GENETICS OF RESISTANCE OF BRASSICA RAPA (L.) AND BRASSICA JUNCEA (CZERN & COSS) TO ALBUGO CANDIDA (PERS.) KUNTZE

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by

Venugopal Kalavacharla

87

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in

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GENETICS OF RESISTANCE OF BRASSICA RAPA (L). AND BRASSICA JUNCEA (CZERN & COSS) TO ALBUGO CANDIDA (PERS.) KUNTZE

BY

VENUGOPAL KALAVACHARLA

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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GENERAL ABSTRACT

Kalavacharla, Venugopal. M.Sc., The University of Manitoba, 1996. Genetics of resistance of *Brassica rapa* (L.) and *Brassica juncea* (Czern & Coss) to *Albugo candida* (Pers). Kuntze Major Professor; Dr. S.R. Rimmer

Plants of two *Brassica rapa* cultivars, UM921 and Horizon were screened against *Albugo candida* (Ac) races Ac7a and Ac7v, and plants that were resistant (R) from the UM921 population and susceptible (S) to both races from the Horizon population were selected and crossed pair-wise within each population. Plants from these crosses were retested for reaction to Ac7a and Ac7v and two plants from each family which were R (UM921) and S (Horizon) were sib-mated. Crosses between selected resistant UM921, and susceptible Horizon plants were made. Most F_1 plants were resistant, indicating that resistance to Ac7a and Ac7v was dominant and nuclearly inherited, while some crosses segregated. F_1 plants resistant to both Ac7a and Ac7v, resistant to Ac7a but susceptible to Ac7v, and susceptible to Ac7a but resistant to Ac7v, were sib-mated to each other to produce F_2 generations. F_1 plants were also backcrossed to both parents. All F_2 families were tested for a one-gene or all possible two-gene models and two families fit a dominant-recessive epistasis model. According to this model, resistance and susceptibility is controlled by two genes with complete dominance at both gene pairs. Assuming that these two genes are A and B determining the genetics of resistance and susceptibility. Gene A when dominant (AA or Aa) produces a product which is epistatic to the product produced by

gene B and resistance is conferred to the plant. When the recessive aa is present with the dominant gene B (BB or Bb), the product produced by aa is not epistatic to the product of gene B and the plant is susceptible. When the homozygous recessive of the second, B gene (bb), is present, the product produced by the recessive bb is epistatic to that of aa and confers resistance to the plant. Therefore resistance is conferred when the genotype of the plant is either A_B_, A_bb or aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb. Applying this model for Ac7a, the two genes are designated as A^a and B^a while for Ac7v, they are designated as A^v and B^v. Since phenotypic linkage of reaction to Ac7a and Ac7v was seen, it is suggested that there may be present a total of four genes (A^a=A gene for Ac7a, B^a=B gene for Ac7a, A^v=A gene for Ac7v and B^v=B gene for Ac7v) of which A^a and A^v are tightly linked and which independently act as inhibitor genes to B^a and B^v. It is also possible that there are only three genes (A, B^a and B^v), with a common A gene for Ac7a and Ac7v, which acts as an inhibitor gene to B^a as well as B^v, with B^a and B^v being unlinked to each other.

The differential cultivar, Burgonde-A (*B. juncea*) is susceptible to race Ac2 (its homologous race) but is resistant to Ac7a (heterologous race), while the differential cultivar, Torch (*B. rapa*) is susceptible to Ac7a (homologous race) but resistant to Ac2 (heterologous race). The inheritance of resistance of Burgonde-A to Ac7a and Torch to Ac2 is not known. The cultivars, UM3512 (*B. juncea*) and CRGC1-18 (*B. rapa*) are susceptible to both races Ac7a and Ac2. Plants that were resistant and susceptible to

Ac7a from Burgonde-A and UM3512 respectively were selfed for two generations to increase homozygosity and to produce parental generations for making F_1 crosses, while for Torch and CRGC1-18, plants that were resistant and susceptible to Ac2 respectively were crossed pair-wise for one generation. Plants from each family of Torch and CRGC1-18, giving resistance and susceptibility respectively were sib-mated for one generation within themselves, to produce parental generation plants. All F_1 plants tested from crosses and reciprocal crosses of Burgonde-A × UM3512 were found to be resistant indicating that resistance is dominant and nuclearly inherited. Plants from each F_1 family, were selfed to produce F_2 generations, while F_1 plants were also backcrossed to both parents. F_2 data from one cross and two reciprocal crosses fitted a dominant-recessive epistasis model. This showed that resistance to Ac7a in *B. juncea* for some crosses is controlled by two genes interacting in a dominant-recessive epistasis model. Other families did not fit a one-gene or two gene-model.

For Torch × CRGC1-18, although most plants were resistant, indicating that resistance is dominant and nuclearly inherited, F_1 crosses and reciprocal crosses segregated for resistance and susceptibility, showing that the parents involved in F_1 crosses were not homozygous for resistance and susceptibility. Resistant F_1 plants from each F_1 family were sib-mated to produce F_2 generations and also backcrossed to both parents. F_2 and backcross data were tested for a one-gene and all possible two-gene models, and data from four F_2 families, fitted a duplicate dominance model. Theoretical crosses of all eight resistant genotypes to the one susceptible genotype gave F_2 ratios such as 15:1, 7:1, 3:1 and 1:1. Data from the four F_2 families which did not fit any of the

models were reanalysed, and one family fitted a 7:1 ratio, while the other families did not fit a one gene or any other two gene models. This suggests that resistance to Ac2 in *B. rapa* is controlled by duplicate dominant genes. The presence of dominant genes for resistance in *B. juncea* cultivar Burgonde-A to Ac7a and in *B. rapa* cultivar Torch to Ac2 confirms the finding of complementary dominant genes for avirulence to *B. juncea* and *B. rapa* to races Ac7a and Ac2.

FOREWORD

This thesis is written in a manuscript style as outlined by the Department of Plant Science, University of Manitoba. A general abstract, a general introduction and a review of literature are presented first. These are followed by two manuscripts each of which include an abstract, introduction, materials and methods, results and discussion. Finally a general discussion, literature cited and appendices end the thesis.

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CHAPTER 1

GENERAL INTRODUCTION

Oilseed turnip rape, *Brassica rapa* L. (syn. *B. campestris* L.), and summer rape *B. napus* (L.) are important *Brassica* species which contribute to the production of canola oil in western Canada. Of these two species, Canadian *B. napus* cultivars are resistant to Canadian isolates of the biotrophic, oomycete, *Albugo candida* (Pers.) Kuntze. *Albugo candida (Ac),* is the causal agent of the disease,white rust of crucifers (Petrie, 1973, 1988; Verma and Petrie, 1975). Yield losses in Alberta and Saskatchewan due to this disease on species of *Brassica* were reported to be between 1.2 and 9.0 percent (Berkenkamp, 1972; Petri, 1973). Yield reductions of 30 to 60 percent have been reported in heavily infected fields of *B. rapa* in Manitoba (Bernier, 1972).

The inheritance of resistance of some *Brassica* species to different races of *A. candida* (Ac) has been previously studied and there is evidence of resistance being dominant. Fan et al. (1983) studied the inheritance of resistance to race 7 (Ac7) in *B. napus* cultivar Regent and found that white rust resistance is conditioned by independent, dominant genes at three loci. Liu et al. (1988) confirmed this observation. The inheritance of resistance to race 2 (Ac2) in a resistant cultivar of brown mustard (*B. juncea*) was studied by Tiwari et al. (1988) who found that resistance was monogenic and they suggested that it could be easily transferred to adapted susceptible genotypes. Quantitative resistance to Ac2 in a rapid-cycling population of *B. rapa* has also been reported by Edwards and Williams (1987). *B. rapa* cv. Reward was selected for

resistance against race Ac7. However some new isolates of Ac7 are virulent on Reward and these have been termed Ac7v, with the initial race non-virulent to Reward being termed Ac7a. The breeding population UM921 was re-selected from Reward for resistance to Ac7v. Thus UM921 is generally resistant to both Ac7a and Ac7v meaning that when plants from the UM921 population were screened for reaction to white rust, they showed interaction phenotypes (IP) of 0 and 1 on a white rust rating scale (Williams, 1985) of IP 0 to IP 9 where IP 0 are plants which are resistant and IP 9 are plants which are susceptible to white rust. Although cultivars of *B. rapa* with resistance to *A. candida* have been developed, it is necessary that the genetic basis of this resistance be determined to facilitate continued breeding for resistance, incorporation of this resistance into commercially available cultivars and for identification of new sources of resistance.

Races of *A. candida* are generally more virulent on the *Brassica* species which they normally infect (homologous host species) but are also capable of infecting some genotypes of other closely related *Brassica* (heterologous host species). *A. candida* race 2 (Ac2) is generally virulent on *B. juncea* but is also capable of infecting some populations of *B. rapa* while Ac7 is virulent on *B. rapa* but can also infect some populations of *B. juncea.* The cultivar Burgonde-A (*B. juncea*) is susceptible to Ac2 but resistant to Ac7a while the cultivar Torch (*B. rapa*) is susceptible to Ac7a but resistant to Ac2, and both of these cultivars are part of a set of differential cultivars which are used for identifying and classifying new isolates of *A. candida* into the different races. Using metalaxyl insensitivity and variation in pathogenicity as genetic markers, Liu and Rimmer (1993) found sexual recombinants in oospore progeny of crosses between Ac2 and Ac7a.

Results obtained suggest that, although these races of *A. candida* are generally more virulent on their respective homologous hosts, cross fertilization can occur in nature between isolates of Ac2 and Ac7 when they simultaneously infect the same host. Rimmer et al. (1995) studied the genetics of virulence of *A. candida* to *B. rapa* and *B. juncea* in crosses of Ac2 and Ac7a. Sexual progenies from crosses between two metalaxyl insensitive isolates of race Ac2 and one metalaxyl sensitive isolate of race Ac7a were tested on Burgonde-A and Torch. Four of twelve single pustule isolates were considered recombinants, and F_2 segregation data of these recombinants were analyzed. Avirulence to *B. rapa* was found to be dominant with three families fitting a 3 avirulent : 1 virulent model and two families fitting a 15 avirulent : 1 virulent model. In *B. juncea,* avirulence was found to be dominant, and fitted a 3 avirulent : 1 virulent model for some families. Therefore, these results suggest the presence of complementary alleles for resistance in the above-mentioned heterologous hosts to isolates of Ac2 and Ac7.

The objectives of this study were to determine the inheritance of resistance to *A. candida* races Ac7a and Ac7v using the *B. rapa* cultivars UM921 (resistant parent) and Horizon (susceptible parent) and to determine the genetics of resistance of the differential cultivars, Burgonde-A (*B. juncea*) to Ac7a and Torch (*B. rapa*) to Ac2.

CHAPTER 2

REVIEW OF LITERATURE

2.1 The Host:

2.1.1 Oilseed brassicas

Oilseed brassicas are one of the important sources of edible oil in the world and are cultivated predominantly in Canada, China, the Indian Subcontinent and Western Europe and include *Brassica napus* L. (Argentine rape or summer rape) and *Brassica rapa* L. (Polish-type rape or summer turnip rape). The seed contains 33 to 50 percent oil on a dry weight basis while the meal contains 38 to 41 percent protein, which makes it a feedstock of high nutritive value. In Canada, rapeseed oil is produced from cultivars of two *Brassica* species, *B. napus* and *B. rapa* (syn. *B. campestris* L.), while other *Brassica* species are used as vegetables or condiments (Liu, 1987). The advent of rapeseed breeding in Canada took place during World War II, when H.G. Neufeld made selections of *B. napus* from seed stocks introduced from Argentina, and the first commercial production of rapeseed oil in Canada occurred in 1943. The term "canola" has been coined to describe cultivars of *B. rapa* and *B. napus* which meet specific requirements for erucic acid (less than two percent erucic acid taken as a percentage of the total fatty acids in the extracted seed oil) and glucosinolate content (less than 30 µmol/gm in the residual meal) [Downey and Rimmer, 1993]. Commercial cultivation of *Brassica juncea* Czern & Coss began in Canada in 1936 with a planting of only 40 hectares (Statistics Canada, 1976), and presently it is grown for condiment purposes on

approximately 80,000 ha with strong potential as an oilseed crop for the prairies (Woods et al. 1991). Cultivars of *B. juncea* grown in Canada are high yielding with maturity periods intermediate to *B. napus* and *B. rapa* (Pawlowski, 1970; Woods et al. 1991).

2.1.2 Botanical relationships:

The botanical relationships among the cultivated *Brassica* species were first demonstrated by Morinaga (1934) who showed through cytological evidence that some *Brassica* species [*B. napus* L. (n=19), *B. juncea* Czern & Coss (n=18) and *B. carinata* Braun (n=17)] were amphidiploids and had arisen through natural interspecific hybridization between diploid *Brassica* species with lower-chromosome numbers, *B. nigra* (L.) Koch. (n=8), *B. oleracea* L. (n=9) and *B. rapa* L. (n=10). U (1935) confirmed this observation, by synthesizing artificially, *B. napus* from crosses of *B. rapa* and *B. oleracea* (Fig 1). Later, Downey et al, (1975) and Olsson and Ellerstrom (1980) obtained synthetic *B. juncea* and *B. carinata* from crosses of *B. nigra* and *B. rapa* or *B. oleracea.* This knowledge of the inter-relationships within the *Brassic*a genus has helped the transfer of valuable characteristics such as early maturity, cytoplasmic male sterility, self-incompatibility etc., from *B. rapa* to *B. napus* (Liu, 1985).

2.1.3 Sib-mating:

B. rapa exhibits sporophytic self-incompatibility. As such, it is difficult to produce seed by selfing as in self-compatible species like *B. juncea* or *B. napus.* Sib-mating to produce F_2 populations has been routinely used by a number of workers while conducting studies on the genetics of inheritance of various characters in *B. rapa.* Stringam (1973) while studying the inheritance and allelic relationship of seven

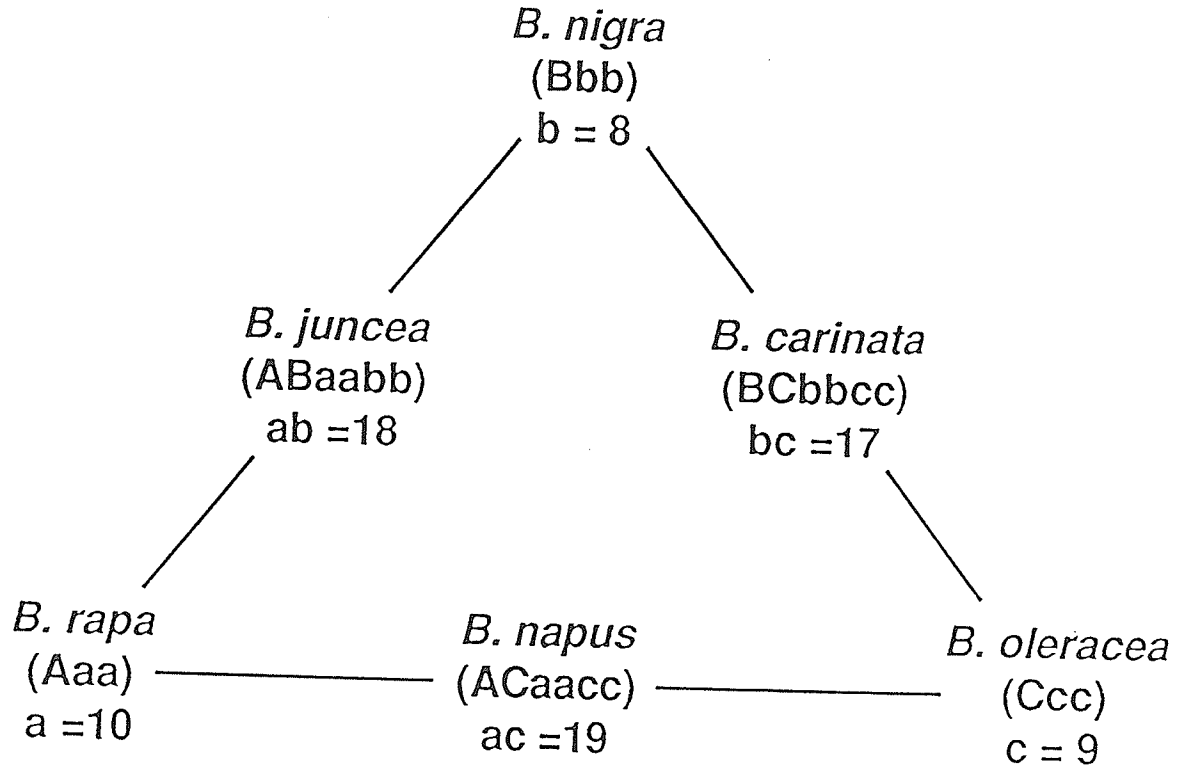


Figure 1. Genome relationship of *Brassica* species (according to U 1935).

Capital letter refers to cytoplasm; small letter refers to nulcear genome.

chlorophyll-deficient mutants used sib-mating of F_1 plants to produce F_2 generations where one of the parents involved in the study was a self-incompatible low erucic acid cultivar. Hawk and Crowder (1978) studied the inheritance of four mutants in early-flowering *B. rapa* and routinely sib-pollinated F_1 plants to produce F_2 populations.

2.2. The pathogen:

2.2.1. The disease:

The disease, white rust (white blister) is caused by several species of *Albugo* which is the only genus in the Family *Albuginaceae* of the Order *Peronosporales* of Class *Oomycetes* and causes diseases on several economically important crops. The disease on crucifers is caused by *Albugo candida* (Pers.) Kuntze, on spinach by *A. occidentalis* Wilson (Wilson, 1907), on sweet potato by *A. ipomoeae-pandurateae* (Schw.) Swingle (Harter and Weimer, 1929; DeMelo, 1947), and on sunflower by *A. tragopagonis* (DC) S.F. Gray (Safeeulla and Thirumalachar, 1953; Ho and Edie, 1969). *A. candida* has been reported to attack 241 species in 63 genera of *Cruciferae* (Biga, 1955). White rust is considered a major problem in cruciferous crops in many countries with the "staghead" phase of the fungus causing heavy yield losses. Verma and Bhowmik (1989) reported a white rust epidemic in a 120,000 hectare crop of *B. juncea* in north western India reducing the yield by about 20 percent. In Canada, average yield losses on *B. rapa* and *B. napus* between 1.2 and 9 percent have been reported (Berkenkamp, 1972; Petrie, 1973), whereas Bernier (1972) reported yield reductions of 30 to 60 percent in severely infected fields. Yield losses on mustard (*B. juncea*) have also been reported by Kumari et al.

(1970) in India.

2.2.2 Host Range:

A. candida (Ac) affects a large number of plants in the *Cruciferae*, including economically important crops like *B. rapa L. (syn. campestris), B. juncea* Czern & Coss, *B. nigra* (L.) Koch, *B. oleracea* (L.) and *Raphanus sativus* L. (Petrie, 1988).

2.2.3 Symptoms:

A. candida can cause local as well as systemic infections. Local infection is characterized by raised white pustules generally formed on the abaxial surface of the leaves. These pustules may be present individually or may coalesce to form large patches (Walker, 1969). Secondary pustules may develop around the original pustules giving rise to concentric rings (Endo and Linn, 1960). When the fungus infects meristems of young stems or inflorescences, hypertrophy and hyperplasia are observed (Walker, 1969). When this takes place, "stagheads" are formed, which are composed of hypertrophied plant tissue containing many brown thick-walled oospores, the over-wintering stage of the fungus. The hypertrophies of host tissue caused by *A. candida* seem to be a favourable site for the growth of secondary microorganisms. Petrie and Vanterpool (1974) have observed over 20 species of fungi to be associated with these hypertrophied inflorescences and stem and pod blisters.

2.2.4.Life cycle:

The pathogen produces oospores in hypertrophied tissues, and other infected plant parts. Oospores may overwinter in the soil and serve as primary inoculum for development of the disease. Oospores may survive for more than twenty years stored in

the laboratory (Verma and Petrie, 1975) and Tiwari and Skoropad (1977) suggest that long-term survival is probably due to the highly differentiated, five-layered cell wall possessed by the oospores. In some perennial hosts such as horseradish, the pathogen can also overwinter in infected crowns and lateral roots (Kadow and Anderson, 1940; Walker, 1957).

2.2.5 Physiologic specialization of *Albugo candida*

Physiological specialization or race specificity in *A. candida* has been recognized for a long time. Eberhardt (1904) grouped *A. candida* into two groups, based on different host plant infectivity. Savulescu and Rayss (1930) identified eight morphological forms within *A. candida* and later, Savulescu (1946), differentiated forms within *A. candida* based on host specialization and morphology of the fungus. Hiura (1930) identified three distinct forms of *A. candida* based on host preference, the first on *R. sativus*, the second on *B. juncea* and the third on *B. rapa.* Togashi and Shibasaki (1934) classified *Albugo* into macrospora and microspora types based on sporangial size. Ito and Tokunago (1935) classified the forms with larger spores to *Albugo macrospora* (Togashi) Ito. Biga (1955), recognized Ito and Tokunago's (1935) classification but renamed them as *A. candida microspora* and *A. candida candida* respectively. He further reported that *A. candida microspora* is restricted to *Armoracia*, *Brassica*, *Erucastrum*, *Raphanus* and *Rapistrum*, whereas *A. candida candida* has a wider range of cruciferous hosts. He also identified interspecific taxa of *A. ipomoeae-panduratae* and *A. tragoponis.* Pound and Williams (1963) identified six races of *A. candida* using a set of six differential cultivars from six different species namely, race 1 (Ac1) from *Raphanus sativus* var. Early Scarlet Globe,

race Ac2 from *B. juncea* var Southern Giant Curled, race Ac3 from *Armoracia rusticana* (Gaertn., Mey., & Scherb) var. common, race Ac4 from *Capsella bursa-pastoris*, race Ac5 from *Sisymbrium officinale* (L.) Scop., and race Ac6 from *Rorippa islandica* (Oeder) Borba's. In addition to these races, four new races were subsequently identified, race Ac7 on *B. rapa* (Verma et al, 1975; Pidskalny & Rimmer, 1985), race Ac8 on *B. nigra* (L.) Koch (Delwiche & Williams, 1977), race Ac9 on *B. oleracea* (L.) and race Ac10 on *Sinapsis arvensis* (Hill et al, 1988). Nine races (Ac1 through Ac8 and Ac10) occur in North America.

Pidskalny and Rimmer (1985) studied the virulence of isolates of *A. candida* from *B. rapa* and *B. juncea* and reported that these isolates infected only the hosts from which they were obtained, confirming the classification proposed by Pound and Williams (1963). Pidskalny and Rimmer (1985) observed that the present race concept in *A. candida* is based on species relationship, and suggested that a situation could arise where cultivars of *B. rapa* or *B. juncea* may be used to differentiate isolates of a pathogen within what are now accepted as races.

2.3. Genetics of Host-Pathogen interaction:

2.3.1 Genetics of Resistance

Resistance to race Ac1:

Williams and Pound (1963) screened 283 radish (*R. sativus*) accessions and 14

commercial varieties, and found two varieties CRW (China Rose Winter) and RBS (Round Black Spanish), resistant to race Ac1, with resistance in both cases being governed by a single dominant gene. Under normal conditions, a hypersensitive reaction occurred on CRW after initial contact between host and parasite. But, under certain environmental conditions, this resistance changed to tolerance with the production of discrete white pustules on the adaxial surface of the cotyledons. This suggested the presence of genes with minor effects to modify the resistance reaction of the major gene. Humadayan and Williams (1976) confirmed the monogenic resistance in CRW and found that monogenic resistance was also present in two cultivars of small radish, "Biser" (white bulgarian radish) and "Rubiso" (round scarlet radish).

Resistance to Race Ac2

Ac2 mainly infects *B. juncea* but can also infect *B. rapa*, *B. nigra* and *B. carinata* (Pound and Williams, 1963; Petrie, 1988). Parui and Bandyopadhyay (1973) found that a line Yellow Rai T4 was immune to natural infection by Ac2. Tiwari et al. (1988) studied the inheritance of resistance to Ac2 in mustard (*B. juncea*) using one resistant and two susceptible cultivars. F_2 segregation data of resistant and susceptible plants showed that resistance was monogenic, dominant and controlled by nuclear genes, and that it could be easily transferred to adapted susceptible genotypes via backcrossing. The inheritance of resistance to white rust in the accession P.I. 347618 was reported by Ebrahimi et al. (1976), wherein F_1 progenies were observed to give the same reaction as the resistant parent, but no F_2 data were reported. In contrast to the above results, Edwards and

Willams (1982; 1987) found that reaction to this race varied from low to high infection type, suggesting that resistance was governed by both major and minor genes. Working with a rapid-cycling *B. rapa* line CRGC-1, they found that resistance which was conditioned by minor genes, could be effectively enhanced by mass selection or half-sib family selection. Verma and Bhowmik (1989) found that two lines of *B. napus*, BN-Sel, and BN-38 Sel, showed a resistant and susceptible reaction respectively to a *B. juncea* pathotype of *A. candida.* They found that F_1 plants showed a resistant reaction, and F_2 plants segregated in a 15 resistant (R) : 1 susceptible (S) ratio, while the backcross between F_1 and the susceptible parent segregated in a 3R : 1S ratio confirming that resistance of BN-Sel to *A. candida* is controlled by dominant duplicate genes.

Kole et al. 1996 studied the inheritance of resistance to race Ac2 in an F_2 population and a set of F_3 families, and found that resistance was dominant and controlled by a dominant allele at a single locus. The resistance locus (ACA1) was found to be linked to the leaf pubescence locus (PUB1). 144 restriction fragment length polymorphism (RFLPs) loci were used to map the ACA1 locus to linkage group 4. The authors suggest that these RFLP markers and the morphological leaf pubescence marker can be used to tag the ACA1 gene for introgressing and map-based cloning of white rust resistance in *B. rapa* and other *Brassica* species.

Resistance to Race Ac3

Studies on the resistance of horseradish (*A. rusticana*) to race Ac3 have been limited because of male sterility seen in common horseradish. Bohemian horseradish resistant to *A. candida* can sometimes provide functional pollen and this discovery prompted Hougas et al. (1952) to study the inheritance of resistance of horseradish to *A. candida* race Ac3. They found that F_1 progenies segregated into three categories: highly resistant without sporulation, resistant with limited sporulation and highly susceptible with abundant sporulation. No F_2 data were reported.

Resistance to Race Ac7

Fan et al. (1983) studied the inheritance of resistance to *A. candida* race Ac7 in *B. napus*, using one resistant Canadian variety, Regent, and two susceptible Chinese lines, 2282-9 and Green Cup Leaf (GCL). F_2 progenies from both crosses, (2282-9 × Regent) and (GCL × Regent), and their reciprocals segregated in the ratio of 15R : 1S, suggesting that resistance was governed by two independent dominant genes. According to this model, resistant plants resulted from the presence of a dominant allele at either of the two loci, and susceptible plants would be seen when alleles at both loci were homozygous recessive. Some of the F_2 progenies, from the GCL × Regent cross were observed to segregate in a 63R : 1S ratio, suggesting the presence of a third dominant resistance gene. This meant that all Regent plants were homozygous and homogeneous for alleles conferring resistance to white rust at two loci, while some plants also had a resistance allele at a third locus. These three resistance genes were designated Ac7-1, Ac7-2 and Ac7-3 according to an earlier proposal of Humaydan and Williams (1976) to designate

white rust resistance genes. Liu (1987) confirmed the above observation that Ac7-1 and Ac7-2 were two independent dominant genes conferring resistance to race Ac7 in *B. napus*.

Races of *A. candida* have generally been seen to be more virulent on the species that they normally attack (homologous host species), but are also capable of infecting other related hosts (heterologous hosts). Liu and Rimmer (1991) studied the inheritance of resistance in *B. napus* to an Ethiopian isolate of *A. candida* from *B. carinata* and found that the line 2282-9 (*B. napus*) possessed a single dominant gene for resistance to that Carinata isolate of *A. candida.* The *B. rapa* cultivar, Reward was selected for resistance to race Ac7. Initially plants of the cultivar Reward were screened for reaction to white rust and plants that were resistance were selected and taken to the next generation. Screening for white rust and selection of resistant plants was carried out for two more cycles to obtain a population of Reward that was generally resistant to race Ac7. But it was observed that new isolates of Ac7 were virulent to the previously resistant Reward (termed as Ac7v) and the initial race, non-virulent to Reward was termed as Ac7a. Further, the breeding population UM921 was reselected from Reward for two selection cycles for Ac7v, so as to obtain a population of UM921 which was generally resistant to both Ac7a and Ac7v with IP of 0 and 1. The genetic basis of resistance of this population and of *B. rapa* in general has not yet been determined. This information is important to continue breeding for resistance to white rust and to incorporate this resistance into commercial *B. rapa* cultivars.

2.3.2 Genetics of Virulence

Although a number of studies on the genetics of resistance to *A. candida* have been reported, corresponding studies on the genetics of virulence of *Albugo* to different *Brassica* species have not been made due to a lack of reliable genetic markers for the pathogen. Liu and Rimmer (1993) developed a procedure to produce and germinate oospores of *A. candida* under controlled environmental conditions. They were able to successfully germinate more than 80 percent of oospores produced in (hypertrophic) stems artificially inoculated with races Ac2 or Ac7. Briefly, this was done by agitating oospores for 24 hours in sterile distilled water containing a 1 to 2 percent mixture of β-glucuronidase and aryl sulfatase followed by 3 days of washing on a rotary shaker at room temperature and 15 hours of chilling at 13°C. The development of this procedure has made it easier to undertake genetic studies in *A. candida.*

Liu (1992) found sexual recombination from progenies derived from crosses of races Ac2 and Ac7 and suggested that, in nature when both of these races simultaneously infect a common host, there is a possibility that a new recombinant race virulent on both the original homologous hosts of the two progenitor races may arise. Rimmer et al. (1995) tested sexual progenies from crosses of two metalaxyl insensitive isolates of race Ac2 and one metalaxyl sensitive isolate of race Ac7 on the differential cultivars, Burgonde-A (*B. juncea*) and Torch (*B. rapa*). Four of twelve single pustule isolates were considered to be recombinants as they were either avirulent to both Burgonde-A and Torch or caused only a low infection on Burgonde-A and showed insensitivity to metalaxyl. F_2

segregation data of five of the recombinants mentioned were analyzed and avirulence to *B. rapa* has been found to be dominant with three families fitting a 3 avirulent : 1 virulent model and two families fitting a 15 avirulent : 1 virulent model. Avirulence to *B. juncea* was found to be dominant in two families and fitted a 3 avirulent : 1 virulent model, while three other familes tested did not support this model. Studies on the inheritance of resistance of the differential cultivars Burgonde-A to Ac7 and Torch to Ac2 may yield more information about these host-pathogen interactions.

2.5. Genome evolution of *B. rapa*

Numerous studies on the construction of linkage maps in various crops have been conducted, using restriction fragment length polymorphisms (RFLPs), isozymes and DNA karyotyping, most significant of which are those on tomato, potato, maize, lettuce and rice. In *Brassic*a, a linkage map of *B. oleracea* using RFLP loci has been constructed by Slocum et al. (1990) which revealed a high level of duplication within the genome. Landry et al. (1987) also constructed a genetic map of lettuce, using RFLP, isozyme, disease resistance and morphological markers.

Röbbelen (1960) analyzed pachytene chromosomes of *B. rapa* and proposed that the genome constitution of *B. rapa* is AABCDDEFFF representing its ten chromosomes. Tang and Williams (cited in Chyi et al. 1992) using improved techniques for karyotyping mitotic chromosomes came to similar conclusions as Röbbelen (1960). These studies suggest that *B. rapa* contains replicated chromosomes. Prakash and Hinata (1980) hypothesized that the diploid *Brassica* species (n=7, 8, 9, 10 and 11) have evolved as an

ascending aneuploid series from a prototype species of n=6. This theory was supported by Attia and Röbbelen (1986). Kole et al. 1996 using RFLP markers mapped a resistance gene in *B. rapa* to race Ac2. They found that the linkage relationship of RFLP loci used in their study were conserved across other *Brassica* species, indicating that these different *Brassica* species had homologous chromosomal regions, which lends support to the suggestion to the *Brassica* have evolved from a common ancestor. Song et al. (1990) suggest that chromosome duplication may have occurred by aneuploidy within the genome or by introgression from closely related genomes. This might have led to meiotic instability such as abnormal chromosome pairing in the newly formed aneuploid leading to structural rearrangement. One reason why frequent and rapid rearrangement, after duplication of chromosomes or large chromosome fragments, may have taken place is that it might provide a mechanism for stabilization of aneuploids. Song et al.'s study (1991) using RFLP data supported the cytogenetic findings of chromosome duplication and suggested structural re-arrangement during the evolution of *B. rapa*. They also found three linkage groups involved in significant duplicate linkage, and these observations taken together imply that many mutations or rearrangements have accumulated since duplication had occurred. Chyi et al. (1992) constructed a genetic linkage map based on RFLP markers for *B. rapa*, using segregating F_2 progeny from the yellow sarson type 'R500' and the Canola-type 'Horizon' and found a large number of duplicated RFLP loci which indicated divergence from common ancestral sequences during evolution of the *B. rapa* genome. They also found that the largest duplicated region involved eight loci between linkage groups 1 and 7, but that one of these eight loci was not present in a

conserved fashion. They also concluded that their RFLP banding patterns revealed evidence for two mechanisms of subchromosomal rearrangement viz., localized sequence duplication as noted by Song et al. (1991) and sequence transposition as noted by McCouch et al. (1988) in rice. Helentjaris et al. (1988) working with RFLP markers in maize suggested a similar event, thus supporting the hypothesis that maize originated from a chromosome number of n=5.

CHAPTER 3

Inheritance of resistance to races 7a and 7v of *Albugo candida* in *Brassica rapa*

3.1. Abstract

Plants of two *Brassica rapa* cultivars, UM921 and Horizon were screened with *Albugo candida* (Ac) races Ac7a and Ac7v. Plants that were resistant (R) from the UM921 population and susceptible (S) to both races from the Horizon population were selected and crossed pair-wise within each population. Plants from these crosses were retested for reaction to Ac7a and Ac7v and two plants from each family which gave the interaction phenotypes (IP) of 0 or 1 (on a scale of IP 0 to IP 9, where 0 is resistant and 9 is susceptible) for UM921 and IP 7 through IP 9 for Horizon were sib-mated to each other. Crosses between selected resistant UM921, and susceptible Horizon plants were made. Most F_1 plants were resistant (IP 0 through IP 5), indicating that resistance to Ac7a and Ac7v was dominant and nuclearly inherited, while some crosses segregated. F_1 plants resistant (IP 0 or IP 1) to both Ac7a and Ac7v, resistant to Ac7a but susceptible (IP 7 to IP 9), to Ac7v, and susceptible to Ac7a but resistant to Ac7v, were sib-mated to each other to produce F_2 generations. F_1 plants were also backcrossed to both parents. All F_2 families were tested for a one-gene or all possible two-gene models and two families fit a dominant-recessive epistasis model. According to this model, resistance and susceptibility is controlled by two genes with complete dominance at both gene pairs. Assuming that these two genes are A and B determining the genetics of resistance and

susceptibility. Gene A when dominant (AA or Aa) produces a product which is epistatic to the product produced by gene B and resistance is conferred to the plant. When the recessive aa is present with the dominant gene B (BB or Bb), the product produced by aa is not epistatic to the product of gene B and the plant is susceptible. When the homozygous recessive of the second, B gene (bb), is present, the product produced by the recessive bb is epistatic to that of aa and confers resistance to the plant. Therefore resistance is conferred when the genotype of the plant is either A_B_, A_bb or aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb. Applying this model for Ac7a, the two genes are designated as A^a and B^a while for Ac7v, they are designated as A^v and B^v. Since phenotypic linkage of reaction to Ac7a and Ac7v was seen, it is suggested that there may be present a total of four genes (A^a=A gene for Ac7a, B^a=B gene for Ac7a, A^v=A gene for Ac7v and B^v=B gene for Ac7v) of which A^a and A^v are tightly linked and which independently act as inhibitor genes to B^a and B^v. It is also possible that there are only three genes (A, B^a and B^v), with a common A gene for Ac7a and Ac7v, which acts is an inhibitor gene to B^a as well as B^v, with B^a and B^v being unlinked to each other.

3.2. Introduction:

Oilseed turnip rape, *Brassica rapa* L. (syn. *B. campestris* L.), and summer rape *B. napus* (L.) are important *Brassica* species for production of canola oil in western Canada. Of these two species, Canadian *B. napus* cultivars are resistant to Canadian isolates of the biotrophic, oomycete, *Albugo candida* (Pers.) Kuntze, the causal agent of white rust of crucifers (Petrie, 1975, 1988; Verma et al., 1975). Yield losses in Alberta and Saskatchewan due to this disease on species of *Brassica* were reported to be between 1.2 and 9.0 % (Berkenkamp, 1972; Petri, 1973). Yield reductions of 30 to 60 % have been reported in heavily infected fields of *B. rapa* in Manitoba (Bernier, 1972).

A. candida (Ac) infects many cruciferous species and in North America, nine biological races of *A. candida* (races 1 through 8 and race 10) have been identified and classified on the basis of a set of differential cultivars of the various *Brassica* species which are infected. These are: race 1 on *Raphanus sativus* L., race 2 on *B. juncea* Czern & Coss, race 3 on *Armoracia rusticana* Gaetn., Mey., & Scherb., race 4 on *Capsella bursa-pastoris*, race 5 on *Sisymbrium officinale* (L.) Scop., race 6 on *Rorippa islandica* (Oeder) Borba's (Pound & Williams 1963), race 7 on *B. rapa* (Verma et al.. 1975; Pidskalny & Rimmer, 1985), race 8 on *B. nigra* (L.) Koch (Delwiche & Williams, 1977), race 9 on *B. oleracea* L., and race 10 on *Sinapsis arvensis* (Hill et al.., 1988).

The inheritance of resistance of some *Brassica* species to different races of *A. candida* has been previously studied and there is evidence of resistance being dominant with monogenic or digenic inheritance. Fan et al. (1983) studied the inheritance of resistance

to race 7 (Ac7) in *B. napus* cultivar Regent and found that white rust resistance is conditioned by independent, dominant genes at three loci. The inheritance of resistance to race 2 (Ac2) in a resistant cultivar of brown mustard (*B. juncea*) was studied by Tiwari et al. (1988) who found that resistance was monogenic. Verma and Bhowmik (1989) studied the inheritance of resistance to a *B. juncea* pathotype of *A. candida* in the *B. napus* line BN-Sel and found that resistance was governed by duplicate dominant genes. Quantitative resistance to Ac2 in a rapid-cycling population of *B. rapa* has been reported by Edwards and Williams (1987). *B. rapa* cv. Reward was developed by three selection cycles for resistance to Ac7 but new isolates of Ac7 virulent on Reward have been seen and these were termed Ac7v, with the initial isolates non-virulent to Reward being termed Ac7a. The breeding population UM921 was reselected from Reward after two cycles of selection for resistance to Ac7v. This was carried out by screening plants from the cultivar Reward for Ac7v and selecting resistant plants. These resistant plants were taken over to the next generation and subjected to another selection cycle, and resistant plants thus obtained were mass pollinated to get the breeding population UM921. But because of the out-crossing nature of *B. rapa*, homozygosity for resistance to Ac7a and Ac7v could not be achieved for the UM921 population.

Although cultivars of *B. rapa* with resistance to both races Ac7a and Ac7v of *A. candida* have been developed, it is necessary that the genetic basis of this resistance be determined to facilitate continued breeding for resistance, incorporation of this resistance into commercially available cultivars and for identification of new sources of resistance. This study therefore examines the inheritance of resistance to *A. candida* races Ac7a and

Ac7v using the *B. rapa* cultivars UM921 (resistant parent) and Horizon (susceptible parent).

3.3. Materials and Methods:

3.3.1: Inoculum Preparation:

Mature zoosporangia of Ac7a and Ac7v were collected separately from *B. rapa* cv. Torch in gelatin capsules (Parke-Davis Size 00) and stored in glass screw-cap vials at -10 °C. Inoculum preparation was according to the methods of Liu et al. (1988). Briefly, zoosporangia of both races were placed in Erlenmeyer flasks containing distilled water, sealed with Parafilm^{tm}, and shaken gently. Flasks were incubated at 12 °C for 3 to 3.5 hours for induction of zoosporogenesis and then placed on ice to avoid zoospore encystment and the number of zoospores were quantified using a haemocytometer and inoculum concentration adjusted to 2×10^5 zoospores per millilitre.

3.3.2: Plant Preparation and Inoculation:

Experiments were conducted in the growthrooms and greenhouses at the Department of Plant Science, University of Manitoba from May 1994 through April 1996. Selected plants of the cultivars UM921 and Horizon which were resistant and susceptible respectively to both Ac7a and Ac7v were used in this study. Flats were seeded containing Metro mix (W.R. Grace & Co. Canada Ltd. Ajax, Ontario), and placed under fluorescent lights in a growth room with 22/17 °C day/night temperatures and a 18 hour photoperiod. 10 µl each of a zoospore suspension of Ac7a or Ac7v was inoculated onto

the adaxial surface of each cotyledon of six-day old seedlings using an Eppendorf repeater pipette. One cotyledon was pierced with an inoculation needle and Ac7a was inoculated onto the marked cotyledon. The other cotyledon was inoculated with Ac7v. Care was taken to avoid mixing of the races on each cotyledon by inoculating on the edge of the cotyledon. In cases where inoculation droplets of the two races were seen to coalesce, plants were discarded. This procedure gave an easily discernible difference between the two cotyledons at the time of inoculation and thus enabled infection types to both races to be scored on the same plant. Inoculated plants were placed in a humidity chamber for 24 hours at 100 percent humidity, and were then moved to the growth room until evaluation for white rust infection.

3.3.3: Screening and Plant Selection:

Thirteen to fourteen days from seeding, plants were scored for interaction phenotypes (IP) on a scale of 0 to 9 (Williams, 1985; Figure 2). Those plants which gave IP of 0 or 1 (resistant, R) for cultivar UM921 and 7 or 9 (susceptible, S) for cultivar Horizon for both Ac7a and Ac7v were selected (Table 1) and transplanted into fibre pots containing a 2:1:1 (v/v/v) mixture of soil, sand and peat and transferred to the greenhouse. Generally, the range of reactions for cv. UM921 were from IP 0-5 and for cv. Horizon from IP 6-9.

3.3.4: Establishment of Parental Populations:

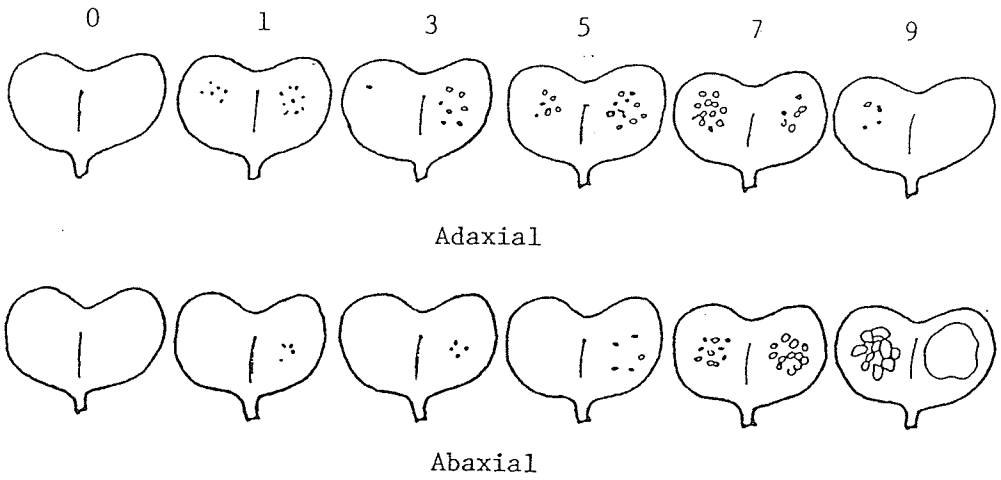
Homozygosity of parental material used for genetic studies is advantageous for

easier interpretation of data. This is easily achieved in self-pollinated crops by selfing, but since *B. rapa* is a naturally out-crossing crop exhibiting sporophytic incompatibility, a sib-mating approach (Figure 3) was used to achieve homozygosity in the parental populations. Plants of UM921 and Horizon were screened for reaction to Ac7a and Ac7v, and plants which were resistance (IP 0 or 1) or susceptibility (IP 7 or 9) respectively to both races were selected. Selected plants were crossed pair-wise (i.e., resistant plants were crossed to resistant plants and susceptible plants were crossed to susceptible plants), to produce generation 1 seed (G1). Approximately 25 such crosses were made and seed from each cross designated as a family. Fifteen families (each of UM921 and Horizon) were chosen for further study and retested for reaction to races Ac7a and Ac7v and 2 plants from 8 families each of UM921 and Horizon in which all/most plants tested showed the required reaction were sib-mated to produce generation 2 seed (G2). Screening as above was carried out and 1 or 2 plants from each family of UM921 and Horizon were selected as parental generation (PG) plants for crossing.

3.3.5: Crossing Pattern:

In total, twelve crosses and reciprocal crosses of PG plants of UM921 × Horizon were made. Pollen of the parents involved in these crosses were collected on bee-sticks and stored at -14 °C in the freezer (Williams, 1980). On average, 35 to 40 F_1 plants from ten different UM921 × Horizon crosses and reciprocal crosses were evaluated for IP. Three classes of F_1 plants (Table 2) were observed and used for producing F_2 seed. Two pairs of F_1 plants resistant to both Ac7a and Ac7v (designated as RR) were selected from

Figure 2. The white rust rating scale



White rust rating scale. Symptoms and signs for the interaction phenotype ratings on the adaxial/abaxial cotyledon surfaces are: 0 = no symptoms on either cotyledon surface, 1 = necrotic flecks/none to few necrotic flecks, 3 = few, minute pustules/none to very few pustules, 5 = few to many small pustules/few small pustules, 7 = many to few small pustules/many large pustules, 9 = very few to no pustules/large coalescing pustules (Williams 1985).

Figure 3. Procedures for populations establishment for Generation 1, Generation 2/Parental Generation, F_1, F_2 and BC1 plants for *B. rapa*.

1. **PARENTAL SELECTION**
Screen 100 & select 10 Resistant (R) & 10 susceptible (S) plants
UM921 Horizon

2. **GENERATION 1 FAMILIES**
Pairwise Crosses within UM921 & Horizon

3. Screen & Select 2R (UM921) & 2S (Horizon)

4. **GENERATION 2 FAMILIES**
Sibmate within UM921 & Horizon

5. Repeat step 3
PARENTAL GENERATION

6. **F_1 PLANTS**
Cross Selected UM921 × Horizon

7. **F_2 GENERATION**
F_1 sibmated to produce F_2 (UM921 × Horizon)

8. **BC1 PLANTS**
F_1 backcrossed to UM921 & Horizon

crosses and reciprocal crosses 1 through 9 only as these crosses had the most seed, while F_1 plants resistant to Ac7a but susceptible to Ac7v (RS) from Cross 1 and F_1 plants susceptible to Ac7a but resistant to Ac7v (SR) were selected from Cross 4. These selected F_1 plants were sib-mated to produce F_2 generations. One F_1 plant used in the sib-matings for F_2 generation plants for all types of selected F_1 plants (RR, RS and SR) used above were also back-crossed to both UM921 and Horizon.

3.4. Data Analysis:

The chi-square test was used to analyze data from segregating F_2 and backcross populations. The chi-square independence test for linkage was conducted to determine linkage of reaction to Ac7a and Ac7v in F_2 data. For data analysis, and segregation of plants into resistant and susceptible classes, IP 0-5 were considered to be resistant and IP 6-9 were considered susceptible. The rationale for this is that, plants with IP 5 show symptoms but sporulation is negligible. Also when the frequencies of IP of more than eight hundred F_2 plants were examined, a bimodal distribution with modes at IP 0 and IP 6 was seen (Figure 4).

3.5. Results:

The four classes of disease reactions observed were (a) resistant to both Ac7a and Ac7v (b) resistant to Ac7a but susceptible to Ac7v (c) susceptible to Ac7a but resistant to Ac7v and (d) susceptible to both Ac7a and Ac7v. For analysis of data for segregation to Ac7a, classes (a) and (b) were taken as those for resistance and classes (c) and (d)

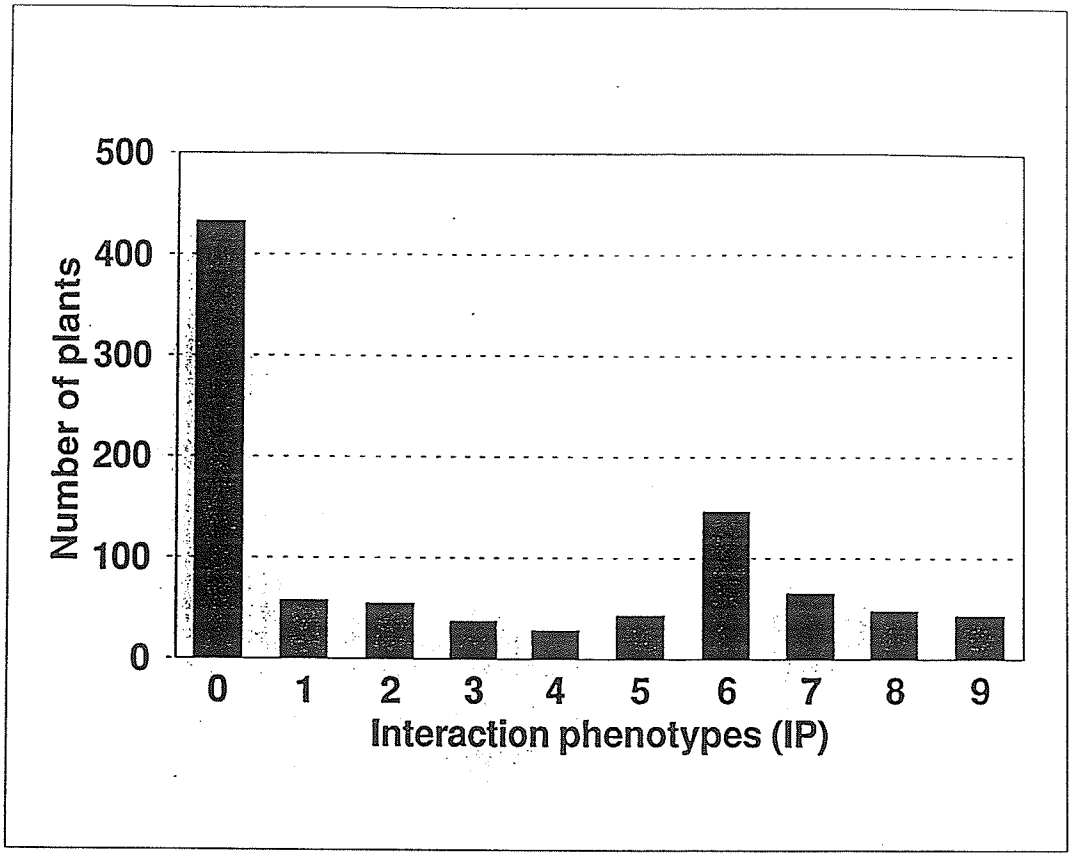
were taken as those for susceptibility, while for analysis of data for segregation to Ac7v, classes (a) and (c) were taken for resistance and classes (b) and (d) were taken as those for susceptibility. For conducting the independence tests for linkage, all four classes of reactions were used (Table 3). Generally, in the breeding population UM921 as well as the F_1 population, it was easier to get plants which were resistant to Ac7a and susceptible to Ac7v, than plants which were susceptible to Ac7a but resistant to Ac7v, indicating the heterozygosity of the parental UM921 population.

F_1 plants from 10 crosses and reciprocal crosses were tested for reaction to white rust races Ac7a and Ac7v (Appendix 7.1). The majority of the F_1 plants were resistant, segregation was observed in all crosses except crosses 5, 7 and 8. Plants were resistant to Ac7a and Ac7v, while some were susceptible to both. Only a few plants were resistant to one race and susceptible to the other. F_1 results also confirmed that resistance to *A. candida* in *B. rapa* is nuclearly inherited with resistance dominant to susceptibility.

3.5.1 F_1 data analysis

F_1 plants from crosses 5, 7 and 8 did not segregate for both Ac7a and Ac7v. All F_1 plants tested from the respective crosses and reciprocal crosses were resistant while for cross 2, only 1 of 34 F_1 plants from the cross and reciprocal cross for Ac7a and 5 of 34 plants from the cross and reciprocal cross for Ac7v were susceptible (Appendix 7.1). Because of low segregation of F_1 plants from these families, it can be assumed resistance to races Ac7a and Ac7v is dominant to susceptibility in *B. rapa.*

Figure 4. Distribution of interaction phenotypes of F_2 *B. rapa* plants to races Ac7a and Ac7v



3.5.2 F_2 data analysis

Of the 9 F_1 crosses that were taken to the F_2 generation, only F_2 families from crosses 1 through 5 were tested for reactions to Ac7a and Ac7v. This was because these families had the most seed, as seed from other families was either lost due to infestation by aphids or was very less to constitute a reasonable size for a F_2 population. Hypotheses for the inheritance of resistance to races Ac7a and Ac7v in *B. rapa* are best postulated based on the F_2 data from crosses 2 and 5 as corresponding F_1 crosses showed least segregation as mentioned above for cross 2 and did not segregate at all for cross 5, and from which it can be assumed that the parental plants for these F_1 crosses were homozygous for resistance and susceptibility to races Ac7a and Ac7v. F_2 segregation ratios in these families gave the best fit to a 13:3 ratio of a dominant-recessive epistasis model with X^2=0.07 and 2.45 and p=0.70-0.80 and 0.10-0.20 for Ac7a and Ac7v respectively (Table 4).

The F_2 families derived from crosses 2 and 5 were tested for other possible segregation ratios for a one-gene or a two-gene model, but the best fit was the above-mentioned dominant-recessive epistasis model.

It is possible that the parents involved in cross 2 and cross 5 are $A^abb \times aaBB$ (5 × 7). Since this model seems best to explain the genetics of resistance, F_2 data from crosses 1, 3 and 4 were examined to test whether these data could also fit a dominant-recessive epistasis model. This was done by applying the conditions of a dominant-recessive epistasis to all genotypes of a classical Mendelian di-hybrid heterozygote, and

all possible theoretical F_1 crosses considered, (of dihybrid genotypes), using resistant and susceptible genotypes and F_2 segregation ratios calculated for each of these crosses by applying the same conditions of a 13:3 model. Table 5 shows the 9 possible genotypes of the dominant-recessive epistasis model. F_2 ratios such as 13:3, 12:4 (3:1), 10:6 (5:3), 8:8 (1:1) can occur dependent on parental and F_1 genotypes. Although for some of the crosses, ratios that could be obtained by theoretical crosses of the resistant and susceptible genotypes from the dominant-recessive epistasis model were seen, genotypes could not be assigned to the parents of these crosses, as these ratios were derived from the sibmating of two F_1 plants one of which was resistant and the other susceptible to Ac7a and Ac7v, whereas, the F_1 plants that were actually sibmated for obtaining F_2 generations were both resistant to Ac7a and Ac7v.

From Table 4, it is seen that Cross 1 fit a 5R:3S ratio for Ac7a and Ac7v with X^2=0.04 and 0.19; p=0.80-0.90 and 0.50-0.70 for Ac7a and Ac7v respectively, but it was not possible to assign genotypes to the parents for these ratios. Chi-square values of 8.72 and 10.24 with p=0.01-0.001 for Ac7a and Ac7v respectively were obtained when the same F_2 data from Cross 1 were tested for a 3R:1S model.

Cross 3 did not fit any ratios, but for a 5R:3S ratio gave X^2=5.69 and 4.70 with p=0.01-0.02 and 0.02-0.05 respectively for Ac7a and Ac7v. No genotypes could be assigned for this cross either. Data from Cross 3 also gave X^2=4.70 and p=0.02-0.05. when tested for a 3R : 1S ratio for Ac7a only. For Ac7v, the data gave X^2=4.34 and p=0.02-0.05 for a 1R : 1S ratio.

When F_2 data for Cross 4 were examined, it was seen that for Ac7a, the data did not fit any ratio applicable to the dominant-recessive epistasis model. When tested for a 1R:1S ratio, the X^2 value obtained was 11.37 with $p<0.001$, but for Ac7v when tested for the same ratio, a fit was obtained with $X^2=0.23$ and p=0.50-0.70.

Cross A was obtained by sib-mating two F_1 plants (from Cross 1) resistant to Ac7a but susceptible to Ac7v (RS) and 235 F_2 plants were tested. For Ac7a, there were 89 resistant and 146 susceptible plants. These plants did not fit any ratio tested, and for a 1R:1S segregation ratio for Ac7a, a $X^2=13.82$ with $p<0.001$ was obtained. Ninety-seven of these 235 F_2 plants were also inoculated simultaneously with Ac7v, and plants segregated for a 1R:1S ratio with $X^2=4.54$ and p=0.02-0.05. Cross B involved sib-mating F_1 plants from Cross 4 which were susceptible to Ac7a but resistant to Ac7v (SR) and F_2 populations segregated for Ac7v in a 13R:3S fashion with $X^2=0.0025$ and p=0.95-0.98. The parental genotypes for this cross would be $A^aBb \times aaBb$ (2×8). Proposed genotypes of the parents and F1 plants from which the observed ratios have been obtained are summarized in Table 6.

3.5.3 Backcross data analysis:

Plants derived from backcrosses of four F_1 plants (from four different crosses : Cr1, Cr2, Cr4 and Cr5) to the susceptible parent, Horizon of each cross were tested for reaction to Ac7a and Ac7v (Table 7). In some cases, few BCF_1 plants were available for screening because of seed abortion due to high temperatures at the time of crossing in the greenhouse. In cases where BCF_1 data corresponding to the F_2 data from the particular cross was unavailable, data from the reciprocal cross is presented. Only the BCF_1 data

from Crosses 2 and 4 are discussed as the F_1 plants used in these crosses correspond to one each of the F_1 plants used in producing F_2 generations from corresponding crosses and reciprocal crosses. B^{a}ckcross 2 (BC-2) fitted both a 3R : 1S and a 1R : 1S ratios, while the BC-4 did not fit either a 3R : 1S or a 1R : 1S ratio.

Data from BC-2 (B^{a}ckcross 2) fit a 3:1 ratio with X^2=0.06 and 3.62 with p=0.80-0.90 and 0.05-0.10 for Ac7a and Ac7v respectively (Table 7), but no genotypes could be assigned for the parents of such a backcross. Data from BC-2 also fit a 1:1 model with X^2=3.54 and 0.22 with p=0.05-0.10 and 0.50-0.70 respectively for Ac7a and Ac7v, and could arise from a cross of (A^{a}bb × aaBB) × aaBB [(5×7) × 7].

Seventy-four plants from BC-4 were tested for segregation for Ac7a and Ac7v but these plants gave X^2=5.40 and 4.36 and p=0.02-0.05 and 0.02-0.05 respectively for Ac7a and Ac7v respectively when tested for a 1R : 1S ratio. Since no genotypes could be assigned for the parents involved, the genotypes involved in the backcross cannot be predicted.

3.5.4 Analysis of linkage observed between reactions to Ac7a and Ac7v

Another aspect of the results of this research was the observed linkage of reaction between Ac7a and Ac7v. The independence tests for linkage of reaction to Ac7a and Ac7v was conducted for five F_2 crosses (cross 1 through cross 5) and chi-square values obtained were highly significant with p<0.001 (Table 3).

3.6. Discussion

According to the dominant-recessive epistasis model, resistance and susceptibility is controlled by two genes with complete dominance at both gene pairs. Assuming that these two genes are A and B determining the genetics of resistance and susceptibility, gene A when dominant (AA or Aa) produces a product which is epistatic to the product produced by gene B and resistance is conferred to the plant. When the recessive aa is present with the dominant gene B (BB or Bb), the product produced by aa is not epistatic to the product of gene B and the plant is susceptible. When the homozygous recessive of the second, B gene (bb), is present, the product produced by the recessive bb is epistatic to that of aa and confers resistance to the plant. Therefore resistance is conferred when the genotype of the plant is either A_B_, A_bb or aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb.

This model can be confirmed by taking susceptible F_2 plants to the F_3 generation, which should segregate in a 1R:3S or a 0R:1S fashion. If both the F_2 plants are homozygous susceptible (aaBB), then F_3 progeny will show no segregation for resistance i.e., all progeny will be susceptible. If at least one or both the parents are heterozygous for the second, non-inhibiting gene (aaBb), then it is possible that progeny will segregate in a 1R:3S fashion, which would confirm the dominant-recessive epistasis model. Although 235 F_2 plants derived from CrA were inoculated with Ac7a, 97 of these plants were also inoculated with Ac7v, in order to examine if these F_2 derived from F_1 plants which were resistant to Ac7a but susceptible to Ac7v would segregate for resistance to Ac7v. From Table 4, it is seen that 38 of 97 plants were resistant to Ac7v, indicating that

sibmating of two susceptible plants can give rise to resistant progeny. This result is comparable to what could be expected from sibmating of susceptible F_2 generation plants as described above, and lends support to the suggestion that the inheritance of resistance in *B. rapa* to Ac7a and Ac7v is governed by two dominant resistance genes interacting in a dominant-recessive epistasis.

Applying this model for Ac7a, the two genes are designated as A^a and B^a while for Ac7v, they are designated as A^v and B^v. Since phenotypic linkage of reaction to Ac7a and Ac7v was seen, it is suggested that there may be present a total of four genes (A^a=A gene for Ac7a, B^a=B gene for Ac7a, A^v=A gene for Ac7v and B^v=B gene for Ac7v) of which A^a and A^v are tightly linked and which independently act as inhibitor genes to B^a and B^v. It is also possible that there are only three genes (A, B^a and B^v), with a common A gene for Ac7a and Ac7v, which acts is an inhibitor gene to B^a as well as B^v, with B^a and B^v being unlinked to each other.

With respect to the linkage of reactions to Ac7a and Ac7v, it is possible that the genes controlling resistance to Ac7a and Ac7v are different, because of the abundance of RS and SR phenotypes which suggests presence of linkage at different loci (Table 3). Kole et al. (1996) have mapped a resistance gene, ACA1 in *B. rapa* controlling resistance to race Ac2 by linkage analysis using RFLP markers. They have found that the ACA1 locus was linked to the leaf pubescence locus (PUB1) and that the RFLP markers used in this study were conserved across other species of *Brassica.* A similar approach can be undertaken to map the resistance genes found in this study, and the map thus obtained

can be correlated to maps for *B. rapa* and other closely-related species to find out the position and linkage relationships of these genes with each other as well as to other molecular and morphological markers. Röbbelen (1960) analyzed pachytene chromosomes of *B. rapa* and proposed a genome constitution of AABCDDEFFF representing the ten chromosomes. These results were confirmed by Tang and Williams (cited in Chyi et al. 1992). Prakash and Hinata (1980) have hypothesized that the diploid Brassica species (n=7,8,9,10 and 11) have evolved by chromosome duplications. This theory is also supported by Attia and Röbbelen (1986) and Song et al. (1990). Song et al. (1991) supported the contention of the cytogenetic studies mentioned previously that *B. rapa* has evolved by chromosome duplications and suggested chromosomal rearrangement. Similar results were also seen by Chyi et al. (1991). Since phenotypic linkage of reactions to Ac7a and Ac7v was seen, it would be interesting to see if the proposed genes for resistance to Ac7a and Ac7v would map to the duplicated regions of the *B. rapa* genome.

The dominant-recessive epistasis model is proposed for explaining the genetics of resistance of *B. rapa* to *A. candida* races Ac7a and Ac7v. Further studies to confirm this model, and to clarify the inter-relationships of the genes mentioned in this study, are necessary.

Table 1. Reactions of selected *B. rapa* plants for producing Generation 1 and Generation 2/Parental Generation plants for resistance (R) and susceptibility (S) to races Ac7a and Ac7v.

Race\Cultivar	UM921	Horizon
Ac7a	[1]R	[2]S
Ac7v	R	S

Notes: 1:R=resistant; 2:S=susceptible

Table 2. Reactions of three classes of F_1 plants, selected to produce F_2 generations plants, to races Ac7a and Ac7v for resistance (R) and susceptibility (S).

Class of F_1\Race	Ac7a	Ac7v	Family of Crosses in which reaction occurred
[1]RR	[4]R	R	1-9
[2]RS	R	[5]S	1
[3]SR	S	R	4

Notes: 1:RR=resistant to both Ac7a and Ac7v; 2:RS=resistant to Ac7a but susceptible to Ac7v; [3]SR susceptible to Ac7a but resistant to Ac7v; 4:R=resistant; 5:S=susceptible

Table 3. Two-way tables for the independence tests for linkage of reactions to races Ac7a and Ac7v for F_2 plants from crosses 1 through 5.

Cross	[1]RR	[2]RS	[3]SR	[4]SS	Total	Chi-square
Cr 1	45	9	8	26	88	28.70
Cr 2	203	4	7	43	257	184.88
Cr 3	141	54	17	69	281	64.81
Cr 4	75	6	27	103	211	100.23
Cr 5	179	8	6	26	219	117.63

Notes:1:RR=resistant to both Ac7a and Ac7v; 2:RS=resistant to Ac7a but susceptible to Ac7v; [3]SR susceptible to Ac7a but resistant to Ac7v and [4]SS susceptible to both Ac7a and Ac7v

Table 4: Observed segregation for resistance (R) and susceptibility (S) for F_2 plants UM921 × Horizon inoculated with races Ac7a and Ac7v.

	Ac7a				Ac7v			
Cross	**Observed**	**Ratio**	**X^2**	**P**	**Observed**	**Ratio**	**X^2**	**P**
	[1]R : [2]S				**R : S**			
$^{RR}Cr1(r^4)$	54:34	5:3	0.04	0.80-0.90	53:35	5:3	0.19	0.50-0.70
		3:1	8.72	0.01-0.001		3:1	10.24	0.01-0.001
$^{RR}Cr2(r)$	207:50	13:3	0.07	0.70-0.80	210:47	13:3	0.026	0.70-0.80
$^{RR}Cr3(r)$	195:86	3:1	4.70	0.02-0.05	158:123	1:1	4.34	0.02-0.05
		5:3	5.69	0.01-0.02		5:3	4.70	0.02-0.05
$^{RR}Cr4(c^3)$	81:130	1:1	11.37	<0.001	102:109	1:1	0.23	0.50-0.70
$^{RR}Cr5(c)$	187:32	13:3	2.45	0.10-0.20	185:34	13:3	1.50	0.20-0.30
^{RS}CrA	89:146	1:1	13.82	<0.001	38:59	1:1	4.54	0.02-0.05
						1:3	10.38	0.01-0.001
^{SR}CrB	31:16	1:0	5.44	0.01-0.02	163:38	13:3	0.002	0.95-0.98

Notes: 1:R=resistant; 2:S=susceptible; 3:c=cross and 4:r=reciprocal cross; RR: derived from sib-matings of F_1 plants resistant to Ac7a and Ac7v.
RS: derived from sib-mating two F_1 plants resistant to Ac7a but susceptible to Ac7v; SR: derived from sib-mating two F_1 plants susceptible to Ac7a but resistant to Ac7v.

Table 5: The dominant-recessive epistasis model.

	AB	**Ab**	**aB**	**ab**
AB	AABB (1)	AABb (2)	AaBB (3)	AaBb (4)
Ab	AABb (2)	AAbb (5)	AaBb (4)	Aabb (6)
aB	AaBB (3)	AaBb (4)	**aaBB (7)**[1]	**aaBb (8)**
ab	AaBb (4)	Aabb (6)	**aaBb (8)**	aabb (9)

Note:Numbers in parentheses are the 9 genotypes of the dominant-recessive epistasis model. Genotypes in bold are the 2 susceptible genotypes, and the other 7 genotypes are resistant
Adapted from Strickberger (1985).

Table 6. Proposed genotypes of parents and F_1 plants and observed F_2 ratios for seven UM921 × Horizon crosses, for resistance (R) and susceptibility (S) inoculated with races Ac7a and Ac7v, segregating in a dominant-recessive epistasis model.

Cross	Races	Parents UM921 × Horizon	F1 genotypes	F2 ratios	Parental genotypes from table 6
^{RR}Cr1	Ac7a & Ac7v	-	-	-	-
^{RR}Cr2	Ac7a & Ac7v	AAbb × aaBB	AaBb × AaBb	13:3	5 × 7
^{RR}Cr3	Ac7a Ac7v	-	-	-	-
^{RR}Cr4	Ac7a Ac7v	-	-	-	-
^{RR}Cr5	Ac7a Ac7v	AAbb × aaBB	AaBb × AaBb	13:3	5 × 7
^{RS}CrA	Ac7a Ac7v	- aaBb × aaBb	- aaBb × aaBb	- 1:3	8 × 7/8
^{SR}CrB	Ac7a & Ac7v	AABb × aaBb	AaBb × AaBb	13:3	2 × 8

Notes: RR: derived from sib-matings of F_1 plants resistant to Ac7a and Ac7v.
RS: derived from sib-mating two F_1 plants resistant to Ac7a but susceptible to Ac7v.
SR: derived from sib-mating two F_1 plants susceptible to Ac7a but resistant to Ac7v.

Table 7: Observed segregation for resistance (R) and susceptibility (S) for backcross plants from (UM921 × Horizon) × Horizon inoculated with races Ac7a and Ac7v.

Cross	Ac7a				Ac7v			
	Observed [1]R : [2]S	Ratio	X^2	P	Observed R : S	Ratio	X^2	P
^{RR}BC-1	5:29	1:3	1.92	0.10-0.20	3:21	1:3	4.73	0.02-0.05
^{RR}BC-2	13:5	3:1	0.06	0.80-0.90	10:8	1:1	0.22	0.50-0.70
		1:1	3.54	0.05-0.10		3:1	3.62	0.05-0.10
^{RR}BC-4	27:47	1:1	5.40	0.02-0.05	28:46	1:1	4.36	0.02-0.05
^{RR}BC-5	40:39	1:1	0.01	0.90-0.95	35:44	1:1	1.02	0.30-0.50

Notes: 1:R=resistant; 2:S=susceptible; RR: derived from BC of 1 F_1 plant resistant to Ac7a and Ac7v with Horizon.

CHAPTER 4

Inheritance of resistance of the differential cultivars, Burgonde-A (*B. juncea*) to race Ac7a and Torch (*B. rapa*) to race Ac2.

4.1. Abstract

The differential cultivar, Burgonde-A (*B. juncea*) is susceptible to race Ac2 (its homologous race) but is resistant to Ac7a (heterologous race), while the differential cultivar, Torch (*B. rapa*) is susceptible to Ac7a (homologous race) but resistant to Ac2 (heterologous race). The inheritance of resistance of Burgonde-A to Ac7a and Torch to Ac2 is not known. The cultivars, UM3512 (*B. juncea*) and CRGC1-18 (*B. rapa*) are susceptible to both races Ac7a and Ac2. Plants that were resistant and susceptible to Ac7a from Burgonde-A and UM3512 respectively were selfed for two generations to increase homozygosity and to produce parental generations for making F_1 crosses, while for Torch and CRGC1-18, plants that were resistant and susceptible to Ac2 respectively were crossed pair-wise for one generation. Plants from each family of Torch and CRGC1-18, giving resistance and susceptibility respectively were sib-mated for one generation within themselves, to produce parental generation plants. All F_1 plants tested from crosses and reciprocal crosses of Burgonde-A × UM3512 were found to be resistant indicating that resistance is dominant and nuclearly inherited. Plants from each F_1 family, were selfed to produce F_2 generations, while F_1 plants were also backcrossed to both parents. F_2 data from one cross and two reciprocal crosses fitted a dominant-recessive

epistasis model. This showed that resistance to Ac7a in *B. juncea* for some crosses is controlled by two genes interacting in a dominant-recessive epistasis model. Other families did not fit a one-gene or two gene-model.

For Torch × CRGC1-18, although most plants were resistant, indicating that resistance is dominant and nuclearly inherited, F_1 crosses and reciprocal crosses segregated for resistance and susceptibility, showing that the parents involved in F_1 crosses were not homozygous for resistance and susceptibility. Resistant F_1 plants from each F_1 family were sib-mated to produce F_2 generations and also backcrossed to both parents. F_2 and backcross data were tested for a one-gene and all possible two-gene models, and data from four F_2 families, fitted a duplicate dominance model. Theoretical crosses of all eight resistant genotypes to the one susceptible genotype gave F_2 ratios such as 15:1, 7:1, 3:1 and 1:1. Data from the four F_2 families which did not fit any of the models were reanalysed, and one family fitted a 7:1 ratio, while the other families did not fit a one gene or any other two gene models. This suggests that resistance to Ac2 in *B. rapa* is controlled by duplicate dominant genes. The presence of dominant genes for resistance in *B. juncea* cultivar Burgonde-A to Ac7a and in *B. rapa* cultivar Torch to Ac2 confirms the finding of complementary dominant genes for avirulence to *B. juncea* and *B. rapa* to races Ac7a and Ac2.

4.2. Introduction:

Albugo candida (Pers.) Kuntze is a biotrophic, obligate fungus of the class Oomycetes capable of infecting several species of the family *Brassicaceae*. In North America, nine physiological races (race 1 through race 8 and race 10) of *A. candida* (Ac) have been identified and classified on the basis of a set of differential cultivars belonging to the various *Brassica* species which are infected. These are: race 1 on *Raphanus sativus* L., race 2 on *B. juncea* Czern & Coss, race 3 on *Armoracia rusticana* Gaertn., Mey., & Scherb., race 4 on *Capsella bursa-pastoris*, race 5 on *Sisymbrium officinale* (L.) Scop., race 6 on *Rorippa islandica* (Oeder) Borba's (Pound & Williams 1963), race 7 on *B. rapa* L. (Verma et al. 1975; Pidskalny & Rimmer, 1985), race 8 on *B. nigra* (L.) Koch (Delwiche & Williams, 1977), race 9 on *B. oleracea* L., and race 10 on *Sinapsis arvensis* (Hill et al., 1988).

Races of *A. candida* are generally more virulent on the *Brassica* species which they normally infect (homologous host species) but are also capable of infecting some genotypes of other closely related *Brassica* (heterologous host species). *A. candida* race 2 (Ac2) is generally virulent on *B. juncea* but is also capable of infecting some populations of *B. rapa* while Ac7a is virulent on *B. rapa* but can also infect some populations of *B. juncea*. The cultivar Burgonde-A (*B. juncea*) is susceptible to Ac2 but resistant to Ac7a while the cultivar Torch (*B. rapa*) is susceptible to Ac7a but resistant to Ac2, and both of these cultivars are part of the set of differential cultivars mentioned above. Using metalaxyl insensitivity and variation in pathogenicity as genetic markers, Liu and Rimmer (1993) found sexual recombinants in oospore progeny of crosses between

Ac2 and Ac7a. Results obtained suggest that, although these races of *A. candida* are generally more virulent on their respective homologous hosts, cross fertilization can occur in nature between isolates of Ac2 and Ac7a when they simultaneously infect the same host. Rimmer et al. (1995) studied the genetics of virulence of *A. candida* to *B. rapa* and *B. juncea* in crosses of Ac2 and Ac7a. Sexual progenies from crosses between two metalaxyl insensitive isolates of race Ac2 and one metalaxyl sensitive isolate of race Ac7a were tested on Burgonde-A and Torch. Four of twelve single pustule isolates were considered recombinants, and F_2 segregation data of these recombinants were analyzed. Avirulence to *B. rapa* was found to be dominant with three families fitting a 3 avirulent : 1 virulent model and two families fitting a 15 avirulent : 1 virulent model. In *B. juncea,* avirulence was found to be dominant, and fitted a 3 avirulent : 1 virulent model for some families. Therefore, these results suggest the presence of complementary alleles for resistance in the above-mentioned heterologous hosts to races Ac2 and Ac7a. The present study was undertaken to determine the genetics of resistance of Burgonde-A to Ac7a and Torch to Ac2.

4.3. Materials and Methods:

4.3.1 Inoculum Preparation:

Mature zoosporangia of Ac7a and Ac2 were collected separately from *B. rapa* cv. Torch and *B. juncea* cv. Burgonde-A in gelatin capsules (Parke-Davis Size 00) and stored in glass screw-cap vials at -10 °C. Inoculum preparation was according to the methods of Liu et al. (1988). Briefly, zoosporangia of both races were placed in Erlenmeyer flasks

containing distilled water, sealed with Parafilm^{tm}, and shaken gently. Flasks were incubated at 12 °C for 3 to 3.5 hours for induction of zoosporogenesis and then placed on ice to avoid zoospore encystment and the number of zoospores were quantified using a haemocytometer and inoculum concentration adjusted to 2×10^5 zoospores per millilitre.

4.3.2 Plant Populations:

The cultivars UM3512 [rapid cycling *B. juncea* selected from CRGC4-1 (Crucifer Genetics Cooperative, Madison, Wisconsin.), University of Manitoba, Winnipeg] and CRGC1-18 (rapid cycling *B. rapa*, Crucifer Genetics Cooperative, Wisconsin, Madison) are susceptible to both races Ac2 and Ac7a. To study the inheritance of resistance of the differential cultivar Burgonde-A to Ac7a, Burgonde-A (resistant parent) was crossed to UM3512 (susceptible parent) and crosses of Torch (resistant parent) and CRGC1-18 (susceptible parent) were made to study the inheritance of resistance of the differential cultivar Torch to Ac2 (Table 8). Plants were grown in conditions as described in Chapter 3.

4.3.3 Screening and Plant Selection:

Plants of the four cultivars were screened against appropriate pathogen races and 10 plants of Burgonde-A and Torch which were resistant to Ac7a and Ac2 respectively and 10 plants of UM3512 and CRGC1-18 which were susceptible to Ac7a and Ac2 respectively were selected for further study. Selected plants of Burgonde-A and Torch were transplanted into fibre pots containing a 2:1:1 (v/v/v) mixture of soil, sand and peat

and transferred to the greenhouse. Plants of UM3512 and CRGC1-18 were allowed to remain in the growth room at day/night temperatures of 22/17 °C with 18-h illumination and 6-h darkness.

4.4.4 Establishment of Parental Populations:

***B. juncea* (Burgonde-A and UM3512)**

The procedures for populations establishment are illustrated in Fig 5. Race Ac7a was used in this study. Selected plants of cultivars. Burgonde-A and UM3512 were bagged and allowed to set seed. Plants derived from this seed were termed Generation 1 (G1) seed and were screened against Ac7a. Two plants each from families in which most plants screened gave resistance or susceptibility for Burgonde-A and UM3512 respectively, were selfed to set seed giving rise to Generation 2 (G2). G2 plants of both Burgonde-A and UM3512 were screened and these plants designated as parental generation (PG) plants.

Crossing Pattern:

Seven crosses and reciprocal crosses of PG plants were made between Burgonde-A and UM3512 to generate F_1 seed. At the time of crossing, pollen of both Burgonde-A and UM3512 were collected on bee-sticks and stored in the freezer at -10 °C (Williams, 1980). F_1 plants were selfed to produce F_2 populations while F_1 plants were simultaneously back-crossed to both the parents using pollen as stored above.

Figure 5. Procedures for populations establishment for Generation 1, Generation 2/Parental Generation, F_1, F_2 and BC1 plants for *B. rapa* and *B. juncea*.

1. **PARENTAL SELECTION**
Screen 100 & select 10 Resistant (R) & 10 susceptible (S) plants
Burgonde-A UM3512
Torch CRGC1-18

2. **GENERATION 1 FAMILIES**
Selfing of Burgonde-A & UM3512
Pairwise Crosses within Torch & CRGC1-18

3. Screen & Select 2R (Burgonde-A) & 2S (UM3512)
Screen & Select 2R (Torch) & 2S (CRGC1-18)

4. **GENERATION 2 FAMILIES**
Self Burgonde-A & UM3512
Sibmate within Torch & CRGC1-18

5. Repeat step 3
PARENTAL GENERATION

6. **F_1 PLANTS**
Cross Selected Burgonde-A × UM3512
Cross Selected Torch × CRGC1-18

7. **F_2 GENERATION**
F_1 selfed to produce F_2 (Burgonde-A × UM3512)
F_1 sibmated to produce F_2 (Torch × CRGC1-18)

8. **BC1 PLANTS**
F_1 backcrossed to Burgonde-A & UM3512
F_1 backcrossed to Torch & CRGC1-18

***B. rapa* (Torch and CRGC1-18):**

The procedure for generating G1, G2, PG, F_1, F_2 and BC populations is given in figure 5 race Ac2 was used in this study. Plants of Torch and CRGC1-18 which showed resistance and susceptibility to Ac2 respectively were crossed pair-wise within cultivars and seed produced was termed as G1 seed. Plants from each G1 family were screened against Ac2, and 2-4 plants from families which had the most plants showing the required reaction were selected and sib-mated to produce G2 seed. G2 plants were screened and those plants which gave the required reaction were selected to constitute PG plants.

Crossing Pattern:

Eight crosses and reciprocal crosses of selected PG plants representing eight families of Torch and CRGC1-18 were made. Pollen of the parents involved in these crosses was stored as above. Two resistant F_1 plants were sib-mated to each other to produce F_2 generations while one plant was backcrossed to both the parents involved in the cross.

4.4. Data Analysis:

The chi-square test was used to determine segregation ratios of F_2 and backcross populations. The distribution of IP of F_2 *B. juncea* plants to Ac7a (Figure 6) and the distribution of IP of F_2 *B. rapa* plants to Ac2 (Figure 7) were plotted. Bimodal distributions with modes at IP 0 and 4 for *B. juncea* and IP 0 and 6 for *B. rapa* were observed. IP of 0 through 5 inclusive were taken as resistant, while IP 6 through 9

inclusive were taken as susceptible. Data were taken to fit the model at p=0.05 for which the tabulated chi-square value for one degree of freedom is 3.84. Any chi-square values that were equal to or less than the tabulated value were taken as statistically non-significant and the null hypothesis was accepted, and those above this value were taken to be significant and the null hypothesis was rejected.

4.5. Results:

4.5.1. Burgonde-A × UM3512

F_1 data analysis:

Plants from 7 F_1 crosses and reciprocal crosses of Burgonde-A × UM3512 were screened against Ac7a. All plants tested were found to be resistant suggesting that resistance is dominant and under nuclear control (Appendix 7.3).

F_2 data analysis:

F_2 data from Crosses (Cr) 1 through Cr6 and two reciprocal crosses (RCr) i.e., RCr 1 and RCr3 (Table 9), were analyzed for all possible segregation ratios for a one-gene and all possible two-gene models. None of the F_2 data from Burgonde-A × UM3512 fitted a one-gene model except Cr3, while data from two F_2 families (reciprocal crosses 1 and 3) fitted a 13R : 3S model. Data from Cr1 did not fit a 13R : 3S model.

Data from Cr2 fit a 5R : 3S ratio with X^2=0.04 and p=0.80-0.90, but no parental genotypes could be assigned to this ratio. Data from Cr3 for a 3R:1S ratio gave a

Figure 6. Distribution of interaction phenotypes of F_2 *B. juncea* plants to race Ac7a.

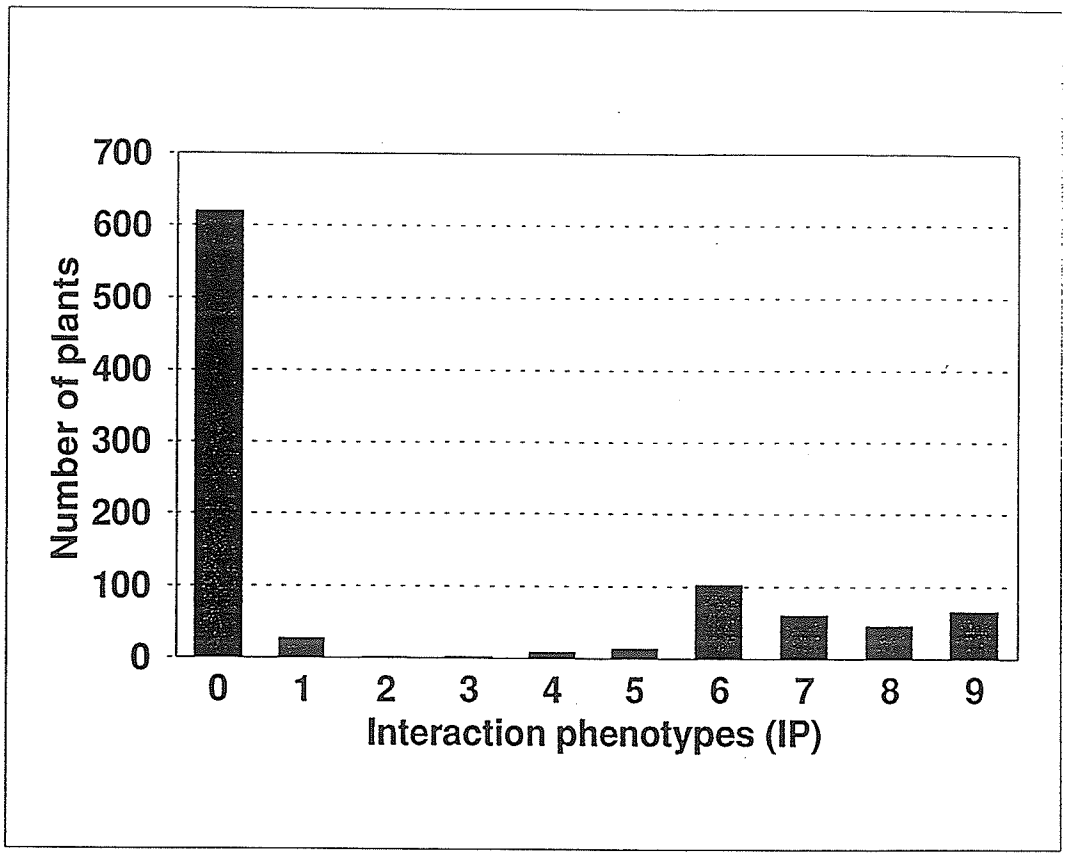
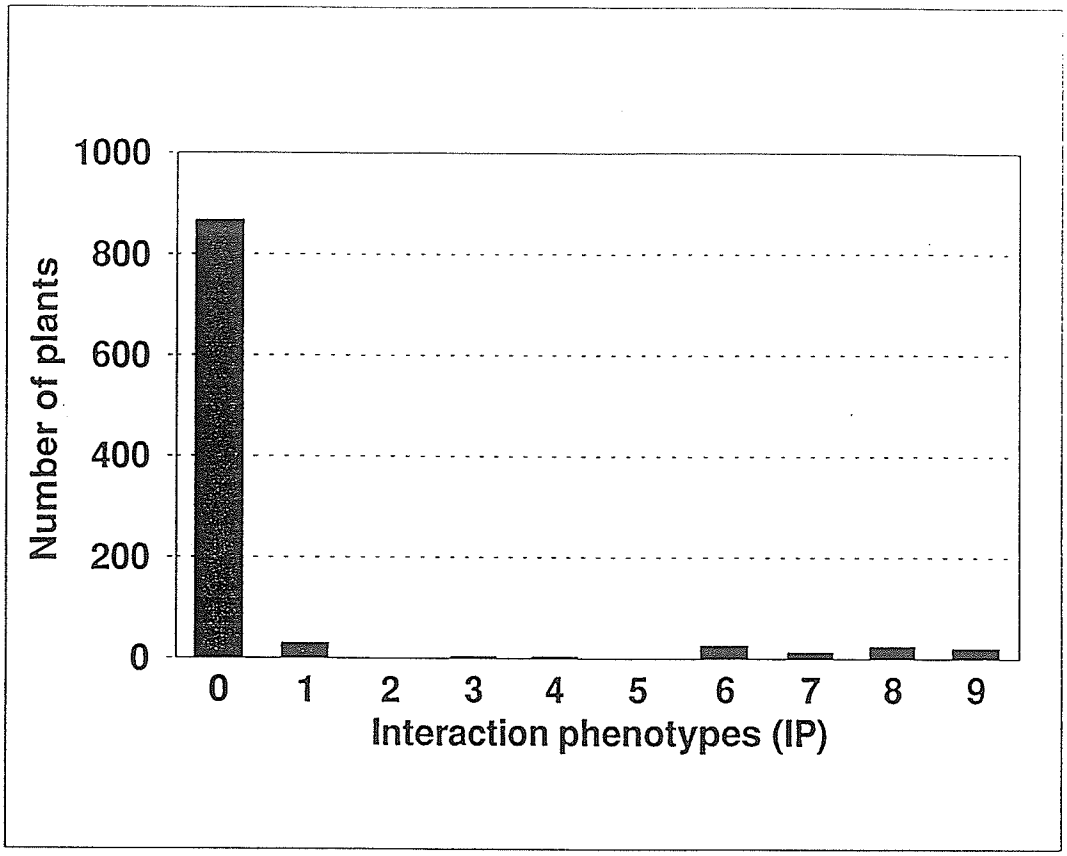


Figure 7. Distribution of interaction phenotypes of F_2 *B. rapa* plants to race Ac2



X^2=1.28 and p=0.20-030 which could be derived from crossing of genotypes derived from a dominant-recessive epistasis model. Genotypes of the parents for this cross could be AABB × aaBB (1 × 7 from table 3).

Data from Cr4, Cr5 and Cr6 fit a 7R : 1S ratio with X^2=0.52, 0.50 and 0.035 with p=0.30-0.50 for Cr4 and Cr5 and p=0.80-0.90 for Cr6, but no genotypes could be assigned to these ratios for a dominant-recessive epistasis model.

Data from RCr1 fit a 13R : 3S ratio (X^2=2.66 and p=0.10-0.20), and genotypes of the parents of this cross could be aaBB × AABb. Data from RCr3 fit a 13R : 3S ratio (X^2=0.37 and p=0.50-0.70) but the genotypes of the parents could not be fit for this ratio.

Analysis of Backcross Data:

Backcross data from only three crosses is presented, (Table 10), because of unavailability of sufficient seed due to seed abortion at the time of crossing. Backcross data from Cr1 × Burgonde$_1$ (B_1) fitted a 1R : 0S ratio with X^2's=1.88 and p=0.10-0.20. This ratio could arise from a cross of parents with genotypes (AABb × aaBB) × AABb [(2×7) × 2]. Cr3 × B3 segregated for a 1R : 0S model with X^2=1.96 and p=0.10-0.20 which is possible if the parents involved in the cross were [(AABB × aaBB) × aaBB]. A summary of the proposed genotypes of parents and F_1 plants with observed F_2 ratios for *B. juncea* segregating for Ac7a is given in Table 11.

4.5.2 Inheritance of resistance to Ac2 in *B. rapa* involving the cultivars Torch × CRGC1-18

F_1 data analysis:

F_1 plants from eight crosses of Torch × CRGC1-18 were screened against Ac2. Almost all crosses and reciprocal crosses segregated for resistance and susceptibility to Ac2 (Appendix 7.4).

F_2 data analysis:

Data from eight F_2 families (Cr1 to Cr6, and RCr2 and RCr3) were analyzed for a one-gene and all possible two-gene models (Table 12). Data from four F_2 families (Cr1, Cr2, Cr3 and Cr6) fit a 15R : 1S ratio with X^2=0.0003, 0.004, 1.20 and 0.54 and p=0.95-0.98 for Cr1 and Cr2, 0.20-0.30 for Cr3 and 0.30-0.50 for Cr4 respectively, while F_2 data from Cr5 did not fit a 15R : 1S ratio. These crosses did not fit a one-gene or any other two-gene model. The genotypes of the parents involved in the crosses Cr1, Cr2, Cr3 and Cr6 could be AABB × aabb (1×9 in Table 13).

Data from other F_2 families were examined to see if they fit a 15R : 1S model for two dominant genes. This was carried out similar to the analysis of the inheritance of resistance to Ac7a in *B. juncea*. Ratios obtained were varied and included ratios such as 15R : 1S, 14R : 2S (7R : 1S), 12R : 4S (3R : 1S) and 8R : 8S (1R : 1S).

Data from Cr4 fit a 7R : 1S ratio with X^2=2.43 and p=0.10-0.20 (genotypes of parents possibly being AaBB × aabb (3×9). Both RCr2 and RCr4 did not fit a one gene or any possible two gene models.

Backcross data analysis:

Table 14 shows the results of backcrosses of the resistant F_1 plants to the resistant parent (Torch) and the susceptible parent (CRGC1-18) wherever available. For the backcross plants from Cr_1 × CRGC $1\text{-}18_1$ (C_1), 16 plants were analyzed and they did not segregate for susceptibility, i.e., they gave a 1R:0S ratio (Table 14) but this data also fits a 3R : 1S ratio with X^2=1.33 and p=0.20-0.30. The latter ratio is more likely if the parents involved in this cross were (AABB × aabb) × aabb.

Backcrosses of Cr2, Cr3, Cr4, Cr5 and Cr6 to their respective resistant parents segregated for a 1R : 0S ratio. Cr2 × T_2 and Cr3 × T_3 gave X^2's=0.40 and 0.08 with p's=0.50-0.70 and 0.70-0.80 respectively, while data from Cr4 × T_4, Cr5 × T_5 and Cr6 × T_6 gave X^2's=0.00 with p's>0.99, as would be expected of crossing resistant F_1 plants to the resistant parent. These ratios could be obtained if the parents involved in the cross were (AABB × aabb) × AABB (1×9×1). Data from Cr3 × C3, Cr5 × C5 and Cr6 × C6 segregated in a 3R : 1S fashion with X^2s=0.09, 0.60, and 4.50 with p's=0.70-0.80, 0.30-0.50, and 0.02-0.05 respectively, confirming the results obtained from corresponding F_2 families. The genotypes of parents involved would be the same as in Cr1 × C1 segregating for a 3R : 1S ratio.

4.6. Discussion:

Inheritance of resistance of Burgonde-A to Ac7a:

Assuming from the F_2 data for RCr1 and RCr3, that resistance to Ac7a in *B. juncea* is controlled by two genes with a dominant-recessive epistatic interaction, resistance and susceptibility is controlled by two genes (A and B) with complete

dominance at both gene pairs. Gene A when dominant (AA or Aa) produces a product which is epistatic to the product produced by gene B and resistance is conferred to the plant. When the recessive allele aa is present with the dominant gene B (alleles BB or Bb), the product produced by allele aa has no effect on that produced by alleles BB or Bb, and susceptibility is seen. When the homozygous recessive of the second, B gene (bb), is present, the product produced by the recessive bb is epistatic to that of aa and confers resistance to the plant. Therefore resistance is conferred when the genotype of the plant is either A_B_, A_bb or aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb.

F_2 data from the other five families were examined to see whether they fit a dominant-recessive epistasis model. This was tested by applying the rules of the dominant-recessive epistasis model to all nine F_2 genotypes obtained by selfing of the classical di-hybrid Mendelian heterozygote, AaBb × AaBb (Table 5). Next, theoretical F_1 crosses between all possible combinations of resistant and susceptible parents were considered, and the segregation of the F_2 genotypes thus obtained from each of these theoretical crosses was examined by applying once again, the rules of the dominant-recessive epistasis model. Segregation ratios such as 14R : 2S (7R : 1S), 10R : 6S (5R : 3S), 12R : 4S (3R : 1S) and 13R : 3S were obtained. Data from Cr3 gave a 3R : 1S ratio which could be derived from the dominant-recessive epistasis model. Data from other crosses did not fit any of these ratios. This may be because, for UM3512 (the susceptible parent), although for Generation 1 plants plants with IP 7-9 were selected, even after one generation of selecting and selfing plants with such genotypes,

susceptibility was not seen and plants from Generation 2 families tended to have IP 5 or IP6. Therefore, a *B. juncea* population more susceptible to Ac7a and Ac2 is needed for a comprehensive understanding of the inheritance of resistance of the differential cultivar Burgonde-A to Ac7a.

Inheritance of resistance of Torch to Ac2:

Assuming from the F_2 segregation data for Cr1, Cr2, Cr3 and Cr6 that resistance to Ac2 in *B. rapa* is controlled by duplicate dominant genes (A and B), resistance would be expressed when either both or only one of the genes is present, either in a homozygous (AABB) or heterozygous form (A_bb or aaB_ or A_B_). Susceptibility will be seen only when the plant has no dominant alleles, i.e., when both genes are present in a homozygous recessive form (aabb). A summary of the proposed genotypes of the parents and F_1 plants producing the various ratios above mentioned is given in Table 15. Data from the other F_2 families were analysed according to the method of theoretical crosses mentioned earlier to see if they could be explained by a duplicate dominace model, and it was seen that only data from Cr4 could be explained by this method. Both of the models proposed for explaining the inheritance of resistance of the differential cultivars Burgonde-A and Torch to Ac7a and Ac2 respectively need to be confirmed. The presence of dominant genes for resistance to Ac7a and Ac2 in Burgonde-A and Torch respectively confirm Rimmer et al. (1995)'s suggestion of the presence of genes for resistance complementary to the dominant genes for avirulence present in races Ac2 and Ac7a of *A. candida.*

Table 8. Reactions of selected *B. juncea* and *B. rapa* plants for producing Generation 1 and Generation 2/Parental Generation plants for resistance (R) and susceptibility (S) to Ac2 and Ac7a.

Race	Host species			
	B. juncea		*B. rapa*	
	Burgonde-A	UM3512	Torch	CRGC1-18
Ac2	[2]S	S	[1]R	S
Ac7a	R	S	S	S

Notes: 1:R=resistant; 2:S=susceptible

Table 9. Observed segregation for resistance (R) and susceptibility (S) for F_2 plants from Burgonde-A × UM3512 inoculated with race Ac7a.

Cross	Observed [1]R : [2]S	Ratio	X^2	P
[3]Cr1	257:41	13:3	4.86	0.02-0.05
Cr2	190:111	5:3	0.04	0.80-0.90
Cr3	229:88	3:1	1.28	0.20-0.30
Cr4	279:35	7:1	0.52	0.30-0.50
Cr5	321:51	7:1	0.50	0.30-0.50
Cr6	250:37	7:1	0.03	0.80-0.90
[4]RCr1	268:48	13:3	2.66	0.10-0.20
RCr3	261:55	13:3 3:1	0.37 9.72	0.50-0.70 0.001-0.01

Note: 1:R=resistant; 2:S=susceptible; 3:Cr=cross; 4:RCr=reciprocal cross

Table 10. Observed segregation for resistance (R) and susceptibility (S) for backcross plants from (Burgonde-A × UM3512) × Burgonde-A or (Burgonde-A × UM3512) × UM3512 inoculated with race Ac7a.

Cross	Observed [1]R : [2]S	Ratio	X^2	P
Cr1 × $^3B_1$	26:7	1:0	1.88	0.10-0.20
Cr2 × B_2	28:21	1:1	1.00	0.30-0.50
Cr2 × $^4U_2$	23:7	3:1	0.04	0.80-0.90
Cr3 × B_3	41:10	1:0	1.96	0.10-0.20

Notes: 1:R=resistant; 2:S=susceptible; 3:B=Burgonde-A; 4:U=UM3512

Table 11. Proposed genotypes of parents and F_1 plants and observed F_2 ratios for six Burgonde-A × UM3512 crosses, segregating for resistance (R) and susceptibility (S) inoculated with race Ac7a.

Cross	Parents Burgonde-A × UM3512	F_1 genotypes	F_2 ratios	Parental genotypes from table 5
[1]Cr1	-	-	-	-
[2]RCr1	aaBB × AABb	AaBb	13:3	2 × 7
Cr2	-	-	5:3	-
Cr3	AABB × aaBB	AaBB	3:1	6 × 8
Rcr3	-		-	
Cr4	-	-	7:1	-
Cr5	-	-	7:1	-
Cr6	-	-	7:1	

Note: 1:Cr=cross; 2:RCr=reciprocal cross

Table 12. Observed Segregation for resistance (R) and susceptibility (S) for F_2 plants from Torch × CRGC1-18 inoculated with race Ac2.

Cross	Observed [1]R : [2]S	Ratio	X^2	P
[3]Cr1	196:13	15:1	0.003	0.95-0.98
Cr2	132:09	15:1	0.004	0.95-0.98
Cr3	318:16	15:1	1.20	0.20-0.30
Cr4	257:47	7:1	2.43	0.10-0.20
Cr5	259:05	15:1	8.53	0.001-0.01
Cr6	144:12	15:1	0.54	0.30-0.50
[4]RCr2	193:44	3:1	5.22	0.02-0.05
RCr3	220:48	3:1	7.17	0.001-0.01

Notes: 1:R=resistant and 2:S=susceptible; 3:Cr=cross; 4:RCr=reciprocal cross

Table 13. The duplicate dominance model.

	AB	Ab	aB	ab
AB	AABB (1)	AABb (2)	AaBB (3)	AaBb (4)
Ab	AABb (2)	AAbb (5)	AaBb (4)	Aabb (6)
aB	AaBB (3)	AaBb (4)	aaBB (7)	aaBb (8)
ab	AaBb (4)	Aabb (6)	aaBb (8)	**aabb (9)**[1]

Note: 1: Genotype in bold is the single susceptible genotype, and the other genotypes are resistant
Adapted from Strickberger (1985).

Table 14. Observed segregation for resistance (R) and susceptibility (S) for backcross plants from (Torch × CRGC1-18) × Torch or (Torch × CRGC1-18) × CRGC1-18 inoculated with race Ac2.

Cross	Observed [1]R : [2]S	Ratio	X^2	P
[3]Cr1 × [4]C_1	16:0	1:0		
		3:1	1.33	0.20-0.30
Cr2 × [5]T_2	84:06	1:0	0.40	0.50-0.70
Cr3 × T_3	42:05	1:0	0.08	0.70-0.80
Cr3 × C_3	41:15	3:1	0.09	0.70-0.80
Cr4 × T_4	58:0	1:0	0.00	>0.99
Cr4 × C_4	62:26	3:1	0.96	0.30-0.50
Cr5 × T_5	85:0	1:0	0.00	>0.99
Cr5 × C_5	53:14	3:1	0.60	0.30-0.50
Cr6 × T_6	68:04	1:0	0.22	0.50-0.70
Cr6 × C_6	44:06	3:1	4.50	0.02-0.05

Notes: 1:R=resistant; 2:S=susceptible; 3:Cr=cross; 4:C=CRGC1-18; 5:T=Torch

Table 15. Proposed genotypes of parents and F_1 plants and observed F_2 ratios for eight Torch × CRGC1-18 crosses for resistance (R) and susceptibility (S) inoculated with race Ac2, segregating in a duplicate dominance model.

Cross	Parents Torch × CRGC1-18	F_1 genotypes	F_2 ratios	Parental genotypes from table 13
[1]Cr1	AABB × aabb	AaBb × AaBb	15:1	1 × 9
Cr2 [2]RCr2	AABB × aabb	AaBb × AaBb	15:1	1 × 9
Cr3	AABB × aab	AaBb × AaBb	15:1	1 × 9
Rcr3	-	-	3:1	
Cr4	AaBB × aabb	AaBb × aaBb	7:1	3 × 9
Cr5	AABB × aabb	AaBb × AaBb	15:1	1 × 9
Cr6	AABB × aabb	AaBb × AaBb	15:1	1 × 9

Notes: 1:Cr=cross; 2:RCr=reciprocal cross

CHAPTER 5

GENERAL DISCUSSION

The inheritance of resistance of *Brassica* species to different races of *Albugo candida* has been previously studied by various workers (Fan et al. 1983; Tiwari et al. 1988; Verma and Bhowmik, 1989; Edwards and Williams, 1987), with resistance being dominant and under the influence of one or two genes. In the present study, resistance races 7a and 7v of *Albugo candida* in *Brassica rapa* was seen to be controlled by two genes interacting in a dominant-recessive epistasis model for some crosses. According to this model, resistance and susceptibility is controlled by two genes with complete dominance at both gene pairs. Assuming that these two genes are A and B determining the genetics of resistance and susceptibility, gene A when dominant (AA or Aa) produces a product which is epistatic to the product produced by gene B and resistance is conferred to the plant. When the recessive aa is present with the dominant gene B (BB or Bb), the product produced by aa is not epistatic to the product of gene B and the plant is susceptible. When the homozygous recessive of the second, B gene (bb), is present, the product produced by the recessive bb is epistatic to that of aa and confers resistance to the plant. Therefore resistance is conferred when the genotype of the plant is either A_B_, A_bb or aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb.

This model can be confirmed by taking susceptible F_2 plants to the F_3 generation, which should segregate in a 1R:3S or a 0R:1S fashion. If both the F_2 plants are

homozygous susceptible (aaBB), then F_3 progeny will show no segregation for resistance i.e., all progeny will be susceptible. If at least one or both the parents are heterozygous for the second, non-inhibiting gene (aaBb), then it is possible that progeny will segregate in a 1R:3S fashion, which would confirm the dominant-recessive epistasis model. Although 235 F_2 plants derived from CrA were inoculated with Ac7a, 97 of these plants were also inoculated with Ac7v, in order to examine if these F_2 derived from F_1 plants which were resistant to Ac7a but susceptible to Ac7v would segregate for resistance to Ac7v. From Table 4, it is seen that 38 of 97 plants were resistant to Ac7v, indicating that sibmating of two susceptible plants can give rise to resistant progeny. This result is comparable to what could be expected from sibmating of susceptible F_2 generation plants as described above, and lends support to the suggestion that the inheritance of resistance in *B. rapa* to Ac7a and Ac7v is governed by two dominant resistance genes interacting in a dominant-recessive epistasis. Applying this model for Ac7a, the two genes are designated as A^a and B^a while for Ac7v, they are designated as A^v and B^v. Since phenotypic linkage of reaction to Ac7a and Ac7v was seen, it is suggested that there may be present a total of four genes (A^a=A gene for Ac7a, B^a=B gene for Ac7a, A^v=A gene for Ac7v and B^v=B gene for Ac7v) of which A^a and A^v are tightly linked and which independently act as inhibitor genes to B^a and B^v. It is also possible that there are only three genes (A, B^a and B^v), with a common A gene for Ac7a and Ac7v, which acts is an inhibitor gene to B^a as well as B^v, with B^a and B^v being unlinked to each other.

Kole et al. (1996) have mapped a resistance gene, ACA1 in *B. rapa* controlling resistance to race Ac2 by linkage analysis using RFLP markers. They have found that

the ACA1 locus was linked to the leaf pubescence locus (PUB1) and that the RFLP markers used in this study were conserved across other species of *Brassica*. A similar approach can be undertaken to map the resistance genes found in this study, and the map thus obtained can be correlated to maps for *B. rapa* other closely-related species to find out the position and linkage relationships of these genes with each other as well as to other molecular and morphological markers. Since phenotypic linkage of reactions to Ac7a and Ac7v was seen, it would be interesting to see if the proposed genes for resistance to Ac7a and Ac7v would map to the duplicated regions of the *B. rapa* genome.

The dominant-recessive epistasis model is proposed for explaining the genetics of resistance of *B. rapa* to *A. candida* races Ac7a and Ac7v. Further studies to confirm this model, and to clarify the inter-relationships of the genes mentioned in this study, are necessary.

Liu and Rimmer (1993) have found sexual recombinants between oospore progeny in crosses of *A. candida* races Ac2 and Ac7a by using metalaxyl sensitivity and variation in pathogenicity as genetic markers. Results obtained from that study suggest that although these races are generally more virulent on their respective homologous hosts, cross-fertilization can occur in nature between isolates of Ac2 and Ac7a when they simultaneously infect the same host, giving the recombinants the ability to infect heterologous host species. Rimmer et al. (1995) studied the genetics of virulence of these races to *B. juncea* and *B. rapa*, and analyzed F_2 segregation data from these recombinants. They found that avirulence to *B. juncea* and *B. rapa* was dominant and controlled by one

or two genes, suggesting the presence of complementary alleles for resistance in the hosts to these heterologous races. In the present study, the inheritance of resistance of Burgonde-A to Ac7a was seen to be controlled by a single gene or two genes in a dominant-recessive epistasis for some crosses. Analysis of data from two reciprocal crosses show that, that resistance to Ac7a in *B. juncea* is controlled by two genes with interacting in a dominant-recessive epistatic model. These genes are, A and B, with complete dominance at both gene pairs. Gene A when dominant (AA or Aa) produces a product which is epistatic to the product produced by gene B and resistance is conferred to the plant. When the recessive allele aa is present with the dominant gene B (alleles BB or Bb), the product produced by allele aa has no effect on that produced by alleles BB or Bb, and susceptibility is seen. When the homozygous recessive of the second, B gene (bb), is present, the product produced by the recessive bb is epistatic to that of aa and confers resistance to the plant. Therefore resistance is conferred when the genotype of the plant is either A_B_, A_bb or aabb. The plant is susceptible only when the genotype of the plant is aaBB or aaBb.

F_2 data from the other five families were examined to see whether they fit a dominant-recessive epistasis model. Theoretical F_1 crosses between all possible combinations of resistant and susceptible parents were considered, and the segregation of the F_2 genotypes thus obtained from each of these theoretical crosses was examined by applying once again, the rules of the dominant-recessive epistasis model. Data from one cross could fit a dominant-recessive epistasis model. Data from other crosses did not fit any of these ratios. This may be because, for UM3512 (the susceptible parent), althogh

for Generation 1 plants plants with IP 7-9 were selected, even after one generation of selecting and selfing plants with such genotypes, susceptibility was not seen and plants from Generation 2 families tended to have IP 5 or IP6. Therefore, a *B. juncea* population more susceptible to Ac7a and Ac2 is needed for a comprehensive understanding of the inheritance of resistance of the differential cultivar Burgonde-A to Ac7a.

Resistance to Ac2 in *B. rapa* is seen to be controlled by duplicate dominant genes (A and B), for four F_2 families. Resistance would be expressed when either both or only one of the genes is present, either in a homozygous (AABB) or heterozygous form (A_bb or aaB_ or A_B_). Susceptibility is seen only when the plant has no dominant alleles, i.e., when both genes are present in a homozygous recessive form (aabb). Data from the other F_2 families which did not fit a duplicate dominance model were analysed according to the method of theoretical crosses mentioned earlier to see if they could be explained by a duplicate dominace model, and it was seen that only data from Cr4 could be explained by this method. Both of the models proposed for explaining the inheritance of resistance of the differential cultivars Burgonde-A and Torch to Ac7a and Ac2 respectively need to be confirmed. The presence of dominant genes for resistance to Ac7a and Ac2 in Burgonde-A and Torch respectively confirm Rimmer et al. (1995)'s suggestion of the presence of genes for resistance complementary to the dominant genes for avirulence present in races Ac2 and Ac7a of *A. candida.*

CHAPTER 6

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CHAPTER 7

Appendix 7.1 Reaction of 10 F_1 crosses and reciprocal crosses involving UM921 and Horizon, and their reaction to Ac7a and Ac7v for resistance and susceptibility.

	Ac7a R:S	Ac7v R:S
Cross1:	15:0	15:0
Recip:	15:5	12:8
Cross2:	19:1	16:4
Recip:	14:0	13:1
Cross3:	16:4	11:9
Recip:	10:5	10:5
Cross4:	14:1	14:1
Recip:	12:8	14:6
Cross5:	13:0	13:0
Recip:	20:0	20:0
Cross6:	13:1	14:0
Recip:	20:0	19:1
Cross7:	18:0	18:0
Recip:	15:0	15:0
Cross8:	19:0	19:0
Recip:	14:0	14:0
Cross9:	25:0	25:0
Recip:	19:1	19:1
Cross10:	21:3	19:5
Recip:	14:3	12:5

Note: R:Resistant and S:Susceptible plants

Appendix 7.2

Table 7.2.1:Two-way table for the Independence test for linkage of reaction to Ac7a and Ac7v and chi-squares for F_2 data from Cross 1.

Ac7v/Ac7a	R	S	Total	X^2
R	45 (a)	09 (b)	54	
S	08 (c)	26 (d)	34	
Total	53	35	88	28.70

Table 7.2.2: Two-way table for the Independence test for linkage of reaction to Ac7a and Ac7v and chi-squares for F_2 data from Cross 2.

Ac7v/Ac7a	R	S	Total	X^2
R	203(a)	04 (b)	207	
S	07 (c)	43 (d)	50	
Total	210	47	257	184.88

Table 7.2.3: Two-way table for the Independence test for linkage of reaction to Ac7a and Ac7v and chi-squares for F_2 data from Cross 3.

Ac7v/Ac7a	R	S	Total	X^2
R	141(a)	54 (b)	195	
S	17 (c)	69 (d)	86	
Total	158	123	281	64.81

Table 7.2.4: Two-way table for the Independence test for linkage of reaction to Ac7a and Ac7v and chi-squares for F_2 data from Cross 4.

Ac7v/Ac7a	R	S	Total	X^2
R	75(a)	06 (b)	81	
S	27 (c)	103(d)	130	
Total	102	109	211	100.23

Table 7.2.5: Two-way table for the Independence test for linkage of reaction to Ac7a and Ac7v and chi-squares for F_2 data from Cross 5.

Ac7v/Ac7a	R	S	Total	X^2
R	179(a)	08 (b)	187	
S	06 (c)	26(d)	32	
Total	185	34	219	117.63

Appendix 7.3. Reactions of 7 F_1 crosses and reciprocal crosses of Burgonde-A X UM3512 to race Ac7a.

	R:S
Cross1:	20:0
Recip:	10:0
Cross2:	20:0
Recip:	19:0
Cross3:	20:0
Recip:	20:0
Cross4:	20:0
Recip:	13:1
Cross5:	13:0
Recip:	15:1
Cross6:	20:0
Recip:	19:0
Cross7:	19:0
Recip:	19:0

Appendix 7.4. Reactions of 8 F_1 crosses and reciprocal crosses of Torch X CRGC1-18 to race Ac2.

	R:S
Cross1:	19:6
Recip:	20:3
Cross2:	15:7
Recip:	16:0
Cross3:	11:4
Recip:	19:1
Cross4:	16:6
Recip:	11:5
Cross5:	21:1
Recip:	27:0
Cross6:	17:0
Recip:	18:4
Cross7:	21:0
Recip:	24:0
Cross8:	08:11
Recip:	13:14