mildew

Table 1 Disease reactions of spinach differentials for determining the race identification of isolates of the spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae*

Differential cultivar	Race[1]												
	1	2	3	4	5	6	7	8	9	10	11	UA12	UA13
Viroflay	+	+	+	+	+	+	+	+	+	+	+	+	+
Resistoflay	–	–	+	+	+	+	+	+	+	+	+	+	+
Califlay	–	+	–	+	–	+	+	–	–	+	–	–	+
Clermont	–	–	–	–	+	+	+	+	+	+	+	+	+
Campania	–	–	–	–	–	+	–	+	+	+	–	+	–
Dolphin	–	–	–	–	–	–	–	+	–	+	–	+	–
Avenger	–	–	–	–	–	–	–	+	–	+	–	+	–
Lion	–	–	–	–	–	–	–	–	–	+	–	–	–
Lazio	–	–	–	–	–	–	–	–	–	–	+	+	+
Whale	–	–	–	(–)	–	(–)	(–)	–	–	+	–	+	+

[1] Races of the downy mildew pathogen as of October 2010. UA12=isolate UA2209 identified in 2009, and UA13=UA0510C indentified in 2010 are deviating isolates but not currently recognized as a bonified race by the International Working Group on Peronospora. Adapted from Irish et al. 2008. +=Susceptible reaction; –=Resistant reaction; (–)=reduced level of infection often referred to as field resistance

resistance of spinach as "*RPF*" loci (for genes involved in the **R**esistance against ***P****eronospora* ***f****arinosa*). Thus, the locus first described as *Pfs-1* will henceforth be referred to as the *RPF1* locus. In addition, two alleles were identified at this locus, *Pfs-1* and *pfs-1*, for the dominant resistance allele and the recessive susceptible allele, respectively. Thus, these two alleles have been renamed *RPF1-1* and *RPF1-2,* corresponding to the dominant and recessive alleles, respectively.

Table 2 Reaction of line NIL1 containing the RPF1 locus introgressed from the hybrid Lion into the susceptible cultivar Viroflay background to different races of *Peronospora farinosa* f. sp. *spinaciae*

Race	Reaction
1	–
2	–
3	–
4	–
5	–
6	–
7	–
8	+
9	nt
10	+
11	–
UA12	+
UA13	–

+=a susceptible reaction
–=a resistant reaction
nt=not tested
UA12 and UA13 are reaction types (see Table 1) caused by strains UA2209 and UA0510C

Although 6 genetically distinct loci have been hypothesized to control resistance to *Pfs*, considerable genetic analysis is needed to verify this hypothesis. In fact, an alternate hypothesis is that there is a single locus with multiple alleles that govern resistance in spinach to *Pfs*. Before naming additional resistance loci, experimental validation through genetic mapping is required.

There is a need to identify additional novel sources of resistance to *Pfs*. However, screening needs to be complemented with studies of the genetics of resistance as one cannot conclude which locus governs resistance in a particular accession to a given race as a locus can control resistance to multiple races (Irish et al. 2008). For example, the *RPF1* locus controls resistance to races 1–7, 9, 11, and UA13 (Irish et al. 2008; Correll et al. *unpublished*) whereas *RPF2* controls resistance to races 1–10 of *Pfs* (Correll et al. *unpublished*).

The Center for Genetic Resources in the Netherlands (CGN) (http://www.cgn.wur.nl/UK/CGN+Plant+Genetic+Resources/Collections/) and the Germplasm Resources Information Network in the U.S. (GRIN) (http://www.ars-grin.gov/) house collections of spinach germplasm, some of which have been screened for responses to the various races of *Pfs*. A consortium of E.U. companies has embarked on an effort through the CGN to collect and screen spinach germplasm for

Table 3 The 6 hypothesized downy mildew pathogen resistance (*RPF*) loci in spinach

	Resistance locus						
	Viroflay	*RPF5*	*RPF4*	*RPF3*	*RPF1*	*RPF2*	*RPF6*
Resistant to races	None	1,2	1-4	1,3,5,8, 9,11,UA12	1-7, 9,11, UA13	1-10	1-5, 7,11, UA13
1		■	■	■	■	■	■
2		■	■		■	■	■
3			■	■	■	■	■
4			■		■	■	■
5				■	■	■	■
6					■	■	
7					■	■	■
8				■		■	
9				■	■	■	
10						■	
11				■	■		■
UA0209D					■	■	■
UA12 (UA2209)				■			
UA13 (UA0510C)					■		■
Super Race?							

Designation	Expected Disease Reaction
	Susceptible
■	Resistant

resistance to various races of *Pfs*, but these data likely will be embargoed until 2015 (Jan deVisser, *personal communication*). The GRIN collection also has acquired new wild spinach germplasm including several collections of *S. tetrandra* and *S. turkistanica.* These lines are particularly difficult to maintain as significant seed dormancy issues must be overcome (Brenner 2009). Lines of several genera related to spinach, namely *Monolepsis, Micromonolepsis,* and *Suckleya* are also in the process of being acquired and should be evaluated for resistance to *Pfs*.

Population diversity of the downy mildew pathogen

Despite the rapid emergence of new races in recent years, little is known about race specificity or genetic diversity in *P. farinosa* f. sp. *spinaciae*. Genomic studies on downy mildew pathogens in general, and *Pfs* in particular, have lagged behind other oomycete pathogens such as *Phytophthora infestans, P. sojae, P. ramorum*, and *P. capsici,* in part due to the inherent difficulties associated with studying obligate plant pathogens. *Pfs* is likely heterothallic and thus requires opposite mating types to produce dormant resting spores (Inaba and Morinaka 1984). Studies with *Pfs* isolates from the U.S. indicate that, although perhaps rare, both mating types may occur as oospores have been observed in inoculation tests with bulk field isolates (Correll et al. *unpublished*). In addition, preliminary analyses of isolates collected from different geographical locations representing diverse races indicated that considerable genetic diversity is present in U. S. populations (Correll et al. *unpublished*). However, a thorough understanding of pathogen diversity is lacking and will ultimately be required to manage downy mildew.

There are two hypotheses regarding the origin of new races of the downy mildew pathogen. One is predicated on the premise that "new" races are already present in natural populations of the pathogen and are ascending due to intense selection via the deployment of certain resistance specificities in commercial spinach production. When the "new" race emerges, it is then moved throughout the world on seed as mycelium, viable sporangia, and/or dormant oospores on seed. Strong circumstantial evidence supports this hypothesis in that one new race was found almost simultaneously in the U.S. and E.U. in 2008. Additional support for this hypothesis is that resistance loci identified decades ago confer resistance

against "new" races. For example, the *RPF3* locus, originally identified in Califlay, confers resistance to races 11 and UA12 (Table 3). However, a second possibility is that new isolates are emerging from endemic populations due to rapid, adaptive evolution of the pathogen. Both hypotheses could be tested by examining the molecular diversity of *Pfs* isolates, conducting surveys for the presence of *Pfs* on commercial seed, and identifying genes encoding effectors/avirulence gene products that are recognized by specific spinach resistance loci.

If new races of *Pfs* continue to emerge at the rate witnessed during the past decade, all currently known sources of genetic resistance could be overcome. This stark possibility serves as an impetus for a more thorough understanding of pathogenesis in *Pfs*, especially a molecular-level understanding of what constitutes distinct races of the pathogen. Today, in the era of next-generation DNA sequencing, the question of how new races emerge so rapidly can finally be answered through comparative genomics. Although very little genomic sequence information is currently available for any isolate or race of *Pfs*, the genome of the closely related species *Hyaloperonospora parasitica* (which causes downy mildew of *Arabidopsis thaliana*) has recently been sequenced (http://genome.wustl.edu). This provides a crucial resource for the sequencing of the *Pfs* genome. Once a reference genome sequence of *Pfs* is created, assembled, and annotated, representative isolates from other races can be sequenced, and comparative analyses can highlight likely sources of race-specificity. With this information in hand, race-specific detection with molecular techniques should be possible, and if the number of loci underlying race specificity is relatively limited, it may be possible to screen pathogen populations to predict the emergence of new races years before they reach ascendance and hinder commercial production.

White rust and the genetics of resistance

White rust of spinach, caused by the oomycete pathogen *Albugo occidentalis*, is an economically important disease in the U.S. The white rust pathogen was originally reported on beetberry (*Blitum capitatum*) in Colorado in 1907 (Wilson 1907) and on spinach in Virginia in 1910 (Walker 1952), but took on commercial significance when it was found at several New York vegetable markets on spinach that originated from Texas (Wiant 1937). Although *A. occidentalis* has not been reported outside the United States, white rust remains an economically important disease in Texas, Arkansas, Colorado, and East Coast production areas (Correll et al. 1994; Morelock and Correll 2008). Goode was the first to demonstrate field resistance to white rust in spinach (Goode et al. 1988), and Brandenberger et al. (1994) demonstrated parallels between quantitative resistance to both the white rust and downy mildew pathogens. Resistance to *A. occidentalis* is presumed to be multigenic and quantitative, but has not been studied in a systematic manner. A USDA collection of spinach germplasm was screened for resistance to *A. occidentalis* and, although quantitative differences in resistance were observed, no lines were identified that had complete resistance to the white rust pathogen (Correll *unpublished*).

Even though white rust and downy mildew are both caused by obligate parasites, breeding approaches to improve resistance are quite different. Unlike downy mildew resistance, spinach does not have single gene immunity to white rust; resistance is polygenic (quantitative) although the exact number of genes involved is unknown (Goode et al. 1988; Morelock and Correll 2001, 2003, 2005). The first spinach cultivars with at least some degree of white rust resistance were Crystal, Jewel, and Wintergarden, which were released by the USDA in 1973 (Morelock 1999) but were not widely grown. The partially resistant cultivars Ozarka and Greenvalley were released in 1980 (Bowers and Goode 1980a,b; Morelock 1999). In 1987, the first cultivar with high levels of white rust resistance, Fallgreen, was released (Goode et al. 1988; Morelock 1999). Interestingly, some cultivars that are resistant to white rust also have partial resistance, or "field" resistance, to some races of *Pfs* (Brandenberger et al. 1992; 1994). The genetic or molecular basis of this resistance is not known, but could be exploited to improve overall resistance to *Pfs* by complementing major genes for resistance.

Spinach genetics and genomic resources. What is available and what is needed?

Due to the historical size of spinach production and its prior status as a minor crop, few genomics

resources have been developed to improve important horticultural traits. Spinach is a diploid with $2n=12$ chromosomes (Ellis and Janick 1960). The chromosomes of spinach have been identified based on their morphological characteristics, i.e., total length, arm ratio, and presence of satellites (Ellis and Janick 1960). The inheritance of common traits in spinach, such as spiny vs. smooth seed, smooth vs. savoy leaves, and short vs. long petiole, are controlled by single genes (Sneep 1958). Spinach is dioecious, with separate male and female plants, although occasionally monoecious plants can be found that contain both male and female flowers (Morelock and Correll 2008). No sex chromosome has been found in spinach; sex determination is likely controlled by either a sex determination locus or chromosomal segments sharing a mechanism similar to X and Y determination in animals (Bemis and Wilson 1953; Janick and Stevenson 1954).

The genomes of spinach organelles have been sequenced and characterized. Spinach has a tripartite mitochondrial genome that is 327 kb in size (Stern and Palmer 1986). The spinach chloroplast genome has been completely sequenced and is a circular molecule of 150 kb (Schmitz-Linneweber et al. 2001). Sequence homologs have been found among chloroplast, mitcochondrial, and nuclear genomes (Timmis and Scott 1983; Whisson and Scott 1985).

Ito et al. (2000) applied fluorescence in situ hybridization (FISH) and computer-aided karyotyping to identify and characterize spinach chromosomes. Three loci of 45S rDNA were found at the nucleolar organizing region (NOR) of Chromosome 5, and at terminal positions of the short arms of Chromosome 2 and 6. Also, three loci of 5S rDNA were found, one located at the subtelomeric region of the long arm of Chromosome 2, and the other two located at the proximal region of the long arm of Chromosome 5. This was the first physical mapping of genes using chromosome condensation patterns (CP) and FISH in spinach.

A high-density genetic map is essential for mapping new genes or markers, map-based gene cloning, and marker-assisted selection (MAS). Thousands of markers, either morphological or molecular, have been used to construct high-density genetic maps in major crops, such as maize, rice, and wheat. However, the genetic map of spinach is currently far from saturated. Groben and Wricke (1998) developed 13 SSR markers based on the spinach sequence information in Genbank and EMBL databases. Khattak et al. (2006) constructed a genetic map of spinach with 101 amplified fragment length polymorphism (AFLP) markers and nine single sequence repeat (SSR) markers. Seven linkage groups were established, with a total length of 585 cM, and an average distance between the markers of 5.18 cM. The sex determination gene is closely linked to SSR marker SO4 with a distance of 1.9 cM. The lack of a high-density genetic map of spinach results from shortages of (1) appropriate mapping populations, and (2) suitable molecular markers. A good mapping population requires high levels of variation between parents, and the population should be able to be saved for future use or used by other researchers. Furthermore, the markers used for mapping should be reproducible and reliable. Although AFLP markers are a type of PCR-based marker, their development is time consuming and laborious. SSR markers are a better choice for mapping but the current pool of available SSRs is far from sufficient to construct more than a rudimentary genetic map. Spinach sequences deposited into public databases are limited, and many of them correspond to physiological genes that are relatively conserved compared to non-coding regions.

The genome size of spinach has been estimated to be 989 Mb (Arumuganathan and Earle 1991). A spinach bacterial artificial chromosome (BAC) library was recently built (Correll et al. 2009). This library can be used to create a physical map of the spinach genome, to fine map genes of interest, and for map-based gene cloning. We have developed approximately 100 SSRs out of 2000 BAC end sequences (BES) (Feng et al. 2010). A preliminary test of these SSRs showed higher levels of polymorphism among commercial spinach cultivars or hybrids compared to SSRs developed from coding regions.

Although none of the six hypothesized loci underlying resistance to downy mildew (Correll et al. 2007) has been cloned or characterized in spinach, the molecular basis of resistance to downy mildew in Arabidopsis (caused by *Hyaloperonospora arabidopsidis*; formerly *Peronospora parasitica* syn. *Hyaloperonospora parasitica*) is increasingly well understood. To date, 28 distinct resistance genes (designated *recognition of Peronospora parasitica*; *RPP*) have been identified in Arabidopsis (Slusarenko

and Schlaich 2003), and several have been cloned and characterized (e.g., *RPP1*, *RPP5*, and *RPP13*; Botella et al. 1998; Bittner-Eddy et al. 2000; Parker et al. 1997). To date, all cloned *RPP* genes are members of the plant disease resistance (*R*) gene family, typified by encoding proteins with an extracellular nucleotide binding site (NBS) fused to a leucine-rich repeat (LRR) with an N-terminal sharing similarity with a Drosophila Toll/mammalian interleukin 1 receptor (TIR) or a coiled-coil domain (CC). Intriguingly, many *RPP* genes are clustered in complex loci known as major recognition gene complexes (MRCs; Holub 1997) and are hypothesized to have originated primarily via duplications and rearrangements (Baumgarten et al. 2003; Meyers et al. 2003). For example, the *RPP5* locus contains seven TIR-NBS-LRR class *R* genes, three related sequences, and two non-*R* genes (Noël et al. 1999). In an evolutionary context, the dynamic nature of MRCs provides Arabidopsis an invaluable resource for rapid co-evolution when threatened by a genetically nimble pathogen. The conveyance of multiple race specificities by *RPF1* in spinach is suggestive of a complex locus analogous to the *RPP* MRCs of Arabidopsis, and such a complex locus for *RPF1* (formerly *Pfs1*) has been hypothesized (Irish et al. 2007).

R-gene mediated resistance to *H. arabidopsidis* is initiated when the product of a given *R* gene recognizes a distinct effector or pathogen-associated molecular pattern (PAMP), which triggers a classic hypersensitive response (HR) of programmed cell death localized at the site of infection. The identification of effector genes from *H. arabidopsidis* that are specifically recognized by characterized *RPP* genes provides unique insight into the Arabidopsis downy mildew pathosystem. For example, the *Arabidopsis thaliana recognized 13* (*ATR13*) effector is recognized by a CC-NBS-LRR class *R* gene encoded by *RPP13* (Allen et al. 2004; Bittner-Eddy et al. 2000). In an additional layer of complexity, *ATR13* also induces resistance against bacterial and viral pathogens (Rentel et al. 2008), which suggests a considerable level of cross talk is interwoven into Arabidopsis downy mildew defense responses. Both *ATR13* and *RPP13* are highly polymorphic in *H. arabidopsidis* and Arabidopsis, respectively, providing evidence of diversifying selection as would be predicted to arise from an ongoing, co-evolutionary battle of pathogenesis (Allen et al. 2004). Whether or not a similar co-evolutionary mechanism at least partially explains the rapid emergence of new races of *Pfs* is a fascinating, yet unanswered, question.

Certain components of *R*-gene mediated signaling pathways are broadly conserved across the plant kingdom. For example, *NPR1* is a central regulator of many, but not all, *R*-gene mediated defenses in Arabidopsis, and represents a point of convergence for pathogen-specific defences and salicylic acid (SA)-dependent systemic acquired resistance (SAR) (Dong 2004). Among characterized *RPP* genes of Arabidopsis, however, downstream signaling pathways appear to be remarkably divergent. For example, *RPP4*-mediated defence requires a suite of well-described signaling components, including *NPR1*, *SID1*, *SID2*, *EDS1*, *PAD4*, and SA (Delaney et al. 1994; Aarts et al. 1998; McDowell et al. 2000; Bittner-Eddy and Beynon 2001; van der Biezen et al. 2002). At the other extreme, *RPP13-ND* functions independently of most signalling components that typify *R*-gene mediated defenses (Bittner-Eddy and Beynon 2001). Where *RPF1* falls in the spectrum of reliance on known resistance signalling intermediaries is currently unknown.

Other factors in addition to *R*-gene mediated resistance play important roles in the suppression of downy mildew in Arabidopsis. For example, resistance to *H. arabidopsidis* is developmentally regulated in ecotype Columbia-0, in that seedlings are highly susceptible, whereas adult plants display substantially greater resistance (McDowell et al. 2005). Intriguingly, downy mildew-resistant (*dmr*) mutants were created by ethyl methane sulphonate mutagenesis in the highly susceptible Landsberg erecta eds1-2 mutant (Parker et al. 1996; Van Damme et al. 2005), providing insight into loci required for susceptibility. Similar approaches could be employed to define the molecular underpinning of resistance conveyed by the *RPF1* locus in spinach.

Genomics-based approaches to elucidate spinach disease interactions: looking to the future

Presently, many of the crucial tools and resources required to define the molecular underpinnings of disease are unavailable for either spinach or its pathogens. New resources and information for spinach genomics would jumpstart ongoing efforts to

define (and deploy) genetic resistance against downy mildew and white rust. Most immediately pressing is the need for a detailed physical and genetic map of the spinach genome, which will facilitate positional cloning of genes and development of markers linked to economically important traits. The impact of these resources would be immediate and substantial for public and private breeding efforts. For example, spinach breeding programs currently rely heavily on pathogenicity tests to identify resistance to downy mildew (Morelock and Correll 2008). However, pathogenicity tests are laborious and variable, even in experienced hands. Marker-assisted selection for resistance would circumvent technical difficulties associated with pathogenicity tests (Irish et al. 2007).

After the development of physical and genetic maps, a critical, foundational resource needed for spinach is a sequenced, assembled, and annotated genome. To this end, a spinach BAC library of approximately 75,000 clones was recently constructed from a single plant from line NIL1 containing the *RPF1* downy mildew resistance locus (Correll et al. 2009; Irish et al. 2008; Feng et al. 2010). Given that the genome size of spinach is ~989 Mb, and the library contains 73,728 clones with an average insert size of 183 kb, coverage of the spinach genome should be ~13×. The construction of the BAC library provides a crucial resource for physical mapping, which in turn will facilitate genome sequencing and assembly.

New genomic resources would also significantly augment white rust management, which could indirectly increase downy mildew resistance. The polygenic nature of white rust resistance in spinach necessitates QTL mapping, which will be possible upon the completion of a high-density genetic map. As genes underlying QTL for white rust resistance are identified and characterized, possible interactions between genes conveying resistance to white rust should be explored thoroughly given the known overlap between resistance to white rust and downy mildew (Brandenberger et al. 1992; 1994).

The possibility that *Pfs* is a seed-borne pathogen is cause for considerable concern. However, the impact of seed-borne transmission on the dispersion of races throughout geographically distinct spinach production areas is unknown. Oospores have been demonstrated to be seed-borne and can serve as a primary source of inoculum to initiate seedling infections (Inaba et al. 1983). However, no systematic studies have been conducted to determine the frequency at which oospores contaminate commercial seed produced in either Denmark (where approximately 75% of commercial spinach seed for U.S. and E.U. markets is produced) or the Pacific Northwest (where approximately 15% of commercial spinach seed for U.S. and E.U. markets is produced) (Daehnfeldt 2007; Foss and Jones 2005). Furthermore, no studies other than the initial studies in the early 1980's have examined populations of *Pfs* for the presence of both mating types. To protect spinach production in the immediate future, a rapid, cost-effective, and specific assay to monitor seed transmission would be extremely valuable. The increased availability of sequence information will facilitate the development of real-time PCR assays to detect *Pfs*. Eventually, when race specificity is understood at the molecular level, PCR assays that distinguish races could be developed as a tool for disease management.

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