Inheritance of resistance in wheat to Septoria tritici

by Maarten van Ginkel

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology
Montana State University

Abstract:
Mycosphaerella graminicola, teleomorph of Septoria tritici, incites a disease of increasing importance in certain wheat growing areas of the world. In the last twenty years septoria tritici blotch has brought about substantial yield losses in the Mediterranean region, due to the susceptibility of the cultivars grown.

The reaction was studied of thirteen durum wheat cultivars used in breeding programs for the Mediterranean basin to thirty-four S. tritici isolates collected in seven countries of the region. Substantial differences in infection levels were observed between cultivars and between isolates. No significant differential interaction between cultivars and isolates could be demonstrated. On a country basis, the durum wheat derived isolates from Syria, where durum wheat is the predominant wheat species cultivated, effected the highest disease infection levels. Generation mean analyses indicated that additive gene effects were of prime importance, although dominance effects were often present. Epistasis seemed negligible. In the combining ability analyses, additive genetic variance proved of much greater magnitude than non-additive variance. Considering the combining ability effects, it appeared that different isolates in their encounter with the set of cultivars initiated the expression of similar genetic systems governing reaction to disease in the aegricorpus. Broad-sense heritabilities estimated for a large number of crosses averaged 38 percent. The average number of effective factors involved in the disease expression of a cultivar was seven.

The analyses did not indicate the presence of classical gene-for-gene relationships, but rather implied host-species specialization. Isolates ranked cultivars in a similar fashion. Cultivars thus appear to differ in horizontal resistance and isolates in aggressiveness. Pure line breeding and selection under disease pressure should result in raising levels of resistance to S. tritici in durum wheat, although progress may be slow.

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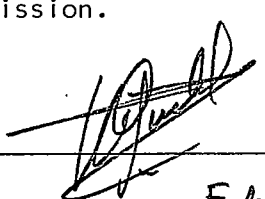
Signature 

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ABSTRACT

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CHAPTER 1

INTRODUCTION

In 1919, Walter Spurgeon Beach wrote in the first review of the septoria disease complex: "The genus is the more worthy of study on account of its high economic importance."

This insight was not immediately apparent from most other reports published prior to the mid-Sixties. *Septoria tritici* is one of those pathogens that is omnipresent, even where conditions are only marginally conducive. However, it often remains inconspicuous, affecting only the lower leaves in the early part of the growing season, when conditions are more humid and cooler. The amount of loss resulting from such low infection levels is unknown but since they occur as they often do almost every year in certain regions, such losses may be of economical significance.

In the last twenty to twenty-five years, attention to *S. tritici* on cereals has intensified. Reports of increased occurrence and disease levels, with occasional larger publicity surrounding regional epidemics, have led to a preeminent place for *S. tritici* in a number of research and breeding programs world-wide (Brown and Rosielle, 1980; Debela and Pinto, 1974; Dubin and Rajaram, 1981; Dutlu and Şölen, 1985; Ephrat et al., 1972; Eyal, 1972; Forni and Zitelli, 1979; Krupinsky et al., 1977; Mann et al., 1985; Mehta, 1976; Saari, 1974; Stewart et al., 1972; Tyagi et al., 1969).

Severe epidemics in the late Sixties, particularly in the North African and Middle Eastern countries bordering the Mediterranean Sea, have promoted the disease to one of continuing and prime importance in the national wheat breeding programs (Deghais and Maamouri, 1982; Djerbi et al., 1974a; El Ahmed et al., 1984; Ephrat et al., 1972; Saari, 1974; Stewart et al., 1972). Cooperative programs were organized and steady progress was achieved in raising the levels of resistance to *S. tritici* in the Mediterranean region (Anonymous, 1984; Ghodbane et al., 1976; Mann et al., 1985; Saari, 1974; Scharen, 1975; Stewart et al., 1972).

Yield losses from a few percent up to 60 percent have been attributed to natural infection by *S. tritici* (Brownell and Gilchrist, 1979; Debela and Pinto, 1974; Eyal, 1972; Mehta, 1976; Shipton, 1968). Using artificial inoculation procedures virtual crop destruction can be obtained, resulting in grain totally unfit for milling. Attempts have been made to develop predictive disease and loss models (Coakley et al., 1985; Eyal and Ziv, 1974; Forrer and Zadoks, 1983; Shaner and Finney, 1976).

More than half of the world's durum wheat is grown in the Mediterranean region, 44 percent in the Middle East and North Africa, with an additional 11 percent in Mediterranean Europe, the total accounting for 32 percent of the total wheat area in that region. Eighty percent of the durum wheat area in developing countries is in the Mediterranean region. In a number of these countries durum wheat is grown on more than two-thirds of their wheat area. More than 60 percent of the world durum wheat production is consumed in the Mediterranean area (Srivastava, 1984; Varughese et al., 1982).

The inheritance of resistance to *S. tritici* in the bread wheats had been elucidated to some extent by the early 1980's, although the combined behavior of a number of genes for resistance was largely unknown (Rosielle and Boyd, 1985).

The situation concerning durum wheats was different. While substantial and effective progress has been made on increasing levels of resistance and releasing improved cultivars and germplasm, no in-depth genetic study has been published centering on the inheritance of resistance in a number of durum wheat cultivars to an array of *S. tritici* isolates. The importance of utilizing various isolates has become especially important since a report of physiologic specialization in *S. tritici* was published by Eyal et al. (1973), bearing clear implications for breeding research (Eyal, 1981b; King et al., 1983).

The objectives of this research were to study the disease reaction in several durum wheat cultivars of importance in North Africa and the Middle East to endemic *S. tritici* isolates, to interpret the observed infection levels of the cultivars in terms of relative additive, dominance and epistatic genetic components of inheritance as well as combining abilities, to determine the order of magnitude for heritabilities of disease expression, to estimate the number of genes involved and to compare the effects of *S. tritici* isolates from varying origins on genetic components of the cultivars.

CHAPTER 2

LITERATURE REVIEW

The Pathogen

Nomenclature and Taxonomy

In 1842, Desmazieres reported the occurrence of *Septoria tritici* on wheat in France. Apparently the name was suggested by Roberge and thus the correct designation of the asexual state of the organism that incites the disease commonly known as speckled leaf blotch is *Septoria tritici* Rob. ex Desm. (Shipton et al., 1971).

Beach (1919) reported that the Septoria genus contained more than 1200 species. Most of these are known to be plant parasites, and about one hundred attack gramineous hosts, including cultivated cereals (Scharen and Sanderson, 1985).

More than a hundred years after the naming of the anamorph Sanderson (1972, 1976, 1977) identified *Mycosphaerella graminicola* (Fuckel) Schroeter as the teleomorph of *S. tritici*.

S. tritici belongs to the form class Fungi Imperfecti and the order Sphaeropsidales. *Mycosphaerella graminicola* is placed in the subdivision Ascomycotina, the class Loculoascomycetes, the order Dothideales and in the family Dothideaceae.

Although the taxonomic classification and nomenclature of the teleomorph and anamorph state have generally been accepted, some confusion

remained concerning the English common names for the two major septoria diseases involving cereal crops. Since 1922, the name "Speckled leaf blotch," suggested by Weber (1922) had been the most widely accepted for *S. tritici*.

The second International Septoria Workshop held at Montana State University in 1983 provided a unique opportunity for resolving this question and the following motion was passed by the participants:

> The taxonomic names of the fungi involved in the septoria disease complex would be based on their perfect state, namely *Leptosphaeria nodorum* E. Muller, *L. avenaria* Weber, f.sp. *triticea* T. Johnson, and *Mycosphaerella graminicola* (Fuckel) Schroeter, and that the common names of the diseases be septoria nodorum blotch, septoria avenae blotch and septoria tritici blotch. The lower case "s" will be used for septoria, and septoria nodorum, etc., would not be written in italics (Scharen, 1985).

Conditions required for the formation of the perfect state are not yet well understood and defined, and its occurrence has only been shown in New Zealand, Australia, the United Kingdom and the U.S.A. (Sanderson et al., 1985).

In this study the anamorph state was utilized and therefore the pathogen designation throughout this thesis will be *Septoria tritici*.

Disease Development

The main economic host of *S. tritici* is wheat (*Triticum* spp.), on which the symptoms develop seven to twenty-one days after fungal penetration. Other gramineae are also attacked (Brokenshire, 1975). Despite extended fungal growth, the onset of actual visible damage is generally delayed (Ride, 1975). All above-ground parts may be infected and develop characteristic lesions, although the symptoms and signs are

usually confined to the foliage. Root production may be reduced (Gough, 1976; Prestes, 1976).

The necrotic lesions, colonized by intercellular hyphae and occurring on adult green leaves, are often delimited by the larger veins. Under conditions of high humidity, asexual fruiting bodies called pycnidia may develop following hyphal aggregation in the substomatal cavities within the necrotic areas on both sides of the leaves. These structures are embedded in the host tissue and produce pycnidiospores which are released in a sticky suspension through the slightly raised ostiole (Hilu and Bever, 1957; Weber, 1922).

Wetting of the tissue leads to spore release. Once on the surface of the leaf the spores may be dispersed by rainsplash, presumably the major mechanism of inoculum spread during the growing season. Conidia expulsed from the pycnidia show a certain tolerance to desiccation for more than two months (Gough and Lee, 1985). Although spores may be sequentially exuded over a period of several weeks upon drying and rewetting, Eyal (1971) found no evidence of new pycnidiospore production. In Tunisia, Djerbi et al. (1977) observed that while the pycnidiospores do not survive the hot summer months, the conserved pycnidia, once again in favorable conditions, regenerate new pycnidiospores. As Shaner (1981) correctly states, additional work is needed on this aspect of fungal biology. This is especially important since it will have ramifications for our understanding of disease build-up over time and space.

Where the sexual state occurs ascospores may be a source of primary inoculum and be more widely spread by wind than pycnidiospores

(Sanderson and Hampton, 1978; Sanderson et al., 1985). Pycnidiospores may produce small colonies of micro- or secondary conidia on the leaf surface and thus give rise to an extra cycle of multiplication. These secondary and infective conidia may subsequently be dispersed by wind (Djerbi, 1972; Harrower, 1978). A similar phenomenon in artificial culture has been described by Djerbi et al. (1974b) and Jones and Lee (1974).

Infection ensues when free water is available for spore germination and fungal penetration (Brown and Rosielle, 1980). Spore germination was observed after twelve hours on a susceptible cultivar and penetration after twenty-four hours. Penetration may be stomatal or direct (Hilu and Bever, 1957). High humidity also enhances disease development and pycnidial formation (Hess and Shaner, 1985; Renfro and Young, 1956).

In the mediterranean region disease incidence is highest where rainfall exceeds 700 mm (Saari, 1974). Minimum, optimum and maximum temperatures for spore germination are 2-3°C, 22-26°C and 32°C, respectively (Weber, 1922). Infection is optimum at temperatures around 20-21°C. Increased temperature may partly compensate for a short period of high humidity. Similarly, a prolonged wet period has been shown to result in high disease levels despite relatively low temperatures (Hess and Shaner, 1985).

Although cultivar dependent, relatively low light intensities stimulated pycnidia production and pycnidiospore discharge (Benedict, 1971).

Inheritance of Resistance

Among *Triticum* Species.

Beach (1919) first reported on resistance in various *Triticum* species. An isolate collected from winter wheat did not produce characteristic symptoms in *T. compactum, T. dicoccum, T. polonicum* or *T. durum*. These same species plus *T. monococcum, T. spelta* and *T. turgidum* were shown to be susceptible to wheat isolates by Weber (1922). While reportedly finding no immune *T. vulgare* lines among the many tested, Mackie (1929) observed many resistant entries among other wheat species. Unfortunately these species are not further specified. While *T. compactum* and *T. vulgare* were susceptible, *T. durum* was virtually immune to isolates tested in Punjab, India (Luthra et al., 1938).

A majority of the *T. durum* cultivars tested by Hilu and Bever (1957) were very resistant, though not immune. Two of these were subjected to more detailed studies and had incubation periods four to ten days longer than the *T. vulgare* check.

Resistant entries were found among a total of 7500 wheats in the following species: *T. carthlicum, T. dicoccum, T. durum, T. polonicum* and *T. pyrimidale*, besides many in *T. aestivum* (Rosielle, 1972).

In California, many durum wheats and triticales were immune (Gul et al., 1975). *T. durum, T. polonicum, T. timopheevi* and a hexaploid triticale selection were shown to be more resistant than the *T. aestivum, T. compactum, T. dicoccum* and *T. spelta* entries tested (Brokenshire, 1976).

Resistant entries of *T. dicoccum, T. spelta* and *T. timopheevi* were identified after seedling inoculations by Krupinsky et al. (1977).

Also in Israel more resistance was available among *T. durum* and triticale entries than in spring bread wheat (Eyal, 1981b). Various sources of resistance were identified among wild *Triticum* species using seven isolates in seedling studies (Yechilevich-Auster et al., 1983).

In general, durum wheat entries tested in Tunisia were more susceptible than the bread wheat entries to the local *S. tritici* isolates (Djerbi and Ghodbane, 1975; El Ahmed et al., 1984; Ghodbane et al., 1976).

Although not a *Triticum* species, *Agropyron elongatum* entries exhibiting resistance have been used in crosses with *T. aestivum* to transfer observed resistance (Gough and Tuleen, 1979; Rillo et al., 1970). Other related genera have also been studied for resistance to *S. tritici* (Brokenshire, 1975; May, 1978).

Within *Triticum aestivum* and *T. durum*.

In most studies discussed above, various levels of resistance were identified within the bread wheat and durum wheat collections screened. Bread wheat cultivars with a winter growth habit appear generally to be more resistant than the spring types. In Israel the winter wheats tested, on average, even surpassed the durum wheats and triticales in resistance (Eyal, 1981b).

The earliest published genetic study on resistance to *S. tritici* was performed by Mackie (1929). He obtained 3:1 ratios of susceptible-to-resistant plants in F_2 progenies of crosses between, unfortunately, unidentified parents.

Rosen (1947) was able to transfer resistance by hybridization and to increase resistance levels by selection.

Using five disease reaction classes, Narvaez and Caldwell (1957) identified a single dominant gene operating in Lerma 52 and P14, and two partially dominant additive genes in Nabob.

The high resistance of the soft red winter wheat cultivar Bulgaria 88 appeared to be based on the presence of a single dominant gene. Almost three weeks after inoculation, gene expression seemed one of complete dominance. Seven to ten days later incomplete dominance was expressed, leading to a 1:2:1 segregation of resistant-intermediate-susceptible. Modifier genes were proposed to explain the appearance of some homogenous segregants with an intermediate disease reaction (Rillo and Caldwell, 1966, 1968).

Incorporation of the Bulgaria 88 resistance into the fairly susceptible background of Arthur 71 resulted in the cultivar Oasis, which was shown to yield about fifteen percent more than Arthur 71, when challenged with *S. tritici* (Shaner et al., 1975).

Resistance in the three cultivars studied by Rosielle and Brown (1979) was also oligogenic, depending on at least three recessive genes in the more resistant Seabreeze and possibly on only one gene in Veranopolis and IAS-20. The single dominant gene in Veranopolis was confirmed and a single dominant gene was proposed for Israel 493 by Wilson (1979). Of the twenty-eight sources of resistance studied in subsequent trials, the majority again appeared governed by single dominant genes with a maximum of two. Dominant, incomplete dominant and recessive genes were identified (Wilson, 1985).

Similarly, Danon et al. (1982) showed that the resistance in ten winter and spring wheats appeared to be based on relatively few genes,

in this case less than four or five, assuming additive inheritance and the presence of modifying genes. Resistance in Carifen 12 seemed conditioned by only one incompletely dominant gene (Lee and Gough, 1984).

Between one and eight resistance components were present in the sixty-three wild and cultivated wheats tested with seven fungal isolates, following a computer data analysis based on an incomplete Person's scheme (Yechilevich-Auster et al., 1983). The same computer program (Kampmeijer, 1981) was used to estimate the number of hypothetical genes in fourteen spring bread wheats, thirteen winter bread wheats, six durum wheats and three triticale lines, following testing with ninety-seven isolates. The number of genes again varied between one and eight (Eyal et al., 1985).

Many of the experiments quoted above have been executed using seedlings in the greenhouse. The question may be raised as to the relevance of these data to the reaction of adult plants and to the response under field conditions. Brokenshire (1976) has studied this aspect systematically and found that significant and quite large interrelationships existed between seedling and adult plant disease parameters. For example, the correlation coefficient between seedling disease score (percentage leaf area diseased) and flagleaf disease score was +0.71 in the greenhouse and +0.70 in the field. The correlations between greenhouse and field disease parameters were of similar magnitude and mostly significant. For example, the correlation coefficient between seedling disease score in the greenhouse and in the field was +0.66 and for flagleaf disease score the value was +0.79. The correlation of +0.85 between greenhouse seedling disease score and field

flagleaf disease score is especially encouraging, as greenhouse seedling tests are comparatively rapid, may be done in the off-season and procedures and environment can be largely standardized. Nevertheless, Brokenshire, noting a few exceptions, recommended subsequent field testing of promising material selected in the greenhouse. Scharen and cooperators follow this procedure (Krupinsky et al., 1977). A high correlation, +0.71, between greenhouse seedling disease scores and field adult plant reactions was also reported by Rillo and Caldwell (1968).

In order to conduct some of the qualitative analyses discussed above, many researchers have had to make somewhat arbitrary cut-points in essentially continuous scales describing percentage leaf area infected, pycnidial density or symptom severity.

Rosielle defined his 0-5 scale in 1972 based on symptom descriptions in which values of two or less are considered resistant. Wilson (1978, 1979, 1985) classified his material according to this 0-5 scale.

Narvaez separated resistant and susceptible at the 30 percent point of surface killed measured for the upper five leaves and furthermore used a four reaction class system (Narvaez and Caldwell, 1957).

Varying cut-points have been employed by Eyal and cooperators: 10 percent disease severity when selecting resistant, early maturing, short-strawed lines (Danon et al., 1982); 30 percent based on a regression of pycnidial coverage on isolate and cultivar variables (Yechilevich-Auster et al., 1983); 15 percent combined with a low Septoria Progress Coefficient (Eyal et al., 1983); and 16.6 percent based on cluster analysis of a 34 cultivars x 97 isolates matrix. Rillo and Caldwell (1966), Rillo et al. (1970) and Krupinsky et al. (1977)

distinguished on the basis of lesion size and shape plus pycnidia presence or absence.

In some of the genetic studies, disease expression in segregating material was evaluated relevant to the reaction of the two parents. The establishment of the total number of classes and the designation of intermediate segregants, however, remained somewhat arbitrary. Attempting to trace individual genes becomes especially awkward if the F_2-distribution is essentially continuous (Scott, cited in Wilson, 1985).

Other disease assessment scales have been used, such as the 0-9 Saari-Prescott scale or the enlarged Saari-Prescott scale (Anonymous, 1981; Saari and Prescott, 1975). Occasionally incubation period (Brokenshire, 1976), latency period (Brokenshire, 1976; Shearer, 1978), mycelial content of leaves (Harrower, 1977), pycnidiospore production (Gough, 1978), and sporulation intensity (Brokenshire, 1976) have been measured as an indication of disease.

Previous work indicates that the inheritance of resistance to *S. tritici* is relatively simple and oligogenically governed. Postulation of modifier genes, lack of clear discontinuities in segregating populations, evidence of transgressive segregation, disagreement on cut-points and environmental sensitivity, however, indicate that the search for single genes may be relatively ineffective, if not inappropriate.

If a more complex system is assumed to be the cause of varying levels of resistance, it may be more practical from a breeding standpoint and realistic from a genetical standpoint to employ techniques of

quantitative analysis, with an emphasis on obtaining estimates of parameters and mechanisms that can be manipulated by crossing and selection in order to raise the level of resistance.

Statistical and Genetical Analyses

Generation Mean Analysis

When traits are presumed to be controlled by several genes and variation is continuous, statistical procedures can help us gain information about their action. Using the means of several specific generations or populations derived from a cross, genetic effects can be calculated.

According to Hayman (1958, 1960), the expectations of the generation means of the six populations -- parent 1 (P_1), parent 2 (P_2), F_1, F_2, backcross P_1 x F_1 (BC_1) and backcross P_2 x F_1 (BC_2) -- may be expressed as follows, using the notations proposed by Gamble (1962a, 1962b).

$$P_1 = m + a - 0.5d + aa - ad + 0.25dd$$

$$P_2 = m - a - 0.5d + aa + ad + 0.25dd$$

$$F_1 = m + 0.5d + 0.25dd$$

$$F_2 = m$$

$$BC_1 = m + 0.5a + 0.25aa$$

$$BC_2 = m - 0.5a + 0.25aa$$

The gene effects, signifying specific sources of deviation from the designated mean, used in the above equations are defined as follows:

m = mean based on the F_2

a = pooled additive gene effects

d = pooled dominance gene effects

aa = pooled additive x additive epistatic gene effects

ad = pooled additive x dominance epistatic gene effects

dd = pooled dominance x dominance epistatic gene effects

The estimates of the six gene effects are obtained as:

$$m = \bar{F}_2$$

$$a = \overline{BC}_1 - \overline{BC}_2$$

$$d = -\tfrac{1}{2}\bar{P}_1 - \tfrac{1}{2}\bar{P}_2 + \bar{F}_1 - 4\bar{F}_2 + 2\overline{BC}_1 + 2\overline{BC}_2$$

$$aa = -4\bar{F}_2 + 2\overline{BC}_1 + 2\overline{BC}_2$$

$$ad = -\tfrac{1}{2}\bar{P}_1 + \tfrac{1}{2}\bar{P}_2 + \overline{BC}_1 - \overline{BC}_2$$

$$dd = \bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 + 4\bar{F}_2 - 4\overline{BC}_1 - 4\overline{BC}_2$$

Rowe and Alexander (1980) have published a procedure with which the weighted least squares analysis can be analyzed by computer using multiple linear regression. The various generation means not having equal variances are weighted using as weights the reciprocals of the squared standard errors of the generation means (Mather and Jinks, 1971).

To test if the terms included are sufficient to explain the observations, the deviation of expected from observed values can be tested on the assumption that the sum of squared deviations minimized in the fitting process has a chi-square distribution. Degrees of freedom are the number of population means available minus the number of gene effects fitted (Mather and Jinks, 1971). If the chi-square value is significant, that is, the particular model insufficiently fits the data, this may be due to random variation or it may be improved by extending

or modifying the model. Significance and standard errors of the gene effects can be calculated using regression techniques (Snedecor and Cochran, 1967).

If epistasis is significant, the additive and dominance gene effects are not uniquely measured due to interactions (Hayman, 1958).

Combining Ability Analysis

The analysis of a diallel involving crosses between all parents has often been used in order to evaluate the importance of specific genetic effects and the role of the parents in crosses, especially in corn and several other cross-pollinating crops.

Two kinds of combining ability estimates can be made. General combining ability (GCA) describes the average performance of a line in hybrid combinations, while specific combining ability (SCA) is a measure of the deviation of a particular cross from the value expected on the basis of the performance of the parents (Sprague and Tatum, 1942).

These values, their relative importance and some additional comparisons obtainable from the analysis enable breeders to select parents and breeding-selection strategies. Thus the absence of a significant SCA would indicate that performance is predictable on the basis of GCA values. A high GCA value of a parent does not necessarily indicate that the parent itself is a high performer (Gilbert, 1958).

According to Matzinger and Kempthorne (1956), GCA estimates contain additive and additive x additive genetic variances plus more complex epistatic variance involving additive effects. SCA includes dominance, additive x dominance, dominance x dominance and more complex epistatic

variance. Sokol and Baker (1977) showed that other than simple and higher order additive effects contribute to GCA. Thus dominance x dominance genotypic effects appear to be always included, while dominance effects are present in specific cases.

Baker (1978) suggested that because of the strict assumptions required concerning epistasis and independent gene distribution, and the sensitivity of the analysis to failure of these assumptions, diallel analyses should be restricted to estimating GCA and SCA mean squares and GCA and SCA effects.

Johnson (1963), discussing yield in barley, states that the assumptions of parental homozygosity, normal diploid segregation and no difference between reciprocal crosses in all probability apply, while the assumption of no multiple alleles and no linkage are probably unimportant. The sixth assumption, that of no non-allelic genic interaction, may be estimated.

Heritabilities

Heritabilities in which the individual plant is taken as the reference unit can be calculated using pooled variances among plants as population variances.

Environmental variance may be estimated using the genetically homogeneous populations, P_1, P_2 and F_1 (Allard, 1960).

Broad-sense heritability is then estimated using Allard's approach (1960):

$$h^2_{bs} = \frac{\sigma^2 F_2 - (\sigma^2 P_1 + \sigma^2 P_2 + \sigma^2 F_1)/3}{\sigma^2 F_2}$$

$\sigma^2 P_1$ = variance of the P_1 population

$\sigma^2 P_2$ = variance of the P_2 population

$\sigma^2 F_1$ = variance of the F_1 population

$\sigma^2 F_2$ = variance of the F_2 population

McNew (personal communication, 1986) supplied a formula to estimate the standard error of the h^2_{bs} equation:

$$S.E.h^2_{bs} = \left\{ \frac{1}{9} * \frac{2}{(\sigma^2 F_2)^2} * \left(\frac{(\sigma^2 P_1 + \sigma^2 P_2 + \sigma^2 F_1)^2}{dfF_2} + \frac{(\sigma^2 P_1)^2}{dfP_1} + \frac{(\sigma^2 P_2)^2}{dfP_2} + \frac{(\sigma^2 F_1)^2}{dfF_1} \right) \right\}^{\frac{1}{2}}$$

dfP_1 = degrees of freedom of the P_1 population

dfP_2 = degrees of freedom of the P_2 population

dfF_1 = degrees of freedom of the F_1 population

dfF_2 = degrees of freedom of the F_2 population

Narrow-sense heritability is often estimated using Warner's (1952) approach:

$$h^2_{ns} = \frac{2\sigma^2 F_2 - (\sigma^2 BC_1 + \sigma^2 BC_2)}{\sigma^2 F_2}$$

$\sigma^2 BC_1$ = variance of the BC_1 population

$\sigma^2 BC_2$ = variance of the BC_2 population

Ketata et al. (1976a) derived the following formula for the standard error of the h^2_{ns} equation.

$$S.E.h^2_{ns} = \left\{ \frac{2}{(\sigma^2 F_2)^2} * \left(\frac{(\sigma^2 BC_1 + \sigma^2 BC_2)^2}{dfF_2} + \frac{(\sigma^2 BC_1)^2}{dfBC_1} + \frac{(\sigma^2 BC_2)^2}{dfBC_2} \right) \right\}^{\frac{1}{2}}$$

In the above formulas the environmental components of variance are assumed of similar size in all plant generations used.

Effective Factors

An equation for the estimation of the minimum number of effective factors operating in a cross has been published by Burton (1951) and Burton and Fortson (1966), and was attributed to Sewall Wright.

$$n = \frac{0.25(0.75 - h + h^2)\ D^2}{\sigma^2 F_2 - \sigma^2 F_1}$$

n = minimum gene number

$h = \dfrac{\bar{F}_1 - \bar{P}_1}{\bar{P}_2 - \bar{P}_1}$

$D = \bar{P}_2 - \bar{P}_1$

$\bar{P}_1$ = mean of the smallest parent

$\bar{P}_2$ = mean of the largest parent

$\bar{F}_1$ = mean of the F_1 population

$\sigma^2 F_1$ = variance among F_1 plants

$\sigma^2 F_2$ = variance among F_2 plants

An unbiased estimate of n is obtained if the effective factors involved are not linked, of equal effect, not epistatic and one parent supplies only plus factors and the other only minus factors of those in which they differ, while the degree of dominance is the same for all plus factors. Gene number will be underestimated if assumptions do not hold and n is thus a conservative estimate.

The value $\sigma^2 F_1$ is used as an estimate of $\sigma^2 E$, the environmental variance, which, however, is more accurately estimated by $(\sigma^2 P_1 + \sigma^2 P_2 + \sigma^2 F_1)/3$. The value D, also sometimes called R, represents the range between extreme genotypes. If parents are used that both show some resistance, be it at different levels, they may both contain plus factors not present in the other and thus their difference does not represent that between the extreme genotypes possible with the genes involved in which they vary. The relevant assumption will be more closely adhered to if the extreme genotypes are selected from the F_2 and used to estimate D (Wright, 1968). The modified equation for the total number of effective factors is:

$$n_T = \frac{0.25(0.75 - h + h^2)\ (Y-X)^2}{\sigma^2 F_2 - [(\sigma^2 P_1 + \sigma^2 P_2 + \sigma^2 F_1)/3]}$$

X = lowest segregate in F_2

Y = highest segregate in F_2

The total number of effective factors involved, having been calculated on the basis of the range in the F_2, can be divided between the two parents of the relevant cross using a suggested relation published by Lawrence and Frey (1976):

$$n_{P_1} = \frac{(Y-P_1) + (P_2-X)n_T}{2(Y-X)}$$

$$n_{P_2} = n_T - n_{P_1}$$

A quite different approach to calculating the number of effective factors in a host-pathogen system is to subject a cultivar x isolate disease reaction matrix to a Person-analysis for incomplete Person

schemes (Person, 1959; Robinson, 1976). A major assumption is that classical gene-for-gene interactions operate in the *S. tritici* - *T. durum* aegricorpus. For the execution of the analysis it is necessary to ascribe a single value to each cell in the matrix; either the reaction is resistant or it is susceptible. This requires that, in the case of an essentially continuous scale, such as percentage leaf area affected, a cut-point must be established below which disease levels are designated resistant and above which they are classified as susceptible. Eyal et al. (1985) and Scharen et al. (1985) have used a cluster analysis of the cultivar x isolate matrix, based on Euclidean distance, to group cultivars in similar disease-response classes. The median between the two moderate response classes minus a standard error estimate obtained from the analysis of variance was used as the cut-point. Their percentage value, below which entries were termed resistant, was 16.6 percent in the case of *S. tritici*.

The computer program GENEALOGY analyzes the cultivar x isolate matrix, with each cell containing either a susceptible or a resistant designation or code. Smaller complete matrices are identified from the entire matrix, which represent interacting genes. The linked smaller matrices necessary to identify the entire matrix indicate the total number of resistance and virulence genes operating and how many each cultivar or isolate respectively contains (Kampmeijer, 1981). The program has been used by Eyal and cooperators to estimate the number of components and their assignment to cultivars and isolates of *S. tritici* and *S. nodorum* (Eyal et al., 1985; Scharen et al., 1985; Yechilevich-Auster et al., 1983).

CHAPTER 3

COMPARISON OF CULTIVARS AND ISOLATES

Introduction

The objective of this research was to study the inheritance of resistance to *S. tritici* in several durum wheat cultivars used in the Tunisian breeding program.

Requirements for the durum wheat cultivars to be studied were one or more of the following:

(a) field resistance to *S. tritici*,

(b) high yielding,

(c) expressing locally desired agronomic traits,

(d) proven wide adaptation in the region,

(e) good combining ability, based on frequent successful use in crosses.

Dr. H. Ketata, at the time working with INAT, Tunisia, supplied us with seed of entries considered of value to the program.

A large number of *S. tritici* isolates had been collected by Dr. A. L. Scharen over the years. Several isolates were available from the Mediterranean region, including a number from Tunisia.

The reaction of the selected durum wheat cultivars to inoculation with Mediterranean *S. tritici* isolates was studied in replicated greenhouse experiments.

Materials and Methods

The Hosts

The ten durum wheat entries from the Tunisian breeding program selected for further study are listed in Table 1. The additional three durum wheat entries are of Mediterranean origin and have been shown to contain some resistance in earlier studies.

Table 1. Durum wheat entries selected for study of inheritance of resistance to *S. tritici* and description.(1)

Entries	Description
Kyperounda	old resistant cultivar
Badri	grown to some extent
BD 2131	high yielding
BD 2127	high yielding
65150-Lds	not high yielding
D75-9-6B-5B-4B-10B	high yielding
D75-40-11B-4B-2B	high yielding
Ben Bechir 79	widely grown semidwarf cultivar
Karim 80	widely grown semidwarf cultivar
Maghrebi 72	widely grown semidwarf cultivar

Etit 38	land cultivar, Israel
Volcani 447	Israel
Zenati Bouteille	Tunisia

(1) Source: Ketata (personal communication, 1982).

Although not all durum wheat entries listed have cultivar status, the general term "cultivar" will be used throughout this text.

The Pathogen

Isolates were selected from seven countries in the Mediterranean area, including the Iberian Peninsula, and are listed in Table 2.

Table 2. Countries of origin, *S. tritici* isolate codes, and collection sources.

Country	Isolate Code	Source[1]
Tunisia	TUN 8201	Jori C69 (DW)[2]
	TUN 8202	$By_E x_E$/Tc//Zb/10/3 (DW)
	TUN 8202-1	DW
	TUN 8204-1	Swan "S"(DW)
	TUN 8205	Nr/Cno-Pj * Gll (BW)[3]
	TUN 8206	Ato "S"//Aa"S"/Plc"S"/3D67.2 (DW)
	TUN 8206-2	DW
Turkey	TKY 81218	N.A.[4]
	TKY 81262	N.A.
	TKY 81282	N.A.
	TKY 8201	Cakmak 79(DW); location: Kazan
	TKY 8202	Cumhuriyet '75 (BW); location: Pamukaren
	TKY 8205	Berkmen 469 (DW); location: Izmir
	TKY 8209	Hersonkaja 64 (DW); location: Izmir
Israel	ISR 8036 B1R1	N.A.
	ISR 8036 B1R2	N.A.
	ISR 8036 B1R3	N.A.
	ISR 8036 B1R4	N.A.
	ISR 80-6	Composite; location: Volcani-Bet Dagan
	ISR 80-8	BW; location: Volcani
	ISR 80-11	Lakhish (BW); location: Valley of Yisrael
Syria	SYR 8202	Plc"S"/Cr/Mca/31D673/Cit 71 (DW); location: N.E. Syria

Table 2--continued

Country	Isolate Code	Source[1]
	SYR 8205	Haurani (DW); location: N.E. Syria
	SYR 8206	Ful "S" (DW); location: Middle West Syria
	SYR 8207	Eider "S" (DW); location: Middle West Syria
	SYR 8209	By_E/Tc*//Gdo v 2512/Gdo (DW); location: Tel Hadya
	SYR 82-6	Location: Latakia
Portugal	POR 3	N.A.
	POR 8	N.A.
	POR 10	N.A.
	POR 11	N.A.
	POR 81199-1	N.A.
Italy	ITL 82024	N.A.
Spain	SPN 81299	N.A.

(1) As described by collector of infected leaf sample

(2) DW = durum wheat

(3) BW = bread wheat

(4) N.A. = information on origin of isolate not available

Each isolate was cultured from spores obtained from a single pycnidium on a leaf and maintained on solid yeast-malt agar in test tubes. The cultures were transferred to a fresh tube every fourteen days (Eyal, 1981a). Prior to inoculation of seedlings, the isolate was grown on liquid yeast extract medium for seven days. The solid and liquid media recipes are described in Table 3.

Table 3. Media recipes for yeast-malt agar and yeast extract shake flasks, and procedures, used for artificial culturing of *S. tritici*.

Yeast-malt agar	Yeast extract shake flasks
1) Add to 1000 ml flask: a) 2 g yeast extract, b) 2 g malt extract, c) 2 g sucrose, d) 10 g agar, e) Antibiotic: Kanamycin sulfate (0.66 g) and/or Gentamycin (5 ml) may be added prior to autoclaving. 2) Add 500 ml distilled water. 3) Thoroughly mix with magnetic stirrer. 4) Cover and autoclave for 20 minutes. 5) With syringe, add 5 cc to each test tube. 6) Cap and reautoclave for 20 minutes. 7) Allow to solidify in slanted position.	1) Add to 1000 ml flask: a) 5 g yeast extract, b) 5 g sucrose, 2) Add 500 ml distilled water. 3) Thoroughly mix with magnetic stirrer. 4) Pour 100 ml into 300 ml flasks. 5) Cover and autoclave for 20 minutes.

Disease Assessment

Ten seeds of each of the thirteen cultivars and of the check bread wheat cultivar, Fortuna, were sown in 21 x 21 x 6 cm square aluminum trays, containing a 1:1 mixture of sterilized soil and sand. The trays were set on greenhouse benches containing sand for germination. Ten days after planting, the seedlings had developed fully extended first leaves and partially emerged second leaves. The seedlings were then inoculated using the quantitative inoculation method described by Eyal

and Scharen (1977) with a 10^7 spores/ml suspension, containing a trace of ivory soap, functioning as surfactant and sticker. Each of the thirty-four isolates, listed in Table 2, was used to inoculate a separate tray with the fourteen cultivars. The trays were placed in a high humidity incubation chamber constructed in the greenhouse. Two cold-water humidifiers within the plastic enclosure misted the seedlings for a period of forty-eight hours. Following this period of exposure to water-saturated atmosphere, which facilitates spore germination and fungal infection, the trays were moved to the greenhouse benches. The temperature in the greenhouse was kept at 19-21^{o}C.

Twenty-one days after inoculation the disease infection of the first leaf of each seedling was visually assessed as percentage necrotic tissue of the total leaf area according to Eyal and Scharen (1977). *S. tritici* lesions are distinct and characteristic and may coalesce to some extent.

The trial as outlined above was run four consecutive times.

The data analyses of this trial and all other trials discussed in the following chapters were executed using BMDP-statistical programs on a CP-6 (Honeywell) system and MSUSTAT-programs on a Superbrain II (Intertec Data Systems) and an IBM Personal Computer XT.

Results

The mean value of each set of ten seedlings representing a particular cultivar in a tray was calculated. These values were used to execute an analysis of variance of the 14 cultivars x 34 isolates x 4

replications trial. Percentage necrotic leaf area over four replications is summarized in Table 4.

Significant differences ($P<0.001$) were determined between cultivars and between isolates with mean square values of 67790 and 2663, respectively. The cultivars, especially, covered a wide range of infection levels. No significant interaction could be shown between cultivars and isolates (mean square value 198). When the analysis was executed for individual countries similarly the interaction component remained small and insignificant, with mean square values ranging between 167 and 244. In Table 4 are also listed the results of a least significant difference pairwise multiple comparison of means at the 5 percent level of probability.

Discussion

Although tested with a large number of isolates originating from diverse locations, the cultivars showed distinct, often easily separable identities as to their overall reaction to infection by *S. tritici*. No complete resistance was observed, thus confirming a conclusion arrived at by many researchers. The lowest levels of infection were obtained on four recently developed cultivars, BD 2131, Karim 80, Volcani 447 and BD 2127, a testimony to successful breeding for resistance.

The check cultivar, Fortuna, showed a moderately high level of infection, while three other cultivars obtained higher mean percentages of infection. As the majority of *S. tritici* isolates originated from durum wheat cultivars, the intermediate reaction of Fortuna, a bread wheat cultivar, was not unexpected and confirmed earlier observations

Table 4. Mean percentage necrotic leaf area of the thirteen selected durum wheat cultivars and a bread wheat check cultivar infected with thirty-four *S. tritici* isolates, averaged over four replications.

	Cultivars														
Isolates:	Kyperounda	Badri	BD 2131	BD 2127	65150-Lds	D75-9-6B-5B-4B-10B	D75-40-11B-4B-2B	Ben Bechir 79	Karim 80	Maghrebi 72	Etit 38	Volcani 447	Zenati Bouteille	Fortuna	$\bar{x}_i$
TUN 8201	24	66	14	5	77	21	63	18	12	12	22	11	39	43	30 BCDEFGH[1]
8202	28	65	13	19	80	37	71	14	3	14	30	5	55	46	34 DEFGHIJ
8202-1	28	24	3	7	62	22	48	6	3	1	17	1	23	48	21 A
8204-1	20	64	2	3	69	23	65	23	1	8	20	4	20	32	25 ABC
8205	56	85	8	15	96	44	74	25	12	24	41	3	51	59	42 JKLM
8206	31	52	5	19	84	40	49	11	8	25	22	6	48	45	32 CDEFGHI
8206-2	38	66	20	32	79	26	68	30	28	40	31	23	55	51	42 JKLM
TKY 81218	22	51	3	3	63	33	71	19	7	10	10	6	30	50	27 ABCDE
81262	31	47	4	13	70	41	46	19	3	15	21	2	32	41	27 ABCDE
81281	36	77	21	24	94	31	61	35	21	34	31	21	53	68	43 KLM
8201	39	53	7	4	93	13	61	5	6	19	21	6	44	43	29 BCDEFG
8202	25	40	6	9	71	22	51	4	5	5	18	4	21	39	23 AB
8205	40	69	4	28	73	33	54	33	16	32	37	12	47	41	37 GHIJKL
8209	40	77	5	22	79	29	51	27	5	27	29	31	55	55	38 HIJKL
ISR B1R1	42	54	8	11	71	19	50	17	12	10	22	3	25	33	27 ABCD
B1R2	38	56	3	5	71	27	69	7	7	27	31	11	44	49	32 CDEFGHI
B1R3	22	53	3	4	72	9	57	19	1	8	22	5	32	42	25 ABC
B1R4	23	67	18	20	92	24	74	23	14	15	24	18	42	52	36 FGHIJKL
80-6	40	51	12	14	66	33	61	29	4	26	27	2	35	48	32 CDEFGHI
80-8	43	51	9	8	86	39	73	17	6	32	30	3	47	50	35 EFGHIJK
80-11	45	74	9	4	89	21	64	17	2	28	29	3	54	51	35 DEFGHIJ
SYR 8202	44	68	19	23	86	40	74	24	19	23	45	10	57	58	42 JKLM
8205	55	59	5	14	73	50	76	41	12	40	53	16	51	67	44 LM
8206	36	63	14	27	80	30	68	25	16	24	36	12	55	50	38 HIJKL
8207	42	79	30	26	91	43	83	43	25	41	39	26	55	74	50 M
8209	36	80	9	16	77	25	65	27	10	20	29	3	45	59	36 FGHIJKL
82-6	45	62	8	21	96	32	62	23	6	29	34	5	51	58	38 HIJKL
POR 3	43	64	18	21	77	30	70	16	12	31	28	10	47	49	37 GHIJKL
8	24	64	4	8	85	24	68	17	7	6	20	3	32	51	29 BCDEFG
10	22	60	3	5	68	14	37	12	4	19	14	5	37	51	25 ABC
11	47	69	3	6	70	36	61	35	1	24	29	14	39	51	35 DEFGHIJ
81199	26	41	3	19	72	28	60	7	2	29	17	5	42	48	28 ABCDEF
ITL 82024	56	66	7	6	88	38	73	30	8	24	33	14	59	46	39 IJKL
SPN 81299	29	45	2	13	52	22	54	18	2	23	25	2	39	53	27 ABCD
$\bar{x}$	36	61	9	14	78	29	63	21	9	22	27	9	43	50	
	D	G	A	A	H	C	G	B	A	B	C	A	E	F	

[1] Means not followed by the same letter are significantly different as tested by L.S.D. (0.05).

and research (Djerbi and Ghodbane, 1975; Eyal et al., 1985; Scharen and Mork, 1983). Eyal et al. (1985), in fact, assigned one hypothetical gene for resistance to Fortuna after analyzing a 35 cultivars x 97 isolates trial, in which most isolates were obtained from bread wheat cultivars.

On average the isolates collected in Syria from durum wheats showed a 10 percent higher infection level than those from other countries, averaging 32 percent necrotic leaf area. About two-thirds of the wheat area in Syria is devoted to durum wheat cultivation and the fungus may have become specialized in respect to its host species.

The cultivar x isolate interaction mean square value, 198, was less than 0.3 percent of the sum of the mean square values due to cultivars and due to isolates, 70423. The interaction component with a mean square value of 1514 constituted an identical proportion in the data published by Eyal et al. (1985). Their study included more cultivars and isolates, respectively 35 and 97, than the present study and, as the authors indicated, the large number of trials and plants recorded would easily result in statements of significance.

There was a lack of significant interaction in the present trial in which mainly durum wheat cultivars and many durum wheat derived isolates were used. In the trial by Eyal et al. (1985) involving bread wheat and durum wheat cultivars and isolates, the significance of the interaction component was largely due to the size of the experiment. In both trials, said component explained only an extremely small proportion of the variance. Combined, these factors may indicate little more than some adaptation of certain isolates to the wheat species from

which they were obtained. The true presence or absence of classical gene-for-gene relationships with physiologic specialization operating in the system studied by Eyal et al. (1985) cannot be easily further ascertained by inspecting and comparing smaller data sets involving cultivars and isolates from only one of the wheat species, since the mean data are not published as they are in Table 4 of this study.

If, indeed, cultivar x isolate interaction is absent when only one species is studied, but appears when both are included, then this may indicate that interspecific crosses which are generally possible between *Triticum* species can, in principle, be exploited to raise resistance levels.

The objective to study and distinguish the cultivars on the basis of their reaction to the different isolates showed the presence of a wide range of disease levels in the cultivars investigated. A similar array of infection levels due to the various isolates collected from distinct regions in the Mediterranean area was demonstrated.

CHAPTER 4

GENERATION MEAN ANALYSIS

Introduction

In order to obtain an understanding of the inheritance of resistance to *S. tritici* with direct implications to the breeder as to potentially successful breeding strategies, gene action was studied using a technique stemming from quantitative genetics, generation mean analysis.

This approach would not be hindered or confused by choices concerning disease reaction classes since infection was estimated on a continuous scale and analyzed as such, while polygenically governed resistance could be partitioned into components with practical relevance. Advantages of the analysis itself are that first order statistics with mean comparisons are used, which can be estimated with greater precision than higher order statistics. Thus a smaller number of experiments may theoretically be run. A disadvantage is that positive and negative gene effects may cancel one another out.

Several generations derived from each cross would be tested simultaneously and in replications, so environmental effects could be both minimized and estimated.

In order to elucidate resistance expression in various genetic backgrounds, generation mean analysis was not only applied to all

"resistant" x "susceptible" crosses, the common approach, but also to all other possible crosses, and their related populations, between the ten cultivars from the Tunisian breeding program plus some additional crosses. Since crosses were now included of parents with very similar disease levels, it was expected that in some cases no significant differences and gene effects would be detectable.

Materials and Methods

In 1983 and 1984, both in the field and in the greenhouse, all possible crosses and backcrosses between the ten cultivars were made, except for the reciprocals, and F_2 generations were obtained. Thus six generations were available for seedling testing in the greenhouse: parent 1 (P_1), parent 2 (P_2), F_1, F_2, backcross P_1xF_1 (BC_1) and backcross P_2xF_1 (BC_2).

Given the lack of cultivar x isolate interaction discussed in Chapter 3, and the desire to run as many replications as feasible, a single *S. tritici* isolate was selected for these trials. Requirements of the isolate to be chosen were:

(a) wide range of infection levels on the cultivars involved, based on the data points discussed in Chapter 3,

(b) good and consistent spore production in liquid yeast extract medium,

(c) of Tunisian origin, to ensure regional compatibility with the plant material, mainly obtained from Tunisia,

(d) above average ability to produce pycnidia in the relatively dry greenhouse environment.

One isolate in particular combined all the above requirements, TUN 8204-1.

Generation mean analysis was executed on $n(n-1)/2 = 45$ crosses and their related generations, F_2's and backcrosses. Additionally, the required six generations were tested for twenty other crosses, involving the ten "Tunisian" cultivars plus Etit 38, Volcani 447 and Zenati Bouteille, three additional sources of some resistance.

The amount of seed obtained from the sixty-five crosses plus progenies varied and thus more replications could be tested for certain crosses than for others. The minimum amount of replications was three, the maximum six. The amount of seeds sown for each generation per replication and the total are listed in Table 5.

Table 5. Number of durum wheat seedlings tested for each plant generation in the generation mean analysis.

Generation	No. of seedlings per replication	Range of total number of seedlings
P_1	5	15- 30
P_2	5	15- 30
F_1	5	15- 30
F_2	20	60-120
BC_1	7	21- 42
BC_2	7	21- 42

Since all seeds did not germinate or produce healthy seedlings deemed suitable for further uniform testing, the number of seedlings was occasionally somewhat less than indicated in Table 5.

One cross plus related progeny was planted per square aluminum pan. Inoculation procedures were as described in Chapter 3. Disease notes on percentage necrotic tissue of the first leaf were taken twenty-one days after inoculation. One week later presence or absence of pycnidia on the individual F_2 seedlings was recorded. These observations will be discussed in Chapter 5 in context with results on combining abilities.

For each cross the mean of the 5 to 20 plants representing a specific generation per replication was calculated and the general mean per generation over all replications was obtained.

Overall generation means were used in the generation mean analysis. After removal of replication effects, variance estimates of the generation means were calculated and their reciprocals used as weights in the multiple regression analyses.

Executing the analysis on every subset of the variables m, a, d, aa, ad and dd is the most thorough way of selecting the relevant variables. The size of deviation from regression mean squares indicates lack of fit and should be minimized to better fit the model. The distribution of the deviations sum of squares is assumed to follow a chi-square distribution. Low chi-square values coincide with high probabilities of greater values, indicating increased fit (the goodness-of-fit test).

The weighted generation means were regressed on the variable subset m, a and d, and subsequently on all seven extensions of this subset, involving one or more of the epistatic effects, aa, ad and dd. Thus for every cross, eight variable subsets were used for regression.

Initially the subset with the highest chi-square associated P-value for deviations from regression was selected for each cross. The associated deviations sum of squares was subtracted from the deviations sum of squares of the next simpler model or models and the result tested assuming a chi-square distribution, with the appropriate degrees of freedom. If the initially selected model was thus shown not to be significantly different at the 5 percent level from a simpler model, then the latter was selected as sufficiently explanatory, a procedure recommended by Snedecor and Cochran (1967). Significance and R^2 values were calculated. Standard errors of the gene effects were estimated by taking the root of the multiplication between the appropriate Gauss multipliers from the inverse of the weighted matrix containing the genetic expectations and the appropriate mean square error term (Snedecor and Cochran, 1967).

Results

The subset of variables m, a, d, aa, ad and dd showing the best fit and being the most simple was selected for each cross. The gene effects of these subsets, their standard errors and levels of significance, and the P-value associated with the chi-square value of the deviations sum of squares along with the R^2 values are presented in Table 6.

The relevant values for the additional crosses involving Etit 38, Volcani 447 and Zenati Bouteille are listed in Table 7.

Table 6. Estimates, standard errors and levels of significance of gene effects for the 10 x 10 diallel cross, plus the P-value associated with the chi-square value of the deviations sum of squares for the model and the R^2 values, for percentage necrotic leaf area of durum wheat seedlings due to infection by *S. tritici* isolate, TUN 8204-1.

	m	a	d	aa	ad	dd	P-value	R^2
Kyperounda x								
Badri	84.3± 7.9***	-16.7±10.8	-72.7± 42.7	-78.8± 41.3		153.3± 65.7*	>.95	1.00
BD 2131	64.3± 3.5***	10.5± 5.1	-115.1± 2.7***	-109.1± 27.4***		148.1± 57.0*	.83	1.00
BD 2127	59.0± 5.4***	28.4± 8.3***	-2.3± 17.8				.30	0.98
65150-Lds	64.9± 3.6***	-13.2± 6.8	22.0± 11.1				.21	0.99
D75-9-6B-5B-4B-10B	45.6± 7.1***	5.7± 8.0	-9.0± 19.7				.23	0.93
D75-40-11B-4B-2B	72.7± 3.2**	-8.1± 4.7	-10.3± 9.3				.84	1.00
Ben Bechir 79	50.8± 5.7***	1.5± 6.8	-4.4± 11.9			99.7± 32.4**	.29	0.98
Karim 80	51.7± 2.7***	15.1± 4.8**	15.9± 7.1*				.41	0.99
Maghrebi 72	58.8± 3.9***	17.9± 7.3*	21.2± 10.9				.23	0.96
Badri x								
BD 2131	60.3±13.7***	18.3±59.3	-109.7±168.1	-91.4±131.8		199.4±322.5	>.95	1.00
BD 2127	50.4± 2.7***	14.9± 5.2*	9.3± 11.4				.36	0.99
65150-Lds	89.6± 1.7***	-6.5± 3.4	-10.5± 10.5				.87	1.00
D75-9-6B-5B-4B-10B	60.4± 2.9***	16.4± 8.0	-12.5± 12.3				.08	0.97
D75-40-11B-4B-2B	68.4± 3.6***	-4.9± 5.1	-18.0± 11.5				.57	1.00
Ben Bechir 79	63.5± 6.4***	20.9±27.3	23.6± 55.1				.64	1.00
Karim 80	45.3±14.5*	29.6±28.7	-18.0± 73.9				.29	1.00
Maghrebi 72	63.3± 3.9***	27.1± 6.1***	18.3± 11.4				.27	0.97
BD 2131 x								
BD 2127	49.2± 3.2***	-11.1± 6.8	-31.2± 13.2*				.24	0.96

Table 6--continued

	m	a	d	aa	ad	dd	P-value	R^2
65150-Lds	81.7± 3.4***	-39.7± 3.5***	-40.4±15.8*	-45.5±11.2***			.16	0.99
D75-9-6B-5B-4B-10B	18.3± 1.8***	12.1± 5.5	-7.7± 4.6		21.3±6.3**		.08	0.93
D75-40-11B-4B-2B	52.4± 3.6***	-26.7± 6.2***	-9.7±10.0				.40	0.97
Ben Bechir 79	18.7± 3.6***	1.0± 6.9	-3.9±13.2				.68	0.97
Karim 80	21.3± 5.1***	0.6±10.0	-29.0±24.4				.34	0.91
Maghrebi 72	21.8± 3.7***	-0.1± 8.8	-7.1± 9.4				.49	0.95
BD 2127 x								
65150-Lds	62.6± 3.7***	27.9± 6.5***	-2.5± 9.7				.69	0.99
D75-9-6B-5B-4B-10B	31.8± 3.2***	0.6± 5.3	-25.6± 9.7*				.64	0.96
D75-40-11B-4B-2B	66.6± 2.7***	27.7± 4.9***	-9.4± 6.9				.13	0.98
Ben Bechir 79	45.3± 2.6***	-4.9± 2.9	-4.6± 6.7			67.9±17.0***	.36	0.99
Karim 80	32.7± 2.4***	3.6± 4.4	-17.3±11.7				.22	0.95
Maghrebi 72	49.9± 3.3***	-13.7± 6.7	-5.8±11.3				.32	0.97
65150-Lds x								
D75-9-6B-5B-4B-10B	87.7± 8.5***	36.7± 8.8***	-162.9±48.3**	-168.1±40.6***		277.8±76.7***	.16	0.99
D75-40-11B-4B-2B	71.8± 4.4***	6.8± 7.4	7.9±17.1				.25	0.97
Ben Bechir 79	75.5± 2.9***	25.4± 5.3***	-13.4± 9.6				.34	0.98
Karim 80	67.2± 4.7***	27.3± 6.7***	-2.4±13.3				.79	0.99
Maghrebi 72	97.4± 4.0***	21.4±12.6	-6.4±13.5	-22.0±16.0			.30	1.00
D75-9-6B-5B-4B-10B x								
D75-40-11B-4B-2B	51.5± 4.2***	-21.2± 6.0***	-29.9±12.6*				.11	0.97
Ben Bechir 79	40.7± 6.7***	8.4± 9.5	-88.3±38.1*	-90.4±34.9*		172.7±60.2*	.88	1.00

Table 6--continued

	m	a	d	aa	ad	dd	P-value	R^2
Karim 80	36.4± 2.8***	-3.5± 4.7	-13.6± 8.1				.15	0.93
Maghrebi 72	25.4± 3.9***	4.4± 7.6	-11.5±20.9				.16	0.92
D75-40-11B-4B-2B x								
Ben Bechir 79	73.6± 5.9***	17.4±11.7	12.5±12.2				.21	0.99
Karim 80	56.2± 6.0***	28.0±12.3*	-16.0±14.1				.71	0.99
Maghrebi 72	81.3± 7.6***	31.0± 5.9***	-157.8±47.6**	-161.9±46.1**		278.2±79.0**	.74	1.00
Ben Bechir 79 x								
Karim 80	53.7± 2.8***	8.6± 7.5	-13.4±15.4				.35	0.99
Maghrebi 72	30.0± 4.1***	1.8± 6.1	-5.4±11.6				.46	0.92
Karim 80 x								
Maghrebi 72	52.8± 5.6***	-4.7± 4.4	-118.8±27.3***	-105.8±26.1***		168.1±38.6***	>.95	1.00

*, **, ***: Significant at the 0.05, 0.01, and 0.005 level of probability, respectively.

Table 7. Estimates, standard errors and levels of significance of gene effects for twenty crosses involving Etit 38, Volcani 447 and Zenati Bouteille, plus the P-value associated with the chi-square value of the deviations sum of squares for the model and the R^2 values, for percentage necrotic leaf area of durum wheat seedlings due to infection by *S. tritici* isolate, TUN 8204-1.

	m	a	d	aa	ad	dd	P-value	R^2
Badri x								
Etit 38	64.0±28.2*	4.5± 6.6	-7.0±57.5				>.95	1.00
Zenati Bouteille	69.3± 3.0***	-1.7± 5.7	-9.7±12.8				.50	1.00
BD 2131 x								
Etit 38	60.3± 4.5***	-30.8± 8.5***	10.6±12.6				.49	0.98
Zenati Bouteille	40.7± 2.5***	-30.2± 4.4***	-17.3± 5.8**				.26	0.97
65150-Lds x								
Etit 38	68.2± 5.1***	22.8± 7.2**	-14.1±12.0				.77	0.99
Zenati Bouteille	77.1± 3.6***	13.3± 6.2*	-23.3±16.6				.80	1.00
D75-9-6B-5B-4B-10B x								
Etit 38	64.5± 4.8***	-10.3± 6.0	-2.5±13.5				.49	0.98
Volcani 447	29.4± 3.1***	6.9± 4.8	-19.6± 9.6				.28	0.88
Zenati Bouteille	67.8± 7.4***	-20.1± 8.1*	-112.2±44.0*	-105.8±36.0*		181.4±71.4*	.75	1.00
D75-40-11B-4B-2B x								
Volcani 447	50.9± 5.2***	30.7± 7.4***	-14.3±20.0				.33	0.99
Zenati Bouteille	66.4± 5.0***	0.7± 9.9	-38.5±11.9**				.37	1.00
Ben Bechir 79 x								
Etit 38	45.1± 5.3***	25.5±10.6*	2.4±22.6				.36	0.95

Table 7--continued

	m	a	d	aa	ad	dd	P-value	R^2
Volcani 447	52.6± 4.0***	19.6±10.9	-145.9±40.8***	-122.5±35.4***		178.2±76.7*	.86	1.00
Zenati Bouteille	59.2± 3.3***	-20.0± 6.4**	-5.4±10.9				.53	0.99
Karim 80 x								
Etit 38	65.3± 3.4***	-13.9±10.7	-110.7±47.9*	-105.2±40.5*		148.1±92.9	>.95	1.00
Volcani 447	39.0± 3.4***	-7.2± 7.1	-31.6± 8.9***				.79	0.98
Zenati Bouteille	54.9± 4.7***	-21.3± 7.3*	-30.9±14.9				.56	0.99
Maghrebi 72								
Etit 38	39.8± 2.8***	-9.7± 4.6	-28.4± 9.5*	-24.5± 8.6*			.81	1.00
Zenati Bouteille	63.2± 3.3***	-20.8± 8.0*	-5.0±15.3				.20	0.98
Etit 38 x								
Volcani 447	50.0± 6.1***	14.4± 3.6***	-121.7±28.7***	-110.7±27.1***		179.4±39.1***	.02	0.96

*, **, ***: Significant at the 0.05, 0.001, and 0.005 level of probability, respectively.

Discussion

The fit of the models listed in Tables 6 and 7 was, in almost all cases, very close as indicated by the high R^2 values and high P-values associated with the deviations sums of squares. The proportion of variance explained by the respective models was high and the probability of having residual sums of squares of the small size observed was similarly high.

In very few cases were epistatic gene effects required to explain differences among the generation means. It may be concluded that overall epistatic effects were of minimal importance. In the cases where epistasis did prove to play a significant role, it was additive x additive and/or dominance x dominance in nature, except for one case, where additive x dominance epistasis was present. The latter component estimates digenic interaction effects between the heterozygous condition at one locus and the alternate homozygous conditions at another locus. Some gene effects may also be cancelled to a certain extent due to the simultaneous presence of positive and negative components.

Since in a number of crosses the generation means did not vary much, presumably due to similar components of resistance in the parents, significant gene effects could not always be detected.

Significant additive gene effects occurred more frequently than dominance gene effects. Including some cases where the 5 percent level of significance was closely approached, more than 50 percent of the crosses had a significant additive gene effects component. In about one-third of the crosses dominance gene effects were important. The

size of the significant additive gene effects determined in the various trials varied in absolute values between 6.5 and 39.7 with an average of 22.0 percent necrotic leaf area. Dominance gene effects were negative in most cases and varied greatly between 15.9 and 162.9 with an average of 67.5 percent necrotic leaf area. The mostly negative sign of the dominance component indicated that in certain hybrid combinations disease levels could be decreased relative to the mid-parent. The additive x additive epistatic gene effects operated in reducing disease infection levels, while the dominance x dominance component enhanced necrotic leaf area.

Because six generations were used, fitting the model involving m, a, d, aa, ad and dd would, of necessity, result in a perfect fit, thereby not allowing a normal estimation of significance of the gene effects. In all but one cross, the P-values associated with deviations sums of squares were so large that less than six parameters adequately described the observations. The last cross listed in Table 7, Etit 38 x Volcani 447, had a significant deviations component remaining after fitting a five parameter model and it would have been desirable if all parameters could have been fitted simultaneously and studied for significance. However, even if an acceptable estimate of the variance were available, the matrix with six parameters was found singular in this case, within rounding error and thus the six parameters could not have been solved. Therefore, the results for this cross as obtained by fitting the five parameters using the previously explained procedure of comparison to simpler models is presented in Table 7.

In summary, the additive gene effects component, a, was of prime importance, but the dominance component, d, was also often significant, while negative in sign. Epistatic effects were mainly of the additive x additive and/or dominance x dominance type and were overall of little importance.

The consequence of the various gene effects on the choice of breeding strategies is that line selection following repeated self-fertilization is expected to raise levels of resistance, due to the additive gene effects. The occasional additive x additive epistatic components are likewise fixable in inbred lines. Lawrence and Frey (1976) suggested that the allopolyploid nature of a crop may furnish an explanation for the additive x additive epistasis due to duplicate gene action. Dominance effects may only be exploited, should hybrid durum wheat be the purpose of the breeding program. The sporadic dominance related epistatic effects were positive and are thus undesirable in the process of selecting for resistance. The simple and epistatic dominance effects, respectively decreasing and increasing infection levels, may require, due to their magnitude, that selection intensity is lenient in early selfed generations, while maximized when almost entirely pure lines are secured (Ketata et al., 1976b).

The inheritance of resistance to the other main member of the septoria disease complex, *Leptosphaeria nodorum* (imperfect state, *S. nodorum*) with a similar etiology and symptomology in two bread wheat crosses studied by Mullaney et al. (1982) also appeared to be governed by primarily additive gene effects, followed by dominance and lastly epistatic gene effects. Similarly, Nelson and Gates (1982) found these

three gene effects to be significantly present in their study of inheritance of resistance to *S. nodorum*.

CHAPTER 5

COMBINING ABILITY ANALYSIS

Introduction

Another biometrical method estimating additive and non-additive components was employed to increase understanding of the inheritance of resistance to *S. tritici*. A diallel analysis was executed on the ten cultivars from the Tunisian breeding program in order to estimate the general combining ability (GCA) and specific combining ability (SCA) from the analysis of variance and the GCA and SCA effects. These data would give an indication of the relative roles of additive and non-additive effects and could be particularly informative for a diallel analysis of entries that are themselves the reference genotypes and thus cannot be considered a random sample from a reference population. This is the case here, since, as described earlier, the cultivars were "hand-picked" according to various stated requirements.

Materials and Methods

The parents and F_1 and F_2 generations, excluding reciprocals, were included in the analyses, bringing the total number of entries based on ten cultivars to $n(n+1)/2$, being 55.

Each entry was represented by four F_2 seedlings per replication. A total of twelve replications was run for the diallel analysis involving a single *S. tritici* isolate.

One isolate was chosen from each of the seven countries listed in Table 2. These selected isolates are presented in Table 8.

Table 8. Selected *S. tritici* isolates for combining ability analyses.

Country	Isolate code
Tunisia	TUN 8202
Turkey	TKY 8201
Israel	ISR 80-8
Syria	SYR 8209
Portugal	POR 81199-1
Italy	ITL 82024
Spain	SPN 81299

Besides an analysis on F_2 seedlings, a similar diallel analysis was run on F_1 seedlings. Each of the fifty-five entries was now represented by two F_1 seedlings and the experiment replicated four times. Again, seven separate sets of trials were executed using the isolates listed in Table 8.

F_1 and F_2 seedling reactions to the isolate from Tunisia, TUN 8204-1, used in the generation mean analyses, were also analyzed for combining abilities. Each entry was represented by $20(F_2)$ or $5(F_1)$ seedlings and this trial replicated six times.

For the latter analysis, involving the isolate TUN 8204-1, data were collected on presence or absence of pycnidia for every F_2 seedling

observed. The percentage of seedlings with pycnidia out of twenty seedlings per entry per replication was calculated. These data, available for six replications, were also analyzed for combining abilities.

For the trials where disease reaction was measured as percentage necrotic leaf area, means were calculated per generation per replication over the number of seedlings used, four or twenty in the case of F_2's and two in the case of F_1's.

Overall means over all replications involved were used in a multiple regression analysis to determine variance components and combining ability effects.

Planting, inoculation and disease assessment were as previously described.

The relative importance of GCA and SCA was determined by calculating ratios of relevant mean squares and sums of squares as suggested by Baker (1978) and Auld et al. (1983):

Baker: $2\,MS_{GCA}/(2MS_{GCA} + MS_{SCA})$

Auld et al.: $SS_{GCA}/(SS_{GCA} + SS_{SCA})$

Ranking of the cultivars for GCA effects by the eight isolates was tested using the Friedman test and Kendall's coefficient of concordance between isolates. Similarly, the SCA effects of the forty-five crosses of the 10 x 10 diallel were tested for possible systematic ranking by the eight isolates using these two analyses.

Results

The mean disease reaction values (percentage necrotic leaf area) of parents and F_2 progenies over twelve replications and means over crosses are presented for each of the seven selected isolates separately in Tables 9 through 15. F_2 data for the isolate used in the generation mean analysis, TUN 8204-1, are listed in Table 16. Similarly, data on F_1 seedlings are listed in Tables 17 through 24.

The values relating to pycnidia presence on F_2 seedlings infected with *S. tritici* isolate, TUN 8204-1, are presented in Table 25.

The analyses of variance for combining abilities, listing degrees of freedom, mean squares, levels of significance and relative importance of GCA and SCA for the eight selected isolates are listed in Table 26 for the F_2 seedlings and in Table 27 for the F_1 data.

Specific GCA effects and their standard errors are presented in Tables 9 through 25.

Correlation coefficients between GCA effects calculated on the basis of F_2 seedling reactions and those from F_1 seedling analyses were calculated and are presented in Table 28.

The forty-five individual SCA effects of the seventeen separate analyses are not presented here because of the minor importance of SCA, which will be discussed later.