Effects of cultivar, isolate and environment on resistance of wheat to septoria tritici blotch in Kenya

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Author's abstract

The research described in this thesis focused on the characterization of some of the factors that influence disease assessment, development and expression of resistance in wheat cultivars to septoria tritici blotch. Earliness appeared to have a strong effect and tallness a small effect on disease severity (DS). A regression equation derived was used to correct the DS in the entries. Another method that gave good disease assessment was to group the cultivars according to their earliness. It appeared that the disease developed in each earliness group at the same rate. The importance of interplot interference in assessing septoria resistance in wheat was studied. There was no indication of interplot interference. The Nitrogen (N) level is another factor that may affect disease assessment. In Kenya there was an increase in DS on cultivars exposed to more N while in The Netherlands there was no similar increase in DS.

When an inoculum mixture or single isolates are used for inoculations, the ranking of the cultivars was essentially not affected, indicating that inoculum mixtures can be used effectively in screening wheat genotypes. The correlation coefficients between the DS at the seedling and the adult plant stages was low. Thus resistance assessed at the seedling stage could not fully explain adult plant resistance. Isolates from Kenya and The Netherlands were tested on wheat seedlings. It was concluded that there was variation in virulence (and so in race-specific resistance in the host) of *Septoria tritici* populations within both countries. The strong cultivar x isolate interactions observed on wheat seedlings was also observed on adult plants in the field.

F6 lines of 36 crosses were evaluated in the field. Transgressive segregation towards more resistance and or more susceptibility occurred in most crosses. It can be said that a fair number of genes operating in an additive manner and epistasis are involved.

Additional Keywords: *Mycosphaerella graminicola, Triticum aestivum.*

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Contents

Chapter 1

General introduction

Wheat area and production

Wheat is grown as a rainfed crop in Kenya at altitudes ranging from 1800 to 3000 m and occupied about 150,000 ha in 1994. Yields improved nearly threefold from 0.63 t/ha in 1921 to 1.76 t/ha in 1981-1990 (Anon., 1991). Wheat is cultivated by both small and large scale farmers. The area under wheat cultivation by small scale farmers is increasing. Hassan et al. (1993) reported that in 1960 only 7% of the total wheat area was under small scale farming, but this had increased to 20% by the 1970's. Between 1987-1990 wheat accounted for about 3% of the total value of marketed agricultural production (Anon., 1991). Today wheat is the second most important cereal crop, after maize, in Kenya.

Until 1974 Kenya was a net exporter of wheat but since then the country had to import wheat every year to meet rapidly increasing local demand. This local demand is growing at the rate of 7% per year and considerably exceeds the increase in domestic production. As a result, wheat imports rose from about 33,000 t in 1977 to approximately 218,000 t in 1987 (Hassan et al., 1993). This is largely due to the rapidly growing population, increased urbanisation and changing food preferences. Currently local production meets only 50% of domestic demand. To meet these challenges the government has proposed several approaches to stimulate production. One of these approaches is to reduce pre-harvest losses caused by disease through breeding for more resistant cultivars.

History of septoria tritici blotch resistance breeding

Septoria tritici, the causal organism of septoria tritici blotch (STB), is at present the second most important wheat pathogen after yellow rust (*Puccinia striiformis*) reducing yields in Kenya. The disease is also of major importance in the highlands of Ethiopia and Tanzania, in the coastal areas of the Mediterranean, in South America, in Australia and in Western Europe (Saari and Wilcoxon, 1974; Rajaram and Dubin, 1977). Almost a complete crop failure was reported from Kenya as early as the mid-twenties by Burton (1927) due to STB and in the following year, he (Burton, 1928) noted that especially plots under irrigation

were severely infected. The importance of STB in Tanzania was highlighted by Riley (1960) and in Ethiopia by Pinto (1972). Efforts were made in the sixties to identify sources of resistance (Saari and Wilcoxson, 1974). A Regional Disease and Insect Screening Nursery (RDISN) was subsequently established. Some of the best sources of resistance to *S. tritici* in the 1971-1972 nurseries were Kenya wheat lines selected in Ethiopia (Pinto, 1972) and submitted for regional testing in the RDISN.

After this initial work in Kenya, little was done on STB over the next two decades. This was probably due to the importance of stem rust (*Puccinia graminis*) in the 1970's and that of yellow rust (*Puccinia striiformis*) in the 1980's (Danial, pers. comm.). To improve stem rust resistance and yielding capacity, early maturing semi-dwarf lines from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico were introduced into the breeding programme. Because of the relationship between plant stature, maturity and resistance to STB (Danon et al., 1982; Eyal et al., 1983) the more STB resistant germplasm were unconsciously discarded. Incidences of STB increased gradually and initially unnoticed. Favourable weather conditions prevailing in Kenya in the 1985 and 1986 growing seasons led to severe STB epidemics and a renewed interest in STB resistance.

The pathogen

Desmazieres (1842) in France published a description of *Septoria tritici*, which he attributed to Roberge. *Septoria tritici* Rob. ex Desm. the causal organism of septoria tritici blotch represents the conidial state of *Mycosphaerella graminicola* (Fuckel), Schroeter. The connection between the two stages, for many years supposed, was proven by cultural experiments by Sanderson (1972). *M. graminicola* is a hemi-biotrophic parasite belonging to the family Mycosphaerellaceae (Muller, 1989). Its asexual state produces pycnidia embedded in the substomatal cavity.

Pycnidiospores germinate on a suitable substrate, following release from the pycnidium when plants are wet. Moisture is required for all stages of infection: germination, penetration, development of the mycelium within the plant tissue and subsequent pycnidial formation (Shaner and Finney, 1976; King et al., 1983). Cardinal temperatures reported for germination of *S. tritici* pycnidiospores are a minimum of 2-3^{0}C and a maximum of 33-37^{0}C with an optimum of 20-25^{0}C (Georghies, 1974a). Symptoms generally appear after

14-21 days. The time from infection to production of pycnidia depends on environmental conditions (moisture, temperature and light), the cultivar and isolate.

The first symptoms of infection on wheat leaves are expressed as irregular chlorotic lesions that usually appear 5-6 days after inoculation. Three to six days later, at 18-24^{0}C and high relative humidity, necrotic lesions develop at the chlorotic sites. Necrotic lesions appear sunken and greyish-green at first. By holding the leaf against the light, the beginning of pycnidia formation can often be seen, usually after 18 days. The pycnidia, ranging in colour from light to dark brown, develop in the necrotic lesion. The pycnidia are scattered within the lesion, and can be on both sides of the leaf surface. The size of pycnidia may vary among cultivars and is also affected by the density. As the number of pycnidia on the leaf increases, the size becomes smaller (Eyal and Brown, 1976).

Severe epidemics in the late sixties, particularly in North Africa, and the spread of *S. tritici* to areas where wheat was not previously commercially grown, initiated increasing attention in national and international breeding programs (Eyal et al., 1973; Mann et al., 1985). The spread of the disease has partly been caused by changing cultivation practices such as reduced crop rotation and leaving infested straw on the soil surface (Brokenshire, 1975), and the widespread introduction of early maturing dwarf cultivars which are generally more susceptible to *S. tritici*.

Many aspects of the septoria diseases of wheat have been discussed in three literature reviews (Shipton et al., 1971; Beggren, 1981; King et al., 1983) and four international workshops (Cunfer and Nelson, 1976; Scharen, 1985; Fried, 1989; Arseniuk et al., 1994). Eyal et al. (1987) have described concepts and methods of disease management of the septoria diseases. The intensity of research worldwide on STB over the last two decades highlights the importance of the disease worldwide.

Pathogenic variation

There are conflicting reports on the issue of physiologic specialisation in *S. tritici*. Significant variation for pathogenicity in *S. tritici* populations can influence how to best breed for resistance. Eyal et al. (1973) and Saadaoui (1987) suggested that true physiologic specialisation existed. Other workers (Prestes and Hendrix, 1977; Ballantyne, 1985; Perello et al., 1989; Kema et al., 1996) too reported the existence of physiologic specialisation.

Significant isolate x cultivar interactions have been reported (Eyal et al., 1985; van Silfhout et al., 1989). Similar results of Eyal and Levy (1987) suggested geographic distribution of specific virulences in *S. tritici*.

However, Marshall (1985) assessed STB severity in the field and in the greenhouse and found a wide variation for aggressiveness but no significant isolate x cultivar interaction. Van Ginkel (1986) suggested that *S. tritici* isolates were specifically adapted to either bread or durum wheat but that cultivar specificity was not significant.

From these studies it seems that specificity does exist in *S. tritici*. However, no clear races have as yet been identified and described. This may be due to: lack of stability of the host-pathogen interaction, smallness of the race-specific effects, lack of a good differential set of cultivars with known specific resistance genes, and/or lack of a good assessment method.

Disease assessment

A reliable assessment of disease severity is essential if resistance to the pathogen is to be effectively achieved. Various authors have discussed the proper measurements of disease severity caused by *S. tritici* (Eyal, et al, 1987). There is at present not a single uniform assessment method accepted by all septoria workers for either controlled studies in the greenhouse or for field evaluation. The severity of *S. tritici* is usually evaluated either by assessing the percentage coverage of the leaf by pycnidia or by determining the percentage necrotic leaf area. Host response to the pathogen and therefore disease assessment may be greatly influenced by factors such as plant growth stage at the time of assessment, inoculum composition, plant growth habit (tallness and maturity), plant nutrition especially nitrogen availability and interplot interference.

i) Plant growth stage

Experiments on the inheritance of resistance in wheat to STB are often carried out on adult plants under field conditions (Jlibene et al., 1992; Shaner and Buechley, 1994). Field experiments have inherent problems regarding plant growth habit (Tavella, 1978; Danon et al., 1982; van Beuningen and Kohli, 1990; Arama et al., 1994). To avoid these problems some investigators have evaluated resistance on seedlings under controlled conditions (van Silfhout et al, 1989; Cohen and Eyal, 1993). Little work has been carried out to compare

resistance at the seedling and adult plant stages. Kema and van Silfhout (1996) found no significant correlations between the disease severities of seedlings and adult plants of 22 wheat cultivars tested against two isolates. Arama (1993) found low correlations between seedling and adult plant resistance. Low correlations indicate that the resistance at the adult plant stage is difficult to predict from the seedling assessments. However, Brokenshire (1976) observed a high correlation between seedling and adult plant resistance.

ii) Inoculum composition

In breeding programs where breeding materials are evaluated under field conditions, STB epidemics are initiated or enhanced by artificial inoculation with a mixture of isolates of *S. tritici* of national origin (Zelikovitch et al., 1986). Studies by Zelikovitch and Eyal (1989) and Zelikovitch and Eyal (1991) suggested that mixing two *S. tritici* isolates differing in virulence in certain combinations resulted in a marked reduction in pycnidial coverage as compared to pycnidial coverage obtained when each isolate was applied separately on wheat seedlings. Gilchrist and Velazquez (1994) did not observe a reduction of pycnidia coverage from the mixture of three isolates under field conditions on adult plants of a set of cultivars differing in resistance to the isolates.

iii) Plant growth habit (tallness and maturity)

Reduced disease severity is often associated with late maturity and tall stature (Eyal, 1981; Danon et al., 1982; van Beuningen and Kohli, 1990; Eyal and Talpaz, 1990; Arama et al., 1994)). Many susceptible semi-dwarf cultivars introduced in the sixties possess one or both of the Norin 10 height reducing genes (Rht1 and Rht2) in their parentage (Gale et al., 1981; Gale and Youssefian, 1985). It has been suggested that short strawed wheat cultivars get higher disease severities to STB because reduced distances between consecutive leaves facilitate the ladder effect of the pathogen progress up the plant (Bahat et al., 1980). However, experimental results have been inconsistent when comparisons were made between plant height and disease severity (Tavella, 1978; Danon et al., 1982; Scott and Benedikz, 1985). Genetic associations between short stature and susceptibility to STB has also been suggested (Rosielle and Brown, 1979; Danon et al., 1982).

Early maturity has been associated with high disease severity (Arama et al., 1994). Genetic

linkages between earliness and susceptibility to STB have also been mentioned (Eyal, 1981; Rosielle and Boyd, 1985). From these studies it is evident that for a proper evaluation of resistance in wheat germplasm one has to take into account the difference in maturity and tallness. These conflicting reports reflect one of the difficulties in making a proper disease assessment.

iv) Plant nutrition

It is common practice for farmers to apply fertilisers to optimise grain yield. Fertiliser and especially nitrogen applications might increase the susceptibility of wheat to *S. tritici*. Increased N levels have been reported to increase septoria severity (Georghies, 1974b). Fellows (1962) found that more lesions developed on leaves and that a greater percentage of the leaf was destroyed when plants were fertilized, with 'Vigoro', 7-13 days before inoculation than when fertiliser was applied at the time of inoculation. However, in other experiments, Johnson et al., (1979) and Tompkins et al (1992) showed that increased N fertilization reduced septoria severity in their trials.

v) Interplot interference

In early stages of a breeding program for resistance to *S. tritici* the breeder is limited in space and seed. The practice is to evaluate large numbers of lines adjacent to each other. Under these conditions susceptible entries often are planted next to resistant entries. Susceptible entries may export far more inoculum to their resistant neighbours than they receive from these neighbours, while the resistant lines may export far less inoculum to the adjacent plots than they receive from these susceptible neighbours. Assessment of disease resistance on the resistant lines could result in a too high disease severity assessment and so is an under-estimation of resistance. This interplot interference has been investigated and found to occur in varying magnitude in different crop/pathogen systems (van der Plank, 1963; James et al, 1973; Parlevliet and van Ommeren, 1984; Broers and Lopez-Atilano, 1995). Little information has been published on interplot interference in wheat-STB system. Burleigh and Loubane (1984) showed that severity of *M. graminicola* was not significantly different in large plots 40 x 40 m and small plots of 10 x 10 m. The area under disease progress curve (AUDPC) from 40 x 40 m plots were significantly different from 20 x 20

m and 10 x 10 m plots but that final disease severities were only 2-10% greater. However, these plots were large compared to the breeders' plots in most stages of a breeding program.

Resistance

Most of the high yielding wheat cultivars grown in Kenya today are quite susceptible to STB. Breeding for resistance is the economically most feasible control measure for many wheat diseases. Septoria tritici blotch has been no exception. Undoubtedly most breeders and pathologists want a form of resistance that keeps its effectiveness over time, and which is easy to transfer across genotypes, easy to identify in segregating progenies and effective under disease conducive conditions. However, germplasm resistant to STB is rather scarce, and little is known about the types of resistance and the mode of inheritance (Eyal, 1981). Conflicting reports are found in the literature regarding the nature of genetic resistance to STB. These range from simple Mendelian genetics to complex quantitative inheritance patterns. Mackie (1929) found by analyzing F2 populations that a single recessive gene provided resistance in an unidentified cultivar. Single dominant genes for resistance have been reported to be present in Lerma'50' and P14 (Narvaez and Caldwell, 1957), Bulgaria 88 (Rillo and Caldwell, 1966), Veranopolis (Rosielle and Brown, 1979; Wilson, 1979), Carifen 12 (Lee and Gough, 1984), Vilmorin (Gough and Smith, 1985) and IAS20/#567.1 (Jlibene , 1990).

Wilson (1985), in evaluating 28 sources of STB resistance found that a single dominant gene was the most common type of genetic resistance. However, there were some exceptions including duplicate dominant, single incomplete dominant models, and for the cultivar Seabreeze, a two recessive gene model was suggested. In other studies Camacho-Casas et al., (1995) reported that additive and dominance effects were responsible for the resistance to STB in II50-18/VGDWF/3/PMF. Wilson (1985) proposed three different genes conferring resistance to STB. These were designated Slb1, Slb2 and Slb3 for the genes in Bulgaria 88, Veranopolis and Israel 493 respectively. Van Ginkel (1986) suggested that the search for single gene resistance to STB may be ineffective because of the presence of modifier genes, lack of discrete classes in segregating populations, evidence of transgressive

segregation, disagreements on where to place the segregation between resistance and susceptibility and environmental influence.

The experiments reported here were aimed at investigating several pathogen, cultivar and environmental factors that affect STB epidemics in Kenya. These included studies on resistant wheat genotypes, inheritance of resistance, influence of maturity and tallness, interplot interference, nitrogen fertilisation, inoculum composition and isolate virulence.

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Chapter 2

[1]Effect of plant height and days to heading on the expression of resistance in *Triticum aestivum* to *Septoria tritici* in Kenya

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Summary

The effect of plant height (HT) and days to heading (HD) on disease severity was studied in the field using 57 wheat cultivars. The linear correlation between the area under disease progress curve (AUDPC) and HD was -0.68 and between AUDPC and HT was -0.25. A multiple regression equation $\sqrt{AUDPC} = 145.5 - 1.5HD + 0.0056HT$ was derived from the data to correct the observed disease severity for the effects of HD and HT. After the correction for differences in HD the differences between the earliness groups had disappeared to a large extent.

Introduction

Septoria tritici blotch of wheat, anamorph of *Mycosphaerella graminicola* (Fuckel) Schroeter is a major wheat disease in many parts of the world (Eyal et al., 1987). Resistance to the pathogen is often associated with undesirable late maturity and tallness (Brokenshire, 1976; Danon et al., 1982; Eyal et al., 1983). Also Tavella (1978) concluded that the taller the wheat cultivar the lower its disease severity tended to be. The objective of this study was to investigate the combined effects of plant height and maturity on disease severity, caused by *Septoria tritici*, under field conditions in Kenya.

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Materials and Methods

From a large number of entries, 57 wheat genotypes varying in disease severity for Septoria tritici blotch, heading date, tallness and resistant to yellow rust were selected for this study. The entries were planted at Timau and Njoro in 1990 main season and off-season (Njoro only) and 1991 main season. The five trials were planted in randomized complete block designs with three replications. Plots were planted three rows, 2 m long. Observations started about 65 days after seedling emergence and thereafter every 14 days for three consecutive times. Septoria infection was visually estimated on the three upper leaves (flag leaf and the two leaves below the flag leaf) of ten tillers taken at random from each plot using a modified Cobb scale. The area under disease progress curve (AUDPC) was calculated as 7DS1 + 14DS2 + 7DS3, where DS (disease severity) refers to the mean percentage of the three upper leaves affected by septoria tritici blotch at the first, second and third observation date. This AUDPC represents the surface under the straight line which connects the three observation dates. For statistical analysis the AUDPC data were square root transformed.

Results

The AUDPC is considered to represent the degree in which the entry was diseased. The data over the five trials were taken together because the variance for host genotype x trial interaction was small for DS at any date, for AUDPC, days to heading from seedling emergence (HD) and plant height (HT) in cm to flag leaf basis. AUDPC, HD and HT varied greatly between entries and appeared associated with each other. The linear correlation coefficient between square root transformed AUDPC and HD, square root transformed AUDPC and HT, and HD and HT were -0.68***, -0.25* and 0.37** respectively (*, **, *** significant at the 10%, 1% and 0.1% level).

Table 1 gives the data for 11 entries, that represent the range of variation for HD, HT and relative AUDPC. As the three variables are correlated the AUDPC should be corrected for the differences in HD and HT. A multiple regression equation was derived from the data to correct the observed AUDPC for the effects of HD and HT. The equation derived was:

$\sqrt{AUDPC} = 145.5 - 1.50HD + 0.0056HT$

Using the multiple linear regression equation, the AUDPC of all entries was corrected to

Table 1. Heading date (HD), plant height (HT) and the relative area under the disease progress curve (AUDPC) before and after correction for differences in HD and HT of 11 wheat entries (observed AUDPC of entry 48 is set at 100%).

Entry	HD (days)	HT(cm)	AUDPC before correction	AUDPC after correction
48*	58**	36	100.0 (1)	69.2 (1)
41	78	59	42.9 (3)	59.0 (2)
14	98**	77	4.2 (10)	35.8 (3)
12	60	73	43.5 (2)	26.9 (4)
47	76	62	15.8 (5)	23.3 (5)
33	88	91	2.6 (11)	17.1 (6)
22	59	33**	28.0 (4)	14.0 (7)
50	70	97**	10.3 (7)	10.3 (8)
54	61	53	14.8 (6)	6.7 (9)
11	64	48	7.6 (9)	3.6 (10)
8	60	52	8.4 (8)	2.2 (11)

* An extremely susceptible entry, which reached a disease level of 70% to 90% affected leaf area in most trials.

** Extreme values, indicating the range in HD and HT among the 57 entries.

a HD of 70 days and to a plant height of 60 cm. The last column of Table 1 shows the corrected relative AUDPC, which is assumed to represent the real level of susceptibility/resistance. The linear correlation coefficient between the observed AUDPC and the corrected one (after square root transformation) was 0.735***, which means that about 50% of the observed variance in AUDPC (square root transformed) is explained by differences in HD and HT.

Tables 2 and 3 show the mean AUDPC of four maturity groups and four plant height groups before and after correction for heading date and plant height respectively.

Table 2. Mean relative area under disease progress curve (AUDPC) of four heading date groups before and after correction for differences in days to heading (relative to most susceptible entry, see table 1).

Group	Days to heading	No. of entries	AUDPC before correction	AUDPC after correction
I	Up to 60	8	35.5	20.3
II	61 - 70	27	21.2	15.6
III	71 - 80	16	10.0	10.0
IV	Above 80	6	2.2	15.2

Table 3. Mean relative area under disease progress curve (AUDPC) of four plant height groups before and after correction for differences in tallness (relative to most susceptible entry, see table 1).

Group	Plant height (cm)	No. of entries	AUDPC before correction	AUDPC after correction
I	<51	8	33.7	33.5
II	51 - 60	21	17.1	17.1
III	61 - 70	14	12.9	12.9
IV	>70	14	16.9	17.1

Maturity had a pronounced effect on the AUDPC (Table 2). The group means for AUDPC were highly significant (***). After correction for differences in HD the differences between the groups had disappeared to a large extent. After correction some differences between the four groups remained, which did not seem to be related to differences in maturity. Such differences are expected when the number of entries per group are fairly

small and when the individual differences are large (Table 1, last column) as is the case here. Plant height, on the contrary, had hardly an effect, if at all (Table 3). The few very short entries appeared really more susceptible than the other entries.

After correction for differences in days to heading and plant height large differences in AUDPC between entries remained. The relative values ranged from 69.2 for the most susceptible entry to 2.2 for the most resistant one, a large difference (Table 1). Between these extremes there was a continuous variation in the AUDPC.

Discussion

The present set of experiments confirmed the strong influence of heading date on the observed disease severity but not of the effect of tallness reported by others (Rosielle, 1972; Tavella, 1978; Danon et al, 1982). The equation derived in this study deviates from those of Eyal and Talpaz (1990) and van Beuningen and Kohli (1990). The equations of Eyal and Talpaz (derived in Israel) and of van Beuningen and Kohli (derived in Paraguay), though somewhat different from one another, both showed significant effects of heading date and plant height. There can be several reasons why in this study hardly any effect of tallness was observed. It could be due to the inoculation procedure used, which involved infested straw spread between the rows and spore suspension inoculation at the tillering stage. Other reasons of the absence of a tallness effect might be in the sample of entries used. The range in this population was 33 to 97 cm (measured to the flag leaf), which means that even the tallest entry could not be considered tall. The population of entries started from and the rather vigorous selection applied (for yellow rust resistance) may have resulted in a sample not fully representative for bread wheat. A third reason could be the climatic conditions in Kenya.

The observations reported here suggest that studies into the effect of days to heading and plant height should not be used indiscriminately. One has to correct the observed disease level of the entries for the effect of heading date and tallness when one is selecting for resistance to Septoria tritici blotch, but the correction should be based on ones own data and not on a formula derived from another population under other conditions.

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Chapter 3

Influence of assessment date in relation to heading date on the evaluation of cultivar response to septoria tritici blotch in the field

Summary

Nineteen cultivars were evaluated for their response to septoria tritici blotch in two experimental setups. All cultivars were evaluated for disease severity at the same time irrespective of the developmental stage in Experiment 1 while in Experiment 2 the cultivars were evaluated at the same developmental stage. Measured at the same time, the disease severity was highest in the early maturing cultivars and lowest in the late maturing cultivars. When assessed at the same development stage the disease buildup was independent of earliness but depended on resistance level. This is expressed in the correlation coefficients between disease severity and heading date , which was -0.78 when disease was assessed at the same time and -0.10 when assessed at the same developmental stage.

Introduction

Septoria tritici is a major foliar pathogen of wheat in many parts of the world (Saari and Wilcoxson, 1974; Rajaram and Dubin, 1977; King et al., 1983). Increased severity of septoria tritici blotch (STB) is thought to be due to the widespread replacement of tall, late maturing local cultivars by high yielding early maturing semi-dwarf wheats (Eyal et al., 1987; Saadaoui, 1987). Many reports have been published associating reduced disease severity with tall stature and late maturity (Shaner et al., 1975; Tavella, 1978; Rosielle and Brown, 1979; Eyal, 1981; Danon et al., 1982; Eyal et al., 1983; Rosielle and Boyd, 1985; Jlibene et al., 1992; Camacho-Casas et al., 1995).

Many susceptible semi-dwarf cultivars introduced in the 1960's possessed one or both of the Norin 10 height reducing genes (Rht1 or Rht2) in their parentage (Gale et al,. 1981; Gale and Youssefian, 1985; Baltazar et al., 1990). It has been suggested that short strawed wheat cultivars get higher disease severities to STB because the reduced distances between consecutive leaves facilitate the pathogen progress up the plant (Bahat et al., 1980).

However, experimental results have been inconsistent when comparisons were made between plant height and disease severity (Tavella, 1978, Danon et al., 1982). Genetic associations between short stature and susceptibility to STB have been suggested (Rosielle and Brown, 1979; Danon et al., 1982). Early maturity has also been associated with high disease severity (Eyal et al., 1983; van Beuningen and Kohli, 1990; Arama et al., 1994; Camacho-Casas et al., 1995). Genetic linkages between earliness with high disease severity have been mentioned (Eyal, 1981; Rosielle and Boyd, 1985).

To help interpret the effect of days to maturity and tallness, it becomes necessary to measure the pattern of variation of these factors together with that of the disease severity. Some methods are available enabling a proper interpretation of the data collected. This in turn then gives insight into the effect of each of the two variables (earliness, plant height) on the disease severity (Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990; Arama et al., 1994). From the data collected by Arama et al. (1994) it appeared that the differences in earliness had a much stronger effect on disease severity than differences in plant height. An experiment was designed to investigate the effect of earliness on disease severity in more detail in order to assess the true resistance of a cultivar. Four groups of cultivars were chosen. Within each group the cultivars had a similar plant height and a similar earliness. The groups differed in their mean earliness. The experiment was designed in such a way that the disease severity of the cultivar groups could be assessed either at the same time or at the same stage.

Materials and Methods

The experiments were planted in 1992 at the experimental farm of the National Plant Breeding Research Centre, Njoro (Altitude 2160 m). Nineteen cultivars and breeding lines were chosen from the 1991 septoria observation nursery maintained at the Centre. Selection was primarily based on days to heading (HD) and secondly on plant height to flag leaf. The cultivars were classified in four heading date groups differing from each other by approximately 10 days: Group I, about 55 days (cv. nrs. 1-5); Group II, about 65 days (cv. nrs. 6-10); Group III, about 75 days (cv. nrs. 11-15); Group IV, about 85 days (cv. nrs. 16-19) (Table 1). Within each group the cultivars had a similar plant height to flag leaf (F).

Experiment 1.

The cultivars were planted in a randomized complete block design without regard for maturity (days to heading) or tallness. Three replicates were planted and these were separated with 2 m of oat. Plots within replicates consisted of four rows of 2 m with a row distance of 20 cm. The plots within a replicate were spaced 30 cm from one another. At the seedling stage septoria infected straw was spread between the rows. Additional artificial inoculation with a spore suspension was done at growth stage 30 (GS 30) (Tottman and Makepeace, 1979). Inoculum preparation was as described by Eyal et al., (1987). Leaf segments 3-5 cm long were placed on a microscope slide in a petri-dish on moistened filter paper for 2 hrs. Pycnidiospores were extruded from pycnidia in cirri. A single cirrus was removed from the leaf segment and inoculated onto freshly prepared Malt Yeast Agar medium. Sub-cultures were made to increase inoculum. At the time of field inoculation, the cultures were scraped off the petri-dishes into 1 L of distilled water. Spore suspension was filtered through cheese cloth to remove agar fragments. The spore suspension was diluted with distilled water and the concentration adjusted to 10^6 spores/ml. A 15 L CP15 knapsack sprayer was used for inoculations. After the inoculations, sprinkler irrigation was applied every three days whenever there was no rainfall in that time interval. Irrigation was applied until the time of last observation. The first observation was made 55 days after planting when the earliest maturing cultivars were at the heading stage. Four consecutive observations were made at an interval of 10 days. The last observation was made 85 days after planting. For each observation twenty main tillers were sampled at random from the inner two rows. Percentage leaf area necrotic due to STB was estimated on the upper two leaves that were fully developed. Data were also taken for heading date in each plot. The plant height to the flag leaf of five representative main tillers in each plot was measured 90 days after planting for the early maturing cultivars and 120 days for the late maturing cultivars.

Experiment 2

The cultivars were grouped into four heading date groups differing by approximately 10 days. The experiment was planted in three replicates adjacent to Exp. 1. Plot size and spacing was as in Exp. 1. The cultivars were planted according to heading date groups with

five cultivars per group. The heading date group position in the replicates was randomized. The cultivar position was randomized within the groups. A 2 m crop of oat was planted to separate the replicates. Straw spread, inoculum preparation and inoculations were as described in exp.1. Overhead sprinkler irrigation was supplemented as in exp.1 until the time of last observation.

Disease assessments were made according to the heading date group so that the first assessment was made when the cultivars within a group were at heading stage and had fully expanded flag leaves. Thus the first disease observations were made 55, 65, 75, and 85 days after planting in Group I, Group II, Group III and Group IV respectively. Four assessments were made in each group at an interval of 10 days such that the last assessment was made 115 days after planting in group IV. Twenty main tillers were sampled as described in Exp. 1. Percentage necrosis was estimated on the flag leaf (F) and the first leaf below the flag leaf (F-1).

Data analysis

The percentage necrosis in both experiments were logit transformed using the formula: Logit = Log((%necrosis)/(100 - %necrosis))+7.

Analysis of variance was carried out on the transformed data. Correlations between heading date, plant height and disease severity were determined in both experiments. In group IV one cultivar appeared to have a HD of 100 days, which did not fit into the earliness class meant (about 85 days). This cultivar was excluded from the analysis.

Results

Experiment 1

Table 3a shows that the mean disease severity for cultivars 16-19 (latest maturing) was less than 5% at day 85. At that time, cultivars 1-5 (early maturing) were all 100% necrotic. Disease severity was highest in the early maturing cultivars and lowest in the latest maturing cultivars (Tables 1 and 3a). The cultivars differed considerably in disease severity (DS) within groups (Table 1). The group means for DS were quite similar when compared at a similar stage; for instance observation date 1, 2, 3 and 4 for the groups I, II, III and IV

Table 1. Plant height (HT) in cm to flag leaf, heading date (HD) in days from sowing and percentage necrosis caused by septoria tritici blotch of 19 wheat cultivars grouped into four maturity groups assessed at the same time for four consecutive assessment times.

					Days after sowing			
Cultivar		Grp.	HT	HD	55	65	75	85
1	Frontatch	I	56.3	59	12.8	43.8	95.9	100.0
2	PEL.72380/ART71/3/..	I	59.8	58	8.3	23.3	99.3	100.0
3	COOK/VEE/DOVE/SERI	I	54.3	55	3.5	17.6	95.1	100.0
4	L2266/1406.101//BUC..	I	58.0	56	0.7	7.5	93.7	100.0
5	VEE'S'	I	49.5	59	2.5	7.2	87.1	100.0
6	CMH78.390//MRNG/ALDAN	II	40.0	63	0.0	13.9	48.8	87.6
7	PAK/BJY/GJO/EMU	II	48.4	64	0.3	7.6	48.2	95.9
8	IAC 168	II	58.0	65	0.0	0.6	25.7	87.5
9	TRAP#1*2//ERP/RUSO	II	56.7	63	0.0	0.2	12.3	65.0
10	BUC/BJY	II	54.0	62	0.0	0.0	2.0	21.1
11	Kenya Sungura	III	70.2	75	0.0	7.5	18.6	40.4
12	Selpek	III	68.3	78	0.0	1.8	16.9	48.7
13	YAP/BJY	III	69.7	75	0.0	0.0	0.0	28.9
14	Ning 8331	III	68.8	76	0.0	0.5	1.7	19.1
15	Milan	III	69.0	75	0.0	0.0	0.0	0.4
16	KVZ/3/BB/CHA//TRM..	IV	59.4	85	0.0	0.1	0.0	5.7
17	ND/VG9144/3/KAL/BB..	IV	52.2	85	0.0	0.0	0.5	2.0
18	BOW/NAC	IV	59.2	89	0.0	0.0	0.5	1.8
19	Unknown (8thLACOS246)	IV	53.0	86	0.0	0.0	0.6	1.2

Table 2. Percentage necrosis of 19 wheat cultivars assessed on heading date group (see table 1) basis at the same developmental stage, but different days after sowing.

	Days after sowing						
Cultivar	55	65	75	85	95	105	115
1	11.4	50.5	96.4	99.3			
2	8.8	29.1	63.9	100.0			
3	5.4	21.0	69.6	100.0			
4	1.5	8.4	91.7	100.0			
5	0.4	5.3	71.4	96.9			
6	-	8.5	45.5	98.0	100.0		
7	-	1.0	35.2	96.0	100.0		
8	-	0.2	21.3	84.1	100.0		
9	-	2.3	8.6	61.6	87.9		
10	-	0.0	0.3	22.2	42.2		
11	-	-	9.4	35.7	86.3	100.0	
12	-	-	10.3	49.8	84.6	100.0	
13	-	-	3.7	32.5	63.0	100.0	
14	-	-	4.3	13.7	72.9	100.0	
15	-	-	0.0	0.0	8.4	23.7	
16	-	-	-	3.3	14.6	72.1	100.0
17	-	-	-	0.0	6.8	39.6	96.7
18	-	-	-	1.2	14.3	57.8	100.0
19	-	-	-	0.2	13.8	54.5	96.7

Table 3a. Means for heading date (HD) in days and percentage necrosis due to septoria tritici blotch at four observation dates for the 19 cultivars grouped according to days to heading groups (I-IV) and whose assessments were carried out at the same time.

		Observation date			
Heading date group	HD	1	2	3	4
I	57	5.6	20.5	94.2	100.0
II	63	0.1	4.5	27.4	71.4
III	76	0.0	2.0	7.4	27.5
IV	86	0.0	0.0	0.4	2.7

Table 3b. Means for heading date (HD) in days and percentage necrosis due to septoria tritici blotch at four observation dates for the 19 cultivars grouped according to days to heading groups (I-IV) and whose assessments were carried out at the same stages of development.

		Observation date			
Heading date group	HD	1	2	3	4
I	57	5.5	22.9	78.6	99.2
II	63	2.4	22.2	72.4	85.9
III	76	5.5	26.3	63.4	84.7
IV	86	1.2	12.4	56.0	98.4

Table 3c. Means for heading date (HD) in days and percentage necrosis due to septoria tritici blotch for the 19 cultivars grouped according to days to heading groups (I-IV) at the first, second and third observation dates (1, 2, 3) from heading for two experiments (E1, E2).

		1		2		3	
Heading date group	HD	E1	E2	E1	E2	E1	E2
I	57	5.6	5.5	19.9	22.9	94.2	78.6
II	63	4.5	2.4	27.4	22.2	71.4	72.4
III	76	7.4	5.5	27.5	26.3	-	-
IV	86	2.7	1.2	-	-	-	-

Table 4. Plant height (HT) in cm to flag leaf, heading date (HD) in days and logit transformed area under disease progress curve (AULOGDPC) of 19 wheat cultivars observed at the same development stage.

Cultivar Nr.	HT	HD	AULOGDPC
15	69.0	75	111.0 a
10	54.0	62	126.8 b
17	52.2	85	175.3 c
9	56.7	63	181.4 c
5	49.5	59	183.5 cd
19	53.0	86	187.8 cd
18	59.2	89	196.1 de
16	59.4	85	207.0 ef
14	68.8	76	209.1 ef
3	54.3	55	213.4 f
13	69.7	75	214.5 f
8	58.0	65	214.8 f
4	58.0	56	215.4 f
2	59.8	58	218.0 f
11	70.2	75	234.2 fg
12	68.3	78	239.4 g
7	48.4	64	241.7 g
1	56.3	59	258.6 h
6	40.0	63	259.1 h

Means followed by the same letter in a column are not significantly different using LSD $P \geq 0.05$

respectively, or observation dates 2, 3 and 4 for the groups I, II and III respectively (Table 3a). The correlation for Area under Logit transformed Disease Progress Curve

(AULOGDPC) and heading date was -0.78.

Experiment 2

Disease development in this experiment was similar to that in experiment 1. There was reduced rainfall in August when the group IV cultivars were heading. This necessitated more frequent overhead sprinkler irrigation to maintain conducive conditions for disease development. Results in Table 2 and 3b indicated that the disease buildup in the four heading date groups was similar. The disease started to buildup at or just before heading stage in all groups. As in exp. 1 there were clear cultivar differences within groups. The correlation between assessments in exp. 1 and exp. 2 when measured at heading (first observation in Table 2) was 0.85. When measured 10 days later (second observation in table 2) for groups I, II and III it was 0.96. The disease developed in the same way in all four groups. It started around heading and had progressed to 100% or nearly so after 30 days except for the resistant ones. In table 4 the cultivars are ranked according to their area under disease progress curve. The correlation between this AULOGDPC and heading date was -0.10 (not significant). The cultivars Milan and BUC'S'/BJY showed high levels of resistance. Milan was in Group III and BUC'S'/BJY was in Group II.

Discussion

In exp.1 the start of observations at 55 days after planting had some implications. At that time the earliest maturing cultivars nr. 1-5 (Table 1) had fully expanded F and F-1 leaves and these were assessed. At the same time cultivars nr. 16-19 were at the tillering or stem elongation growth stage. Assessment was made on the second to fourth lower leaves after the flag leaf (F-2 to F-4) leaves. Such assessments were not comparable because plants were assessed at different developmental stages. Fully emerged F and F-1 leaves were observed in these cultivars nr. 16-19 at date 85. The disease exposure was also less than in the early maturing cultivars.

The experimental setup represented by table 1 is the usual approach when comparing resistance of a range of cultivars. In such an approach the differences in disease severity are often for a large part attributed to differences in resistance. In reality the differences in disease severity are far more affected by the differences in days to heading than in

differences in resistance. Table 2 and 3b shows clearly that the four HD groups had the same epidemic development taken from the moment of heading. The epidemic started at the same development stage of the plant in early and in late maturing cultivars and the rate of epidemic development also did not differ. Among the 19 cultivars only two are clearly more resistant than the others; BUC/BJY (nr. 10) and Milan (nr. 15). There are a few others such as nrs. 8, 9, 13 and 14 which appear to have some resistance compared to the more susceptible ones. But as a whole the differences in disease severity due to resistance are small compared to the differences due to HD. The fact that the disease severity started at the same time and developed at a similar rate irrespective of the earliness group means that the late maturing cultivars do not have some inherent resistance to STB.

In exp. 1 the cultivars were fully randomised. Late and early cultivars could be adjacent to one another. In such a case the late cultivar is exposed to the inoculum produced by the early cultivar (where the disease develops much earlier). If there is considerable interplot interference, the later cultivars would be more affected in this randomized setting than in the setting in exp. 2 where the cultivars were planted in maturity groups and therefore not exposed to more diseased earlier cultivars. If interplot interference was of some importance, one should expect the later maturing cultivars more affected in exp. 1 than in exp. 2; especially in the earlier stages of disease development i.e. in the first and second observation after heading. In exp. 1 the average disease severity for the heading date groups II to IV were marginally higher in exp. 1 than those in exp. 2 (Table 3c). This might be due to a slight interplot interference, but this effect is so small that it did not affect the conclusions one should make. The results obtained from exp. 1 and those from exp. 2 when taken at the same stage of plant development were very similar (Table 3c) . For instance the correlation coefficient for the second observation after heading of the cultivars between exp. 1 and exp. 2 was 0.96. This is the stage at which the disease severity is around 20 to 50% for the more susceptible cultivars, a good stage for comparing. From this experiment it can be concluded that one can assess true resistance of a range of cultivars provided one measures the disease severity not at the same moment, but at the same stage of plant development. It becomes easier if one is able to group the genotypes to be tested in maturity groups.

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Chapter 4

[1]Variation in virulence patterns of *Septoria tritici* on *Triticum aestivum* in Kenya.

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Summary

Sixteen isolates of *Septoria tritici* collected from different geographic locations were tested on 43 cultivars of wheat (*Triticum aestivum*) at the seedling stage in a growth room. The interaction between cultivars and isolates was highly significant. Some isolates collected from the same location differed in virulence. Cluster analysis of percentage pycnidia coverage on the first leaves grouped the isolates into six virulence groups and the cultivars into five resistance groups at P=0.05 level of significance. Similar analysis on necrosis grouped the isolates into eight virulence groups and cultivars into six resistance groups. There was an indication that necrosis and pycnidia coverage were independent of each other and may be controlled by different genes.

Introduction

The response of wheat cultivars to isolates of *Septoria tritici* is assessed by the quantification of symptoms (percentage necrosis and pycnidial coverage). The latter has epidemiological significance in the dissemination of the pathogen whereas the former may express a phenomenon associated with response to the toxic products of the pathogen. Knowledge of the physiologic specialization of *S. tritici* is a necessary prerequisite to any

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reliable breeding program for disease resistance (Eyal et al., 1973). Many researchers who studied this matter could not identify distinct races of the pathogen (Narvaez, 1957; Arsenijevic, 1965; Shipton et al., 1971; Perello et al., 1991). Physiologic specialization in *S. tritici* was elucidated by Eyal et al. (1973) on the basis of the interactions between 14 bread and durum wheat cultivars and five Israeli isolates (from bread and durum wheats). The authors concluded that the significant cultivar x isolate interaction reflects specificity and that differentiation occurs also at the species level. Several reports have provided supportive data to indicate that physiologic specialization exists in *S. tritici* and specificity is manifested both by host and pathogen (Yechilevich-Auster et al., 1983; Royle et al., 1987; Saadaoui, 1987; Ballantyne, 1989; van Silfhout et al., 1989; Diaz de Ackermann et al., 1994; Gilchrist, 1994; Kema et al., 1996). In most of these studies the magnitude of the interaction between cultivars and isolates was low and variable but statistically significant. Furthermore, it was strongly affected by the choice of the isolates and wheat differentials, environmental conditions and methodology.

There are reports that aggressiveness rather than virulence explains the differences between isolates of *S. tritici* (Marshall, 1985; van Ginkel and Scharen, 1988). Van Ginkel (1986) used *T. durum* cultivars and isolates collected from both *T. durum* and *T. aestivum* cultivars. Based on percentage necrotic leaf area he could not ascertain a significant isolate x cultivar interaction. In view of the importance of the knowledge of virulence patterns of *S. tritici* for a sound breeding program, the present study was undertaken to determine whether isolates of *S. tritici* in Kenya differ in their virulence.

Materials and Methods

The pathogen

Diseased leaf samples were collected from all major wheat growing areas in Kenya. From the samples, 13 were selected to represent 13 isolates (Table 1). For comparison, three Dutch isolates previously studied and found to differ in virulence on wheat seedlings (Kema pers. comm.) were included. For each isolate a leaf segment was attached to a glass slide and placed in a petri dish with filter paper saturated with sterile water placed on the bottom. The petri dish was closed to provide a moist environment for 4 hrs. The petri dishes were then transferred to a laminar-flow clean air cabinet bench. Oozing pycnidia were located

under the stereoscopic microscope. With the help of a fine-pointed needle, sterilized in a flame and cooled briefly, a single cirrus was picked and transferred to V8 juice agar medium in a petri dish for multiplication. The isolates were transferred to cryo-tubes and stored at -80^{0}C until the time of use. Isolates were grown for 5 days at 20^{0}C on petri plates of V8 juice medium. Before inoculation the isolates were suspended in distilled water and filtered through a double layer of cheese cloth. The concentration was determined and adjusted to 1x10^7 spores/ml.

The host

The differential set used consisted of 43 cultivars. These included 14 cultivars that exhibited varying levels of resistance to septoria leaf blotch in the field in Kenya; 16 old Kenyan commercial cultivars released between 1960 and 1975; 10 cultivars selected from the septoria differential set used at IPO and three Dutch commercial spring wheat cultivars. Cultivars were sown in jiffy pots (7x7 cm) to have 9-15 seedlings/cultivar. The experiment comprised sets of inoculations and were conducted according to a partial balanced incomplete block design with respect to isolates, which allowed the execution of three replicates over time.

Inoculation and disease assessment

Ten days old plants were inoculated with the respective isolates. Trays containing the pots with seedlings were placed on a turntable revolving at 21 RPM and evenly sprayed with 30 ml of spore suspension. After inoculation plants were placed in a growth room at a temperature of 20 to 22^{0}C and 90 to 95% relative humidity. Plastic covers were placed over the plants for the first 48 hrs of incubation to ensure high relative humidity. Plants were incubated for 21 days in the growth room. Percentage necrosis and pycnidia coverage were estimated on the first leaves.

Results

Statistical analyses were conducted using the Genstat 5 package (Genstat 5 Committee, 1990) on the untransformed data. From the analysis of variance (ANOVA) in Table 2 on pycnidia coverage, the main effect of isolates is significant at the 5% level and the effect

Table 1. *Septoria tritici* isolates collected from *Triticum aestivum* used to study possible differences in virulence

Isolate	Acc. No.	Location collected	Cultivar
1	IPO93001	Njoro	?
2	IPO92052	Eldoret	Kenya Fahari
3	IPO92043	Eldoret	Mbuni
4	IPO92045	Endebess	Pasa
5	IPO92046	Endebess	Kenya Paka
6	IPO92047	Naivasha	Mbuni
7	IPO92049	Naivasha	Mbuni
8	IPO92050	Mai Mahiu	Mbuni
9	IPO92062	Mai Mahiu	Mbuni
10	IPO92044	Timau	Morocco
11	IPO92048	Moiben	Mbuni
12	IPO92054	Iten	Mbuni
13	IPO92069	Ngorengore	Mbuni
14	IPO001	Ulrum (Ned.)	?
15	IPO290	Z. Flevoland (Ned.)	Clement
16	IPO323	W. Brabant (Ned.)	Arminda

of cultivars and the effect of interaction between isolates and cultivars is highly significant ($P<0.001$). The covariance efficiency values of 0.63, 1.00 and 1.00 for isolates, cultivars and isolates x cultivars interactions respectively showed that isolates were not completely independent from block effects while the other two factors were independent.

In order to reveal structures of the interactions between host and pathogen genotypes, the tables of means were subjected to a hierarchical agglomerative clustering procedure as described by Corsten and Denis (1990). Cluster analysis of the percentage pycnidia (Fig 1) shows that there are six virulence groups A-F at the $P=0.05$ level of significance. Likewise

Table 2. Analysis of variance of mean pycnidial coverage

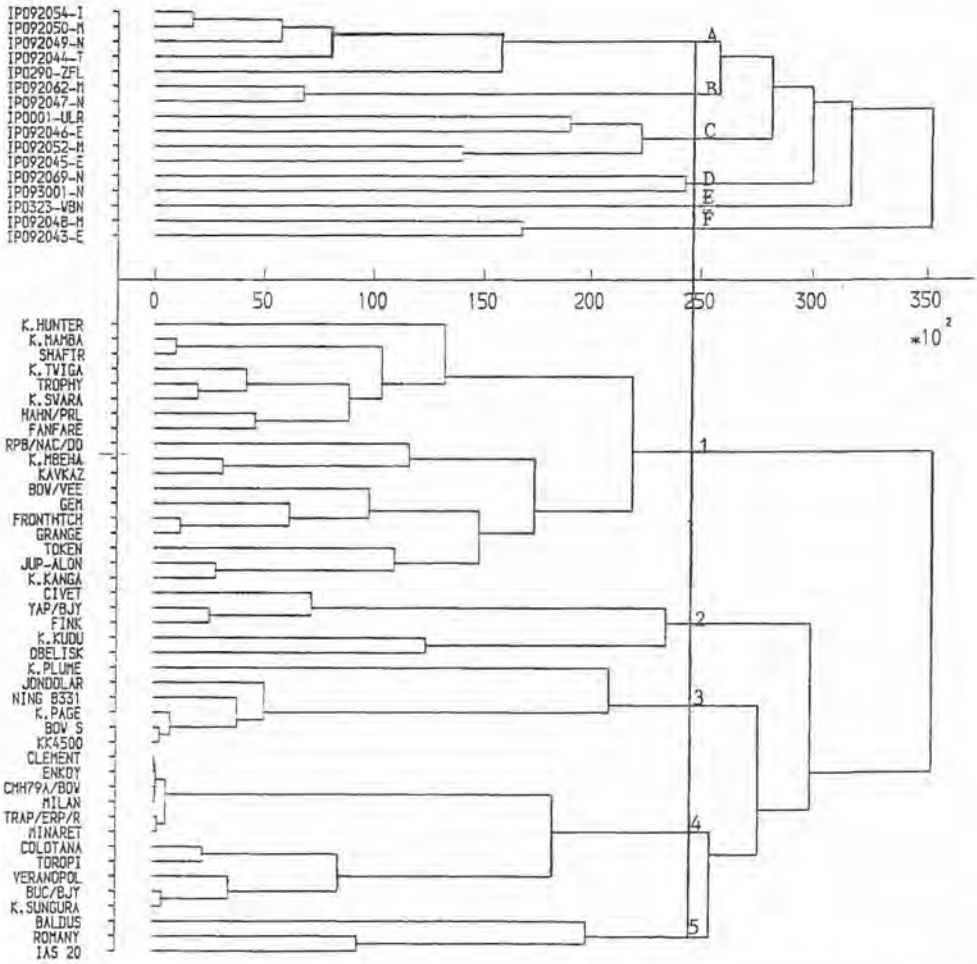
Source of var.	d.f	s.s.	m.s.	v.r.	cov. eff
Isolates	15	26206.4	1747.1	2.84*	0.63
Blocks	15	58866.1	3924.4	6.38**	
Residual	17	10454.9	615.0	5.92	3.52
Cultivar	42	177531.5	4226.9	40.66***	1.00
Isolate*cultivar	630	105622.0	167.7	1.61***	1.00
Residual	1344	139734.6	104.0		1.00
Total	2063	533469.7			

* significant at 5%; ** significant at 1%; *** significant at 0.1%

the 43 cultivars are grouped into at least five resistance groups (1-5). An attempt was made to assign susceptible (S) or resistance (R) reactions based on arbitrary border lines for mean percentage pycnidia coverage (Table 3). From the dendograms presented in Fig. 1 it is shown that isolates in the same virulence group are not necessarily from the same location. For instance isolate IPO92069-N in virulence group D was collected from Narok while IPO93001-N was from Njoro. The same applies to isolates IPO92048-M in virulence group F from Moiben and IPO92043-E from Eldoret. However some isolates collected in the same location are clustered in the same virulence group like IPO92045-E (group C) and IPO92046-E both collected at Endebess. In table 3 it is shown that the isolates in virulence groups B, C, D and F are virulent on cultivars in resistance group 1. Also isolates in virulence groups B and E have virulence for cultivars in resistance group 5. There is similarity in virulence for isolate in groups C and F. The three isolates collected from The Netherlands IPO290-ZFL, IPO001-ULR and IPO323-WBN are clustered in virulence groups A, C and E respectively.

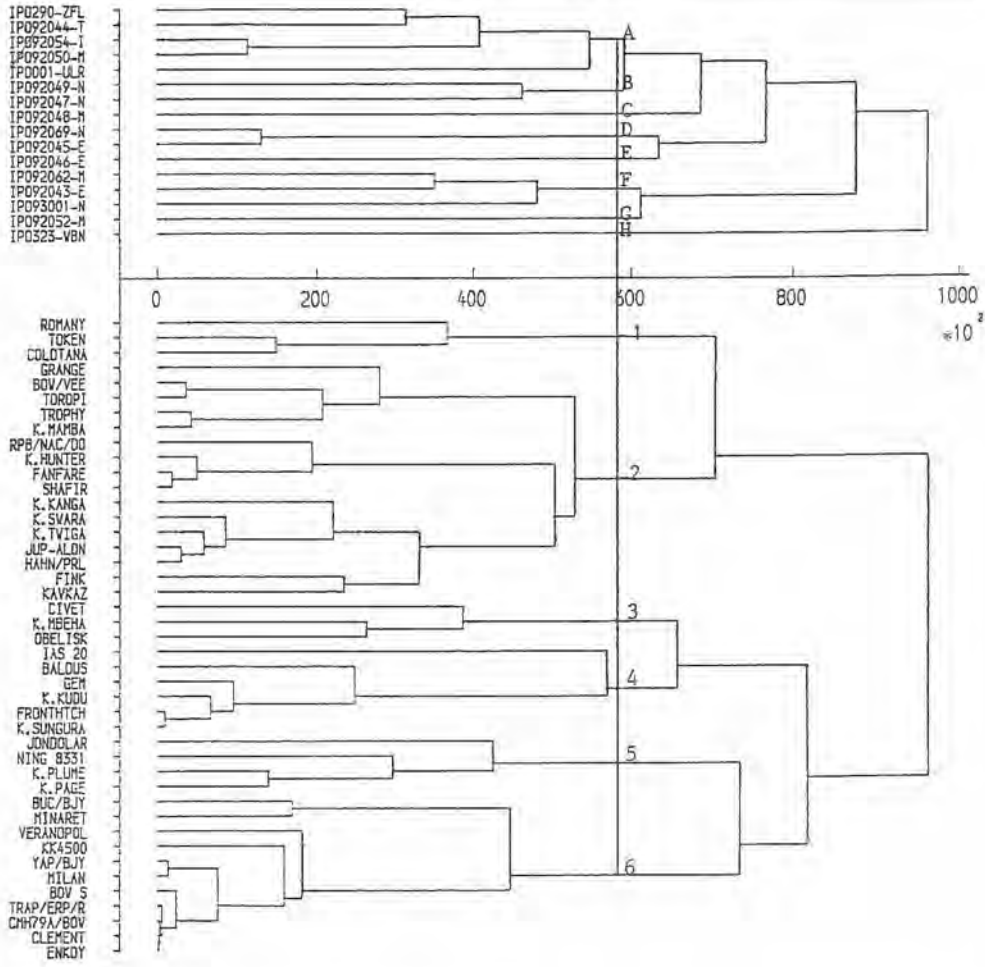
A similar cluster analysis was performed on necrotic leaf area to produce the dendograms in Fig. 2. The isolates are grouped into eight virulence groups and the cultivars into six resistance groups (P=0.05). The assignment of letters A to F for isolate virulence groups

Fig 1. Dendograms of simultaneously clustered *Septoria tritici* isolates (16) and wheat genotypes (43) based on percentage pycnidia coverage on the first leaves.



The area on the left of the vertical line represents non-significant differences at P=0.05

Fig 2. Dendograms of simultaneously clustered *Septoria tritici* isolates (16) and wheat genotypes (43) based on percentage necrosis on the first leaves.



The area on the left of the vertical line represents non-significant differences at P=0.05

Table 3. Response classes derived from means of percentage pycnidia within six isolate virulence groups and five cultivar resistance groups determined by cluster analysis

	Virulence group					
Resist. Grp.	A	B	C	D	E	F
1	MR	S	S	S	R	S
2	MR	MR	MR	S	MR	MR
3	R	R	MR	MR	R	R
4	R	R	R	R	R	R
5	MR	S	MR	MR	S	MR

Resistant (R) 0-10%; Moderately Resistant (MR) 11-20%; Susceptible (S) >20%

Table 4. Response classes derived from means of percentage necrosis within eight isolate virulence groups and six cultivar resistance groups determined by cluster analysis

	Virulence group							
Resist. Grp.	A	B	C	D	E	F	G	H
1	MR	MR	R	R	R	S	S	R
2	MR	S	S	S	MR	S	S	R
3	MR	MR	S	MR	MR	S	S	S
4	S	S	S	S	R	S	S	S
5	MR	R	MR	S	S	R	S	MR
6	R	R	R	R	R	R	R	R

Resistant (R) 0-20%; Moderately Resistant (MR) 21-40%; Susceptible (S) >40%

Table 5. Interactions between percentage necrosis (Necr.) and pycnidia (Pyc.) coverage within the necrotic leaf area of three cultivar/isolate combinations

Cultivar	Isolate	%Necr.	%Pyc.	
Kenya Kudu	IPO92069	82	55	HH
TRAP#1*2//ERP/RUSO	IPO92048	3	1	LL
Kenya Plume	IPO92054	53	3	HL

HH: High necrosis, high pycnidia; LL: Low necrosis, low pycnidia; HL: High necrosis, low pycnidia

and numbers 1 to 5 for cultivar resistance groups in Fig. 1 is independent from those of Fig. 2. It is shown that grouping of individual isolates and cultivars in Fig. 2 is different from that of Fig. 1. Isolates IPO92045-E and IPO92069-N are in virulence group D (Fig. 2) and are in virulence groups C and D respectively in Fig. 1. Another example is isolates IPO92047-N and IPO92062-M in group B (Fig. 1) and are in groups B and D respectively (Fig 2). The most virulent isolates are in group G and the least virulent are in group E (Table 4). Cultivars in group 6 are resistant to all the isolates.

Discussion

The occurrence of pathogenic variation in virulence of *S. tritici* in Kenya suggests that breeding for resistance to the pathogen may not be as straightforward as previously believed. It is therefore highly advisable to intensify research in such areas as physiologic specialization, inheritance of resistance and research for effective sources of resistance in order to control this important disease and hence allow new high yielding wheat cultivars to express their yield potential. The present study was a preliminary study into the *S. tritici* population in Kenya as the sample studied was too small (13) to be representative of the whole pathogen population. From this sample distinct virulence groups were identified. On further examination of these virulence groups the average disease severity was calculated and classified as Susceptible (S) Moderately resistant (MR) or Resistant (R) (Table 3 and

4). The cut-off points for the disease severity categories S, MR and R are arbitrary.

The variation in virulence in the *S. tritici* pathogen population in Kenya may dictate monitoring the pathogen populations to elucidate their relevance to the national program. The choice of isolates for screening germplasm then becomes of special importance. In the germplasm screening for resistance to septoria tritici blotch resistance at Njoro Research Center, an isolate collected from the fields in the area represented by IPO93001 in virulence group F (Table 4) is often used for artificial inoculations. In such a situation the cultivars in groups 5 and 6 will be selected for their high resistance reaction based on percentage necrosis (Fig 2 and Table 4). If these cultivars are later released as commercial cultivars and are grown in areas where isolates in virulence groups D, E and G occur, they will become susceptible.

The resistance provided by a specific cultivar should be investigated under a relevant virulence spectrum over time prior to its incorporation into breeding. The relevance of virulence spectra can be assessed by monitoring the pathogen populations on a standard set of differential cultivars selected from national and international programs. Such a standard set of cultivars has been suggested and composed by Gilchrist (1994) and is called the Septoria Monitoring Nursery (SMN). While this set of cultivars may in the future form the basis for virulence studies, supplemental cultivars of national importance should be included. In a previous study (Arama et al., 1989) no clearly distinguishable pathogenicity groups amongst Kenyan isolates could be detected. This could have been due to the cultivars used in the differential set which was composed of cultivars originating mainly from South America and Israel. It is likely that the pathogen population in Kenya is more adapted to the cultivars grown by the farmers. In that case the larger number of cultivars from Kenya used in this study enabled a better differentiation of the isolates from Kenya than in the previous study.

Apart from lack of an agreed-upon standard differential set of cultivars, there are still a lot of inconsistencies in methodology, environmental conditions, culturing of isolates and cut-off points for separating resistance from susceptibility. This makes comparison between results obtained here and elsewhere to be difficult. The differences in groupings of isolates into different virulence groups when pycnidia and necrosis were analyzed separately in this study suggested that the two parameters were independent of each other and may be

influenced by different genes. Kema, (1996) observed cultivar/isolate interactions with high necrosis-low pycnidia coverage and other interactions of high necrosis-high pycnidia in similar studies. Such interactions were also observed in this study (Table 5). The cultivar/isolate combination of Kenya Kudu and IPO92069 produced high necrosis accompanied with high pycnidia coverage. On the other hand, isolate IPO92054 produced high necrosis with low pycnidia coverage on Kenya Plume.

In conclusion it is advisable to identify an isolate that represents the pathogen virulence of a region. Such an isolate can be effectively used to screen for resistant germplasm in the greenhouse or in the field.

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Chapter 5

[1]Comparison of resistance of wheat cultivars to *Septoria tritici* at the seedling and adult plant stages

P.F. Arama, J.E. Parlevliet and C.H. van Silfhout

Summary

Fourteen wheat cultivars were tested for resistance to six *Septoria tritici* isolates at the seedling and adult plant stage under controlled environmental conditions. Correlations between the disease severity at the seedling and at the adult plant stages ranged from 0.36 to 0.78, indicating that seedling resistance does not predict adult plant resistance very well. Three types of resistance were shown to occur: Resistance in the seedling and adult plant stages (overall resistance), resistance in the seedling stage only (seedling resistance), and resistance in the adult plant stage only (adult plant resistance). Adult plant resistance was the less common phenomenon. In screening nurseries for resistance to *Septoria tritici*, testing both seedlings and adult plants is advisable to discern among the three types of resistance.

Introduction

Evaluation of wheat cultivars for resistance to *Septoria tritici* is often carried out on adult plants under field conditions (Jlibene et al., 1992; Shaner and Buechley, 1994). Field experiments with respect to septoria leaf blotch have inherent problems. It has been shown that the assessment of resistance in adult plants which is deduced from the disease severities in the various genotypes, is influenced by plant height and maturity as these factors too affect the disease severity (Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990; Arama

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et al., 1994). Some researchers have evaluated resistance on seedlings under controlled environmental conditions (Silfhout et al., 1989; Cohen and Eyal, 1993; Kema et al., 1996). It is not known whether resistance expressed in the seedling stage is also reflected in the adult plant stage and vice versa. Kema and van Silfhout (1995) found no significant correlations for two of the three isolates tested on 22 wheat cultivars inoculated at the seedling and adult plant stages. However, Brokenshire (1976) observed a high correlation between seedling and adult plant resistance. In other crop/pathogen systems, Koch (1990) indicated that all cultivars of rice to *Xanthomonas campestris* pv. *oryzae* showed a general trend towards reduced susceptibility with increasing age. Broers and Jacobs (1989) in their studies on wheat/leaf rust reported that partial resistance genes were better expressed in the adult plant stage than in the seedling stage. From such studies it is evident that the association between the resistance in the seedling and adult plant stages can vary with the particular crop/pathogen system studied. The main objective of the present investigation is to study the relation between seedling and adult plant resistance to *S. tritici* in wheat.

Materials and Methods

Seedlings

Fourteen cultivars of spring wheat (*Triticum aestivum*) were used in this study. Susceptible checks were Kenya Sungura and HAHN'S'*/PRL'S'. The resistant check was Milan. The other entries tested (Table 3) were thought to have varying levels of resistance to *S. tritici* based upon their reaction in the field. The entries were sown in jiffy pots (7x7 cm) to have 9-15 seedlings/entry. The trial was designed in a randomized complete block design with three replicates.

Six isolates were used in this experiment. These included three isolates IPO323, IPO001 and IPO290, collected from different locations in the Netherlands, and were found to differ in aggressiveness when tested on wheat seedlings (Kema pers. comm.). Three other isolates IPO92069, IPO93001 and IPO93043 were collected from different locations in Kenya. These isolates were the most aggressive at the seedling stage from the Kenyan septoria isolates collection maintained at the Research Institute for Plant Protection (IPO). Isolates were retrieved from cryo-tubes stored at -80^{0}C and grown on petri plates of V8 juice agar medium. Before inoculation the isolates were scraped off and suspended in distilled water

and filtered through cheese cloth. The concentration was adjusted to $1x10^7$ spores/ml. The plants were inoculated when they were 10 days old with cultures which were grown for five days. Potted seedlings in each tray were placed on a turn-table revolving at 21 RPM and evenly sprayed with 30 ml of spore suspension. After inoculations plants were placed in a growth room at temperatures of 20 to 22°C and 90 to 95% RH. Plastic covers were placed over the plants for the first 48 hours of incubation to ensure 100% RH. Plants were incubated for a total of 21 days in the growth room. The first leaves were visually assessed for percentage necrosis and pycnidia due to septoria leaf blotch.

Adult plants

The same isolates and cultivars as in the seedling experiment were used. Inoculum preparation was as described above. Inoculum was adjusted to $1x10^7$ spores/ml. Entries were planted in plastic pots with two plants per pot, five pots per entry. To synchronize on maturity three plantings were done at an interval of 10 days. Of each entry plants were chosen for inoculation that were in a similar stage (just heading).

The plants were placed on greenhouse benches flooded with water. An Ultra Low Volume (ULV) sprayer was used to inoculate the plants. One liter of the spore suspension was used to inoculate 140 plants until leaf wetness. Plants were incubated under plastic covers for 48 hours at 90-95% RH. and 20-22°C. Thereafter the plastic covers were removed. Observations were made 21 days after inoculations. It was observed that the conditions in the greenhouse were not humid enough to enable proper formation of pycnidia. Thus percentage necrosis was the parameter preferred. For comparison the same parameter was used in the seedling experiment. The percentage necrosis on the uppermost two leaves were visually assessed on each plant. The trial was repeated three times to be analyzed as replicates.

Results

The analysis of variance presented in Table 1 showed that the interaction variance between isolates and cultivars was highly significant ($P<0.001$) in both plant stages. Specific interactions were observed at the adult plant stage such as in the cultivar/isolate combination of RPB.1468/NAC//DOVE and CMH79A.307/BOW'S' with isolates IPO323

Table 1. Analyses of variance of percentage necrosis of 14 wheat cultivars inoculated with six *Septoria tritici* isolates at seedling and adult plant stages.

Source of Variation	Df.	MS. Seedling	MS. Adult
Isolates	5	7269.6 ***	16027.9 ***
Cultivars	13	8857.4 ***	12692.1 ***
Isolate x Cultivar	65	885.0 ***	1558.0 ***

*** = significant at the P< 0.001.

Table 2. Correlation coefficients between seedling and adult plant necrosis of 14 wheat cultivars inoculated with three Dutch (NL) and three Kenyan (KE) *Septoria tritici* isolates.

Isolate	Correlation	Isolate	Correlation
IPO323 (NL)	0.78 ***	IPO92043 (KE)	0.65 **
IPO001 (NL)	0.66 **	IPO93001 (KE)	0.58 *
IPO290 (NL)	0.59 *	IPO92069 (KE)	0.36 ns

*** significant at P< 0.001, ** significant at P< 0.01 and * significant at P< 0.05.

and IPO001 (Table 3). In the seedling stage such interactions were observed on for instance Milan and BUCK'S'/BJY'S' with isolates IPO93001 and IPO92043.

Correlations between disease severities in the seedling and adult plant stages (Table 2) were significant (P<0.05) for all isolates except for isolate IPO92069 which showed a non-significant correlation coefficient of 0.36. These correlations were not high, the highest being 0.78 of IPO323.

Results in Table 3 show that there were cases of combined seedling and adult-plant resistance, seedling resistance/adult-plant susceptibility, seedling susceptibility/adult plant

Table 3. Mean percentage necrosis of 14 wheat genotypes inoculated with six isolates of *Septoria tritici* at the seedling stage (S) and adult plant stage (A).

	Isolate													
	001		323		290		92069		93001		92043		Mean	
Entry	S	A	S	A	S	A	S	A	S	A	S	A	S	A
Frontatch	54	69	31	65	75	79	76	89	89	87	73	98	66	81
K. Sungura	64	92	14	90	74	100	56	100	81	95	65	99	59	96
RPB./NAC..	0	0	77	65	56	86	80	13	63	58	73	38	58	43
Ning 8331	14	66	21	52	57	71	67	85	75	92	69	93	51	77
Jup.-Al.	0	0	58	75	22	85	51	76	73	92	70	85	46	69
BOW'S'/VEE	6	0	21	59	60	82	56	81	60	96	63	86	44	67
HAHN/PRL	0	58	41	82	24	98	17	100	62	100	70	99	36	90
Fink'S'	0	0	25	0	0	50	22	100	71	100	55	87	29	56
BUCK/BJY	0	0	21	75	33	60	27	86	9	75	47	69	23	61
YAP/BJY'S'	0	0	24	0	0	67	19	80	70	90	12	67	21	51
TR/ERP/RUS	27	0	21	21	0	81	13	28	12	11	13	70	14	35
Milan	0	3	0	0	0	5	15	0	41	0	0	0	9	1
Clement	0	0	15	0	0	18	8	66	4	90	3	75	5	42
CMH79/BOW	0	40	0	1	0	58	6	0	9	0	2	15	3	19

resistance and of susceptibility at both plant stages. Entries HAHN'S'/PRL'S', Fink'S', BUCK'S'/BJY'S' and YAP/BJY'S' when inoculated with isolates IPO290 and IPO92069 showed seedling resistance. Adult plant resistance and seedling susceptibility was less common amongst the entries tested. This type of resistance for instance was shown by isolate/entry combination of IPO92069/RPB.1468/NAC//DOVE with 13% necrosis at the adult plant stage and 80% infection at seedling stage. The third type of resistance observed was that of seedling resistance and adult plant resistance. This was exhibited by for instance the isolate/entry combination of IPO323, IPO290 and IPO001 with Milan and Clement. Frontatch, K. Sungura and Ning 8331 were susceptible to IPO290, IPO92069 and IPO93001 at both plant stages.

Discussion

The low correlation coefficients between seedling and adult plant stages indicated that the resistance measured in the seedling stage could not explain the adult plant effect. Kema and van Silfhout (1995) in their studies involving three Dutch *S. tritici* isolates for their virulence on 23 wheat cultivars found significant correlations between seedling and adult plant data for only one isolate but not significant for the other two isolates. This indicated according to them that seedling data had limited value for resistance evaluation at adult plant stage. Arama (1993) reported that seedling reaction was in quite a number of cases not a good predictor of adult plant reaction. However, Brokenshire (1976) observed a high correlation between seedling and adult plant resistance.

From the specific entry/isolate interactions observed at both plant stages it was obvious that certain isolates were able to overcome the resistance of the entries tested. Each of these isolates must have one or more genes for specific virulence which do not occur in the other isolates. In this study it was possible to discern three types of resistance. The first type was resistance that was expressed at seedling and adult plant stage. An example for this resistance was with cultivars Milan and Clement to isolates IPO323 and IPO001. This type of resistance was referred to as 'overall' resistance by Zadoks (1961). In the second type of resistance plants were resistant at the seedling stage and susceptible at adult plant stage. A third type of resistance exemplified by the isolate/cultivar combination of IPO92069/RPB.1468/NAC//DOVE, plants were more resistant in the adult plant stage than

in seedling stage. This adult plant resistance can be explained by the presence of genes that are expressed only in the adult plant stage.

In conclusion, seedling tests with respect to wheat/septoria leaf blotch are only advisable as a rough screening for resistance. For more precise assessments, both seedling and adult plant tests are recommended. The advantage with seedling tests is that they take far less space than adult plant tests and that no difficulties arise with genotypic differences in the development stage.

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Chapter 6

Wheat cultivars response to three isolates of *Septoria tritici* under field condition.

Summary

A field experiment was carried out in Wageningen, The Netherlands to evaluate the resistance in some wheat cultivars to three isolates of *S. tritici* collected from different locations in The Netherlands. The isolate IPO290 collected in 'Zuid Flevoland' was virulent to all the cultivars with the exception of Clement and Milan. Clear isolate x cultivar interactions were shown especially involving the less virulent isolates IPO001 and IPO323. Although the cultivar x isolate interaction variance was highly significant, the variances due to main effects of cultivars and isolates was far greater.

Introduction

The currently grown high yielding wheat cultivars are more susceptible to septoria leaf blotch and utilization of sources of resistance is of a high priority in national and international breeding programs (Eyal et. al., 1983). Identification of different sources of resistance constitutes the foundation of these septoria breeding programs. However, physiological specialization of septoria isolates within a geographic region may pose a problem in designing proper resistance breeding programs (Eyal et al., 1973). Expression of host response to *S. tritici* is usually evaluated by appraising the relative amount of infected tissue either by assessing necrotic leaf tissue or pycnidia coverage (Brokenshire, 1976; Eyal et al., 1987.).

Several sources of resistance have been reported (Eyal et al., 1987; Nelson and Marshall, 1990). Reduced disease development is often correlated with traits like plant height, maturity and canopy structures (Mann et al., 1985; Eyal and Talpaz, 1990; van Beuningen and Kohli, 1990; Arama et al., 1994). Attempts have been made to correct for variation in these characters, especially for heading date and tallness, to determine true disease severity (van Beuningen and Kohli, 1990; Arama et al., 1994;). Within countries differences in *S. tritici* virulence and wheat genotype susceptibilities have been described (Yechilevich-

et al., 1996).

Here the response of a range of wheat genotypes to three *S. tritici* isolates under field conditions was studied.

Materials and methods

Twenty nine spring wheat cultivars originating from Kenya (14), CIMMYT (11), and The Netherlands (4) were chosen. The experiment was planted in a field located at the Department of Plant Breeding, Wageningen in April 1993. Entries were planted in plots of six rows of 1 m long in a randomized complete block design with three replicates. The three blocks to be inoculated with three isolates were separated by 4 m of oat. Isolates used in this experiment were IPO290 collected from Z. Flevoland, IPO323 collected from W. Brabant and IPO001 collected from Ulrum in The Netherlands. These isolates had been tested earlier (Kema, pers. comm.) and were found to differ in their virulence when inoculated on wheat seedlings. Each isolate was inoculated into 12 conical flasks containing 1 L of yeast-glucose liquid medium (Eyal et al., 1987). Flasks were shaken for five days. The liquid medium was decanted and the spores re-suspended in distilled water. Spore concentration was determined and adjusted to $1.3x10^6$ spores per ml. Two inoculations were carried out, one 55 days after planting and the second eight days later. From the first inoculation onward overhead sprinkler irrigation was provided daily for one hour in the morning and again in the evening. Disease assessment was made visually by estimating the percentage necrotic leaf area due to septoria on the flag leaf (F) and the first lower leaf after the flag leaf (F-1). Ten tillers, sampled at random from the inner four rows, were assessed.

The percentage necrosis was arcsine transformed before the data were analyzed. The Data were then analyzed using SAS General Linear Model Procedure (SAS, 1985)

Results

The analysis of variance (Table 1) shows that the main effects of isolates and cultivars and the isolate x cultivar interactions are highly significant. Although the isolate x cultivar interaction variance is highly significant, the variances due to main effects of cultivars and

Table 1. Analysis of variance of percentage leaf necrosis coverage (arcsine transformed) on adult plants of 29 wheat cultivars inoculated with three isolates of *Septoria tritici*.

Source of variation	df.	S.S.	M.S.	F-ratio	Sig. level
Isolates (I)	2	16.9084	8.4542	733.36	P<0.001
Cultivars (C)	28	34.9627	1.2487	108.32	P<0.001
Replicates(Isolates)	6	0.2906	0.0484	4.20	P<0.001
I x C	56	25.7800	0.4604	39.93	P<0.001
Error	168	1.9367	0.0115		
Total	260	79.8785			

Coefficient of variation = 11.0%

isolates is far greater. Isolate IPO290 was highly virulent to all the cultivars with an exception of Milan (26.0%) and Clement (46.4%) (Table 2). The first 11 cultivars from Baldus to Ning 8337 were highly susceptible to the three isolates. The remaining 18 cultivars showed varying levels of resistance or susceptibility to IPO001 and IPO323. Isolate IPO323 had probably the same avirulence to Jupateco-Alondra, Kenya Mamba, RPB/NAC//DOVE, Kenya Hunter, BOW'S/VEE'S', BUC'S'/BJY'S' and Fanfare . Another avirulence factor observed involved IPO001 and the cultivars Enkoy and Kenya Mbweha. Isolates IPO323 and IPO001 were both avirulent to Jondolar, JAP/BJY'S', Fink's' and Clement. A strong isolates x cultivars interaction was shown between IPO001 and IPO323 with Kenya Mamba and Enkoy. A similar interaction was observed between IPO001 and IPO323 with BUC'S'/BJY'S' and CMH79A/BOW'S'. Also between IPO001 and IPO323 with the cultivars Jupateco-alondra and Kenya Mbweha. A weak interaction was observed between IPO290 and IPO001 with Clement and Milan. The cultivars Minaret, Jondolar, JAP/BJY'S', Fink's' Clement and Milan were highly resistant to isolates IPO001 and IPO323.

Table 2: The percentage necrosis on the uppermost two leaves of 29 wheat cultivars inoculated with three isolates of *Septoria tritici* in the field.

	Isolate		
Cultivar	IPO290	IPO001	IPO323
Baldus	100.0	100.0	100.0
Kenya Plume	95.7	100.0	93.2
Kenya Sungura	100.0	84.8	100.0
Kenya Page	97.1	94.9	87.3
Trophy	100.0	94.8	76.9
Frontatch	98.7	84.3	88.4
HAHN'S'*PRL'S'	100.0	99.8	57.5
Grange	100.0	98.3	93.3
Civet	71.7	91.7	97.7
Token	96.5	91.7	54.7
Ning 8331	91.1	64.3	85.3
TRAP//ERP/RUSO	94.7	37.8	98.3
Jupat.-Alond.	100.0	84.3	0.8
Kenya Mamba	100.0	87.2	0.0
RPB/NAC//DOVE	100.0	79.5	7.7
Kenya Hunter	98.7	77.9	0.8
BOW'S'/VEE'S'	94.7	78.8	0.0
BUC'S'/BJY'S'	79.8	89.4	0.0
Romany	97.2	41.0	12.4
Enkoy	71.7	2.3	77.1
Fanfare	75.3	73.8	2.8
CMH79A/BOW	77.2	11.1	59.0
Kenya Mbweha	76.7	1.3	61.1
Minaret	100.0	0.0	22.7
Jondolar	97.8	6.6	1.9
YAP/BJY'S'	65.3	9.3	0.0
Fink's'	67.3	2.3	0.3
Clement	46.4	0.3	4.9
Milan	26.0	0.0	17.2

Discussion

This experiment showed that many clear cultivar x isolate interactions existed when cultivars of diverse origin were inoculated with a small sample of isolates from one country. Avirulence appeared more frequently in two of the three isolates, IPO001 and IPO323. The three isolates have been studied on wheat seedlings under controlled environmental conditions in other experiments by Kema (1996) and Arama (this thesis, Chapter 4) and found to differ in virulence pattern using cluster analysis. Such differences in virulences observed by them on seedlings were also evident under field conditions, albeit on different wheat genotypes. This experiment also highlights the importance of a relevant choice of isolates for screening wheat genotypes in a nursery. Without knowledge of the isolate used, the breeder may use isolates IPO001 or IPO323 for artificial inoculations in The Netherlands. The cultivars such as Minaret, Jondolar, JAP/BJY'S' and Fink's' would be selected (among others) due to their high resistance response to these isolates. If these cultivars were later on released for commercial growing (Minaret and Jondolar are commercial cultivars in The Netherlands) in 'Zuid Flevoland' where the population of isolate IPO290 is found these cultivars may become highly susceptible. This indicates the importance of cultivar x isolate interactions observed in the growth rooms and now also observed in the field.

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Chapter 7

The response of field inoculated wheat cultivars to two *Septoria tritici* isolates and their 1:1 concentration mixture

Summary

Six wheat cultivars of diverse genetical background and response to *Septoria tritici* were inoculated in the field with two isolates, IPO93001 (fairly virulent) and IPO92054 (highly virulent) and the 1:1 concentration mixture. The main effect of cultivars and isolates and the cultivar x isolate interaction were found to be highly significant. In certain cultivar/isolate combinations the isolate mixture had less pycnidia coverage than IPO92054 but more than IPO93001. On the more resistant cultivars Milan and Fink's the isolate mixture produced more pycnidia and leaf necrosis than either IPO92054 and IPO93001.

Introduction

Differentiation of host response to *Septoria tritici* is based on quantitative assessment of symptoms as qualitative assessment of symptoms is rarely possible (Eyal et al., 1987). Based on such quantitative assessments, significant cultivar x isolate interactions have been reported (Eyal et al., 1985; van Silfhout et al., 1989). Similar results of Eyal and Levy (1987) suggested geographic differentiation of specific virulences in *S. tritici*. In host-pathogen systems where specific interactions do occur, testing for resistance needs special attention with respect to selection of isolates and also whether to use single isolates or mixtures.

In breeding programs where host genotypes are evaluated under field conditions, septoria tritici blotch (STB) epidemics are sometimes incited through artificial inoculations with a mixture of isolates of *S. tritici* of national origin (Zelikovitch et al., 1986; Anon, 1988; Smirnova et al., 1990; McKendry and Henke, 1994). Mixing of virulent and avirulent *S. tritici* isolates in certain combinations resulted in reduced pycnidial density as compared to coverage obtained when each isolate was applied separately on wheat seedlings according to Zelikovitch et al. (1986) and Zelikovitch and Eyal (1991). In other experiments, Eyal (1992) observed that, apart from the reduction of pycnidial coverage for mixtures of two

isolates, cultivars exposed to the mixtures expressed differential response in pycnidial coverage compared to the single isolate response. However, Gilchrist and Velazquez (1994) did not observe such a reduction in pycnidial density for the mixture of three isolates under field conditions on adult plants differing in resistance to the isolates.

The observations reported above are of considerable importance in relation to the type of inoculum to be used in screening experiments. The objective of the present study was to evaluate the response of six wheat cultivars to two *S. tritici* isolates, and their 1:1 concentration mixture under field conditions in Kenya.

Materials and methods

Six spring wheat cultivars (Table 1) showing varying levels of resistance to the two isolates were selected on the basis of resistance level in the seedling stage. Cultivar Mbuni is a Kenya commercial cultivar released in 1983. The other cultivars originated from the International Maize and Wheat Improvement Center (CIMMYT), Mexico. These entries were planted at the experimental farm of the National Plant Breeding Research Center, Njoro, Kenya (2160 m altitude) on May 3, 1994 during the main rain season (March-September). Each plot consisted of six rows, 2 m long and a row distance of 0.25 m. The experimental design (18 treatments; 6 cvs. x 3 inoculums) was a randomized complete block with three replicates. Plots were separated by eight rows of 2 m long buffer crop of oat while blocks (replicates) were separated by 16 rows of 2 m long of oat

Isolates and inoculation

Isolates selected for this study were IPO92054 (virulent) collected at Eldoret and IPO93001 (less virulent) collected at Njoro. These isolates had been tested in the seedling stage on a differential set consisting of 43 wheat cultivars and found to differ in virulence (this thesis chapter 4).

Isolates were retrieved from cryo-tubes obtained from the *S. tritici* collection at the Research Institute for Plant Protection (IPO-DLO) in the Netherlands. The two isolates were grown on Malt Yeast Agar (Eyal et al., 1987) in petri dishes for five days. A spore suspension was made for each isolate and used to inoculate cultivar Kenya Kongoni seedlings. Plants were incubated in the greenhouse for 21 days. Leaves containing pycnidia

were harvested, dried and stored in a refrigerator at 4^0C until the time of use.

Leaf segments 3-5 cm long were placed on a microscope slide in a petri-dish on moistened filter paper to create high humidity for two hours. Pycnidiospores were extruded from pycnidia in cirri. A single cirrus was removed from the leaf segment and inoculated onto freshly prepared Malt Yeast Agar. Sub-cultures were made to multiply inoculum.

At the time of use, a loopful of isolate culture was transferred into Pyrex conical flasks containing 1 L of Yeast Sucrose liquid medium (Eyal et al., 1987). The inoculum was shaken for five days on a rotary shaker at room temperature. Inoculum was filtered through cheese cloth to remove large mycelial particles. Spore concentration was determined and adjusted to 1 x 10^7 spores/ml. Isolate mixture of 1:1 concentration ratio was prepared by mixing the individual isolates just before inoculations.

Inoculations were done using separate CP15 knapsack sprayers for the isolates and the mixture. Two inoculations were made at an interval of five days when the plants were at growth stage 31 (Tottman and Makepeace, 1979). Supplemental overhead sprinkler irrigation was applied every three days whenever there was no rainfall in between so as to maintain high humidity conditions in the plots.

Three observations were made at an interval of 14 days from July 27. At each time, ten tillers were sampled at random from the innermost four rows of each plot. The percentage necrotic leaf area and the density of pycnidia within the necrotic area was estimated on the upper two leaves.

The percentage of necrotic or pycnidial coverage was combined for the two leaves in each tiller and averaged for the ten tillers observed in each plot. Area Under Disease Progress Curve (AUDPC) was calculated as (7DS1+14DS2+7DS3) where DS1, DS2, and DS3 are percentage coverage (necrosis or pycnidia) at first, second and third observation dates respectively.

Results

The correlation coefficient between pycnidia coverage and leaf necrosis was 0.97.

The 1:1 isolate mixture produced less necrosis than isolate IPO92054 but more necrosis than IPO93001 when inoculated on Mbuni, RPB/NAC/DOVE, Jupateco-Alondra and Ning 8331 (Table 1). On the cultivars Mbuni and RPB/NAC//DOVE the AUDPC for the isolate

Table 1. Means for area under disease progress curve for leaf necrosis on six wheat cultivars inoculated with isolates IPO93001, IPO92054 and the 1:1 isolate mixture.

	Isolate		
Cultivar	IPO93001	1:1 mix	IPO92054
1 Mbuni	1488 bc*	1499 bc	2024 a
2 RPB./NAC//DOVE	1124 de	1363 cd	1714 b
3 Jupateco-Alondra	872 ef	1364 cd	1430 bc
4 Fink'S'	470 gh	1104 de	755 fg
5 Ning 8331	442 h	755 fg	957 ef
6 Milan	49 i	356 h	255 hi
Mean	744	1073	1189

*Means followed by the same letter are not significantly different (P≥0.05) using Tukey's W method.

mixture was significantly lower than that of IPO92054 but was not significantly higher than that of IPO93001. The AUDPC for the 1:1 mixture on Milan and Fink's was higher than of the two individual isolates. In Table 2, on pycnidia coverage, the isolate mixture produced less pycnidia than IPO92054 on Mbuni and RPB/NAC//DOVE. On cultivars Jupateco-Alondra, Fink's and Milan there were more pycnidia produced by the isolate mixture than by IPO92054. The less virulent isolate IPO93001 produced more pycnidia on Ning 8331 than IPO92054 and the 1:1 mixture. Differential cultivar x isolate interactions were observed such as between Mbuni and Fink's with the 1:1 mixture of isolates and isolate IPO92054 (Table 1 and 2) and between Jupateco/Alondra or Fink's and Ning8331 with isolates IPO93001 and the 1:1 mixture (Table 2). There are more interactions, but these are not differential; such as Mbuni and Milan/IPO93001 and Mix. The Spearman's rank correlations between the AUDPC of the isolate mixture with IPO93001 and IPO92054

Table 2. Means for area under disease progress curve for pycnidia coverage on six wheat cultivars inoculated with IPO93001, IPO92054 and the 1:1 mixture.

	Isolate		
Cultivar	IPO93001	1:1 mix	IPO92054
1 Mbuni	1005 c-e*	1217 bc	1732 a
2 RPB./NAC//DOVE	825 ef	1108 b-d	1286 b
3 Jupateco-Alondra	692 fg	1172 bc	1138 bc
4 Fink 'S'	309 i-k	890 d-f	576 gh
5 Ning 8331	199 kl	156 h-j	186 g-i
6 Milan	14 l	239 jk	112 kl
Mean	507.3	797	838.3

*Means followed by the same letter are not significantly different (P≥0.05) using Tukey's W method.

were between 0.89 and 0.94.

Discussion

The response of wheat cultivars to *S. tritici* was evaluated by assessing the level of necrosis or pycnidia density on the foliage as carried out by others (Eyal et al., 1987). Under optimal environmental conditions, pycnidial formation is usually induced on most wheat cultivars (Eyal et al., 1987). Necrosis without pycnidia formation is mostly expressed under sub-optimal environmental conditions, by resistant cultivars or in certain species, upon inoculation across a range of graminaceous genera and species with various isolates of *S. tritici* (Brokenshire, 1975; Eyal et al., 1987; Kema, pers. comm.).

In this experiment Mbuni, the most susceptible cultivar, produced 100% leaf necrosis and 90% pycnidia coverage when inoculated with the isolate mixture and the individual isolates

IPO92054 and IPO93001 at the time of the third observation date. The high correlation coefficient of 0.97 between necrosis and pycnidia density could be attributed to high humidity conditions that were enhanced with overhead sprinkler irrigation in the plots to supplement rainfall during the experimentation period in 1994 (April - September). This ensured a severe epidemic of septoria tritici blotch in the field. Under these conditions and cultivars, either pycnidia coverage or necrosis could be used to assess host response to the pathogen. Results obtained in Table 1 and 2 on area under disease progress curve for both leaf necrosis and pycnidia density showed that the 1:1 isolate mixture had in some cases more pycnidia than the less virulent isolate IPO93001 and in some cases less than the more virulent isolate IPO92054. There was no significant change in the ranking of the cultivars. This contradicts the observations made by other workers (Zelikovitch et al., 1986; Zelikovitch and Eyal, 1991; Eyal, 1992). In the case of Zelikovitch and Eyal (1991) the conditions in the greenhouse may not have been optimal enough and this could have contributed to the low pycnidia density observed. In that experiment the most susceptible cultivar Shafir produced 45% pycnidia coverage with the most virulent isolate ISR398. To highlight the importance of environmental influence on pycnidia coverage Eyal (1992) recorded significant reductions in pycnidial coverage for mixtures of two or five isolates relative to the virulent isolate ISR8036 under the moderate 1989/1990 epidemic in the field. Under the severe 1990/1991 epidemic due to favourable weather, pycnidia coverage on cultivars inoculated with the mixture of the same two isolates did not differ significantly from that of ISR8036. The data reported here agree with those of Gilchrist and Velazquez (1994) who also did not observe a reduction in pycnidial density for the mixture of three isolates under field conditions on adult plants differing in resistance to the isolates.

In most septoria tritici blotch breeding programs the knowledge of the virulence spectrum of *S. tritici* populations within the mandated research areas where the disease is prevalent is generally insufficient. No standard differential cultivar series has yet been developed for world wide use in monitoring the virulence of local populations of the pathogen. Under these circumstances breeders and pathologists prefer to use mixtures of isolates collected from different locations within the country hoping to incorporate as many pathotypes as possible in the inoculum. More studies especially under field conditions need to be carried out in future so as to understand the interaction between isolate mixtures host genotypes and

environment.

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Chapter 8

Effect of plot size and plot situation on the assessment of resistance in wheat cultivars to *Septoria tritici*

Summary

In small breeders' plots, adjacent to one another, a representational error can be expected when screening for resistance to septoria tritici blotch is carried out. The representational error or interplot interference may occur as an underestimation of resistance or as an error in the ranking of the cultivars tested.

Three experiments were conducted in Kenya (1992 and 1995) and The Netherlands (1993) to study interplot interference in different adjacent and isolated plot situations. Results showed that the mean disease severity, range and standard deviation increased from the hill plot situation to the eight rows isolated plots. Range in disease severity between cultivars in the small, adjacent plots was similar to that in the large, isolated plots indicating that the resistance level was not underestimated. Spearman's rank correlations between the small adjacent plots (hill, one and two rows) and the large isolated and none isolated plots ranged between 0.83 to 1.00. This shows that ranking of cultivars was not seriously affected by the presence or absence of interplot interference despite the increase in disease severity in the large plots. From the breeder's point of view, selection for resistance in small adjacent plots is not affected by interplot interference and is representative of the farmer's situation.

Introduction

Small amounts of seed are a common feature in the early stages of breeding programmes. Combined with the need to test large numbers of entries, the breeder frequently has to set up field experiments consisting of a large number of entries in relatively small plots adjacent to each other. This way of evaluating germplasm for disease resistance may lead to an under-estimation or over-estimation of the level of resistance. Samborski and Peturson (1960) observed that within leaf rust resistant wheat cultivars planted in large plots, the amount of rust spores produced remained low and the total amount of dead tissue resulting from infection was also low. However when such resistant cultivars were grown in small

experimental plots, the proximity of susceptible cultivars provided a constant heavy supply of inoculum and a considerable increase of infection on the resistant cultivars. Apparently the disease situation in experimental plots can differ from the one in farmers' field situations they are meant to represent and this error is called the 'representational or cryptic error' (Vanderplank, 1963) or interplot interference.

The importance of interplot interference in field plot experiments has been recognized and was an object of several studies in different crop/pathogen systems (Vanderplank, 1963; James et al., 1973; Parlevliet and van Ommeren, 1975; Burleigh and Loubane, 1984; Bowen et al., 1984; Parlevliet and van Ommeren, 1984; Randle et al., 1986; Danial et al., 1993; Broers and Lopez-Atilano, 1995). James et al., (1973) in their studies on interplot-interference on late blight of potatoes described two types of interplot effects which they referred to as negative and positive interference. Negative interference occurred when a large proportion of the inoculum produced within a plot was dispersed outside the plot's boundaries. Epidemic development is limited since the lost inoculum cannot contribute to further infections. Conversely, positive interplot-interference occurred when a plot was subject to influx of inoculum from external sources, resulting in increased disease development. They concluded that all experimental plots were subject to both positive and negative interplot interference and that plots were not affected equally.

Parlevliet and van Ommeren (1975; 1984) found that the partial resistance of barley to leaf rust in a 'mosaic of small adjacent plots' was severely underestimated compared to the degree of resistance observed in the same cultivars in isolated plots due to interplot interference. Broers and Lopez-Atilano (1995) observed that the partial resistance of durum wheat to stem rust was slightly underestimated in such a 'mosaic of small adjacent plots'. In yellow rust of wheat there was no measurable interplot interference (Daniel et al., 1993). Negative interplot interference has also been shown to occur in field experiments with leaf rust of wheat (Bowen et al., 1984).

Few experiments have been conducted to study interplot interference in septoria tritici blotch of wheat. Burleigh and Loubane (1984) showed that the severity of *Mycosphaerella graminicola* was not significantly different in plots 40 x 40 m and 10 x 10 m in one site and in plots 40 x 40 m and 20 x 20 m at another site. The area under the disease progress curve (ADPC) from plots 40 x 40 m infected with *M. graminicola* were significantly greater

than ADPC from plots 20 x 20 m and 10 x 10 m but final disease severities were only 2-10% greater. However, these plot sizes are large to those used in breeding programs and may not be representative for small adjacent plot situations.

These studies highlight the importance of interplot interference and that the effects cannot be generalised. The present study was conducted to determine if and to what extent interplot interference can affect the assessment of *S. tritici* resistance in wheat cultivars under different field plot situations

Materials and methods

Experiment 1: Njoro, 1992

This experiment was sown on 10th October at Njoro, Kenya, altitude 2160 m. Four bread wheat cultivars varying in resistance levels were planted. The entries were evaluated for their level of resistance in five plot situations:

H: Hill plots planted in four rows of 2.0 m length

NI1: Adjacent plots of one row of 2.0 m length

NI2: Adjacent plots of two rows of 2.0 m length

NI8: Adjacent plots of eight rows of 2.0 m length

I8: Isolated plots of eight rows of 2.0 m length

The block with isolated plots was planted adjacent to the non-isolated block and separated from it by 4 m of oats. Cultivars were planted in three replicates in a randomized complete block design. The seed rate was 120 Kg/ha at a spacing of 20 cm between rows. Each plot was isolated by 3 m of oat while the replicates were separated from each other by 4 m of oats. The non-isolated block was also planted in three replicates. Within each replicate the position of the plot situation (ie. hills, 1 row, 2 rows and 8 rows) was randomised. The sequence of the cultivars was the same for the four treatments in each replicate. Hills were planted 4-7 seeds at a distance of 10 cm between the hills. Four rows of hill plots were planted in each replicate.

An isolate collected from Njoro was used for artificial inoculations. Inoculations were carried out at growth stage 30 (GS 30) (Tottman and Makepeace, 1979). The isolate was inoculated into Yeast-sucrose liquid medium prepared according to the method described by Eyal et al. (1987) and shaken for 5 days. Spore concentration was determined and

Table 1. The mean, range and standard deviation for area under disease progress curve (AUDPC) of four wheat cultivars in five plot situations in 1992.

	Plot situation					
Cultivar	I8	NI8	NI2	NI1	H	Mean[a]
CMH78.390/4/MRNG..	1306.1	1152.6	1064.6	872.3	865.5	934.1
LOV23/BJY'S'	1042.9	837.7	769.3	747.9	612.9	710.0
BR5/4TP//CNO/INIA..	821.2	837.0	615.6	513.3	612.9	580.6
BUC'S'/BJY'S'	471.1	420.4	249.5	190.0	221.6	220.4
Mean	910.3	811.9	674.5	580.9	578.2	611.2
Range	835	732.2	579.9	397.4	542.2	713.7
Standard deviation	352.6	300.4	249.5	190.0	221.6	298.7

[a]Mean of H, NI1 and NI2 representing breeders' small adjacent plots.

adjusted to $5x10^6$ spores/ml. Before inoculations 10 ml Tween 20 surfactant was added to 15 L of the inoculum. Plots were inoculated twice at an interval of 7 days. Overhead sprinkler irrigation was supplied every two days for a period of two hours between 16.00-18.00 h.

Disease severity (DS) was assessed on three dates at an interval of 14 days. The first observation was done on 10 December. Ten main tillers were taken at random from the plots. Disease assessment was made by estimating the percentage necrosis on the flag leaf (F) and the first leaf below the flag leaf (F-1). The area under the disease progress curve (AUDPC), derived from the three observation dates was calculated as:

7 x DS1 + 14 x DS2 + 7 x DS3 where DS1, DS2, and DS3 refers to percentage disease severity at observation dates 1, 2 and 3 respectively.

Table 2. The mean, range and standard deviation for area under disease progress curve (AUDPC) of six wheat cultivars in five plot situations in 1993.

	Plot situation					
Cultivar	I6	NI6	NI2	NI1	H	Mean[a]
Baldus	57.7	61.0	55.0	51.8	42.3	49.7
Minaret	34.6	38.8	34.3	32.8	28.3	31.8
CMH78.390//MRNG..	46.0	34.0	30.8	28.7	26.1	28.5
Jondolar	30.8	25.6	23.0	21.6	30.9	25.2
LOV23/BJY'S'	2.8	9.9	3.6	0.7	3.0	2.4
BUC'S'/BJY'S'	8.3	3.3	2.8	1.5	1.8	2.0
Mean	30.0	28.8	24.9	22.9	22.1	23.3
Range	54.9	51.1	52.2	51.1	40.5	47.7
Standard deviation	21.2	20.9	19.8	19.6	16.2	18.4

[a]Mean of H, NI1 and NI2 representing breeders' small adjacent plots.

Experiment 2: Wageningen, 1993.

An experiment similar to the one of 1992 was planted in Wageningen on 20 April. Six cultivars with similar maturity were planted in five plot situations:

H: Hill plots planted in four rows of 2.0 m length

NI1: Adjacent plots of one row of 2.0 m length

NI2: Adjacent plots of two rows of 2.0 m length

NI6: Adjacent plots of six rows of 2.0 m length

I6: Isolated plots of six rows of 2.0 m length

The setup of the experiment was as described for experiment 1. The experiment was inoculated with isolate IPO290 collected in The Netherlands. Inoculations were done twice on June 5 and June 12 at the late tillering stage of development. Inoculum concentration

Table 3. The mean, range and standard deviation, for the percentage necrosis of leaves of six wheat cultivars in six plot situations in 1995.

	Plot situation						
Cultivar	I8	NI8	NI4	NI2	NI1	H	Mean[a]
Mbuni	92.9	90.2	95.5	87.2	82.4	75.5	81.7
Jupateco-Alondra	73.2	77.0	68.7	57.1	43.3	36.9	45.8
Minaret	65.7	55.3	53.6	54.3	32.4	42.7	43.1
Baldus	23.3	23.6	16.2	14.6	2.2	3.4	6.7
Jondolar	24.3	24.8	10.0	3.0	1.7	0.3	1.7
BUC'S'/BJY'S'	10.4	14.7	13.0	5.9	4.5	0.3	3.6
Mean	48.3	47.6	42.8	37.0	27.8	26.5	30.6
Range	82.3	76.5	85.5	84.2	80.7	75.2	80.0
Standard Dev.	33.3	31.4	35.3	34.2	32.0	30.6	32.0

[a]Mean of H, NI1 and NI2 representing breeders' small adjacent plots.

was $5x10^6$ spores/ml. Overhead sprinkler irrigation was supplied for one hour in the morning and evening every day after inoculations until observation. Two observations were made on July 8 and july 22. Disease assessment was made as in exp. 1.

Experiment 3: 1995.

The experiment was planted at Njoro, Kenya on April 29 during the main rainy season. Six cultivars were planted in this experiment with six plot situations:

H: Hill plots planted in four rows of 2.0 m length

NI1: Adjacent plots of one row of 2.0 m length

NI2: Adjacent plots of two rows of 2.0 m length

NI4: Adjacent plots of four rows of 2.0 m length

NI8: Adjacent plots of eight rows of 2.0 m length

I8: Isolated plots of eight rows of 2.0 m length

The setup was as described in experiment 1. Infected straw was spread on the plots at the seedling stage. The isolate IPO93001 collected at Njoro was used for the inoculations. Spray inoculations were carried out as in exp. 1. Disease assessment was carried out once on 14 August when cultivars were at the dough stage (GS 85-87) (Tottman and Makepeace, 1979). Assessment was as described in exp. 1.

Data analysis

The H, NI1 and NI2 represents the breeders' small plots in the early part of the breeding program while the NI4, NI6 and NI8 represents the breeders' large plots in the later stages of the program during adaptability and yield trials. The I6 and I8 represents the farmers' field situation. The mean of the small adjacent plots was calculated. Spearman's rank correlation (Sokal and Rohlf, 1980) was used to compare the ranking of the cultivars in the farmers' field situation with the mean of the small breeders' plots and also between the breeders' small and large plots.

The disease mean, range and standard deviation (SD) was calculated for each plot situation. The range determined the difference within a plot situation between the most susceptible and the most resistant cultivar. The standard deviation within a plot situation is a measure for the spread in disease severity among cultivars, which would become smaller in case of interplot interference.

Results

Experiment 1.

Moderate epidemics of septoria tritici blotch (STB) developed in 1992 This was attributed to the dry and warm weather conditions at Njoro during the off-season (October-March). Irrigation was supplemented to create conducive conditions favourable for septoria development. Results presented in Table 1 show that the disease severity increases from the hill plots (H) to the isolated plots (I8); the increase being about 50%. Both the range and standard deviation too increased from the H to the I8 treatments with about 50%. The Spearman's rank correlations between the mean of the small adjacent plots with I8 and NI8

were 1.00 and 1.00 respectively.

Experiment 2

Though two assessments were made, the second assessment was omitted in the analysis because by that time all the cultivars except BUC'S/BJY'S and LOV 23/BJY'S had 100% necrosis. The first observation, which gave much more discrimination among the cultivars, was analyzed. Results are presented in Table 2. There was a similar but less pronounced pattern in this experiment compared with experiment 1. The mean, range and standard deviation tended to increase from H to I6, this increase being in the order of 30%. The spearman's rank correlations between the mean of the small adjacent plots with I6 and NI6 were 0.89 and 1.00 respectively.

Experiment 3.

There was very good disease development during the period the experiment was carried probably because of heavy rainfall for a period of two weeks just before the observations were made. In Table 3 the results are shown. Again the mean disease severity increased strongly from H to I8, but the range and standard deviation did not follow this pattern. They remained more or less similar over the range of treatments. Spearman's rank correlations between the mean of small adjacent plots with the I8, NI8 and NI4 were 0.83, 0.83 and 1.00 respectively.

Discussion

In breeders' screening nurseries two types of errors, underestimation of level of resistance and a wrong ranking error for resistance, can be made when assessing entries in small adjacent plots, but which error and how severe an error one makes depends apparently on the pathosystem (Parlevliet and Danial, 1992). These authors analyzed data on interplot interference in barley-barley leaf rust and wheat-yellow rust and reported that in the former the resistance of partially resistant entries was severely underestimated, while this was not so in the latter. In both pathosystems the cultivars always ranked in a very similar way irrespective of the plot situation or year. Broers and Lopez-Atilano (1995) showed that the genotypic ranking of durum wheat for resistance to stem rust was not affected by interplot

interference, but the disease level on the resistant entries was reduced in the small plots. The results from Njoro and Wageningen gave no evidence of interplot interference in adjacent plots infected with STB. This was seen from the high ranking correlations between the small adjacent plots and the large isolated and non isolated plots in the three experiments and the similarity in range and standard deviations between the breeder's plots and the isolated plots.

The consistent increase in disease severity (averaged over the entries) from hill plots to isolated plots in all three experiments could be seen as an interplot interference too, but not of importance for the breeder. An explanation for this increase in disease severity could be that within the H plots there was more space between individual plants. The spores produced by the individual plants are mostly lost in the relatively large open space around them. In the I8 plot situation the individual cultivar within a plot covered most of the space uniformly and this means that most of the spores produced are retained or have a higher chance to be disseminated to the next plant also the micro-climate within the plots might have changed with increasing plot size and this could also affect the disease situation. Burleigh and Loubane (1984) used plot sizes between 10 x 10 m to 40 x 40 m to study interplot interference in wheat infected with STB and reported no significant interplot interference effect. Results from their experiments showed that septoria tritici blotch severity in larger plots was always higher than in smaller plots. For instance, at Jamaa Shaim, the cultivar Sieta Cerros had 70% and 60% DS in plots of 40 x 40 m and 20 x 20 m respectively. In any case these plot sizes are large and are not representative of breeders' plot situation.

The ranking order remained nearly always the same in the various plot situations and there was no indications that the resistance level is underestimated in the small plots as the standard deviation (seen against the mean disease severity) in these small plots is not significantly smaller than the standard deviation in the large isolated plots.

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Chapter 9

Effect of Nitrogen fertiliser application on the disease severity of septoria tritici blotch on wheat in the field

Summary

Two experiments were carried out in Njoro (Kenya) and Wageningen (The Netherlands) to determine the influence of Nitrogen applied as Calcium Ammonium Nitrate (CAN) [$Ca(NH_4NO_3)_2$] on septoria tritici blotch severity on wheat. Fertiliser was applied by hand as top-dress. At Njoro the soils are Mollic Andosols (volcanic ash) while at Wageningen the soils are sandy. There was a significant increase in disease severity at Njoro on cultivars in plot treatments with increased N from 0 Kg/ha to 60 Kg/ha. At wageningen there was no significant increase in disease severity in plots applied with 65 Kg/ha N compared to those applied with 0 Kg/ha N. The differences in the results between the two experiments could be explained by the frequent irrigation, timing of N application and soil types.

Introduction

Because crops are often fertilized especially with nitrogen (N) to obtain maximum productivity and quality the effect of N fertilisers on disease development becomes an important consideration. Agronomic practices have been reported to influence septoria tritici blotch (STB) severity by modifying the microclimate within the crop canopy (Eyal et al., 1987). The magnitude and direction of these effects have been inconsistent. Fellows (1962) conducted experiments in pots in the greenhouse and reported that application of the N fertiliser 'Vigoro', 7-13 days before inoculation of plants with *Septoria tritici* gave a stronger increase of the percentage of leaves infected and number of lesions per infected leaf compared with the same fertiliser treatment applied at the time of inoculation. In other studies Hayden et al. (1994), using detached leaves in the laboratory, reported a greater development of necrosis and a higher production of pycnidiospores on leaves of plants grown at high N levels. Increase in STB severity associated with increased N application have also been reported by Gheorghies (1974), Prew et al. (1983), Howard et al. (1994) and Leitch and Jenkins (1995).

However, Tompkins et al. (1993) reported that increased N significantly stimulated septoria development in only one of their trials. Greater septoria severity was associated with low N fertility in all the other experiments. No significant STB severity increase was observed under increased N regimes by Wilson and Loughman (1989). Under field conditions in 1993, Hayden et al. (1994) reported that there was little effect on disease incidence due to N regimes. In addition, Huber and Watson (1974) observed that the incidence of several wheat diseases depended on the form of N applied.

To lower the pressure on the use of marginal land, high yields are necessary on suitable wheat areas. Therefore high N inputs have to be given. Because STB could be aggravated by high N, experiments were conducted to assess the influence of N on STB severity in wheat.

Materials and methods.

Experiment 1

The experiment was conducted at the National Plant Breeding Research Centre, Njoro (Alt. 2160 m) in 1992 during the main wheat growing season (March-September). Four wheat genotypes with varying levels of resistance to STB were obtained from the 1991 CIMMYT disease nurseries planted at the Centre. Entries were sown in plots of eight rows 2 m long at a row spacing of 20 cm. Triple Superphosphate (TSP) fertiliser was applied in all plots at the time of planting. The experimental design was a randomised complete block one with individual treatments replicated three times. Plots were separated from one another with 2 m of oat. Soil samples were taken from the experimental block to analyze the amount of N available at planting time.

Treatments comprised of three N rates of 20, 40, and 60 kg/ha and a control treatment with no application of N fertiliser at Growth Stage (GS) 30 (Zadoks et al., 1974). The N fertiliser source was Calcium Ammonium Nitrate (CAN) [$Ca(NH_4NO_3)_2$] which contains 26% N.

STB epidemics were incited through infected straw spread in the plots at the seedling stage. Plots were irrigated by overhead sprinklers every three days in case of no rainfall from the time infected straw was spread until the time of disease assessment. Per plot 20 main tillers were randomly taken from the inner six rows. Disease severity was assessed as the

percentage leaf area necrotic due to septoria on the flag leaf (F), first lower leaf after the flag leaf (F-1) and the second lower leaf after the flag leaf (F-2). The average leaf necrosis over the three leaves was determined.

Experiment II

The trial was planted on April 14 at the Department of Plant Breeding Wageningen on sandy soil. Three commercial Dutch spring wheat cultivars Jondolar, Baldus, and Minaret were planted. The cultivars were planted in plots of six rows 2 m long at a row spacing of 20 cm between rows in a randomized complete block design with three replicates. Each plot was surrounded by 2 m of oat. Nitrogen source was CAN applied at GS 30. Three N rates of 0, 32.5 and 65 kg/ha were applied.

The most virulent Dutch isolate IPO290 (Kema pers. comm.) collected from Zuid Flevoland from the cultivar Clement was used for inoculations. The isolate was increased on five petri plates of V8 juice agar for five days. A spore suspension was made in sterile water. Using a sterile syringe, 4.5 ml of spore suspension was inoculated into 1000 ml conical flasks containing 500 ml yeast sucrose liquid medium (Eyal et al., 1987). Flasks were shaken for five days at a temperature of 20 ^{o}C. The shaker was put off to allow spores to settle at the bottom. The next day, the liquid was carefully decanted. Spores left at the bottom of the flask were re-suspended in distilled water. The spore concentration was estimated using a haemocytometer and adjusted to 5×10^{6} spores per ml. Inoculations were carried out in the evening using a 15 L pressurised container. The experiment was inoculated twice; at GS 39 and eight days later.

The plots were irrigated every day by overhead sprinklers; for one hour in the morning and one hour in the evening to create high humidity and leaf wetness.

Two observations were made at an interval of 14 days. The first disease observation was made on July 8 and the second one on July 22. Ten randomly chosen tillers were taken from the inner four rows. Infection level was estimated as the percentage of the leaf area necrotic due to septoria on F and F-1 leaves.

Table 1. The area under disease progress curve of leaf necrosis of four wheat cultivars at four N fertiliser rates.

	N (Kg/ha)			
Genotype	0	20	40	60
BR5/4/TP//CNO/INIA/..	58.4	61.6	64.1	80.8
PAK/BJY//GJO/EMU	41.7	49.2	56.1	62.6
LOV23/BJY	48.1	46.4	61.3	68.4
TRAP#1*2//ERA/RUSO	2.8	3.5	5.1	14.2
Mean	38.7 a	40.2 a	46.7 b	56.5 c

Means followed by the same letter are not significantly different using LSD (P≥0.05)

Table 2. Percentage necrotic leaf area observed on two observation dates of three wheat cultivars applied with three N fertiliser rates at growth stage, GS 30.

	July 8			July 22		
	N (kg/ha)			N (kg/ha)		
Cultivar	0	32.5	65	0	32.5	65
Baldus	28.4	34.8	34.8	99.7	96.1	99.0
Minaret	14.2	21.6	17.8	91.3	89.3	87.6
Jondolar	8.8	9.1	10.8	63.6	69.6	70.9
Mean	17.1 a	21.8 a	21.1 a	84.9 b	85.0 b	85.8 b

Means followed by the same letter are not significantly different using LSD. (P≥0.05)

Results

Experiment 1

There was a general increase in disease severity on the cultivars tested upon increased N rates (Table 1). Such an increase was observed on the most susceptible cultivar BR/4/TP//CNO//INIA/.. which had 58.4% necrosis at 0 Kg/ha N application and 80.8% necrosis when 60 Kg/ha N was applied. The same increase was observed for the most resistant cultivar TRAP#1*2//ERA/RUSO which had 2.8, 3.5, 5.1 and 14.2% necrosis when 0, 20, 40 and 60 Kg/ha N was applied respectively. The mean disease severity also showed a general trend of increase in disease severity from 38.7% at the 0 Kg/ha N rate to 56.5% at the highest applied N rate of 60 Kg/ha.

Tests on the soil samples taken from the experimental plots just before planting indicated that there was 0.21% N in the soil depth of 0 - 20 cm and 0.20% N in the soil depth of 20 - 40 cm. The pH was 4.90 for the top soil and 5.07 for the 20 - 40 cm depth.

Experiment 2

Severe epidemics of STB prevailed during the experimentation period. It was intended to make three observations at an interval of 14 days. However, the disease developed so rapidly that by the time of the second observation on July 22 Jondolar and Minaret had almost 100% necrosis on F and F-1, making a third observation useless. On July 8, the plots with 0 Kg/ha N had the lowest disease severity. On the cultivar Jondolar disease severity increased slightly from 8.8% necrosis in plots applied with 0 Kg/ha to 10.8% in plots applied with 65 Kg/ha N. Minaret had the highest disease severity of 21.6% necrosis with the applied N rate of 32.5 Kg/ha N. This N rate also had the highest mean disease severity of 21.8% necrosis. The three disease severity means for the N rates were not significantly different.

When plots were observed a second time on July 22, Jondolar showed a similar increase in disease severity upon increase in rates of N applied (Table 2). The same could not be said of the more susceptible cultivars Baldus and Minaret. Minaret showed a decrease in disease severity from 91.3% in plots applied with 0 Kg/ha to 87.6% in plots applied with 65 Kg/ha N. The mean disease severity increased from 84.9% to 85.8% upon increase in N rates from 0 Kg/ha to 65 Kg/ha but the increase was not significant at all.

Discussion

The two experiments represented completely different environments. In Experiment 1 the N rate of 20kg/ha represents the rate recommended to wheat farmers by the National Plant Breeding Research Centre, Njoro in Kenya. Wheat farmers are recommended to apply 125 kg/ha of Diammonium Phosphate (DAP) (18:46:0 of N:P:K) compound fertiliser at planting time which gives the crop 22.5 kg/ha N. Most farmers apply this rate without having the soils tested for the amount of N already available in the soil at planting. Large scale farmers in the high potential areas of Meru, Nakuru, Narok and Uasin-Gishu districts on the other hand apply upto 250 kg/ha DAP (45 kg N/ha). In these areas STB epidemics have been more frequent. Results presented here indicated that the frequent epidemics observed in these districts may be attributed to the high rates of N applied.

Analysis of the soil samples for N content using the Kjeldal method showed that the 0.20 - 0.21% was adequate for the growing of a wheat crop (Mwangi pers. comm.). Detailed survey of the soils at the Centre carried out in the past (Anon., 1979) showed that these soils are Mollic Andosols and had high N content ranging from 0.20% to 0.45% and were slightly acid with pH ranging from 5.6 to 6.4 in the top soil.

Wheat farmers in The Netherlands apply N to their crop in split applications. The rates vary from about 30 to about 120 Kg/ha based on the N still present in the soil. The maximum rate applied in experiment 2 of 65 Kg/ha was equivalent to about half of the highest levels applied in poor soils. The non significant increase in disease severity at Wageningen could be explained by the soil type, the time of N application and the rates applied. The experiment was carried out on a sandy soil which was poor in N content. Combined with the time N was applied at GS 30 (stem elongation) and frequent irrigation, it was expected that part of the CAN fertiliser that was applied was leached before the onset of disease. Under such circumstances, timing of application, split applications and soil types may affect the results considerably. These factors could explain the differences in results obtained in Njoro and Wageningen.

Another aspect that may contribute to the differences in results obtained here and elsewhere is the form of N fertiliser used. It is generally the form of N available to the host or pathogen that affect disease severity or resistance rather than the amounts of N applied (Huber and Watson, 1974). The two forms of N fertiliser commonly applied are the

Ammonium nitrogen (NH_4-N) and the Nitrate nitrogen (NO_3-N). The effect of specific forms of N on disease severity depends on many factors and is not the same for all host-parasite associations. Within the wheat/septoria tritici blotch system such comparative studies on forms of N fertiliser used are scarce. Nitrate nitrogen has been reported to increase stem rust (Daly, 1949), and yellow rust (Huber, 1980) severities in wheat, while Ammonium nitrogen was found to decrease severities of the two diseases by the same authors.

Much of the reported data concerning the effect of N on plant disease is difficult to interpret because soil conditions, form, rate and time of the N application differed or were sometimes not properly described. It shows that in order to elucidate the effect of N on the STB development more sophisticated research is needed.

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Chapter 10

Inheritance of quantitative resistance in bread wheat to *Septoria tritici*

Summary

An experiment was carried out to study the inheritance of resistance in F6 progenies obtained from 36 crosses involving 14 wheat cultivars. Transgressive segregation towards more resistance and/or more susceptibility to septoria tritici blotch in wheat occurred in most of the crosses. With so many parents, most showing transgression, the conclusion is that a fair number of loci is involved. The combination of quantitative resistance and transgressive segregation is indicative of at least some genes operating in an additive way.

Introduction

Septoria tritici, the causal organism of septoria tritici blotch (STB), is at present the second most important wheat pathogen after yellow rust (*Puccinia striiformis*) in reducing yields in Kenya. The disease is also of major importance in the highlands of Ethiopia and Tanzania, in the coastal areas of the Mediterranean, in South America, in Australia and in Western Europe (Saari and Wilcoxon, 1974; Rajaram and Dubin, 1977). Almost a complete crop failure was reported from Kenya as early as the mid twenties by Burton (1927). Efforts were made in the sixties to identify sources of resistance (Saari and Wilcoxson, 1974). A Regional Disease and Insect Screening Nursery (RDISN) was subsequently established. Some of the best sources of resistance to *S. tritici* in the 1971-1972 nurseries were Kenya wheat lines selected in Ethiopia (Pinto, 1972) and submitted for regional testing in the RDISN.

After this initial work in Kenya, little was done on STB over the next two decades. This was probably due to the importance of stem rust (*Puccinia graminis*) in the 1970's and that of yellow rust (*Puccinia striiformis*) in the 1980's (Danial, pers. comm.). To improve stem rust resistance and yielding capacity, early maturing semi-dwarf lines from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico were introduced into the breeding programme. Because of the relationship between plant stature, maturity class and resistance to STB (Danon et al., 1982; Eyal et al., 1983; Arama et al., 1994) the more STB

resistant germplasm was unconsciously discarded. Incidences of STB increased gradually and initially unnoticed. Favourable weather conditions prevailing in Kenya in the 1985 and 1986 growing seasons led to severe STB epidemics and a renewed interest in STB resistance.

Most of the high yielding wheat cultivars grown in Kenya today are quite susceptible to STB. Breeding for resistance is the economically most feasible control measure for many wheat diseases. Undoubtedly most breeders and pathologists want a form of resistance that keeps its effectiveness over time, and which is easy to transfer across genotypes, easy to identify in segregating progenies and effective under disease conducive conditions. However, germplasm resistant to STB is rather scarce, and little is known about the types of resistance and the mode of inheritance (Eyal, 1981).

Conflicting reports are found in the literature regarding the nature of genetic resistance to STB. These range from simple Mendelian genetics to complex quantitative inheritance patterns. Mackie (1929) found, by analyzing F2 populations, that a single recessive gene provided resistance in an unidentified cultivar. Single dominant genes for resistance have been reported to be present in Lerma'50' and P14 (Narvarez and Caldwell, 1957), Bulgaria 88 (Rillo and Caldwell, 1966), Veranopolis (Rosielle and Brown, 1979; Wilson, 1979), Carifen 12 (Lee and Gough, 1984), Vilmorin (Gough and Smith, 1985) and IAS20/#567.1 (Jlibene, 1990). Two to three dominant genes have been found to confer resistance in Thornbird (Jlibene, 1990).

Wilson (1985), in evaluating 28 sources of STB resistance found that a single dominant gene was the most common type of genetic resistance. However, there were some exceptions including duplicate dominant, single incomplete dominant models, and for the cultivar Seabreeze, a two recessive gene model was suggested. In other studies Camacho-Casas et al., (1995) reported that additive and dominance effects were responsible for the resistance to STB in II50-18/VGDWF/3/PMF. Wilson (1985) proposed three different genes conferring resistance to STB. These were designated Slb1, Slb2 and Slb3 for the genes in Bulgaria 88, Veranopolis and Israel 493 respectively. Van Ginkel (1986) suggested that the search for single gene resistance to STB may be ineffective because of the presence of modifier genes, lack of discrete classes in segregating populations, evidence of transgressive segregation, disagreements on where to place the dividing border line for segregation

between resistance and susceptibility and environmental influence. Failure to transfer satisfactory levels of resistance to septoria leaf blotch was also noted by Eyal et al. (1987) who suggested the presence of modifying genes that affect the expression of dominant genes for resistance. In durum wheat, Van Ginkel and Scharen (1987) reported that resistance to septoria tritici blotch was explained by models involving additive and dominant gene effects and that the additive gene effects were more important than dominant gene effects. Epistatic gene effects were of minimal importance.

The objective of this experiment was to study the quantitative inheritance of adult plant resistance of bread wheat to *S. tritici*.

Materials and methods

Fifty seven bread wheat cultivars were evaluated for their resistance to natural inoculum of the *S. tritici* populations in Njoro (2160 m) and Timau (2640 m) in 1988 and 1989. After correcting the disease severity for maturity and tallness, 14 cultivars, which ranged from low to high levels of resistance to STB, were selected (Table 1). A single ear of each cultivar was harvested and planted in a 2 m row. At the heading stage some ears in each row were bagged individually to ensure complete selfing. This was repeated in 1990 and 1991. Cultivars were grouped as early and late maturing. There were seven early maturing and six late maturing entries. The cultivar 343 (TRAP#1*2//ERP'S'/RUSO), a medium maturing cultivar, was included in both groups (Table 1).

The entries were planted four times at an interval of 10 days beginning 23 September 1991. Half-diallel crosses were made within each group. F_1 progenies and F_2 generations realised were planted widely spaced in 2 rows, 4 m long in 1992. Some crosses were discarded in F_3 because they were found to have been mixed up. Only 36 crosses were harvested in the F_3. From each cross about 100 ears were randomly and individually harvested. The ears were planted in rows at a wide spacing to raise F_4 populations. This was repeated to raise the F_5. Three plants from each row were harvested and threshed separately.

In 1995 the seeds of three plants from an F_5 line were planted in three rows to make F_6 plots. Rows were planted 1.5 m long, 20 cm apart. The two parents of the cross were planted in 3 rows after every 20 plots. Because of infrequent rainfall in the months of June and July, irrigation was provided twice a week.

Table 1. Pedigrees of parents used in the study of inheritance of resistance to septoria tritici blotch of wheat caused by *Septoria tritici*.

Code	Pedigree/Name
Early maturing	
396	BOW'S'/VEE'S'
343	TRAP#1*2//ERP'S'/RUSO
327	RPB.1468/NAC//DOVE'S'
303	Clement (CNO-INIA*LFN/TOB*KI.PERAF)
287	Frontatch (FRONTANA/K58/NEWTHATCH)
244	HAHN'S'*/PRL'S'
127	CMH79A.307/BOW'S'
001	BUC'S'/BJY'S'
Late maturing	
343	TRAP#1*2//ERP'S'/RUSO
301	Jupateco-Alondra
282	Fink's'
279	Kenya Sungura (IDAHO 1877.NR.BJx11-53-370=MORRIS)
267	YAP/BJY'S'
106	Ning 8331
019	Milan (VS73.600/MRL'S'/3/BOW'S'//YR/TFR'S'

Inoculum preparation and inoculations

The isolate IPO93001 (previously sampled from Njoro) was selected for the inoculations. An infected leaf segment was attached to a glass slide and placed in a petri dish fitted on the bottom with filter paper saturated with sterile water. The petri dish cover was replaced to provide a moist environment for 4 hrs. It was then transferred to a laminar-flow clean air cabinet bench. Oozing pycnidia were located under the stereoscopic microscope. With

the help of a fine-pointed needle, sterilized in a flame and cooled briefly, a single cirrus was picked and transferred to Yeast-Malt Agar (YMA) (Eyal et al., 1987) medium in a petri dish. The growing colony was spread on the medium and sub-cultured in fresh media for multiplication.

The cultures were inoculated into 1 L Erlenmeyer flasks containing 1 L Yeast sucrose liquid medium (Eyal et al., 1987). Cultures were shaken on a Lab-Line Orbit Shaker at 150 RPM for five days. The shaker was turned off overnight to allow the spores to settle down. The liquid medium was carefully decanted after which spores were re-suspended in distilled water and filtered through a cloth filter. The spore concentration was determined and adjusted to $1x10^6$ spores/ml. Tween 20 surfactant was added into the inoculum just before inoculations. A CP15 knapsack sprayer was used for inoculations. The experiment was inoculated four times at an interval of seven days beginning 30 days after planting.

The heading date for each plot was noted and marked. Plants in plots that had similar heading dates were observed at the same time. Five main tillers were sampled at random from the middle row of each plot. Percentage necrosis and percentage pycnidia coverage were visually assessed on the two uppermost leaves.

As the irrigation was not uniform on all the plots the disease spread was not expected to be uniform. Indeed the disease severity (DS) as measured on the same genotypes (parents) did show clear variations. The mean percentage pycnidia coverage of the two leaves after logit transformation, was therefore corrected for within site variation based on 2-d-polynomials and fitted with the SAS PROC GENMOD method (SAS., 1985) using the DS observed on the manyfold replicated parents in the 36 crosses. The frequency distribution of the progenies was calculated and grouped at 10% DS interval classes.

Results

Testing of the various F6 wheat lines with isolate IPO93001 showed that there was clear transgressive segregation for DS in many of the crosses. Transgressive segregation was observed for increased resistance, increased susceptibility or both.

The extent of transgressive segregation varied among the crosses (Table 2). Cross nrs. 17 and 18 had more than 50% of the population segregating towards more resistance than the most resistant parent. On the other hand, the cross nrs. 8, 10, 20, 21, 22, 23, 26, 27, 28, 30,

Table 2. Frequency distribution of F6 lines in 10 disease severity classes of 36 crosses obtained from 14 parents.

Cross		Mean	Disease severity class (percentage)									
			0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	100
1	F6		2	1	2	5	15	***25***[a]	***43***[a]	11	0	0
	287[b]	52.1										
	244[b]	67.9										
2	F6		0	1	3	8	7	***19***	***25***	38	5	0
	327	50.6										
	244	67.9										
3	F6		7	2	11	11	8	***21***	3	27	12	0
	327	50.6										
	287	52.1										
4	F6		4	2	***5***	6	3	7	1	***19***	1	0
	301	72.9										
	279	29.3										
5	F6		3	9	3	***14***	3	14	***15***	22	15	0
	396	32.9										
	244	67.9										
6	F6		1	2	6	***9***	20	12	***6***	21	4	0
	343	30.4										
	244	67.9										
7	F6		2	6	***8***	28	32	19	***0***	8	0	0
	127	29.8										
	244	67.9										
8	F6		1	2	2	***7***	7	***11***	2	33	42	0
	396	32.9										
	287	52.1										
9	F6		0	3	13	***16***	8	***27***	9	22	8	1
	343	30.4										
	287	52.1										
10	F6		4	3	1	***2***	7	***19***	12	41	16	0
	327	50.6										
	396	32.9										
11	F6		15	12	***12***	12	21	***18***	9	4	0	0
	127	29.8										
	287	52.1										
12	F6		6	***10***	13	17	5	22	***6***	17	2	0
	244	67.9										
	001	13.2										
13	F6		0	5	9	***10***	9	***24***	4	31	0	0
	343	30.4										
	327	50.6										
14	F6		10	8	***12***	10	2	***12***	4	18	15	0
	327	50.6										
	127	29.8										
15	F6		***2***	11	16	23	22	14	***11***	5	2	0
	303	6.6										
	244	67.9										
16	F6		***0***	9	7	20	6	19	8	***25***	0	0
	301	72.9										
	106	1.4										

Table 2. Continued.

Cross		Mean	Disease severity class (percentage)									
			0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	100
17	F6		61	***14***	4	6	1	8	***2***	6	2	0
	244	67.9										
	019	14.1										
18	F6		43	16	***6***	3	***3***	5	0	21	9	0
	267	41.5										
	279	29.3										
19	F6		0	1	***4***	18	***12***	39	8	16	6	0
	282	25.6										
	267	41.5										
20	F6		0	***1***	6	3	4	***7***	7	28	42	7
	327	50.6										
	001	13.2										
21	F6		0	5	11	***14***	19	23	9	32	3	0
	343	30.4										
	396	32.9										
22	F6		7	9	***14***	***18***	21	17	14	11	1	0
	127	29.8										
	396	32.9										
23	F6		1	2	***13***	***16***	20	25	5	25	1	0
	343	30.4										
	127	29.8										
24	F6		***8***	8	13	21	15	***14***	9	3	1	0
	303	6.6										
	287	52.1										
25	F6		***6***	13	12	21	10	***15***	17	8	1	0
	303	6.6										
	327	50.7										
26	F6		2	1	***11***	***19***	10	11	4	20	6	0
	282	25.6										
	343	30.4										
27	F6		6	***18***	8	5	***6***	13	1	20	21	0
	267	41.5										
	019	14.4										
28	F6		6	4	***6***	7	4	21	4	25	16	0
	282	25.6										
	279	29.3										
29	F6		22	***4***	10	***11***	2	5	2	19	19	0
	396	32.9										
	001	13.2										
30	F6		0	***2***	7	***15***	3	21	6	42	5	0
	343	30.4										
	001	13.2										
31	F6		3	***3***	***6***	14	11	18	9	11	4	0
	127	29.8										
	001	13.2										
32	F6		22	***8***	***5***	10	3	10	3	5	1	0
	282	25.6										
	019	14.4										

Table 2. Continued.

Cross		Mean	Disease severity class (percentage)									
			0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	100
33	F6		*7*	7	4	***15***	17	22	13	12	4	0
	303	6.6										
	396	32.9										
34	F6		***28***	21	***29***	11	4	1	1	0	0	0
	303	6.6										
	127	29.8										
35	F6		*3*	16	***19***	11	19	13	0	3	0	0
	282	25.6										
	106	1.4										
36	F6		***29***	***15***	15	15	12	6	6	4	0	0
	303	6.6										
	001	13.2										

[a] Bold and italic numbers indicate the disease severity classes in which the parents occurred.
[b] Parents codes (Table 1) with their mean disease severity

31 33, 35 and 36 showed over 50% segregation towards more susceptibility than the susceptible parent. There was an increase in resistance in relation to the mid-parent (MP) values in five out of the 36 crosses (Table 3). The greatest increase in resistance obtained was 45% in relation to the mid-parent (MP) value from the cross 244 x 019. However, most crosses showed a decrease in resistance compared to the MP-value. The highest decrease of resistance obtained was 179.8% from the cross 303 x 001. The overall mean MP value was 34.8% while the corresponding F6 mean was 47.7%, a considerable increase in susceptibility. The standard deviations for F6 and the MP value were both 12.3. The correlation coefficient between F6 and MP-values was 0.44, while the correlation coefficient between the MP-values and percentage change of the F6 from the MP-values was 0.73, both being highly significant.

Discussion

Genetic studies in inheritance of resistance to STB have generally been conducted on seedlings in the greenhouse and have been oriented towards examining effects of simple Mendelian inheritance. However, in practical plant breeding situations, selection is done in the field for an array of traits, most of which are exposed in later stages of plant development and are quantitatively inherited. Ballantyne (1985) and Camacho-Casas et al.,

Table 3. Mean disease severity of parents (P1, P2), F6 and the midparent (MP), percentage decrease (-) or increase (+) in mean disease severity in the F6 compared to the MP value, and the range in the disease severity, where tr = trace, in the F6 of 36 crosses.

Cross	P1	P2	F6	MP	Change of F6 from MP, %	Range
287 x 244	52.1	67.9	56.9	60.0	-5.2	5 - 75
327 x 244	50.6	67.9	62.5	59.3	+5.4	25 - 85
327 x 287	50.6	52.1	53.7	51.4	+4.5	tr - 85
301 x 279	72.9	29.3	51.7	51.1	+1.2	5 - 80
396 x 244	32.9	67.9	56.3	50.4	+11.7	5 - 85
343 x 244	30.4	67.9	52.9	49.2	+7.5	15 - 85
127 x 244	29.8	67.9	42.4	48.9	-13.3	5 - 75
396 x 287	32.9	52.1	69.4	42.5	+63.3	10 - 80
343 x 287	30.4	52.1	50.1	42.3	+18.4	15 - 85
327 x 396	50.6	32.9	64.1	41.8	+53.3	10 - 85
127 x 287	29.8	52.1	36.8	41.0	-10.2	tr - 75
244 x 001	67.9	13.2	44.6	40.6	+9.9	tr - 75
343 x 327	30.4	50.6	53.9	40.5	+33.1	15 - 75
327 x 127	50.6	29.8	49.0	40.2	+21.9	tr - 85
303 x 244	6.6	67.9	41.5	37.3	+11.3	5 - 85
301 x 106	72.9	1.4	50.2	37.2	+34.9	15 - 75
244 x 019	67.9	14.1	19.8	36.0	-45.0	tr - 85
267 x 279	41.5	29.3	32.6	35.4	-7.9	tr - 85
282 x 267	25.6	41.5	54.4	33.6	+61.9	15 - 85
327 x 001	50.6	13.2	76.6	31.9	+140.1	5 - 85
343 x 396	30.4	32.9	53.4	31.7	+68.5	5 - 85
127 x 396	29.8	32.9	43.3	31.4	+37.9	tr - 80
343 x 127	30.4	29.8	50.7	30.1	+68.4	5 - 85
303 x 287	6.6	52.1	38.7	29.4	+31.6	tr - 80
303 x 327	6.6	50.6	42.0	28.6	+46.9	tr - 80
282 x 343	25.6	30.4	51.1	28.0	+82.5	5 - 85
267 x 019	41.5	14.1	51.1	27.8	+83.8	tr - 85
282 x 279	25.6	29.3	51.6	27.5	+87.6	5 - 85
396 x 001	32.9	13.2	46.2	23.1	+100.0	20 - 95
343 x 001	30.4	13.2	59.3	21.8	+172.0	15 - 85
127 x 001	29.8	13.2	49.8	21.5	+131.6	tr - 85
282 x 019	25.6	14.1	30.5	19.9	+53.3	20 - 85
303 x 396	6.6	32.9	47.8	19.8	+141.4	20 - 85
303 x 127	6.6	29.8	19.7	18.2	+8.2	tr - 55
282 x 106	25.6	1.4	34.6	13.5	+156.3	tr - 75
303 x 001	6.6	13.2	27.7	9.9	+179.8	tr - 75
Mean			47.7	34.8	+51.3	
Std. dev.			12.3	12.3		

Table 4. Mean disease severities (DS) and transgressive segregation in a half diallel of crosses involving five parents.

	Parent	287	327	396	343	127
Parent	Mean DS	52.1	50.6	32.9	30.4	29.8
287		-	+[a]	+	+	+
327			-	++	++	++
396				-	++	++
343					-	++
127						-

[a] + means some transgression; ++ means considerable transgression.

(1995) reported that plant reaction to *S. tritici* in F3 lines did not suggest simple inheritance. Moreover there have been reports of resistant cultivars being obtained from parents of susceptible background (Shaner et al., 1975; Shaner and Finney, 1982; Wallwork and Johnson, 1983; Lee and Shaner, 1985; Milus and Line, 1986; Rose-Fricker et al., 1986; Schultz and Line, 1992; Poysa, 1993; Campbell and Wernsman, 1994; Roumen, 1994; Campbell and White, 1995), suggesting that resistance can result from a combination of genes that individually are ineffective (epistatic effects).

The data from this study show that transgressive segregation towards more resistance and/or more susceptibility to STB in wheat occurred in most of the crosses. Because so many parents were involved, it suggests that transgressive segregation should be obtained from many other crosses as well and is not just an occasional phenomenon. The frequent occurrence of transgressive segregants indicates the multigenic nature of resistance in the cultivars used. As an illustration, Table 4 gives a half diallel crosses involving five parents, from Table 2, with all F6's showing transgressive segregation. It can be deduced that 287 and 327 must differ for at least two genes. In this case recombination is possible and some F6 lines are either more susceptible or resistant than the parents. But then 287 differs also with 396, 343 and 127 for at least two genes and that cannot be the same genes, otherwise

the transgression among the others cannot be explained. So with so many parents, most showing transgression, the conclusion is that a fair number of genes are involved.

Transgressive segregation for higher resistance to *S. tritici* was demonstrated in progenies of a number of crosses. These transgressive segregants clearly originated from a combination of genetic components from both parents of each cross where the phenomenon was occurring. The highest increase in resistance was obtained from the cross 244 x 019 (susceptible x resistant). Of the F6 population, 58% were more resistant than 019 (Table 2). This suggests that there are genes in the susceptible parent 244 that contribute to resistance. This is confirmed from other crosses with 244 (crosses 1, 2, 5 and 7) where transgression beyond the resistant parent occurs also. Moreover, it was observed that in most of the crosses involving 244, the F6 means were almost equal to the MP values. Another interesting increase in resistance was observed in the cross 287 x 244 (susceptible x susceptible). Pope (1968) hypothesized that genes controlled functions in a sequence of events leading to resistance. In this case, each gene alone has no effect, but high levels of resistance can be achieved when the necessary combination of genes is produced by crossing.

The combination of quantitative resistance and transgressive segregation is indicative of at least a few genes operating in an additive way. More work need to be done to identify the resistance factors in the susceptible cultivars 244 (HAHN'S'*/PRL'S' and 287 (Frontatch).

Out of the 36 crosses analyzed, 31 crosses had F6 population means higher than the mid-parent value, indicating greater susceptibility than expected from the parental performance. It was observed that the crosses involving the parents 001, 282 and 343 had most of the transgressive segregants towards more susceptibility than the susceptible parent. It could be said that these cultivars had a poor general combining ability. Their resistance being to a fair extent of a non-additive nature (inter-locus interactions). Such cultivars are of little use in breeding for resistance.

The high correlation between MP and the loss in resistance is interesting. So, the higher the resistance of the parents, the more the F6 tended to less resistance, an observation of importance for breeders.

Here it is clearly shown that resistance to STB can be obtained by crossing commercial wheat cultivars which are considered fairly to moderately susceptible. This not only avoids

introducing undesirable traits which are often associated with resistant parents of exotic or non agronomic types but also increases the probability of obtaining progenies with well adapted agronomic traits suitable for cultivar development. Selection can then be done on resistant transgressive segregants within the F6 population. This reduces the chances of a single gene based resistance, generally vulnerable to adaptation by the pathogen.

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Chapter 11

General discussion

Until 1987 little consistent work on septoria tritici blotch (STB) had been reported as a follow up of Pinto's work (Pinto, 1972) in Kenya. Breeders and Pathologists referred to the disease as the 'septoria complex'. This naming also erroneously referred to disease symptoms caused by stagonospora nodorum blotch (*Stagonospora nodorum*) and tan spot (*Helminthosporium tritici-repentis)* which could not be distinguished from STB. The occurrence of STB in farmers' fields is often manifested by the presence of pycnidia within the necrotic lesions. However, under less humid conditions pycnidia may be absent, sparse or are relatively small in size and are difficult to identify. Under such circumstances proper identification in the field becomes difficult due to the prevalence of other diseases that show similar necrotic symptoms like tan spot. Leaf samples are preferably sent to the laboratory for identification. So far the perfect stage *Mycosphaerella graminicola* have not yet been isolated in farmers' fields; neither has STB been observed to attack the ears. The occurrence of stagonospora nodorum blotch (SNB) in the farmers' fields has also not been reported. No fungicides have as yet been approved for control of the disease. Thus host resistance is the most promising control strategy being pursued at the National Plant Breeding Research Centre, Njoro.

Disease assessment

Proper disease assessment is essential for a reliable breeding program for resistance to STB. Disease assessment in the field is often affected by the cultivar maturity (days to heading) and tallness. Data presented in Chapter 2 suggests that under Kenyan conditions, especially days to heading and not tallness had a strong influence on disease severity (DS). The effect of plant height might have been reduced considerably by the artificial inoculation on the crop canopy. Under natural epidemic conditions, the primary inoculum is from infected straw on the soil. The spores are splashed in raindrops to the lowest leaves, and thereafter progress upwards. In this natural situation plant tallness may play an important role in disease development. The range for tallness (33-97) cm and heading date (58-98) days also

is representative of the situation in Kenya. Therefore the regression equation derived for correction of DS is representative enough for Kenyan conditions. This equation may not be used for a different set of cultivars or isolates in another region. Such an equation therefore must be specifically derived from the data collected on heading dates, plant height and disease severity in the region.

Another method was also investigated to correct for the effects of especially heading date (Chapter 3). In the first experiment, the entries were planted at random as the breeders would have them in a selection field; having no prior information on heading time and plant height. Disease assessment was made on all the entries at the same moment irrespective of the maturity. Then it was shown that the early maturing cultivars tended to be severely diseased and the late maturing cultivars much less so. In the second experiment the cultivars with similar maturity were grouped together and planted in the same block. Disease assessment was made in each block depending on heading time. Then the disease severity was similar in the four heading date groups and the effect of maturity disappeared. The discrepancy in the disease assessment methods in the two experiments can be seen in the differences in leaf age and the duration of time of disease exposure. When the first observation was made 55 days after planting in exp. 1, the flag leaf (F) and the first lower leaf (F-1) were observed. On the late maturing cultivars, the second or third lower leaves were observed as no flag leaves were present yet. On the fourth observation date (85 days), F and F-1 of all were observed. However the F and F-1 of the early maturing cultivars had a much longer exposure to the inoculum. For the late maturing cultivars it meant that at the first observation date, different leaves were observed compared with the early maturing cultivars. This is the situation facing many breeders and could lead to the erroneous selection of late maturing cultivars as resistant. Application of a regression equation similar to the one in Chapter 2 or the classification of cultivars into maturity groups and assessing each maturity group when it has reached a certain development stage (Chapter 3) could enable assessment of resistance through disease severity, independent of the confounding effects of maturity and tallness.

Interplot interference

In breeders' nurseries two types of errors, underestimation of level of resistance and wrong

ranking order for resistance, can be made when assessing in hills or small plots of one or two rows adjacent to each other. But which error and how severe an error one makes depends apparently on the pathosystem (Parlevliet and Danial, 1992). In case of the wheat-*Septoria tritici* pathogen system, there was no evidence of interplot interference in the breeder's point of view (Chapter 8). The cultivars showed nearly always the same ranking order in the small adjacent plots and the standard deviation in these small plots was not significantly smaller than the standard deviation in the large plots four to eight rows (depicting breeders' yield trial plots) and isolated six to eight row plots representing the farmers' fields. This means that satisfactory assessment of resistance in wheat to STB can be carried out in small adjacent plots.

Nitrogen

The effect of nitrogen level on STB was also studied in the field in Njoro (Kenya) and Wageningen (The Netherlands). These experiments represented completely different environments. The data reported in Chapter 9 shows that in Njoro, increase in N level resulted in a clear increase in disease severity, while in Wageningen, such an increase in N did not result into a significant increase in DS. The experiment was planted on sandy soil in Wageningen and on volcanic soil in Njoro. It was expected that part of the CAN fertilizer that was applied at GS30 (stem elongation) in Wageningen was leached long before the onset of disease. Soils in Njoro (Mollic Andosols) had a higher capacity to retain N resulting in less leaching. Under the circumstances as in Wageningen, timing of application may have affected the results considerably. It can be said that the high increase in STB epidemics in Eldoret Timau, Narok and Nakuru can also be explained by the high N application to the wheat crop in these areas especially by the large scale farmers.

Race specific effects

In order to develop a sound breeding program for resistance to STB in Kenya, it is necessary to have an insight into the pathogen population. Some preliminary work on the virulence spectrum was carried out in 1987 (Arama et al., 1989) on a small number of isolates collected from Njoro, Eldoret, Timau and Mau Narok. The isolates were found to be highly virulent on the differential set used. However no attempts were made to separate

the isolates into virulence groups. In chapter 4, more representative isolates from the major wheat growing regions in Kenya were studied. The differential set used this time was composed of selected entries from the standard set used at the Research Institute for Plant Protection (IPO-DLO), and a supplemental set composed of some old Kenyan commercial cultivars. The old commercial cultivars were preferred to the recently released cultivars due to their higher level of resistance in the field in trials conducted between 1988 to 1991. Also it was expected that the Kenyan isolates were more adapted to the commercial cultivars that had been grown in the country than to the cultivars in the standard differential set. Results showed differences in virulence of the isolates sampled from the same location and different locations. In this and the previous study (Arama et al., 1989), isolates collected at Njoro were highly virulent. These isolates may have adapted to the wide range of wheat genotypes that are screened every year in the breeding program. The low virulence shown by the isolates from Timau and Eldoret could be explained by the adaptability to a small number of commercial cultivars grown in those areas each year.

Attempts were made to group the cultivars into resistance groups and the isolates into virulence groups. The disease severity levels used for discerning resistance (R), moderate resistance (MR) and susceptibility (S) were arbitrary as it usually is when a quantitative trait is approached as if it is a qualitative one. The virulence groups differed depending on whether the leaf necrosis or pycnidia coverage was assessed. This suggests that necrosis and pycnidia coverage could be partially independent from each other and may be influenced partially by different genes. However, results from field experiments in Kenya (for instance those presented in Chapter 7) showed that the correlation between necrosis and pycnidia coverage was always high (0.93 - 0.97) under favourable weather conditions. This could be explained by the differences in leaf age. Mamluk et al. (1995) reported that tissue necrosis and pycnidia formation were leaf-age dependent. When wheat seedlings were inoculated at the second leaf stage, they reported that there was higher necrosis formation on leaf 1, compared with leaf 2, while there was a generally higher pycnidial formation in leaf 2 compared with leaf 1. Another possible explanation for the differences could be the long daylight length of 16 h day^{-1} that both the host and pathogen were subjected to in the growth room (Chapter 4). Under field conditions in Kenya, the host and pathogen are adapted to 12 h day^{-1} day length.

It is still difficult to equate the virulence groups obtained from the data in Chapter 4 in the same context as physiologic races in wheat/stem rust, wheat/yellow rust or potato/late blight pathosystems. This is because of the lack of easily recognizable infection types and qualitative differences in disease severity to separate resistant and susceptible genotypes. Agreements have also to be made among septoria workers as to whether necrosis or pycnidia should be preferred in the assessment of disease. Both of them are highly influenced by the environment. From data obtained in his experiments, Kema (1996) reported that pycnidia coverage is more stable and reliable than necrosis. The same author also established a detailed protocol for testing isolates on sets of wheat seedlings. This is a first step in harmonising the work on virulence spectra of STB isolates from different regions which will enable comparison of isolates to be made.

It was shown that the resistance expressed is dependent on the isolate used due to the cultivar x isolate interaction at the seedling stage in Chapter 4. To see whether this was valid in the field in adult plants, three Dutch isolates, found to differ in virulence on wheat seedlings, were selected for inoculations on wheat genotypes in the field. Data presented in Chapter 6 shows that the three isolates IPO290, IPO001 and IPO323 still maintained their difference in virulence on adult plants on which they had not been tested before. The most virulent isolate was IPO290. Very clear cultivar x isolate interactions were shown between a number of moderately resistant cultivars and the two less virulent isolates. Most of the old Kenyan commercial cultivars tested were susceptible to these Dutch isolates. This was not surprising because these isolates were also grouped in virulence groups in which some Kenyan isolates also occurred implying similar virulence (Chapter 4). The Dutch commercial cultivars Jondolar, Minaret and Clement were resistant to IPO323 and IPO001 but susceptible to IPO290. It is suggested that in effective screening for resistance in The Netherlands, it is preferable to use IPO290, due to its wider virulence than IPO001 and IPO323, for artificial inoculations.

In artificial inoculations a breeder may decide to use a singe isolate or a mixture of isolates collected from the region. Zelikovitch et al. (1986) and Zelikovitch and Eyal (1991) reported that there was a considerable reduction of pycnidia coverage on seedlings inoculated with isolate mixtures as compared with the individual isolates, which would have an important bearing on the selection approach. The data presented in Chapter 7 shows that

the 1:1 concentration mixture of the two isolates differing in virulence produced more necrosis and pycnidia than the less virulent isolate and less DS than the more virulent isolate. The high correlation coefficient of 0.97 between pycnidia and necrosis also indicated that the two were not independent. In field experiments conducted in 1989/90, Eyal (1992) again reported significant reductions in pycnidia coverage on cultivars inoculated with mixtures of isolated as compared to the virulent isolate. The environmental conditions were moderately favourable. When he repeated the same experiment in the following year, severe epidemics prevailed due to favourable weather. The pycnidia coverage on cultivars inoculated with the mixture of the same two isolates did not differ significantly from that of the virulent isolate. Likewise, results obtained by Gilchrist and Velazquez (1994) in Mexico showed that there was no reduction in pycnidial density for a mixture of three isolates under field conditions on adult plants. It can be said that under favourable weather conditions in Kenya isolate mixtures can effectively be used in artificial inoculations. Though if the most virulent isolate is identified, this would be preferred.

Cultivar resistance

Evaluation of STB resistance in wheat is often carried out on seedlings under controlled environmental conditions. The advantage of seedling tests is that a large number of genotypes can be evaluated in a relatively small space. The influence of maturity, tallness and other effects that can interfere with the assessment of resistance in the field is also absent. Genotypes selected on the basis of their seedling resistance may be of little benefit to the farmer if the resistance is not also expressed in the adult plant stage. Data presented from experiments described in Chapter 5 shows that it is erroneous for the breeder to extrapolate seedling resistance to adult plant resistance. It was shown that there are three types of resistance operating:

a) Overall resistance; the plants express resistance at all plant stages.

b) Seedling resistance; resistance is only expressed in the seedling stage. The plants become susceptible at the adult plant stage.

c) Adult plant resistance; resistance is expressed at the adult plant stage. Seedlings are susceptible. This kind of resistance appeared to be less common. This indicates that some resistance genes are only expressed at certain development stages of the wheat plant. Of

these three kinds of resistance, the breeder is often interested in overall and adult plant resistance. To be able to identify these, it is advisable to test the genotypes at both seedling and adult plant stages.

Inheritance of quantitative resistance

Genetic studies in the inheritance of resistance to STB have generally been conducted on seedlings and have been focused towards examining effects of simple Mendelian inheritance. Little success has been achieved in transferring high levels of resistance in the susceptible agronomically adapted cultivars in different breeding programs (Eyal et al., 1987). These authors suggested that the presence of modifier genes could affect the expression of dominant genes for resistance. Data presented in Chapter 10 where quantitative resistance was studied shows that transgressive segregation towards more resistance and or more susceptibility to STB in wheat occurred in most of the crosses studied. Because many parents (14) were involved, it suggests that transgressive segregation should be obtained for many other crosses as well and is not just an occasional phenomenon.

Transgressive segregation for higher resistance to STB was demonstrated in progenies of a number of crosses. These transgressive segregants clearly originated from a combination of genetic factors from both parents of each cross where the phenomenon was occurring. This is indicative that additive genes are involved in the resistance. The susceptible cultivars 244 (HAHN'S'*/PRL'S') and 287 (Frontatch) were identified as good combiners. It is interesting to realize that both susceptible cultivars contributed resistance factors to their progenies. On the other hand, crosses involving moderately resistant parents 001, 282 and 343 had most of the transgressive segregants more susceptible than the susceptible parent. This could be an indication that these cultivars have poor combining ability with the other cultivars.

For the Kenyan breeding program cultivars 287 (Frontatch) and 279 (Kenya Sungura) are of interest. These cultivars have been found to be durably resistant to yellow rust (Danial, 1994), and in this case were also selected for their high levels of resistance to stem rust (*Puccinia graminis* f.sp. *tritici*) and leaf rust (*Puccinia hordei*). Breeding for resistance to STB in Kenya can be obtained by crossing the currently high yielding but highly

susceptible cultivars like Mbuni, Pasa, Kenya Fahari, Mulembe and Kenya Kima with Frontatch and K. Sungura. This not only avoids introducing undesirable traits which are often associated with resistant parents of exotic or non agronomic types but also increases the probability of obtaining progenies with adapted agronomic traits suitable for cultivar development. Selection should be done on advanced generations in F6 or F7 on resistant transgressive segregants which are more likely to possess additive genes. This reduces the chances of a single gene based resistance, generally vulnerable to adaption by the pathogen.

How to select for quantitative resistance

In this pathosystem, major gene resistance seems not durable. So, resistance based on several genes with smaller effects has more chance to last. It was shown here that such resistance can be obtained by crossing moderately susceptible cultivars with each other. If a breeder desires to go for quantitative resistance, high yielding and moderately susceptible commercial cultivars could be crossed.

At Njoro, weather conditions are favourable to grow wheat throughout the year. This enables a breeder to realize two generations in a year. In the second year after crossing, F3 progenies are realized. The F3 should be widely spaced so as to get maximum tillering. About 100 to 150 single ears should be harvested to represent all the progenies available. These are planted in 2-3 m ear to row widely spaced F4 lines. A single isolate collected from the region should be used for artificial inoculations at the early tillering stage. Supplemental irrigation maybe necessary to provide a conducive moist environment in case of irregular rainfall. Data should be taken on heading date and plant height to the flag leaf. The extremely susceptible, late maturing and too tall lines should be discarded. Selected lines should then be grouped into heading date groups. From the selected F4 lines, a single plant should be harvested. The selected plants are planted in 2 m rows according to the maturity groups. Several disease assessments are made within the maturity groups independently starting from the date when the genotypes in the respective group is just heading. Extremely susceptible, late and tall lines are again discarded. This is repeated in F6. These are planted in larger plots (4 rows) and are still assessed according to maturity groups. Disease assessment in the breeder's plot situation has been shown to be representative of the farmer's field situation. A multi-locational yield and adaptability trial

can then be started at F8. Representative locations such as Njoro, Narok, Timau, Eldoret and Mai-Mahiu should be planted. The selected lines are likely to be transgressive segregants which are more likely to possess additive genes which would provide durable resistance.

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Summary

Septoria tritici blotch caused by *Mycosphaerella graminicola* (*Septoria tritici)* is one of the important diseases of wheat in Kenya. The disease is mainly splash-borne. Under favourable weather conditions, symptoms appear as grayish green to brown necrotic lesions on leaves. The pycnidia ranging in colour from light to dark brown or black, develop in the necrotic lesions. The pycnidia are scattered within the lesion, and can be on both sides of the leaf. The size of pycnidia may vary with cultivar and pycnidia density. No fungicides have yet been recommended for control in Kenya and resistance in the cultivars is the preferred strategy. Unfortunately, all the high yielding cultivars grown today are susceptible to the pathogen.

The objective of this thesis was to characterize some of the factors that influence disease assessment, disease development and expression of resistance in wheat cultivars.

Disease assessment

There are several factors that may affect disease severity (DS) and so, interfere with the proper assessment of the resistance/susceptibility level of the entries to be evaluated.

i) Earliness (days to heading) and tallness are factors that can affect DS measured at the same moment considerably. To correct for these disturbing effects, one can observe the entries at the same date when the most susceptible (check) cultivars are some 80 - 90% necrotic. A partial regression equation incorporating the heading date, tallness and disease severity (DS) can then be used to correct the DS in the entries. This was done in an experiment in five environments in Kenya with 57 wheat genotypes. Earliness appeared to have a strong effect and tallness a small effect on DS. After correcting for differences in earliness, the ranking order for resistance changed markedly.

Another method that gave good results was to group the cultivars according to their earliness. The entries in each group were assessed four times starting at heading. It appeared that the disease developed in each earliness group at the same rate, starting later when heading was later.

ii) The importance of interplot interference in screening for septoria tritici blotch (STB) resistance in wheat was studied in Kenya and The Netherlands. In three experiments there

was no indication of interplot interference of any significance. The ranking of cultivars was not seriously affected by plot size and whether the plots were neighbouring each other or not. The range in DS between the small, adjacent plots was similar to that in large, isolated plots indicating that the resistance level was not underestimated in the small plots. From the breeder's point of view, selection for resistance in small adjacent plots is not affected by interplot interference and is representative of the farmer's situation.

iii) The Nitrogen (N) level is another factor that may affect the assessment of STB. Two experiments were carried out in Kenya and in The Netherlands under very different environmental conditions. In Kenya there was a considerable and significant increase in disease severity on cultivars exposed to more N. The soil type at the experimental site was volcanic (Mollic Andosols). There was a slight but not significant increase in DS with increase in N level in The Netherlands. That experiment was on sandy soil. The difference in results could be due to environmental conditions and soil types.

iv) In breeding programs where host genotypes are evaluated under field conditions, breeders and pathologists prefer to use mixtures of isolates collected from different locations within the country, hoping to incorporate as many pathotypes as possible in the inoculum. Two single isolates differing in virulence and their 1:1 concentration mixture was used in the inoculation of six wheat cultivars in the field. The correlation coefficient between the area under disease progress curves (AUDPC) of necrosis and pycnidia was 0.97. The mean AUDPC for the 1:1 mixture inoculum was higher than the less virulent isolate of the mixture and lower than the more virulent isolate. The ranking of the cultivars was essentially not affected. This shows that inoculum mixtures can be effectively used in screening wheat genotypes.

v) Assessment of resistance is either carried out on seedlings under controlled environmental conditions or on adult plants in the field. Fourteen wheat cultivars were tested for their resistance to six *Septoria tritici* isolates at the seedling and adult plant stage under controlled and identical environmental conditions. Correlation coefficients between the disease severity at the seedling and at the adult plant stages ranged from 0.36 to 0.78 for the six isolates. This indicated that resistance assessed at the seedling stage could not fully explain adult plant resistance. Three types of resistance were shown to occur: Resistance in the seedling and adult plant stages (overall resistance), resistance in the

seedling stage only (seedling resistance), and resistance in the adult plant stage only (adult plant resistance). Adult plant resistance was the less common phenomenon. In screening nurseries for resistance to *Septoria tritici*, testing of both seedlings and adult plants is advisable to discern among the three types of resistance.

Pathogenic variation

i) Sixteen isolates from Kenya and The Netherlands were tested on 43 wheat cultivars at the seedling stage. Based on the percentage pycnidia coverage, cluster analysis grouped the isolates into six virulence groups and the cultivars into five resistance groups. On the other hand, there were eight virulence groups and six resistance groups based on cluster analysis of leaf necrosis. This indicated that pycnidia and necrosis were partially independent of each other. Some isolates collected from different locations were grouped together and some isolates collected at the same location were grouped in different virulence groups. It was concluded that there was variation in virulence (and so in race-specific resistance in the host) of *Septoria tritici* populations within Kenya and within The Netherlands.

ii) Twenty nine wheat cultivars were tested in the field with three isolates IPO290, IPO001 and IPO323, collected from different locations in The Netherlands. Isolate IPO290 was the most virulent as all the cultivars except Milan and Clement were highly susceptible. Clear cultivar x isolate interactions existed between the moderately resistant cultivars with the less virulent isolates IPO001 and IPO323. Although the cultivar x isolate interaction variance was highly significant, the variances due to main effects of cultivars and isolates was far greater. Breeders should be more aware that race-specificity appears to be more common.

Inheritance of quantitative resistance

Fourteen cultivars ranging from quite resistant to very susceptible were intercrossed in a half diallel scheme. The F_6 single seed descent derived lines of 36 crosses realized were inoculated with a single isolate of *S. tritici*, IPO93001. Transgressive segregation towards more resistance and or more susceptibility occurred in most crosses. Because so many parents were involved, it suggests that transgressive segregation should be obtained from many other crosses as well and is not just an occasional phenomenon.

Transgressive segregation for higher resistance than the mid-parent values was shown in

progenies of a number of crosses. Most of the progenies obtained from crosses involving the susceptible parent 244 (HAHN'S'*/PRL'S') showed this kind of transgression. It can be said that a fair number of genes operating in an additive manner are involved. As many of the F_6 populations had a disease severity mean much higher than that of the their mid-parents, epistasis was clearly involved. For breeders it was interesting to see that from crosses between fairly susceptible cultivars, fairly resistant lines were obtained.

Samenvatting

Bladvlekkenziekte, veroorzaakt door *Mycosphaerella graminicola* (*Septoria tritici*) is één van de belangrijkste ziekten van tarwe in Kenia. Het inoculum verspreidt zich voornamelijk via opspattende regendruppels. Onder gunstige weersomstandigheden ontstaan er grijsgroene tot bruin-necrotische lesies op de bladeren. Pycnidia, die zich als donkere puntjes voordoen, worden verspreid in deze lesies gevormd. De grootte van de pycnidia is afhankelijk van tarweras en pycnidia-dichtheid. In Kenia worden geen fungiciden aanbevolen en wil men de ziekte via resistente rassen bestrijden. Resistente rassen zijn echter nog niet beschikbaar in Kenia.

Het hier beschreven onderzoek richt zich op de analyse van resistentie in tarwe tegen dit pathogeen en op de factoren, die de ernst van de aantasting en daarmee de evaluatie van resistentie kunnen beïnvloeden.

Evaluatie van de mate van aantasting

Er zijn diverse factoren die de mate van aantasting beïnvloeden en daarmee interfereren met een juiste beoordeling van het resistentie niveau van de te evalueren tarwelijnen.

i) Vroegheid (van in aar komen) en plantlengte hebben een duidelijk effect op de mate van aantasting. Gemeten op het zelfde moment zijn vroege en korte rassen doorgaans zwaarder aangetast dan late en lange rassen. Dit werd gedaan bij 57 rassen, die in vijf milieu's werden getoetst. Een partiële regressie analyse toonde aan, dat vroegheid een prominent effect had en plantlengte vrijwel geen. Via de verkregen regressievergelijking kon een inzicht in de werkelijke resistentieniveau's van de rassen verkregen worden.

In Kenia, met geringe temperatuurverschillen, is een andere benadering mogelijk. Negentien rassen werden in vier groepen ingedeeld op basis van hun vroegheid. De aantasting werd diverse malen gemeten vanaf het moment van in aar komen. Het bleek dat de toename in aantasting gelijk was voor alle groepen en dat in feite het begin van de aantasting door de vroegheid werd bepaald. Veredelaars kunnen dus hun selecties naar vroegheid indelen en binnen de groepen vergelijken.

ii) Selectie wordt in kleine veldjes, die naast elkaar liggen, uitgevoerd, terwijl de boer zijn ras of rassen op relatief grote velden teelt. Bij de kweker kan door interplot-interferentie

een vertekend beeld ontstaan. Deze interplot-interferentie werd in drie experimenten (2 in Kenia en 1 in Nederland) bestudeerd. De volgorde en de grootte van de rasverschillen in kleine, naast elkaar liggende veldjes was niet anders, dan die in grotere van elkaar geïsoleerde veldjes. Er werd geen interplot-interferentie van belang waargenomen. De waarnemingen van de kweker in zijn selectieveldjes zijn representatief voor de praktijksituatie.

iii) Het stikstof (N) niveau in de bodem zou de mate van aantasting kunnen beïnvloeden, net zoals bij b.v. gele roest. Er werden twee experimenten, in Kenia en Nederland, uitgevoerd. In Kenia werd een duidelijke toename in de aantasting waargenomen bij alle rassen bij stijgende N-giften. In Nederland werd ook een toename waargenomen, maar deze was klein en statistisch niet betrouwbaar. De bodemverschillen kunnen hiervan de oorzaak zijn.

iv) In de selectievelden worden de te beselecteren lijnen veelal blootgesteld aan mengsels van pathogeen-isolaten. In de literatuur is melding gemaakt van een verminderde werking van zulke mengsels. Een experiment, waarin een mengsel van twee isolaten vergeleken werd met de individuele effecten van die isolaten kon geen mengsel-effect waargenomen worden.

v) Om de resistentie van lijnen te bepalen worden vaak zaailingtoetsen uitgevoerd. Om te bestuderen of dergelijke toetsen representatief zijn voor volwassen planten werden 14 rassen in het zaailing- en volwassen plantstadium onder gecontroleerde en gelijke omstandigheden beproefd met zes pathogeen isolaten. De correlatie coefficient in de aantasting tussen zaailingen en volwassen planten varieerde met de isolaten tussen 0,36 en 0,78. Er werden drie typen resistentie waargenomen; zaailingresistentie, waarbij de resistentie alleen in het zaailingstadium tot expressie komt; volwassen plantresistentie, waarbij de resistentie alleen in het volwassen plantstadium tot expressie komt; en de "overall" resistentie; waarbij de resistentie in alle plantstadia tot expressie komt. Zaailingtoetsen zijn dus niet zonder meer representatief voor volwassen planten.

Pathogeenvariatie

In een zaailingproef met 16 pathogeen-isolaten en 43 tarwerassen werden de pycnidia-aantasting en de mate van necrose gemeten. Clusteranalyse werd op beide waarnemingen

toegepast. Bij de pycnidia-aantasting konden de 16 islolaten in 6 virulentiegroepen worden ingedeeld, de rassen in 5 resistentiegroepen. Op basis van de necrose-waarnemingen waren er 8 virulentie- en 6 resistentiegroepen. Blijkbaar zijn pycnidiavorming en necrose gedeeltelijk onafhankelijk van elkaar. Zowel in Kenia als in Nederland bestaat variatie voor virulentie en dus voor fysiospecifieke resistentie.

In een veldproef in Nederland werden 29 rassen vergeleken bij drie Nederlandse pathogeen-isolaten. Voor één isolaat waren vrijwel alle rassen vatbaar. Alleen de rassen Milan en Clement vertoonden een bescheiden niveau van resistentie. Bij de twee andere isolaten werd veelvuldig fysiospecifieke resistentie waargenomen. Fysiospecificiteit komt dus veel voor en de kwekers moeten daar wel rekening mee houden.

Overerving van quantitatieve resistentie

Veertien rassen, variërend van behoorlijk resistent tot zeer vatbaar werden in vele richtingen met elkaar gekruist. Van 36 kruisingen werd voldoende F_2 zaad verkregen. Deze werden via de "single seed descent" benadering tot de F_6 doorgeteeld. Per kruising werden tot 100 F_6 lijnen te velde met hun ouders vergeleken op hun aantasting na inoculatie met één isolaat. Bijna alle kruisingen vertoonden transgressie. De hoge frequentie van transgressie duidt op de aanwezigheid van op zijn minst een redelijk aantal resistentiefaktoren. Bij de meeste kruisingen was de gemiddelde aantasting van de F_6 hoger tot beduidend hoger dan het oudergemiddelde. Dit duidde op de aanwezigheid van epistasie. Bij de kruisingen met een van zeer vatbare ouder leek deze epistasie niet op te treden en was de overerving voornamelijk van additieve aard. Ook de zeer vatbare rassen bleken nog resistentiefaktoren te bevatten. Kruisingen tussen zulke vatbare rassen leverden zelfs vrij resistente F_6 lijnen op.

Curriculum vitae

Peter Futi Arama was born on October 13, 1958 in Migori district, Kenya. He and his wife Roselyne have three children, Frederick, Robert and Susan. After attending high school education at St. Georges, Giryama and Friends Kamusinga, Arama joined University of Nairobi in 1979 where he completed his B.Sc. (in Agriculture) (Hons.) in 1983. He obtained an M.Sc. (in Plant Pathology) at the same University in 1989.

In 1983, Arama commenced his professional career when he was employed by the Ministry of Agriculture and Livestock Development and was posted to the National Plant Breeding Centre (NPBRC), Njoro as a Research Officer in oil crops breeding. Between 1983 and 1984 he was involved in initiating research on development of Kenyan hybrid sunflower cultivars. From 1984 to 1986 he also worked on barley/barley scald resistance. At the beginning of 1987 he started work on septoria tritici blotch of wheat. He was offered a 12 month research fellowship by the Dutch Minister for International Development Cooperation (DGIS) in 1987 to work on the global virulence of septoria isolates at the Research Institute for Plant Protection (IPO-DLO), Wageningen, The Netherlands. This was under the collaborative program between International Maize and Wheat Improvement Centre (CIMMYT), IPO-DLO and Tel Aviv University, Israel.

From January 1991 to December 1995 he worked on the collaborative research between the Kenya Agricultural Research Institute (KARI) and Wageningen Agricultural University (WAU); and funded by KARI and DGIS under the Durable resistance program. The project was on partial resistance in bread wheat to septoria tritici blotch in Kenya. This work, which resulted in this thesis, was carried out at the NPBRC, IPO-DLO and the Department of Plant Breeding, WAU.