The Russian wheat aphid (*Diuraphis noxia* Mord.): Damage on Kenyan wheat (*Triticum aestivum* L.) varieties and possible control through resistance breeding

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Contents

Abstract

We studied the effect of the Russian wheat aphid (RWA) (*Diuraphis noxia*) infestation on seedlings and adult plants of eight Kenyan wheat (*Triticum aestivum* L.) varieties. The Kenyan varieties were 91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi and Mbega. Two RWA resistant wheats, Halt and PI 294994, were also tested against Kenyan isolates of the aphid.

All the Kenyan varieties were susceptible to RWA when compared with the resistant line PI 294994. Halt, which is a resistant variety developed in the USA, was susceptible to Kenyan isolates of RWA. This indicates that the Kenyan RWA isolates are different from the USA ones. In seedlings, the RWA damage was expressed mainly as leaf chlorosis and leaf rolling, with damage scores increasing with time. Differences among the Kenyan varieties in the extent of leaf chlorosis were observed. The most devastating effect of RWA infestation of adult plants of the Kenyan varieties was the reduction in seed set. The tight rolling of flag leaves caused by the aphid delayed ear emergence, leading to floret sterility. Infestation also reduced the quality of the seeds produced, as shown by increased rate of seed deterioration under accelerated ageing conditions, and reduced seedling vigour. The effect of infestation on seed quality was more pronounced under dry conditions. Morphological and genetic variations within PI 294994 were identified. The PI 294994 plants tested could be separated into three distinct groups, all of which had equally high resistance to Kenyan RWA. One PI 294994 derived line, designated P3, was discovered to require no vernalization and therefore to be suitable for use in a Kenyan breeding programme. Segregation in the F_2 populations indicated that resistance in two PI 294994 derived lines (P1 and P2) was controlled by two genes (one dominant and one recessive). For P3, the results were inconclusive since in one F_2 population the segregation indicated that the resistance was controlled by one dominant gene, whereas in another population the segregation indicated that resistance was due to one dominant and one recessive gene. Work to identify molecular markers linked to RWA resistance gene(s) in P3 was initiated.

CHAPTER 1

General introduction

Bread wheat

Wheat is one of the leading cereal grain crops produced, consumed and traded in the world today. It provides over 20% of the calories for the world population and is a staple food for 35 % of the world's population (FAO, 1998). In Eastern Europe and Russia, over 30 % of the calories consumed come from wheat (Anon., 2002). Wheat is grown on more land area worldwide than any other crop and only competes with maize and rice in total world production (Table 1). In the early 1990s, annual wheat production was averaging more than 500 million metric tonnes, and represented almost one-third of all cereal production (Oleson, 1994). In 1999 the worldwide area planted with wheat was over 212 million ha as compared with about 139 million ha for maize and 156 million ha for rice (FAO, 2001).

Table 1a. Annual wheat, rice and maize production, average for 1994 -1996

	World	Percentage of world production						
	production	North	Latin					
Crop	(Mt)	America	America	EU 15	CIS 12*	Asia	Oceania	Africa
Wheat	552.9	15.5	3.1	16.2	11.0	41.7	3.1	2.5
Rice	553.1	1.4	3.8	0.4	0.2	88.8	0.2	2.7
Maize	515.9	37.7	14.6	5.9	1.4	28.4	0.08	6.9

*Former Soviet Union, except the Baltic States. Source: FAO, 1998.

Table 1b. Annual harvested area for wheat, rice and maize, average for 1994-1996

	Area	Percentage of world production						
	grown	North	Latin					
Crop	(Mha)	America	America	EU 15	CIS 12*	Asia	Oceania	Africa
Wheat	222.3	16.3	3.6	7.5	20.5	39.2	4.2	3.8
Rice	149.2	0.8	4.6	0.2	0.03	87.8	0.08	4.7
Maize	136.3	20.0	21.4	2.8	0.05	30.0	0.05	18.8

*Former Soviet Union, except the Baltic States. Source: FAO, 1998.

Wheat is used mainly as a human food. The cultivated wheats belong to two main classes, common or bread wheat (*Triticum aestivum* L.), which accounts for about 95% and durum wheat (*Triticum durum*), which accounts for 5 % of world wheat production. Common wheat is used to make bread and biscuits, whereas durum wheat is used to make pasta. Unlike any other plant-derived food, common wheat contains gluten protein, which enables leavened dough to rise by forming minute gas cells that hold carbon dioxide during fermentation and enables production of light textured bread.

Common wheat is classified into hard or soft wheat based on its suitability for making bread. Hard wheat has a physically hard kernel that yields flour with high gluten and hence high protein content. This type of flour is more suitable for producing bread. Soft wheats on the other hand have lower protein contents and are more suitable for producing biscuits and cakes, which do not require strong flour (i.e. flour with high gluten content). Wheat is also classified as either red or white wheat depending on the colour of the aleurone layer. Another classification is that based on the growth habit which groups wheat into spring and winter types. Winter types require vernalization at the seedling stage to enable normal development to the reproductive stage.

Wheat is a widely adapted crop. Although it is most successful between the latitudes of 30^{o} and 60^{o} N and 27^{o} and 40^{o} S, respectively, wheat can be grown beyond these limits from within the Actic Circle to higher elevations near the Equator (Nuttonson, 1955, as quoted by Curtis, 2002). In altitude the crop is grown from sea level to more than 3000 m a.s.l. It can be grown in areas ranging in annual precipitation from 250-1750 mm, although most of the world crop is produced in areas with 375-875 mm annually (Leonard and Martin, 1963). Currently, wheat is grown in more than a hundred countries from Finland in the north to Argentina in the south (Oleson, 1994). However, most of the production is centered in the temperate regions of the world.

Through the ages, wheat production increases arose mainly from increased area. However, from the 1950s, world wheat production increased dramatically without a corresponding increase in crop area due to improved yields. In 1951, world production was nearly 1 tonne/ha. It reached 2 tonnes/ha by the early 1980s and climbed to nearly 2.5 tonnes/ha by 1995 (FAO 1996). The increased yields have been

attributed mainly to the green revolution, which was accompanied by the adoption of management responsive, high yielding, disease-resistant semidwarf wheat cultivars throughout much of the world, particularly in developing countries (Curtis, 2002).

As the worlds most important crop, wheat has to meet the demands of the increasing population and changing lifestyles. Although wheat production has generally been increasing gradually in the last 20 years, wheat use has been increasing at a slightly higher rate such that from 1999/2000, wheat use has exceeded production, with the short fall being met by world stocks (Figure 1). The world population growth rate in the 1990s averaged 1.5 %, while the growth rate for wheat production between 1985 and 1995 was 0.9 % (CIMMYT, 1996). If population growth continues to double the growth of wheat production, there will likely be serious difficulties in maintaining future wheat food supply. World population was projected to grow by 35 % between 1997 and 2025 and reach 7.9 billion (United States Census Bureau, 1998). Assuming little or no change in world consumption trends of wheat, a projection of 786 million tonnes of wheat will be required annually for human use in the year 2025, an annual production increase of 204 million tonnes above production in 1997. This underscores the need to rapidly and continuously increase production to match the demand.

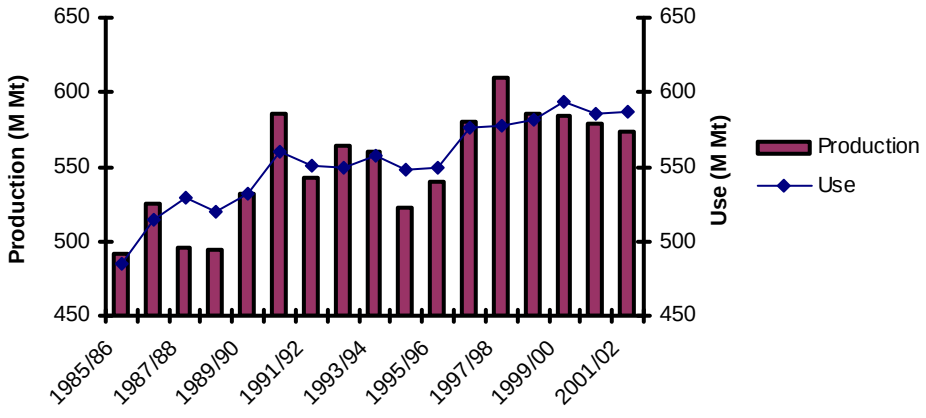


Figure 1. World wheat production and use from 1985/86 to 2001/2002.
Adapted from Iowa State University's Agronomy Website.

The Russian wheat aphid problem

The Russian wheat aphid (RWA) *Diuraphis noxia* Mord. is indigenous to southern Russia, Iran, Afghanistan and countries bordering the Mediterranean Sea. The aphid was first reported by Mordvilko and by Grossheim around 1900 in the Mediterranean Sea region and southern Russia (cited from Jones *et al*. 1989 and Elsidaig and Zwer, 1993, respectively). It is believed that the aphid spread from west Asia to the USA and Canada via South Africa and Mexico (Saidi and Quick, 1996). The aphid has since spread to most of the wheat producing regions of the world. It attacks most of the small grain cereals, including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare*), rye (*Secale cereale*), Triticale (×.*Triticosecale*), and oats (*Avena sativa*).

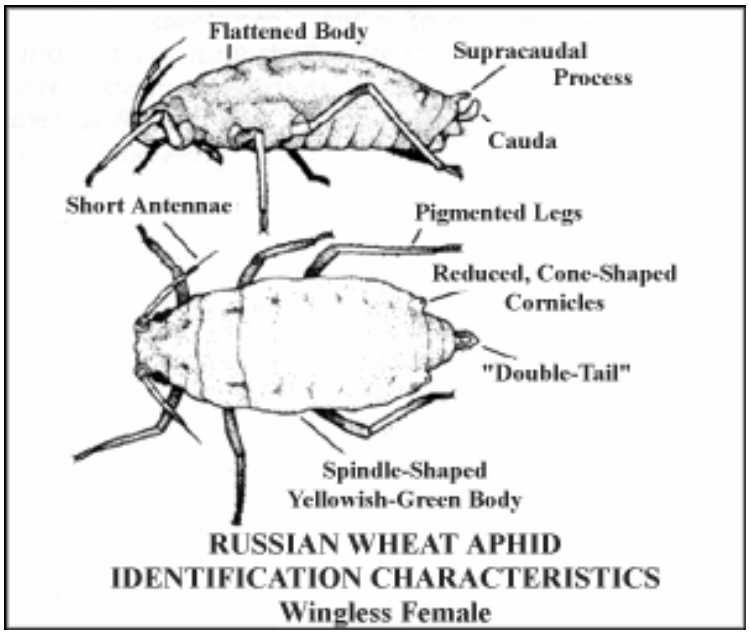


Figure 2. Identification characteristics of RWA.
Adapted from Hein *et al.* 1989.

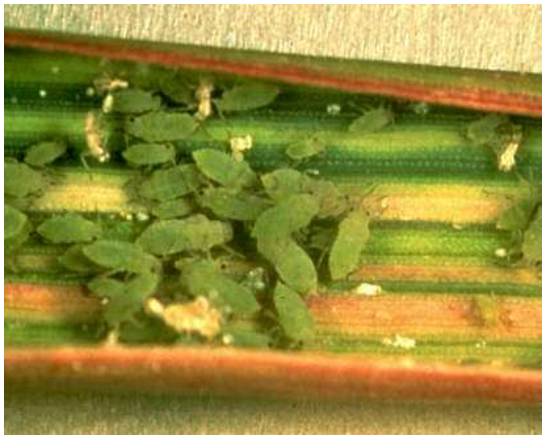
The Russian wheat aphid is pale to light green in colour with an elongated, spindle shaped body and grows to up to 2 mm long. It has short antennae with rounded very short, nearly invisible cornicles (Stoetzel, 1987, Karren, 1993). The feature that easily distinguishes it from other cereal aphids is the presence of an appendage (supracaudal

process) above the cauda, giving the aphid the appearance of having two tails (Figure 2). The Western wheat aphid *Diuraphis tritici* (Gillette) is similar in its shape, size and damage to wheat, but has the more typical single tail and is much waxier in appearance (Peairs, 1998). Its presence in a field is easily detectable through longitudinal leaf rolling with white/yellow (warm weather) or purple (cold weather) streaking on the leaves (Figure 3). This damage is caused by injection of a toxin into the plants during feeding, which prevents the production of chlorophyll and causes leaf curling.

Aphids are characterized by their ability to reproduce either sexually or asexually (parthenogenesis). While some species deposit eggs, others such as RWA retain their eggs inside the female until she 'gives birth' to living young (Hein *et al*., 1989). Under favourable conditions, all RWA are females that do not lay eggs but give birth to live young ones at a rate of 4 to 5 per day for up to 4 weeks. The new young females can mature in as little as 7-10 days. Infestation can thus spread quickly under favourable conditions (Karren, 1993). Overcrowding and adverse weather conditions may stimulate production of winged forms, which are easily dispersed in wind currents. The Russian wheat aphids prefer to live in the leaf whorls or in tightly rolled leaves. They are hardy and can survive extremely low temperatures.

A

B



C

D

E

F

Figure 3. The two forms of RWA A) wingless adult B) winged adult, and symptoms of damage caused in cereal crops C) reddish/purplish streaks D) whitish/yellowish streaks E) severely attacked plant F) patch of wiped out crop.

The super cooling point (temperature at which body fluids freeze and mortality occurs) for this aphid was determined to be −27.6^{o}C, and did not change with rearing environment or insect life stage (Armstrong and Nielsen, 1998). The Russian wheat aphid currently poses a serious threat to the production of wheat and other small grain cereals in many parts of the world (Webster *et al*., 1987; Archer and Bynum, 1992; Porter *et al*., 1993; Zwer *et al*., 1994; Nkongolo, 1996). It causes characteristic longitudinal leaf chlorosis, stunted growth, leaf rolling and leaf folding, spike trapping and sterility (Hewitt *et al*., 1984; Kiriac, 1990; Miller *et al*., 1994; Zwer *et al*., 1994). Extensive chlorosis leads to death of plants while leaf rolling retards plant development. In colder climates, the streaks become reddish or pinkish due to anthocyanic pigments (Kazemi *et al*., 2001). Rolling of the flag leaf causes delayed ear emergence, leading to decreased fertility of florets. Although RWA has variously been reported as a non-transmitter of diseases, a few researchers have reported that the aphid could play a role in transmitting some viruses. Rybicki and Von Wechmar (1984) reported that various aphid species, including *D. noxia*, were capable of efficiently transmitting virus disease complexes. Also, Damsteegt *et al*., (1992) reported a clone of *D. noxia* that could transmit some plant pathogenic viruses including Barley Yellow Dwarf Virus, Barley Mosaic Virus and sugarcane Mosaic Virus.

Significant yield and quality losses attributed to RWA have been documented around the world (Du Toit and Walters, 1984; Pike and Allison, 1991). In South Africa, where the aphid was first reported to be a serious pest of wheat and barley, yield losses of between 35 and 60% were recorded (Du Toit and Walters, 1984). Since its detection in the USA in 1986, it had caused over $850 million damage in wheat and barley by 1991 in the western Great Plains and the intermountain region of the country. By this time RWA had established itself as the primary pest of small grains in the arid and semi-arid areas of the USA (Webster *et al*., 1987; Massey and Amosson, 1991; Legg and Amosson, 1993; Porter *et al*., 1993). There is evidence that the extent of damage resulting from aphid attack on cereals may depend on plant growth stage. Kieckhefer and Gellner (1988) found that plant stage differentially influenced fecundity of four cereal aphids, *Schizaphis graminum* (Rondani), *Rhopalosiphum padi*, *R. maidis* (Fitch) and *Macrosiphum avenae* on spring wheat and

barley. Similarly, Hein (1992) reported a significant cultivar by growth stage interaction for RWA reproduction among three winter wheats and a triticale (Figure 4). He further reported a significant cultivar by growth stage interaction for aphid damage, which he attributed to a decrease in damage rating for the susceptible cultivars of plants in the reproductive stages as compared to plants in the vegetative stages.

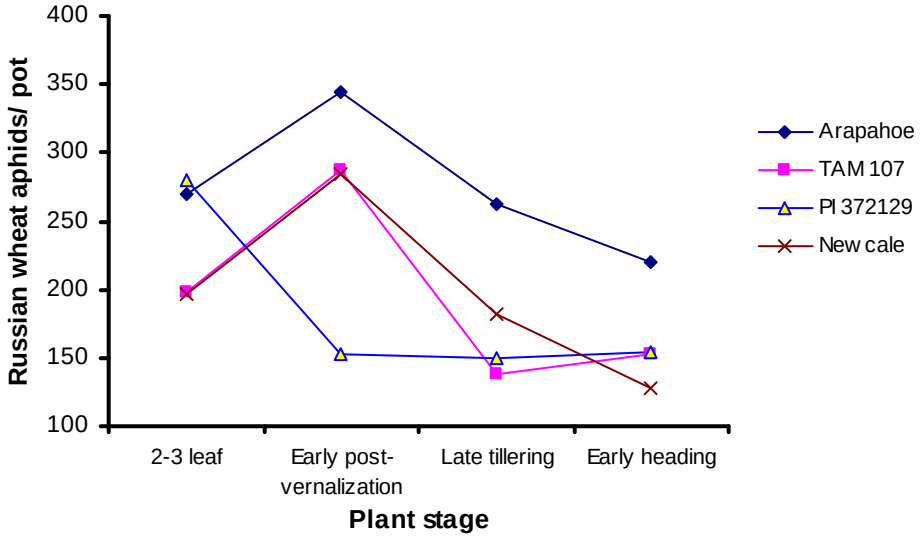


Figure 4. RWA reproduction (number of aphids per pot) on three winter wheats Arapahoe, TAM 107 and PI 372129, and triticale variety Newcale at four growth stages. Adapted from Hein (1992).

Initial efforts to control the RWA were made through the use of insecticides. The characteristic habit of the RWA of rolling cereal leaves, however, makes its control by insecticides difficult. The aphid secludes itself within the rolled leaves. Aphids secluded in the rolled leaves are partially protected from natural enemies and from contact insecticides, thereby necessitating the use of the more expensive systemic insecticides (Du Toit and Walters, 1984).

When RWA first appeared in the USA, the principal management strategies to control it included the use of systemic insecticides, delayed plantings and the growing of non-host crops (Pike, 1988; Elsidaig and Zwer, 1993), while in South Africa large-scale aphicide applications were made annually to protect crops. This was achieved by the application of expensive mixtures of systemic and contact insecticides, supplemented by the eradication of volunteer wheat which served as a host between seasons (Du

Toit and Walters, 1984; Du Toit, 1989a). Systemic aphicides, however, are very expensive. The most effective, economical and environmentally safe option of controlling the RWA is the use of resistant cultivars (Elsidaig and Zwer, 1993; Zhang *et al*. 1998, Tolmay and Mar'e, 2000). Current and future control of the Russian wheat aphid will depend heavily on the development and use of varieties resistant to the aphid.

Search for RWA resistance genes

Ever since the RWA was identified as a serious pest of wheat and barley in South Africa in 1978, plant breeders have been searching for sources of resistance to the aphid. Butts and Pakendorf (1984) and Du Toit and Van Niekerk (1985) demonstrated that potential for *D. noxia* resistance exists in the ancestral diploid wheat species *Triticum monococcum, T. timopheevi, T. dicoccoides* and *T. tauschii,* and amphiploids of *T. monococcum* / *T. durum*. Resistance to the RWA has also been reported in rye (*Secale cereale*) and *Triticale* (Nkongolo *et al*., 1989; Webster, 1990). In a greenhouse experiment involving a number of wheat, rye and *Triticale* lines, Nkongolo *et al*., (1989) found that rye and *Triticale* lines were all moderately resistant to resistant to the aphid, whereas the wheat lines ranged from susceptible to resistant. A high level of RWA resistance was observed in interspecific hybrids, indicating that genes conferring resistance in wheat relatives were accessible for use in wheat improvement by established cytogenetic and plant breeding techniques (Nkongolo *et al*., 1990). Due to its simple inheritance and absence of known unfavourable linkages, transferring RWA resistance from resistant lines to adapted cultivars has posed no serious problems (Saidi and Quick, 1996).

Through increased efforts to find sources of resistance in hexaploid wheat which can be utilized easily in breeding programs, RWA resistance was first reported in two wheat lines, PI 137739 (a hard, white spring wheat from Iran) and PI 262660 from Bulgaria (Du Toit, 1987; 1988). Since then, resistance has been identified in several *T. aestivum* cultivars and unimproved germplasm mainly from southwest and central Asia, and the Middle-East region (Nkongolo *et al*., 1989; Zemetra *et al*., 1990; Quick *et al*., 1991; Harvey and Martin, 1990; Smith *et al*., 1991, Porter *et al*., 1993.). The high number of accessions with RWA resistance from this region is thought to be due

to natural selection pressure as a result of the genotypes being subjected to repeated RWA infestations. All the resistant lines were of poor agronomic quality, necessitating a series of backcrosses to obtain the RWA resistance in an adapted background. RWA resistance is virtually non-existent in improved wheat cultivars and germplasm developed for production areas outside of central Asia (Souza *et al*., 1991).

Apart from PI 137739 and PI 262660, the other plant introductions with high levels of RWA resistance are PI 372129, PI 294994, PI 262605 and PI 243781 (Nkongolo *et al*., 1989; Quick, 1989; Quick *et al*., 1991). Analysis of the inheritance pattern of resistance in the wheat lines PI 137739, PI 262660, PI 372129 and PI 243781 suggested that single dominant independently inherited genes, designated *Dn1*, *Dn2*, *Dn4* and *Dn6* conferred resistance in the four genotypes respectively (Du Toit, 1989b; Nkongolo *et al*., 1991a; Saidi and Quick, 1994). Marais and Du Toit (1993) reported that one dominant gene, *Dn5*, controlled resistance in wheat line PI 294994. However, Liu *et al*. (2001) reported two other resistance genes *Dn8* and *Dn9* in this line. So far, a total of 10 resistance genes have been reported (Table 2).

Studies on the mode of resistance in some of the wheat lines have revealed that antibiosis, antixenosis and/or tolerance are involved. Resistance in PI 147739, PI 262660 and PI 294994 are attributed mainly to antibiosis and antixenosis (Du Toit, 1987; 1989a; Smith *et al*. 1992), although PI 262660 also exhibits some tolerance (Du Toit, 1989a). Resistance in PI 372129 is due mainly to tolerance in combination with a low level of antixenosis (Quick, 1989; Nkongolo *et al*. 1989).

Colorado State University has developed several commercially available RWA resistant varieties of winter wheat such as Halt, Prairie Red, Prowers 99 and Yuma (Thomas *et al*., 2002). All these varieties have the *Dn*4 resistance gene derived from PI 372129 (Turcikum 57). Halt is a semidwarf hard red winter wheat that is well adapted to the production areas of eastern Colorado (Peairs *et al*., 1999). It demonstrates a good level of resistance mainly due to tolerance. Thus Russian wheat aphids may survive in numbers similar to those in susceptible varieties, but the leaves Table 2. Sources of the 10 known Russian wheat aphid resistance genes, their origins and mode of resistance

Source of resistance	Wheat type	Chromosomal location	Origin of accession	Resistance gene	Mode of resistance
PI 137739	Hard White Spring	7D (Schroeder-Teeter *et al*., 1994)	Iran (Du Toit, 1987)	*Dn1*	Antibiosis and antixenosis (Du Toit 1987, 1989)
PI 262660	Hard White Winter	7D (Ma *et al*., 1998)	Bulgaria (Du Toit, 1987)	*Dn2*	Antibiosis and antixenosis (Du Toit 1987, 1989)
Triticum tauschii	-		Nkongolo et al., 1991a	*dn3*	-
PI 372129	Hard Red Winter	1DS	Former Soviet Union (Nkongolo *et al*. 1991b; Saidi and Quick, 1996).	*Dn4*	Tolerance (Meyer *et al*. 1989 as cited by Saidi and Quick, 1996)
PI 294994	Hard Red Winter	7D (Du Toit, 1987; Marais and Du Toit, 1993)	Bulgaria (Marais and Du Toit, 1993)	*Dn*5	Tolerance, antibiosis and antixenosis (Du Toit 1987, 1989 Smith *et al*., 1992)
PI 243781	Winter wheat	-	Iran (Saidi and Quick, 1996)	*Dn*6	Tolerance and Antibiosis (Miller *et al*., 2003
Rye accession	-	Transferred to 1RS in wheat (Liu *et al*., 2001)	-	*Dn*7	-
PI 294994	Hard Red Winter	7D (Liu *et al*., 2001)	Bulgaria (Marais and Du Toit, 1993)	*Dn*8	-
PI 294994	Hard Red Winter	1D (Liu *et al*., 2001)	Bulgaria (Marais and Du Toit, 1993; Liu *et al*., 2001)	*Dn*9	-
PI 220127	Winter wheat	7D (Liu *et al*., 2001)	Afghanistan (Harvey and Martin, 1990)	*Dnx*	-

do not curl or streak (Thomas *et al*., 2002). It therefore had significant yield advantage over the susceptible varieties ‘TAM 107’ and Arapahoe when exposed to RWA

(Figure 5). Currently, large areas are planted with RWA resistant cultivars in the USA and South Africa. Recent reports (Peairs *et al*., 2003), however, indicate that RWA resistant cultivars with the *Dn4* gene are susceptible to a new biotype of RWA designated Biotype B.

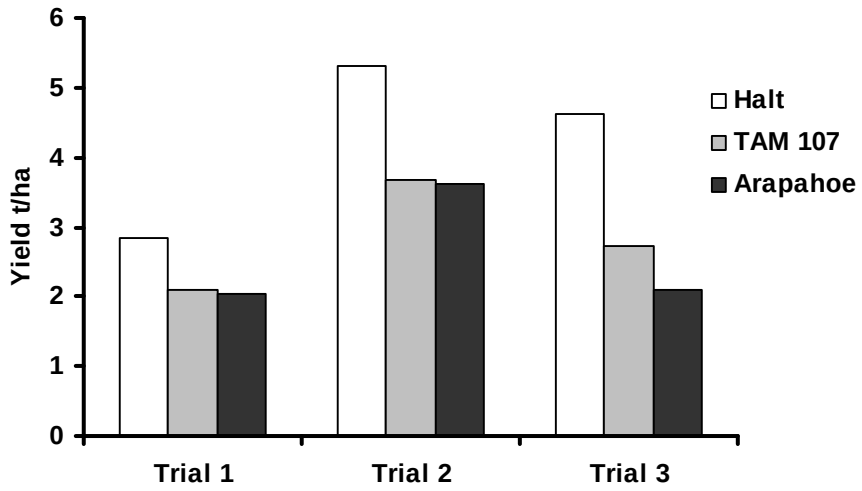


Figure 5. Yields following heavy spring infestations with RWA of winter wheat varieties Halt (resistant), TAM 107 (susceptible)and Arapahoe (susceptible). (Trial 1, High plains Agricultural Laboratory, Sidney, 1994; Trial 2, Panhandle Research and Extension Centre, Scottsbluff, 1994; Trial 3, High plains Agricultural Laboratory, Sidney, 1996). Adapted from Thomas *et al*., 2002.

Wheat in Kenya

Although wheat production is concentrated mainly in the temperate regions of the world, it has also become an important crop in highland areas of some tropical countries such as Kenya and Ethiopia. Wheat was introduced in Kenya towards the end of the 19th century and has since been grown on an increasing scale in the highland areas. The wheat growing areas lie between 1800 and 2900 m a.s.l., and receive more than 750 mm of rainfall per annum. The wheat is grown under rainfed conditions, in small and large farms where nearly all production activities are mechanized. All the wheat is spring wheat and several varieties of both hard and soft wheat are grown.

The first attempt at extensive commercial wheat production was made in 1907. In the initial stages, the crop suffered heavily from diseases, particularly the rusts. The first crop, which was planted using varieties introduced from Australia, was severely attacked by stem rust (*Puccinia graminis*). The next crop planted with the Italian variety Rieti, succumbed to yellow rust (*Puccinia striiformis* Westend.) (Guthrie and Pinto, 1970). The result of these problems was the beginning of a wheat breeding programme whose primary objective was to develop rust resistant varieties. Although the released varieties kept on succumbing to new physiological races of the rust, this breeding programme has evolved over the years into the National Plant Breeding Centre under the Kenya Agricultural Research Institute (KARI). Apart from disease resistance, and particularly rust resistance, other traits such as yield and baking quality also became important objectives.

The earlier emphasis on disease resistance meant that there was a strong tendency in the early-generation selection towards this objective. Consequently, selection for other characters was only practiced on the survivors from the disease screening. This implies that more desirable plants may have been discarded. In recent years, therefore, more attention has been given to yield and baking quality. This has raised yields to an average of 2 tonnes per hectare (Payne *et al*., 1995).

Wheat is currently the most important cereal crop after maize with more than100 varieties having been released by the research centre. The varieties released are suited to the various agro-ecological zones in the wheat growing districts of Nakuru, Narok, Uasin-Gishu, Trans-Nzoia and Laikipia. Due to increasing population and changing lifestyles the demand for wheat has steadily been increasing. By 1993, the demand was growing at 7 % per year and the total production was about 50 % of the national demand (Hassan *et al*., 1993). Currently it is estimated that the country is producing less than 40 % of the national demand with the remaining 60 % being met through imports. Although the annual consumption stood at about 500,000 tonnes, wheat production was 195,000 tonnes in 1991, 76,900 tonnes in 1993 and 128, 600 tonnes in 1995 (Anon. 1999). Wheat is produced in the high potential areas of Kenya, which cover only 20 % of the total land area. It has to compete with other agricultural enterprises such as maize, tea, coffee, barley and dairy, and it is unlikely that the area under wheat will expand in these high potential areas.

Kenya has a well developed wheat seed production system. Most of the wheat breeding and maintenace work is conducted by the Kenya Agricultural Research Institute (KARI). The new varieties are evaluated by the Kenya Plant Health Inspectorate Service (KEPHIS) before release and registration. Two types of tests are done: Distinctness, Uniformity and Stability (DUS) tests and National Performance Trials (NPTs). Wheat seed production in Kenya is currently done by one company, the Kenya Seed Company Ltd. The released varieties are passed to the seed company, which multiplies the seed through 4 generations, namely pre-basic, basic, certified first generation and certified second generation. The last two generations are the ones usually offered for sale to the farmers. At each stage of seed multiplication, field inspections are carried out by KEPHIS. Also during seed processing, samples are drawn from the seed lots and sent to the KEPHIS seed testing lab. The seeds are tested for purity, germination and, if need be, health. These tests are based on the International Seed Testing Association (ISTA) standards. Although there are usually sufficient quantities of certified seed in the market, most farmers still use farm-saved seed because of financial constraints.

To bridge the gap between wheat production and demand it is imperative that bottlenecks hampering production are removed. This requires developing varieties with tolerance to the acid soils which are prevalent in wheat producing areas of Kenya, developing drought tolerant varieties to expand on production area and minimizing losses due to pests and diseases. One important pest that is currently causing heavy losses in wheat fields is the Russian wheat aphid.

The Russian wheat aphid problem in Kenya

The important cereal aphids that attack wheat in Kenya include *Schizaphis graminum, Sitobion avenae, Rhopalosiphum padi, R. maidis, Metopolophium dirhodum* and lately, *Diuraphis noxia* or the Russian wheat aphid (Wanjama, 1990, Macharia *et al*., 1993; 1997). The Russian wheat aphid is a relatively new pest of wheat in Kenya. It was first identified in farmers' fields in 1995 (Macharia *et al*., 1999). It then spread quickly to all the wheat growing areas of the country and it became evident that all the commercial wheat varieties in Kenya were susceptible to RWA (Malinga *et al*.,

2001b). The damage resulting from RWA attack is manifested through leaf chlorosis, leaf rolling, leaf folding and plant stunting. In Kenya, the damage usually appears when crops have attained the tillering stage. Yield losses ranging from 25 to 90% have been reported (Macharia *et al*., 1999). Research towards controlling RWA followed two approaches. One was to test various insecticides to determine the ones that are effective against the aphid, while the other approach was to screen the non-commercial and old germplasm for resistance to RWA. Malinga *et al*. (2001a) evaluated some of the old Kenyan wheat germplasm for RWA resistance and they found 33 out of 190 lines to contain some moderate levels of resistance. The line with the highest level of resistance, Supy, had a damage score of 4 (on a scale of 1-9) compared to scores of 1 and 8 for the resistant and the susceptible checks respectively. They concluded that the levels of resistance available were insufficient for use in a breeding programme.

There were also cases in which some Kenyan wheat germplasm, which showed resistance in other countries, failed to show the same level of resistance in Kenya. Two lines, Kariega and Marico were found to be resistant to RWA at the Small Grain Institute in South Africa. When tested in Kenya however, the two were found to be moderately resistant and moderately susceptible respectively. A variety that is resistant to RWA in one region is not necessarily resistant in another region (Puterka *et al*, 1992). This has been associated with the presence of biotypes of the RWA. To develop locally adapted resistant wheat varieties it is important to identify a suitable source of resistance that is effective against the local biotypes of the aphid.

The current control measures in Kenya involve a combination of seed dressing and spraying of insecticides. Several insecticides were tested to determine their effectiveness against RWA. Seed dressing with Gaucho 350FS, Carbofuran 350 ST or foliar spraying with Brigade increased yields by 175 %, 147% and 123% respectively over the untreated control. Similarly, foliar sprays with 120 ml/ha of Decis 100EC (Deltamethrin 100g/L) and 40 tablets /ha of Decistab (Deltamethrin 0.25 g/tablet) resulted in significant yield increases of 21.8% and 16.8 % respectively (Macharia *et al*., 2001). The yields obtained after seed dressing were significantly higher than the yield of the control. Wheat yield losses associated with RWA, however, are still high since most farmers use non-dressed seeds or fail to use effective sprays due to the

high costs of systemic insecticides. To reduce losses caused by this pest in the long run, these control measures need to be augmented and improved by breeding for resistance to RWA. Development of resistant varieties will minimize chemical control costs and reduce the detrimental effects of extensive spraying on the environment.

Thesis objectives and outline

The main objective of this study was to determine the effect of the Russian Wheat Aphid (*Diuraphis noxia*) on Kenyan bread wheat varieties, identify useful sources of resistance and initiate a breeding programme to develop RWA resistant wheat cultivars.

Specifically, the aims of the thesis were to:

- Study the effect of RWA infestation on young seedlings of eight Kenyan wheat varieties (Chapter 2).
- Determine the effect of late infestation with RWA on plant development and grain yield in the eight Kenyan wheat varieties under well-watered and drought stress conditions (Chapter 3).
- Study the effect of RWA infestation on the quality of seeds produced by wheat varieties under well-watered and drought stressed conditions (Chapter 4).
- Test the RWA resistant winter wheat variety Halt together with single seed derived lines of PI294994 for resistance to Kenyan isolates of RWA and initiate a programme to develop RWA resistant varieties (Chapter 5).
- Study association between AFLP markers and RWA resistance gene(s) to identify possible markers for RWA resistance gene(s) (Chapter 6).

The effect of the RWA on seedlings of Kenyan wheat varieties was studied in greenhouse experiments in which seedlings of eight Kenyan wheat varieties together with two resistant winter wheat varieties were used. Seedlings at the two-leaf stage were infested with 3 adult aphids from a colony tracing back to a single aphid collected from a Kenyan wheat field. Damage on the seedlings was determined in four observations over a period of five weeks by scoring the extent of leaf chlorosis, leaf

folding and leaf rolling. The effect on other growth parameters such as plant height, number of leaves per plant, total leaf length, number of tillers per plant, shoot and root fresh weight together with shoot and root dry weight were also measured. During each observation, the number of aphids per plant was also counted.

To study the effect of drought and late infestation of RWA on the Kenyan varieties, the above varieties were grown in the greenhouse under well-watered and drought-stressed conditions. Infestation was done when the plants attained the tillering stage, the stage at which infestations are usually noticed in Kenyan wheat fields. Observations were made for damage due to RWA infestation at four growth stages, with the last observation coming at the grain filling stage. Yields were determined at harvest.

Most Kenyan farmers use seeds from their previous crop to plant the next wheat crop. Prevalence of RWA in many wheat fields could affect the quality of seed harvested and subsequently wheat yields. Seeds harvested from the experiment in chapter 3 were used to study the effect of RWA infestation on the quality of wheat seed.

The Russian wheat aphid resistant variety Halt, together with the lines of PI 294994 were tested against Kenyan accessions of RWA collected from wheat fields in the different growing regions. One PI 294994 line was selected for crossing with two Kenyan varieties Mbuni and Kongoni. The segregation of the F_2 populations for RWA resistance was studied and a backcrossing programme was started to develop adapted resistant varieties.

Breeding for RWA resistance requires a reliable method of selecting plants containing a resistance gene. Molecular markers such as AFLP markers have been developed and used in the breeding of many crop species. They allow traits of interest to be quickly and efficiently selected early in the breeding programme leading to time saving. They are not affected by environmental conditions and also have the advantage of allowing selection to be performed in areas where the aphid does not exist. To identify possible AFLP markers for RWA resistance, two selfed backcross populations from crosses between an accession of PI 294994 (resistant) and two Kenyan varieties Mbuni and Kongoni were infested with RWA in the greenhouse. The seedlings were scored for

RWA resistance based on expression of leaf chlorosis, leaf rolling and leaf folding. Leaves for AFLP analysis were harvested from individual resistant and susceptible seedlings and their parents.

CHAPTER 2

Varietal differences in response of wheat seedlings to infestation with the Russian wheat aphid (*Diuraphis noxia* Mordvilko)

ABSTRACT

Eight popular Kenyan wheat varieties (91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi and Mbega) were compared for Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), damage in a greenhouse alongside two RWA resistant winter wheat varieties obtained from the USA (Halt and PI 294994). All the Kenyan varieties were susceptible to RWA. However, inspection of leaf symptoms (chlorosis, leaf rolling and leaf folding) revealed significant differences among the Kenyan varieties, with Fahari showing significantly stronger leaf chlorosis than all the other varieties seven weeks after infestation. Mbega and 91B33 were the most affected by RWA infestation with respect to plant growth and development traits, *i.e.* plant height, number of leaves, total leaf length and number of tillers per plant. Halt, which is resistant in the USA and South Africa, was highly susceptible in the present study, suggesting that the Kenyan RWA isolate differs from those in these two countries. The other winter wheat line, PI 294994, was highly resistant and will be an important source of resistance in a breeding programme.

Keywords: breeding for resistance, chlorosis, *Diuraphis noxia*, Kenyan wheat, Russian wheat aphid.

INTRODUCTION

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is an important pest of wheat and barley in many regions of the world (Webster *et al*. 1987; Archer and Bynum, 1992; Porter *et al*. 1993; Zwer *et al*., 1994; Nkongolo, 1996). It causes characteristic longitudinal leaf streaking, stunted growth, leaf rolling and folding, spike trapping and sterility (Hewitt *et al*., 1984; Kiriac *et al*., 1990; Miller *et al*., 1994; Zwer *et al*. 1994). The leaf streaks are usually yellowish or whitish under warm weather conditions, but are purplish in cool weather. The damage is caused by the injection of a toxin into the plant as the aphid feeds. The toxin prevents the production of chlorophyll and causes the leaves to curl (Karren, 1993). Extensive chlorosis leads to death of plants, while leaf rolling retards plant development and partially or fully protects the aphid from parasites, predators and contact insecticides, thereby necessitating the use of more expensive systemic insecticides (Du Toit and Walters, 1984; Webster *et al*., 1987).

Significant yield and quality losses attributed to RWA have been documented around the world (Du Toit and Walters, 1984; Pike and Allison, 1991). In South Africa, where the aphid was first reported to be a serious pest of wheat and barley, yield losses of between 35 and 60% were recorded (Du Toit and Walters, 1984).

Since its detection in the USA in 1986, RWA had caused over $850 million damage in wheat and barley by 1991 in the western Great Plains and the intermountain region of the country. By then it had established itself as a primary pest of small grains in the arid and semi-arid areas of the USA (Webster *et al*., 1987; Massey and Amosson, 1991; Legg and Amosson, 1993; Porter *et al*., 1993). Porter *et al*. (1999) estimated the economic loss from RWA in the western USA for the period 1987 to 1998 to be over $1 billion (cited by Mornhinweg *et al*., 2002).

Studies on seedlings conducted in greenhouses or growth chambers have been the primary means for studying the genetics of resistance of wheat varieties to RWA

(Elsidaig and Zwer, 1993, Du Toit and Van Niekerk, 1985, Webster *et al*., 1987, Nkongolo *et al*., 1990). Although field conditions may affect insect longevity, fecundity and plant reactions differently as compared with controlled environments (Elsidaig and Zwer, 1993), a good correlation has been observed between RWA damage scored in the field and the greenhouse (Du Toit, 1990; Robinson, 1992).

Wheat cultivars have been successfully separated into resistant and susceptible classes based on expression of RWA damage symptoms on their seedlings. However, considerable variation in resistance may occur within each of the two classes. Among susceptible cultivars, for instance, it is possible to detect some with low levels of RWA resistance. Hein (1992) observed that the difference in damage between two susceptible cultivars, Arapahoe and TAM 107, approached statistical significance at $P = 0.06$. Similarly, Smith *et al*. (1991) identified low levels of RWA resistance in some susceptible wheats. Malinga *et al*. (2001) observed some lines among old Kenyan wheat germplasm to contain moderate levels of resistance to RWA.

In Kenya, RWA was first noted in 1995. Since then it has spread to all the wheat and barley producing areas of the country and has established itself as one of the most serious insect pests for both crops. The current control measures in Kenya involve a combination of seed dressing and spraying of insecticides. Wheat yield losses associated with RWA, however, are still high since most farmers spray late or fail to spray due to the high costs of systemic insecticides. Although none of the Kenyan commercial wheat varieties is resistant / tolerant to RWA, studies have not been done to determine if they vary in their levels of susceptibility. Such studies may assist farmers to choose varieties that are less susceptible in order to reduce losses due to this pest.

The objective of this study is to compare the response (at the seedling stage) of eight popular Kenyan wheat varieties and two winter wheats, Halt and PI 294994, to infestation by a RWA accession collected from a Kenyan wheat field.

MATERIALS AND METHODS

Plant material and RWA accession

Seeds of eight popular Kenyan wheat varieties were obtained from the seed quality control station of the Kenya Plant Health Inspectorate Service. The varieties were 91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi and Mbega. As they are all spring wheats, they do not require vernalization. Seeds of two RWA resistant winter wheats, Halt and PI 294994, were obtained from Dr. James Quick of the Department of Soil and Crop Sciences, Colorado State University, USA in 1999. The winter wheats have both been reported to be resistant to RWA in the USA (Quick *et al*., 1996, Peairs *et al*., 1999, Hawley *et al*., 2002, Marais and Du Toit, 1993, Saidi and Quick, 1996). Halt is a semi-dwarf hard red winter wheat that is well adapted to production areas of eastern Colorado. Released in 1994 by the Colorado Agricultural Experimentation Station, Halt was the first RWA resistant wheat variety in the USA (Hawley *et al*., 2002; Peairs *et al*., 1999). It has a single dominant resistance gene (*Dn4*) derived from Turcikum 57 (PI 372129). PI 294994 is a winter wheat accession originally from Bulgaria (Zhang *et al*., 1998). It has excellent resistance to RWA (Du Toit, 1990).

A colony of a RWA accession was developed from a single aphid collected from a wheat field in the Eldoret area. The colony was raised on young potted seedlings of the Kenyan wheat variety Mbuni.

Planting and Experimental Design

The planting medium used in the experiment was a mixture of forest soil and river sand in a volume ratio of 2:1. Planting of the 10 wheat varieties was done in a greenhouse in January 2000. Each replicate had 10 main plots to which the varieties were assigned randomly. The main plots were divided into two sub-plots. The sub-

plots, which were the experimental units, were wooden flats measuring 30 × 20 × 10 cm. Fourty seeds were planted per flat (two per hill). The flats were arranged in a split-plot design with 4 replicates, with the varieties randomized in the main plots and the two RWA treatments, infested or non-infested, randomized across the sub-plots. The 80 flats were watered daily under natural light and temperature conditions. At the one-leaf stage, the plants were thinned to leave one seedling per hill, *i.e*. 20 seedlings per flat. At the two-leaf stage, the seedlings in one sub-plot, *i.e*. one flat, were each infested with 3 adult aphids, while those in the other sub-plot, *i.e*. another flat containing the same variety within the same main plot, were not. Infestation was done by placing the aphids in the whorls of the seedlings, using a small paintbrush. Thereafter all flats, including the ones with non infested plants, were caged to prevent movement of aphids from one flat to another.

Observations

Four observations at intervals of two weeks were made to assess a number of traits of both infested and non-infested plants. The first observation took place one week after infestation. The characters scored were: leaf chlorosis, leaf rolling, leaf folding, plant height (cm) from the base of the plant to the tip of the youngest fully expanded leaf, number of leaves per plant, total leaf length per plant (cm), number of tillers per plant, number of aphids per plant, and shoot and root fresh and dry weight per plant (g). Leaf chlorosis was scored following the 1-9 scale described by Nkongolo *et al*. (1989), where:

1 = no visible chlorotic spots
2 = presence of small isolated spots on some leaves
3 = presence of large chlorotic spots on some leaves
4 = mild chlorotic streaks visible in some leaves
5 = prominent chlorotic streaks present in some leaves
6 = prominent chlorotic streaks present in more than half of the number of leaves
7 = prominent chlorotic streaks present and necrosis appearing in some leaves
8 = severe chlorotic streaks with advanced necrosis in many leaves
9 = severe necrosis with plants beginning to die.

Leaf rolling occurs when the leaf blades of fully emerged leaves fail to open and remain rolled. Leaf rolling was scored on a scale of 1-4 where:

1 = no visible leaf rolling

2 = mild rolling of some leaves

3 = tight rolling of some leaves

4 = tight rolling of more than half of the number of leaves

Leaf folding occurs when the tips of the younger leaves are trapped in rolled older leaves, causing the looping of the blade of the younger leaf. Leaf folding was scored on a scale of 1-3 where:

1 = no leaves folded

2 = one leaf folded

3 = two or more leaves folded

The total leaf length was determined by measuring the lengths of leaf blades of fully emerged leaves together with the length of emerged blades of younger leaves and adding for each plant.

Leaf symptoms and number of aphids per plant were only observed for each individual infested plant. The mean score per flat was determined for further data analysis.

For destructive measurements, such as shoot and root weights, only two plants occupying similar positions in the flats were used in each of the first three observation dates. In the fourth observation, measurements were taken from all the remaining plants.

The damage due to RWA infestation, defined as the difference between the observations for corresponding infested and non-infested sub-plots, was estimated for plant height, number of leaves per plant, total leaf length per plant, number of tillers per plant, and shoot and root fresh and dry weights.

Statistical analysis

The data were analysed using SPSS release 10.0. For leaf chlorosis, leaf rolling, leaf folding and number of aphids per plant, only data from the infested plants were analysed as a Randomized Complete Block design using GLM univariate analysis. Plant height, number of leaves per plant, total leaf length per plant, number of tillers per plant, and shoot and root fresh and dry weights were analysed as split plot using repeated measures. Correlations between traits were tested using Pearson's two-tailed test. Data from infested and non-infested sub-plots were compared on the basis of a paired t-test.

RESULTS

ANOVA results

Table 1 summarizes the ANOVA results for leaf observations and for number of aphids per plant of the infested plants in each of the 4 observations. Varietal differences were highly significant for chlorosis and leaf rolling in the 4 observations. For leaf folding, significant differences among the varieties occurred only in the first 3 observations. Significant differences in the number of aphids per plant occurred in the last 3 observations.

Table 1. Differences among varieties (infested), as obtained by ANOVA, for each of the 4 observations (- = non-significant, *, **, *** = significant at $P < 0.05$, 0.01 and 0.001, respectively).

	Chlorosis	Leaf rolling	Leaf folding	No. of aphids
Observation 1	***	***	*	-
Observation 2	***	***	***	*
Observation 3	***	***	***	***
Observation 4	***	***	-	*

The ANOVA results for plant growth and development traits are summarized in Table 2. Varietal differences were highly significant for plant height, number of leaves per plant and number of tillers per plant in each of the 4 observations. The varieties differed significantly for total leaf length only in observation 4.

Infestation significantly affected the number of leaves per plant in observation 3, whereas in observation 4, significant effects of infestation occurred in plant height, number of leaves per plant and total leaf length. Significant effects of infestation also occurred with respect to shoot and root fresh and dry weights in observations 3 and 4 (except for root fresh weight in observation 3). Infestation had no effect on the number of tillers per plant.

Significant variety by infestation interaction occurred only with respect to the number of leaves per plant and shoot fresh weight in observations 1 and 4, respectively.

Table 2. ANOVA results for plant growth and development traits at each of the 4 observations (- = non-significant, ∗, ∗∗, ∗∗∗ = significant at $P < 0.05$, 0.01 and 0.001, respectively).

Source of variation	Observation no.	Plant height	No. of leaves	Total leaf length	No. of tillers	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.
Variety	1	∗∗∗	∗∗∗	-	∗∗∗	-	-	-	∗
	2	∗∗∗	∗∗∗	-	∗∗∗	-	-	-	-
	3	∗∗∗	∗∗∗	-	∗∗∗	-	-	-	-
	4	∗∗∗	∗∗∗	∗	∗∗∗	-	-	-	-
Infest.	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	∗∗∗	-	-	∗∗	∗∗	-	∗∗∗
	4	∗∗∗	∗∗	∗∗	-	∗∗∗	∗∗∗	∗∗∗	∗∗∗
V ∗ I	1	-	∗∗	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
	4	-	-	-	-	∗	-	-	-

Leaf symptoms of infested plants

Based on the extent of leaf chlorosis, leaf rolling and leaf folding (except Mbuni in observation 1 and Nyangumi in observations 1 and 3), all the Kenyan varieties tested

were found to be susceptible to RWA when compared with the resistant line PI 294994 (Table 3). Generally, the chlorosis and leaf rolling scores increased from the first observation to the fourth observation, whereas leaf folding scores increased till the third observation before decreasing in observation 4.

Table 3. Mean scores for chlorosis, leaf rolling and leaf folding, as well as mean number of aphids per plant, in the infested sub-plots

Variety	Chlorosis				Leaf rolling				Leaf folding				No. of aphids / plant			
	Observation No.				Observation No.				Observation No.				Observation No.			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
91B33	2.80bc	3.88bc	4.78bc	5.16b	1.96bcd	2.47bc	2.69d	2.86b	1.16bc	1.57cd	1.80c	1.11	5.8a	21.4ab	112.0c	161.5b
Fahari	3.32c	4.62d	4.95c	6.50c	2.07cd	2.58bc	2.68d	3.03b	1.28c	1.71d	1.62bc	1.02	13.6ab	26.9ab	128.2cd	165.0b
Kwale	3.25c	4.10bcd	4.63bc	5.44b	1.85bc	2.27bc	2.52bcd	2.88b	1.21bc	1.36bc	1.69bc	1.13	1.9a	27.4ab	63.6b	118.1b
Mbuni	3.36c	4.14cd	4.17b	5.10b	1.99bcd	2.20b	2.32b	2.67b	1.14abc	1.27b	1.48b	1.02	16.0ab	23.1ab	106.3bc	166.8b
Chiriku	3.09bc	4.21cd	4.83c	5.77b	2.07cd	2.58bc	2.67d	3.07b	1.18bc	1.45bcd	1.41b	1.00	25.8b	66.8c	112.5c	124.6b
Kongoni	3.29c	4.34cd	4.87c	5.60b	2.15d	2.44bc	2.58cd	2.92b	1.15bc	1.40bc	1.44b	1.02	14.9ab	20.8ab	79.9bc	126.6b
Nyangumi	3.03bc	4.04bcd	4.43bc	5.27b	1.76b	2.50bc	2.41bc	2.82b	1.13ab	1.26b	1.13a	1.00	6.0a	37.4ab	107.5bc	167.5b
Mbega	2.71bc	3.85bc	4.52bc	5.36b	1.99bcd	2.41bc	2.67d	3.11b	1.18bc	1.47bcd	1.68bc	1.04	11.5ab	59.6bc	171.3d	186.1b
Halt	2.45b	3.52b	4.16b	4.98b	1.73b	2.65c	2.55bcd	2.77b	1.17bc	1.49bcd	1.86c	1.11	13.6ab	27.6ab	63.1b	112.1b
PI 294994	1.53a	1.92a	1.83a	1.87a	1.04a	1.07a	1.00a	1.00a	1.00a	1.00a	1.00a	1.00	5.5a	7.1a	11.0a	9.3a

Means followed by the same letter are not significantly different from each other according to the Duncan Multiple Range Test. P = 0.05.

Significant differences among the Kenyan varieties in the extent of leaf chlorosis emerged three weeks after infestation (observation 2). The differences were also expressed in the third and fourth observations, which occurred five and seven weeks after infestation, respectively. Although the varieties changed their rankings in terms of mean chlorosis score from one observation to another, Fahari was always among the varieties with the highest chlorosis score. Seven weeks after infestation, Fahari had a significantly higher score for chlorosis than all the other varieties. All the varieties showed the highest levels of chlorosis 7 weeks after infestation, but at this time differences among the varieties, excluding Fahari and PI 294994, could not be detected.

For leaf rolling, the observations taken 1 week, 3 weeks and 5 weeks after infestation showed significant differences among the Kenyan varieties. As in the case of chlorosis, differences among the Kenyan varieties were not significant 7 weeks after infestation.

For some varieties leaf folding scores peaked 3 weeks after infestation, while for the others it peaked 5 weeks after infestation. Seven weeks after infestation, however, the leaf folding scores of all the varieties had fallen to the low level of PI 294994.

The number of aphids per plant followed the same trend as leaf chlorosis and leaf rolling. They increased through the experiment duration period, but significant differences among the Kenyan varieties were evident only in the first 3 observations. The number of aphids per plant increased at different rates. Chiriku, which had the highest number of aphids in observation 1, had one of the lowest numbers of aphids in observation 4, while the opposite is true for Nyangumi.

The winter wheats, Halt and PI 294994, reacted differently to infestation. PI 294994 was resistant to RWA infestation and showed significantly lower scores for chlorosis, leaf rolling and leaf folding than Halt and all the Kenyan varieties in each of the 4 observations. Halt, however, was susceptible to RWA infestation and showed scores as high as or even higher than those of the Kenyan varieties. The number of aphids

per plant remained low at about 10 or less in PI 294994, whereas in Halt it increased to levels similar to those in the Kenyan varieties.

Significant phenotypic correlations existed between chlorosis, leaf rolling, leaf folding and number of aphids per plant (Table 4). The correlation between chlorosis and leaf rolling increased from observation 1 to observation 3 before decreasing in observation 4. The same trends were observed in the correlation between leaf rolling and leaf folding. The correlation between chlorosis and leaf folding decreased after observation 2. The number of aphids per plant was significantly correlated with chlorosis, leaf rolling and leaf folding, with the highest correlation in all cases occurring in observation 3.

Table 4. Correlations between leaf chlorosis, leaf rolling, leaf folding and number of aphids per plant in the 4 observations.

Trait	Observation No.	Leaf chlorosis	Leaf rolling	Leaf folding
Leaf rolling	1	0.72**	-	
	2	0.81**	-	
	3	0.92**	-	
	4	0.90**	-	
Leaf folding	1	0.32*	0.49**	-
	2	0.58**	0.62**	-
	3	0.49**	0.63**	-
	4	0.02	0.08	-
No. of aphids per plant	1	0.00	0.32*	0.36*
	2	0.33*	0.37*	0.27
	3	0.58**	0.64*	0.35**
	4	0.51**	0.60*	0.07

General effects of infestation on plant growth and development

Generally, infestation reduced plant growth and development in the Kenyan varieties as shown by reduction in plant height, number of leaves per plant, total leaf length per plant, number of tillers per plant and shoot and root fresh and dry weights (Table 5). Apart from root fresh weight (for observation 1 only), the non-infested plants as a group were always taller, had more leaves per plant, greater leaf length, higher number of tillers, and had higher shoot and root fresh and dry weights than the infested ones. The difference between infested and non-infested plants generally increased from observation 1 to observation 4.

Growth and development of infested plants

Significant varietal differences occurred in the infested sub-plots with respect to plant height, number of leaves per plant, total leaf length and number of tillers per plant (Table 6a). Generally, plant height and number of leaves per plant increased through the four observations. Total leaf length increased to attain the highest values in observation 2, before dropping in observations 3 and 4. Apart from a few cases, the Kenyan varieties became more similar, with time, with respect to plant height, number of leaves and number of tillers per plant. However, differences with respect to total leaf length increased with time to reach significant levels in observations 3 and 4.

The winter wheats differed in their growth and development patterns from the Kenyan varieties. The difference between the two groups with respect to plant height, number of leaves per plant, total leaf length and number of tillers per plant increased with time.

Shoot and root fresh and dry weights of infested plants generally increased through the 4 observations (Table 6b). Significant differences were observed among the Kenyan varieties with respect to shoot and root dry weight (observation 4), root fresh weight (observations 1, 3 and 4). In contrast to other traits, there were no marked differences between the two groups, *i.e*. the Kenyan varieties and the winter wheats, with respect to shoot and root weights.

Table 5. Means of non-infested and infested plants and their differences for plant height (cm), number of leaves per plant, total leaf length per plant (cm), number of tillers per plant, shoot and root fresh and dry weight per plant (g).

Trait	Observation 1			Observation 2			Observation 3			Observation 4		
	Non-infested (NI)	Infested (I)	Difference (NI – I)	Non-infested (NI)	Infested (I)	Difference (NI –I)	Non-infested (NI)	Infested (I)	Difference (NI – I)	Non-infested (NI)	Infested (I)	Difference (NI – I)
Plant height	23.61	23.13	0.48	25.50	25.20	0.30	25.27	24.67	0.60	29.37	26.53	2.84
No. leaves	4.45	4.21	0.24	6.91	6.17	0.74	8.41	7.06	1.35	9.65	7.88	1.77
Leaf length	68.90	67.00	1.90	97.10	91.84	5.26	116.97	107.51	9.46	117.87	103.87	14.00
No. tillers	0.46	0.40	0.06	0.65	0.52	0.13	0.69	0.48	0.21	0.66	0.39	0.27
Shoot FW	0.64	0.56	0.08	1.03	0.88	0.15	1.29	0.95	0.34	1.98	1.29	0.69
Root FW	0.10	0.11	-0.01	0.14	0.12	0.02	0.13	0.10	0.03	0.37	0.19	0.18
Shoot DW	0.120	0.110	0.010	0.249	0.220	0.029	0.356	0.274	0.082	0.513	0.298	0.215
Root DW	0.022	0.021	0.001	0.034	0.031	0.003	0.059	0.033	0.026	0.076	0.039	0.037

Table 6a. Mean scores for plant height, number of leaves per plant, total leaf length per plant and number of tillers per plant, in the infested sub-plots.

Variety	Plant height (cm)				Number of leaves per plant				Total leaf length per plant (cm)				Number of tillers per plant			
	Observation No.				Observation No.				Observation No.				Observation No.			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
91B33	26.5de	27.8bc	28.4bc	28.5bc	3.8ab	5.4a	6.0a	6.7ab	123.5	167.0	99.1ab	105.7abcd	0.65b	0.72bc	0.28a	0.24a
Fahari	27.0e	29.3c	30.0c	29.7bc	3.8ab	5.4a	5.9a	6.2a	159.5	208.0	104.3ab	84.5a	0.18ab	0.14ab	0.10a	0.06a
Kwale	22.4bcd	25.1b	25.1b	26.4b	3.7ab	6.2a	6.0a	6.6ab	119.8	155.0	98.3ab	95.4ab	0.04a	0.04a	0.06a	0.04a
Mbuni	24.0cde	26.0bc	26.3bc	29.8c	3.4a	4.8a	5.6a	6.5ab	114.3	156.8	88.9a	96.6ab	0.10ab	0.10a	0.07a	0.09a
Chiriku	25.5de	27.5bc	27.4bc	28.4bc	3.8ab	5.1a	5.8a	6.5ab	131.8	200.5	113.6ab	96.7ab	0.11ab	0.10a	0.06a	0.00a
Kongoni	24.4cde	26.1bc	25.3b	26.9bc	4.2ab	5.9a	6.6ab	7.6ab	147.3	172.5	108.1ab	100.3abc	0.33ab	0.47ab	0.47a	0.49a
Nyangumi	20.9bc	24.8b	26.5bc	27.4bc	4.5b	6.4a	7.7b	8.6b	120.8	183.0	115.7ab	111.7bcd	0.36ab	0.38ab	0.41a	0.39a
Mbega	24.4cde	27.5bc	27.8bc	26.8bc	3.8ab	5.0a	5.7a	6.4ab	125.3	178.8	95.9ab	99.4abc	0.14ab	0.16ab	0.13a	0.11a
Halt	19.5ab	19.2a	18.7a	21.5a	5.5c	9.5b	11.6d	11.6c	160.3	235.3	129.0b	126.5d	1.11c	1.86d	2.07c	1.29b
PI 294994	16.8a	18.8a	19.6a	19.8a	5.4c	8.1b	9.8c	11.9c	139.0	182.0	123.0ab	121.1cd	1.03c	1.18cd	1.18b	1.20b

Means followed by the same letter are not significantly different from each other according to the Duncan Multiple Range Test. P = 0.05.

Table 6b. Mean scores for shoot and root fresh and dry weights per plant, in the infested sub-plots.

Variety	Shoot fresh weight (g)				Shoot dry weight (g)				Root fresh weight (g)				Root dry weight (g)			
	Observation No.				Observation No.				Observation No.				Observation No.			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
91B33	0.579ab	0.761	1.048	1.236a	0.119a	0.208	0.276	0.304ab	0.106ab	0.109	0.104ab	0.202abc	0.027	0.037ab	0.038	0.053b
Fahari	0.819b	1.294	1.293	0.871a	0.328b	0.284	0.327	0.237a	0.098ab	0.105	0.073a	0.102a	0.019	0.035ab	0.028	0.028a
Kwale	0.565ab	0.788	0.698	1.198a	0.106a	0.195	0.268	0.299ab	0.098ab	0.127	0.135ab	0.138ab	0.017	0.025ab	0.029	0.031a
Mbuni	0.570ab	0.806	0.888	1.288ab	0.099a	0.194	0.259	0.335ab	0.100ab	0.080	0.071a	0.180abc	0.021	0.024ab	0.026	0.038ab
Chiriku	0.545ab	0.916	1.051	1.163a	0.106a	0.247	0.301	0.303ab	0.110ab	0.075	0.229b	0.187abc	0.022	0.032ab	0.044	0.032a
Kongoni	0.601ab	0.770	0.794	1.126a	0.109a	0.217	0.233	0.296ab	0.190c	0.119	0.067a	0.232bc	0.027	0.035ab	0.029	0.045ab
Nyangumi	0.537ab	1.038	1.229	1.602ab	0.100a	0.239	0.346	0.327b	0.183c	0.114	0.135ab	0.258c	0.024	0.034ab	0.044	0.040ab
Mbega	0.533ab	0.966	0.872	1.110a	0.114a	0.230	0.235	0.244a	0.111abc	0.111	0.058a	0.135ab	0.023	0.041b	0.029	0.036ab
Halt	0.488ab	0.654	0.877	1.202a	0.111a	0.189	0.263	0.285ab	0.073a	0.262	0.091ab	0.223bc	0.018	0.026ab	0.046	0.045ab
PI 294994	0.411a	0.773	0.763	2.118b	0.106a	0.199	0.237	0.312ab	0.064a	0.064	0.054a	0.200abc	0.018	0.022a	0.022	0.046ab

Means followed by the same letter are not significantly different from each other according to the Duncan Multiple Range Test. P = 0.05.

Damage due to infestation with respect to growth and development traits

The damage due to RWA infestation, as estimated by the difference between infested and non-infested sub-plots of each variety, increased through the 4 observations. In observation 1, the damages for the measured traits were mostly small and non-significant. Especially for the Kenyan varieties, the damages increased with time until the 4th observation (Table 7) when most of the differences were significant. The damage was highly manifested in the shoot and root fresh and dry weights, in which all the Kenyan varieties exhibited significant weight reduction under infestation.

Table 7. Reduction, due to infestation, of plant height, number of leaves per plant, total leaf length, number of tillers per plant, shoot and root fresh and dry weights in observation 4. Each figure was obtained by subtracting the value for the infested from that of the corresponding non-infested subplot.

Variety	Plant height (cm)	No. of leaves	Total leaf length (cm)	No. of tillers	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
91B33	3.44*	2.83*	39.3*	0.34*	0.598*	0.313*	0.290*	0.053*
Fahari	5.14*	0.72*	–0.3	0.00	0.213*	0.273*	0.177*	0.034*
Kwale	4.57*	2.93*	24.8*	0.27	0.623*	0.287*	0.263*	0.048*
Mbuni	3.31	1.41*	7.0	0.09	0.385*	0.227*	0.195*	0.047*
Chiriku	2.05	1.66*	23.5*	0.18	0.278*	0.202*	0.163*	0.044*
Kongoni	1.46	0.72	8.1	–0.2	0.310*	0.133*	0.079	0.026*
Nyangumi	3.17*	0.82	–4.1	0.08	0.208*	0.235*	0.172*	0.043*
Mbega	6.52*	3.60*	35.7*	0.60*	0.695*	0.419*	0.323*	0.065*
Halt	0.26	3.51	6.1	1.03	0.202	0.053	0.206*	0.046*
PI 294994	1.09	–0.16	–0.3	0.51*	–0.068	0.007	0.018	0.066*

* Difference is significant at P = 0.05

The effect of infestation was also evident in the winter wheats, though not to the same extent as in the Kenyan varieties. Halt showed significant reduction in plant height in observations 2 and 3 and showed significant reduction in root fresh and dry weight in

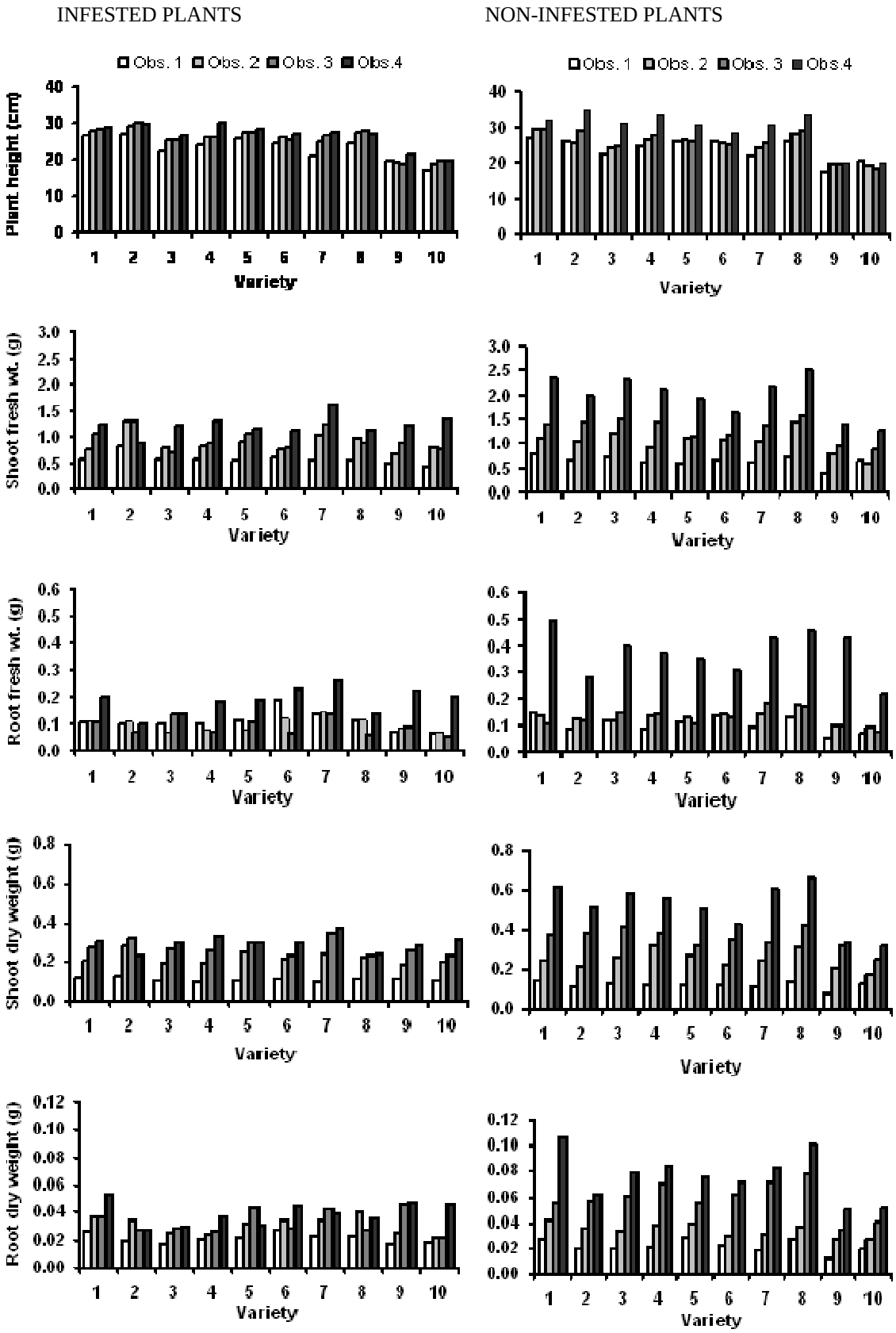


Figure 1. Mean scores for plant height, and shoot and root fresh and dry weight in the 4 observations. The varieties are: 1 = 91B33; 2 = Fahari; 3 = Kwale; 4 = Mbuni; 5 = Chiriku; 6 = Kongoni; 7 = Nyangumi; 8 = Mbega; 9 = Halt and 10 = PI 294994.

observation 4. PI 294994, which showed high RWA resistance based on leaf symptoms, had a significant reduction in root dry weight in observations 2, 3 and 4.

Infestation caused a delayed attainment of the stem elongation stage in the Kenyan varieties. In observation 4, sharp increases in plant height, and shoot and root fresh and dry weight occurred in the non-infested plots but not in the infested plots (Figure 1). This was due to the fact that the plants in the non-infested plots had attained the stem elongation stage, while those in the infested plots had not. In general, differences between infested and non-infested plants with respect to growth and development traits became more pronounced after attainment of the stem elongation stage.

DISCUSSION

The leaf damage symptoms, especially chlorosis and leaf rolling, clearly separated the RWA susceptible (Kenyan varieties and Halt) varieties from the resistant (PI 294994) variety. The difference between resistant and susceptible varieties with respect to chlorosis and leaf rolling increased from observation 1 to 4, suggesting that the accuracy of selection based on these characters increases with time until seven weeks after infestation. Differences, however, become evident much earlier, and many observations are usually made between 3 and 5 weeks after infestation. The significant differences among the Kenyan varieties detected in the first 3 observations appear to concern mainly the rate of expression of the symptoms of chlorosis, leaf rolling and leaf folding. Some susceptible varieties take longer than others to express chlorosis or leaf rolling. This could be the reason why some Kenyan varieties showed more resistance than others in the early observations, although all the varieties were similarly affected 7 weeks after infestation. Leaf folding is expressed over a very short period before its expression ends when the tips of the folded leaves emerge from the rolled leaves and the leaf blades straighten out.

Halt was affected as much as the Kenyan varieties, suggesting that it is susceptible to the accession of RWA used. In the USA, Colorado State University researchers have reported the emergence of a RWA biotype known as biotype B, which attacks previously resistant varieties (Peairs *et al*., 2003). The most strongly affected variety

is Prairie Red, which has the same resistance gene (*Dn4*) as Halt. This may necessitate the pyramiding of resistance genes in breeding programs.

Although the mean scores per plot for chlorosis, leaf rolling and leaf folding were highly correlated in some of the observations, one may not conclude that a plant with a high score for chlorosis would also have a high score for leaf rolling or leaf folding or vice versa. Thus there existed, within the presumably pure lines, plants with high chlorosis scores, but with no rolled or folded leaves. Smith *et al*. (1991) and Souza *et al*. (1991) made a similar observation, which necessitated scoring the characters separately.

The winter wheats developed very differently as compared to the Kenyan varieties. Although they had more leaves and tillers, they were generally slower growing and prostrate rather than upright. This is probably because they were not vernalized. Comparison of the development of the two wheat types in the absence of vernalization is therefore only possible in the very early seedling stages, before the winter wheat attains the stage suitable for vernalization.

The effect of RWA infestation became more pronounced with time. This is shown by the difference between infested and non-infested plants: significant differences are observed in more traits in later observations than in the earlier ones. Shoot and root fresh and dry weight consistently showed significant differences between infested and non-infested plants in all varieties. It is unclear why PI 294994, which had consistently shown resistance to RWA in all the other traits, showed significant reduction due to RWA infestation in root dry weight in observations 2, 3 and 4. This warrants further investigations.

Many researchers have used only the leaf symptoms to characterise wheat varieties for RWA resistance. Results from this study show that RWA damage expressed in the growth and development traits corroborate the results of the leaf symptoms.

CONCLUSIONS

All the Kenyan varieties tested were susceptible to the Russian wheat aphid based on leaf symptoms and plant growth parameters. Fahari had a significantly higher score for chlorosis than all the other varieties and appears to be the most susceptible variety based on leaf symptoms. Leaf folding became less of a problem as the plants advanced beyond the four-leaf stage and hence may only be useful as a damage rating parameter at the very early seedling stage. Based on plant growth parameters, Mbega and 91B33 were the most affected varieties. PI 294994 was highly resistant to RWA and can be used in Kenyan resistance breeding programmes. Halt was susceptible to RWA, suggesting that the RWA accession used is different from to the ones occurring in the USA.

Significant differences among the Kenyan varieties with respect to leaf symptoms and growth and development traits suggest that growers may reduce losses due to RWA by growing certain varieties. The effects of RWA on the best Kenyan varieties, however, were still much higher compared to the effect on the resistant line PI 294994. None of the Kenyan varieties hence has sufficient RWA resistance to justify their utilization in a breeding programme. However, when introducing RWA resistance into Kenyan wheat germplasm by backcrossing, the small differences among the Kenyan varieties observed in this study may still be relevant in the choice of the recurrent parent(s). This is because a low level of RWA resistance in the recurrent may be complementary to the resistance of the donor.

Observation at the seedling stage clearly enables identification of resistant genotypes. The effects of infestation are more clearly manifested in the leaf symptoms at the early seedling stages, whereas at the later stages effects with regard to growth and development traits become more important.

The emergence of a new biotype of RWA implies that breeding programmes may need to consider gene pyramiding in which a number of resistance genes are combined in one variety. This calls for a greater importance of marker assisted selection in breeding for resistance to RWA.

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CHAPTER 3

Effect of late infestation of Kenyan wheat varieties with the Russian wheat aphid (*Diuraphis noxia* Mordvilko) under well-watered and dry conditions

ABSTRACT

Studies were conducted to determine the effect of late infestation with the Russian wheat aphid (RWA) of eight Kenyan wheat varieties (91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi and Mbega) and two RWA-resistant winter wheats (PI 294994 and Halt) under well-watered and dry conditions. All the tested Kenyan varieties were susceptible to RWA, with the damage in most cases being greater under dry conditions. Significant differences in the extent of leaf chlorosis was observed among the Kenyan varieties. Fahari had a significantly higher score for leaf chlorosis than 91B33. There were also significant varietal differences in leaf rolling scores: Mbega had a significantly higher score than 91B33, Nyangumi, Kwale, Chiriku and Fahari. The leaf damage symptoms in the adult plants were not as clearly manifested as in the case of young seedlings and could not be scored after anthesis. The trait that was most seriously affected by infestation was seed set. In the well-watered plants reduction in seed set due to infestation ranged from 33% in Fahari to 84% in 91B33, while in the drought-stressed plants the reduction in seed set ranged from 27% in Fahari to 80% in 91B33. Under RWA infestation, the traits seed set and plant height had a strong positive correlation with grain yield, while the percentage of deformed ears had a strong negative correlation with grain yield. The three traits may therefore be used to select potential varieties in case real yield data are not available.

Key words: drought stress, Kenyan wheat, late RWA infestation, Russian wheat aphid

INTRODUCTION

Ever since it was recognized as a serious pest of wheat in South Africa in 1978, the Russian wheat aphid (RWA) (*Diuraphis noxia* Mordvilko) has been the subject of much research in all areas to which the pest had spread. *D. noxia* injures the cereal plant both directly through the sucking of the sap and indirectly through the effect of a phytotoxin injected during feeding, which causes the breakdown of chloroplasts (Fouche *et al*., 1984). In seedlings, the damage symptoms are manifested mainly through leaf chlorosis, leaf rolling and folding, while in adult plants head trapping may occur, resulting in substantial yield losses (Du Toit and Walters, 1984; Fouche *et al*., 1984; Kriel *et al*., 1986; Kieckhefer and Gellner, 1992). Hewitt *et al*. (1984) reported that reduction of chlorophyll content of up to 85% in infested leaves resulted in yield reductions of 25-50%.

Several studies have shown that plant growth stage in cereals affects aphid reproduction. The Bird-Cherry aphid (*Rhopalosiphum padi* L.) was found to have a higher reproductive rate on headed wheat than on younger wheat plants, while it had a higher reproductive rate on the seedlings as compared to the adult plants in barley and sorghum (Kieckhefer and Gellner, 1988; Dewar, 1977). Watt (1979) reported that the English grain aphid (*Sitobion avenae* Fitch) has a higher reproductive rate on ears of oats and wheat than on their leaves. The Corn-Leaf aphid (*Rhopalosiphum maidis* Fitch) reproduction was higher on barley or sorghum seedlings than on adult plants, while that of the Greenback (*Schizaphis graminum*) was higher on headed than on younger wheat plants (Kieckhefer and Gellner, 1988).

Girma *et al*. (1990) reported that the fecundity of RWA was significantly affected by plant growth stage × temperature interactions, with the highest number of progeny being produced at 19.5^{o}C during the jointing stage (stage 30-36; Zadoks *et al*., 1974). Hein (1992) also reported that reproduction of RWA in wheat is significantly affected by both cultivar and growth stage. He observed a significant cultivar × growth stage interaction for plant damage, which he attributed to a decrease in damage rating for the susceptible cultivars at the reproductive growth stages as compared to vegetative stages.

Apart from the plant growth stage, stress is another factor that has been reported to affect the extent of damage in cereal plants infested by RWA. The damage resulting from RWA infestation is greater in stressed cereal plants than in non-stressed plants. Riedell (1989) reported that RWA infestation in barley disrupted osmoregulatory processes and interfered with plant responses to drought. Similarly, Miller *et al*. (1994) found that drought-stressed barley cultivars exhibited a greater relative loss of chlorophyll upon infestation than non-stressed plants. Mowry (1994) reported that wheat plants infected with Barley Yellow Dwarf Virus expressed less antibiosis to *D. noxia.*

Most of the studies on the effect of RWA infestation in wheat concerned seedlings at the 1-2 leaf stage (Nkongolo *et al*. 1989; Saidi and Quick, 1996; Zemetra *et al*. 1990; Webster *et al*. 1987; Formusoh *et al*. 1992; Souza *et al*. 1991; Baker *et al*. 1992; Smith *et al.* 1992). This is the stage when the plants are most susceptible to RWA attack. In these studies, varieties could be characterized as resistant or susceptible based on visual leaf symptoms of chlorosis, leaf rolling, leaf folding and plant stunting.

In Kenya, RWA infestations in the field are usually noticed when the plants have grown beyond the tillering stage. This could be because the heavy rains that prevail when the crops are at the early seedling stage delay the build up of aphid populations until after the tillering stage. The damage symptoms are usually chlorosis, leaf rolling or head trapping, depending on the severity of the attack and the plant growth stage. The damage and subsequent yield losses seem to be greater during periods of drought.

The objective of this study was to investigate the effect of late RWA attacks on Kenyan wheat varieties under well-watered as well as dry conditions. The main question addressed was whether the adult plants of the Kenyan varieties responded differently to RWA infestation with respect to leaf symptoms and plant damage, observed as reduction in plant growth and development. The number of aphids per plant at various dates of observation and the effect of infestation on yield were also determined.

MATERIALS AND METHODS

Plant material and experimental set-up

Eight popular Kenyan wheat varieties (91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi and Mbega), together with two winter wheat varieties (PI 294994 and Halt), were planted in a greenhouse in May 2000. All the Kenyan varieties had been found to be susceptible to RWA at the early seedling stage. PI 294994 was resistant, whereas Halt was susceptible although it has been reported to be resistant in the USA. The experimental design used was a 3-factor split-split plot design with 3 replicates. The main plots were either well-watered or drought-stressed. The 10 wheat varieties were randomly assigned to the sub-plots, which had two sub-sub-plots each. The two infestation treatments, *i.e.* infested and non-infested, were randomly assigned to the two sub-sub-plots within each sub-plot.

The winter wheat varieties were planted 5 weeks earlier than the Kenyan varieties. Planting was done in $30 \times 20 \times 10$ cm wooden flats containing a mixture of forest soil and sand in a volume ratio of 2:1. For each variety, twelve flats were planted with 40 seeds per flat. All the flats were watered daily to field capacity and left under natural light and temperature. The seedlings were thinned to 20 per flat one week after emergence. After thinning, the flats of the winter wheat varieties were transferred for 6 weeks to a vernalization chamber set at 4^{o}C and a photoperiod of 8 hours. After vernalization, the flats were transferred back to the greenhouse and arranged with the flats of the Kenyan varieties in a split-split plot design. Each flat coincided with a sub-sub-plot.

The Kenyan varieties were planted one week before the winter wheats were to complete vernalization. The planting procedure was as described for the winter wheats.

Aphid infestation and watering interval

Aphid infestation was done when the plants attained the stem elongation stage (stage 30-35; Zadoks *et al*. 1974). The aphids used were from a colony derived from a single aphid collected from a wheat field in the Eldoret area and raised on the Kenyan wheat

variety Mbuni in the greenhouse. Plants in one sub-sub-plot were infested with five adult RWA, while those in the adjacent sub-sub-plot were non-infested. The aphids were transferred into the whorls of the plants using a paintbrush. Immediately after infestation each flat was caged separately to prevent aphid movement from one flat to another. The cages, measuring 20 × 30 × 120 cm, were made from clear polythene paper on two sides and a fine net on the other two sides and the top. Both the net and the polythene paper were supported by wooden planks.

The watering interval treatments were started immediately after infestation: For one main plot (20 flats in total) daily watering was maintained, while the other main plot was watered at two-day intervals to induce drought stress on the plants.

Damage assessment

The plants were rated for aphid damage at 3 developmental stages. These were the early booting stage (Zadoks 40-43), anthesis (Zadoks 60-63) and milk development stage (Zadoks 70-72).

During the first observation (early booting stage), the characters scored were: leaf chlorosis, leaf rolling, plant height (cm), number of leaves per plant, number of tillers per plant, number of aphids per plant and shoot and root fresh and dry weight (g) of some plants. For chlorosis and leaf rolling the scoring of a plant followed the method described by Souza *et al*. (1991), with some modification on the scales. Chlorosis was scored on a scale of 1-9 where:

1 = no visible chlorotic spots
2 = presence of small isolated spots on some leaves
3 = presence of large chlorotic spots on some leaves
4 = mild chlorotic streaks visible in some leaves
5 = prominent chlorotic streaks present in some leaves
6 = prominent chlorotic streaks present in more than half of the number of leaves
7 = prominent chlorotic streaks present and necrosis appearing in some leaves
8 = severe chlorotic streaks with advanced necrosis in many leaves
9 = severe necrosis; plants beginning to die.

Leaf rolling was scored on a scale of 1-4 where:
1 = no visible leaf rolling
2 = mild rolling of some leaves
3 = tight rolling of some leaves
4 = tight rolling in more than half of the number of leaves.

Scores were taken for each individual plant and the mean score per flat (sub-sub-plot) was determined.

For destructive measurements, such as shoot and root weights, only two plants occupying similar positions in all flats were used in the first two observations, at the Zadoks stages 40-43 and 60-63, respectively.

In the second observation, the characters scored were plant height (cm), number of leaves per plant, number of tillers per plant, number of aphids per plant together with shoot and root fresh and dry weight (g).

The characters scored during the third observation were plant height (cm), number of leaves per plant, number of tillers per plant, number of aphids per plant, shoot and root dry weights (g). Also scored per flat were percentage of headed tillers, percentage of deformed ears (number of deformed ears as a percentage of the total number of ears) and seed set (number of spikelets that set seed as a percentage of the total number of spikelets on the ears). At harvest time, shoot dry weight (g) and grain yield (g) per plant were determined.

During the second and third observations chlorosis and leaf rolling could not be scored accurately as some leaves were already ageing.

Statistical analysis

The data were analysed using SPSS release 11.0. For leaf symptoms, number of aphids per plant and percentage of deformed ears, only data from the infested plants were analysed using GLM univariate analysis. For the growth and development traits,

data were analysed according to a split plot design using repeated measures. Infested and non-infested plots were compared on the basis of a paired t-test. Further, correlations between grain yield and other traits were studied by means of biplots to determine which traits can be to select varieties with the lowest yield reduction under RWA infestation used indirectly.

RESULTS

ANOVA results

At the early booting stage (observation 1), highly significant (P = 0.001) differences were observed among the Kenyan varieties for leaf chlorosis, leaf rolling, plant height, number of leaves per plant, number of tillers per plant, and shoot dry weight (Table 1). Infestation and watering interval did not have significant effects on the number of leaves and number of tillers per plant. Among the leaf symptoms, drought significantly (P = 0.01) increased leaf rolling but had no significant effect on chlorosis. Both infestation and watering interval had highly significant effects on shoot and root fresh and dry weights.

Table 1. ANOVA results for observation 1 (plants at early booting stage) (– = non-significant, ∗, ∗∗, ∗∗∗ = significant at P < 0.05, 0.01 and 0.001, respectively).

Source of variation	Chlorosis	Leaf rolling	Plant height	No. of leaves	No. of tillers	Shoot fresh wt.	Root fresh wt.	Shoot dry wt.	Root dry wt.
Variety (V)	∗∗∗	∗∗∗	∗∗∗	∗∗∗	∗∗∗	–	–	∗∗∗	–
Infestation (I)	∗∗∗	∗∗∗	∗∗∗	–	–	∗∗∗	∗∗	∗∗∗	∗∗∗
Watering interval (W)	–	∗∗	–	–	–	∗∗∗	∗∗∗	∗∗	∗∗
V*W	–	–	–	–	–	–	–	–	–
V*I	∗∗∗	∗∗∗	–	–	–	–	–	–	–
W*I	–	∗∗	–	–	–	–	–	–	–
W*I*V	–	–	–	–	–	–	–	–	–

There were highly significant interactions between varieties and infestation with respect to chlorosis and leaf rolling, as well as between watering interval and infestation with respect to leaf rolling.

At the anthesis stage (observation 2), the leaf symptoms, especially chlorosis, could not easily be scored due to interference from ageing symptoms in many leaves. However, varietal differences were observed in all the growth and development traits measured, except shoot and root dry weight (Table 2). The effect of infestation on varieties was highly significant with respect to plant height, number of leaves per plant, number of tillers per plant and shoot and root dry weights. Watering interval significantly affected the number of leaves per plant, root fresh weight and shoot and root dry weights. Both infestation and watering interval did not affect shoot fresh weight.

Table 2. ANOVA results for observation 2 (plants at anthesis) (– = non-significant, ∗, ∗∗, ∗∗∗ = significant at $P < 0.05$, 0.01 and 0.001, respectively).

Source of variation	Plant height	No. of leaves	No. of tillers	Shoot fresh wt.	Root fresh wt.	Shoot dry wt.	Root dry wt.
Variety (V)	∗∗∗	∗∗∗	∗∗∗	∗∗∗	∗∗∗	–	–
Infestation (I)	∗∗∗	∗∗∗	∗∗∗	–	∗	∗∗∗	∗∗
Watering interval (W)	–	∗∗	–	–	∗∗	∗∗∗	∗∗∗
V*W	–	–	–	–	–	–	–
V*I	∗∗∗	∗∗∗	–	–	–	–	–
W*I	–	∗∗	–	–	–	–	–
W*I*V	–	–	–	–	–	–	–

Significant interactions occurred between variety and infestation with respect to plant height and number of leaves per plant and also between watering interval and infestation with respect to the number of leaves per plant.

At the milk development stage, varietal effects were highly significant (P = 0.001) for all the measured traits except the percentage of deformed ears (Table 3). Infestation had significant effects on plant height, percentage of deformed ears, seed set %, shoot dry weight and grain yield. Watering interval significantly affected plant height, percentage of headed tillers, shoot dry weight and grain yield. As in observation 1, infestation and watering interval had no effect on the number of leaves and number of tillers per plant.

Table 3. ANOVA results for observation 3 (plants at milk development stage) (– = non-significant, ∗, ∗∗, ∗∗∗ = significant at P < 0.05, 0.01 and 0.001, respectively).

Source of variation	Plant height	No. of leaves	No. of tillers	Headed tillers	Deformed ears	Seed set (%)	Shoot dry wt.	Grain yield
Variety (V)	∗∗∗	∗∗∗	∗∗∗	∗∗∗	–	∗∗∗	∗∗∗	∗∗∗
Infestation (I)	∗∗∗	–	–	–	∗∗∗	∗∗∗	∗∗∗	∗∗∗
Watering interval (W)	∗	–	–	∗∗∗	–	–	∗∗	∗
V*W	–	–	–	–	–	–	–	–
V*I	–	–	∗	–	–	∗∗∗	–	∗∗∗
W*I	–	–	–	–	–	–	–	–
W*I*V	–	∗∗	∗	–	–	–	–	–

Significant interactions occurred between variety and infestation with respect to number of tillers per plant, % seed set and grain yield. The 3-way interaction between watering interval, infestation and variety was significant for number of leaves and number of tillers per plant.

Leaf symptoms, number of aphids per plant and percentage of deformed ears in infested plots

The extents of leaf chlorosis and leaf rolling at the early booting stage were higher in the drought-stressed plots than in the well-watered plots. This is shown as mainly negative values occur in the table of differences (Table 4). The difference in the

number of aphids per plant fluctuated in the three observations. In the first observation, the number of aphids per plant was higher in the drought-stressed plots than in the well-watered ones. However, this changed gradually such that by the time of the third observation there were, in most varieties, more aphids per plant in the well-watered than in the drought-stressed plots. Nearly all the varieties showed more deformed ears in the drought-stressed plots than in the well-watered ones.

Table 4. Effect of watering interval on chlorosis, leaf rolling, number of aphids per plant and % deformed ears. Leaf symptoms were scored only in observation 1, whereas deformed ears were counted only in observation 3. Each value was obtained by subtracting the value of the drought-stressed plots from that of the corresponding well-watered plots. A negative value means a higher score at dry conditions.

Variety	Observation 1			Observation 2	Observation 3	
	Leaf chlorosis	Leaf rolling	No. of aphids	No. of aphids	No. of aphids	Deformed ears (%)
91B33	-0.30	-0.25	-61.2	113.8	4.1	-28.0
Fahari	-0.45	-0.02	-77.2	86.1	-13.4	-16.0
Kwale	-0.19	-0.30	-19.4	-68.0	33.4	-5.2
Mbuni	-0.17	-0.12	55.7	19.8	71.5	-6.5
Chiriku	-0.78	-0.42	-103.6	63.6	23.6	16.3
Kongoni	0.12	-0.11	59.7	-60.2	-35.3	-6.4
Nyangumi	-0.37	-0.05	-7.1	31.3	100.1	3.8
Mbega	0.55	-0.66	-89.7	-40.7	11.9	-23.1
Halt	-0.22	-0.09	-17.9	-15.2	-15.6	-0.3
PI 294994	-0.20	0.00	-7.0	1.9	2.6	14.8

Damage due to infestation in well-watered and drought-stressed plots

At the early booting stage (observation 1), RWA infestation generally resulted in reduced plant height. The same trend emerged with respect to shoot and root fresh and dry weight as indicated by mainly superiority of non-infested plants (Table 5). In most of the varieties, the reductions in plant height, shoot fresh and dry weight as well as root fresh weight were greater in the drought-stressed plants than in the well-watered ones. This was however not the case for root dry weight.

Table 5. Differences between non-infested and infested plants in plant height, number of leaves per plant, number of tillers per plant and shoot and root fresh and dry weight in well-watered (W) and drought-stressed (D) plots in observation 1. Each figure is obtained by subtracting the mean value for the infested plots from that of the corresponding non-infested ones.

	Plant height (cm)		No. of leaves per plant		No. of tillers per plant		Shoot fresh weight per plant (g)		Shoot dry weight per plant (g)		Root fresh weight per plant (g)		Root dry weight per plant (g)	
Variety	W	D	W	D	W	D	W	D	W	D	W	D	W	D
91B33	7.10*	10.60*	0.60	2.89	0.17	0.83	1.272	3.502	0.209	0.965	0.228	0.245*	0.074*	0.084*
Fahari	1.38	4.03	1.37	2.67	-0.42	0.53	0.014	1.200	0.127	0.631	-0.127	0.114*	0.000	0.073*
Kwale	4.22	6.53*	1.37	0.61	0.00	0.35	2.367*	3.537*	0.573*	1.025*	-0.124*	0.321*	0.093*	0.087*
Mbuni	6.35	16.00*	-0.20	0.37	-0.37	-0.15	3.564	1.345	0.911	0.544	0.281*	-0.038	0.092*	0.007
Chiriku	-1.30	3.45	-2.23	0.67	-0.52	0.55	-0.050	4.539*	0.083	1.075*	0.087	0.368*	0.032	0.076
Kongoni	2.87	9.71*	3.80	-0.82	-0.53	-0.48*	2.147	-0.981	0.711*	0.771*	-0.100	-0.057	0.009	-0.002
Nyangumi	0.83	8.63*	4.67	-0.08	0.22	0.63*	3.925*	2.739*	1.085*	0.952*	0.491*	0.327*	0.158*	0.068*
Mbega	1.06	2.12	1.17	-1.08	0.03	-0.52*	0.956	0.581	0.344	0.253	0.198*	0.339*	0.064*	0.121*
Halt	5.71*	-0.39	-11.97	-7.89*	0.57*	-0.03	-2.598*	-0.720	-0.130	-0.027	0.260	0.125	-0.034	0.013
PI 294994	1.39	-1.17	2.88	-0.73	0.42	-0.03	-0.586	1.924*	0.282	0.173	-0.244	-0.058	0.000	-0.022

Table 6. Differences between non-infested and infested plants in plant height, number of leaves per plant, number of tillers per plant and shoot and root fresh and dry weight in well-watered (W) and drought-stressed (D) plots in observation 2. Each figure is obtained by subtracting the mean value for the infested plots from that of the corresponding non-infested ones.

	Plant height (cm)		No. of leaves		No. of tillers		Shoot fresh weight		Shoot dry weight (g)		Root fresh weight (g)		Root dry weight (g)	
Variety	W	D	W	D	W	D	W	D	W	D	W	D	W	D
91B33	16.93*	10.31*	-0.49	3.14	-0.56	-0.14	3.786*	3.248	1.225*	1.022	0.289	0.449*	0.086	0.156
Fahari	9.26	7.31*	-2.02*	-0.29	-0.63*	-0.39	2.851	0.744	1.331	0.343	0.110	-0.033	0.033	-0.027
Kwale	5.24	13.15*	-1.12	3.31	-0.12	0.37	3.120*	4.711*	1.640*	1.650*	0.229*	0.522*	0.054*	0.184*
Mbuni	9.71	16.95*	-1.05	0.18	-0.21	0.20	4.647	2.663*	1.456*	0.977*	0.238*	0.049	0.089	0.083*
Chiriku	-0.43	11.25*	-2.61	1.96	0.14	0.39	0.106	3.213*	0.063	1.340*	-0.283	0.387*	-0.043	0.152*
Kongoni	17.78*	14.25*	-1.84	-1.69	-0.41	0.23	4.454*	1.739*	1.754*	0.731*	0.096	-0.006	0.065*	0.042*
Nyangumi	7.61	16.33*	-0.27	0.65	-0.06	0.19	1.869	4.307*	0.928	1.403*	-0.115	0.020	0.029	-0.034
Mbega	8.51	10.67*	-0.92	-0.81	0.04	0.04	0.355	2.868*	0.534	0.950*	0.188	0.214	0.051	0.095*
Halt	1.05	6.47*	5.71*	-0.26	0.66	-1.73*	0.427	6.558*	0.053	0.943*	-0.116	0.444*	-0.006	0.126*
PI 294994	-1.50	0.42	1.05	-3.54*	1.40*	-1.39*	4.065	1.762	0.595*	0.296	-0.076	-0.285	0.003	-0.021

Table 7. Differences between non-infested and infested plants in plant height, number of leaves per plant, number of tillers per plant and shoot dry weight in well-watered (W) and drought-stressed (D) plots in observation 3. Each figure is obtained by subtracting the mean value for the infested plots from that of the corresponding non-infested ones.

	Plant height (cm)		No. of leaves per plant		No. of tillers per plant		Tiller height (cm)		Shoot dry weight (g)	
Variety	W	D	W	D	W	D	W	D	W	D
91B33	15.63*	11.71*	-0.86	3.83*	0.00	0.52	5.93*	8.20*	11.69*	12.77*
Fahari	16.53*	2.25*	-1.59*	0.38	-0.40	0.38	20.57*	2.90*	2.16	4.78*
Kwale	13.05*	5.44	-1.89	4.09	-0.21	0.55	12.03	4.12	4.15	12.14*
Mbuni	15.17*	14.05*	-1.26	0.00	-0.32	0.09	-3.34*	-0.41	7.18	8.56*
Chiriku	2.07*	10.59*	-1.07	2.24	-0.46	-0.52*	8.38	3.48	1.84	4.54
Kongoni	16.02*	10.96	-1.48*	-1.53	-0.29	-0.45	20.61	0.11	-3.34	-0.69
Nyangumi	13.74*	1.58	-1.86	0.47	0.19	0.38	9.89	4.36	10.75	9.93
Mbega	13.46*	12.10	-1.14	-1.31*	-0.29	-0.34*	-0.94*	2.84	8.51	4.77*
Halt	7.66	1.67	11.33*	-0.96	2.06*	0.29	3.32	7.16	24.22*	5.42
PI 294994	1.38	2.86	15.38*	-8.89*	1.67*	-0.58	-2.57	-0.31	17.63*	1.32

Infestation had no significant effects on the number of leaves per plant among the Kenyan varieties. Apart from the drought-stressed plants of Kongoni, Nyangumi and Mbega, infestation also had little effect on the number of tillers per plant.

In the winter wheats the effects of infestation with respect to plant growth and development traits was less clear as the differences between infested and non-infested plants didn't show clear trends.

At anthesis (observation 2), the non-infested plants of the Kenyan varieties were generally taller than the infested ones in both drought-stressed and well-watered plots. This is shown as mainly positive values occur in Table 6. The differences in height between infested and non-infested plants was greater in the drought-stressed plots, where the differences were significant for all the Kenyan varieties.

The varieties showed similar trends with respect to biomass accumulation. The non-infested plants generally had higher shoot and root fresh and dry weights than the infested ones.

With respect to the number of leaves per plant and number of tillers per plant, the varieties reacted differently to infestation under well-watered as compared to drought-stressed conditions. Although the differences between infested and non-infested plants were largely not significant, the infested plants generally had more leaves and tillers than the non-infested ones in the well-watered plots, whereas the opposite was true in the drought-stressed plots.

At the milk development stage (observation 3), infestation resulted in reduced plant height in all the Kenyan varieties. Unlike in the previous observations, the difference between infested and non-infested plants was higher in the well-watered plots than in the drought-stressed plots (Table 7). In the drought-stressed plots, significant differences between infested and non-infested plots were observed only in four varieties, whereas significant differences were observed in all the Kenyan varieties in the well-watered plots. The Kenyan varieties appear to react differently with respect to the number of leaves per plant and the number of tillers per plant under well-

watered and drought-stressed conditions. When well-watered, the infested plants tend to have more leaves and more tillers than the non-infested ones; when drought-stressed, however, the non-infested plants tend to have more leaves and tillers than the infested ones. The same results were obtained in observation 2. Infestation reduced shoot dry weight in most of the varieties, albeit rarely significant.

Differences between infested and non-infested plants with respect to plant height and tiller height were not significant in the winter wheats. However, when well-watered, the non-infested plants had significantly higher number of leaves per plant, number of tillers per plant and shoot dry weight than the infested plants.

The winter wheats developed very slowly and had not even set seed by the time the experiment was harvested. For the Kenyan varieties, the non-infested plants had significantly higher seed set than the infested ones in all the varieties (except Fahari when drought-stressed) (Table 8). The non-infested plants generally had higher percentages of headed tillers and higher grain yield than the infested plants, with the differences being greater in the well-watered than in the drought-stressed plots.

Table 8. Differences between non-infested and infested plants in seed set, percentage of headed tillers and grain yield in well-watered (W) and drought-stressed (D) plots. Each figure is obtained by subtracting the value for the infested plots from that of the corresponding non-infested ones.

	Seed set %		Headed tillers %		Yield per plant (g)	
Variety	W	D	W	D	W	D
91B33	57.0*	59.1*	11.5	6.0	1.08*	1.05*
Fahari	26.0*	18.0	12.6	12.1	2.62*	0.43*
Kwale	56.4*	53.3*	-48.1*	2.6	7.99*	1.25*
Mbuni	52.1*	56.1*	-14.2	27.8	1.38*	0.72*
Chiriku	35.3*	50.3*	31.4	14.3	2.70*	0.35
Kongoni	59.7*	60.9*	20.9*	10.7	2.65*	0.65*
Nyangumi	58.0*	55.6*	-2.6	20.0	-0.82*	0.71*
Mbega	38.4*	50.2*	-16.3	-2.4	-3.73*	0.76*

The biplot analysis showed that % seed set and plant height were strongly and positively correlated with grain yield (Figure 1). The percentage of headed tillers was also positively correlated with grain yield, whereas shoot dry weight was not correlated. The number of leaves and number of tillers per plant, together with the percentage of deformed ears, were all negatively correlated with grain yield.

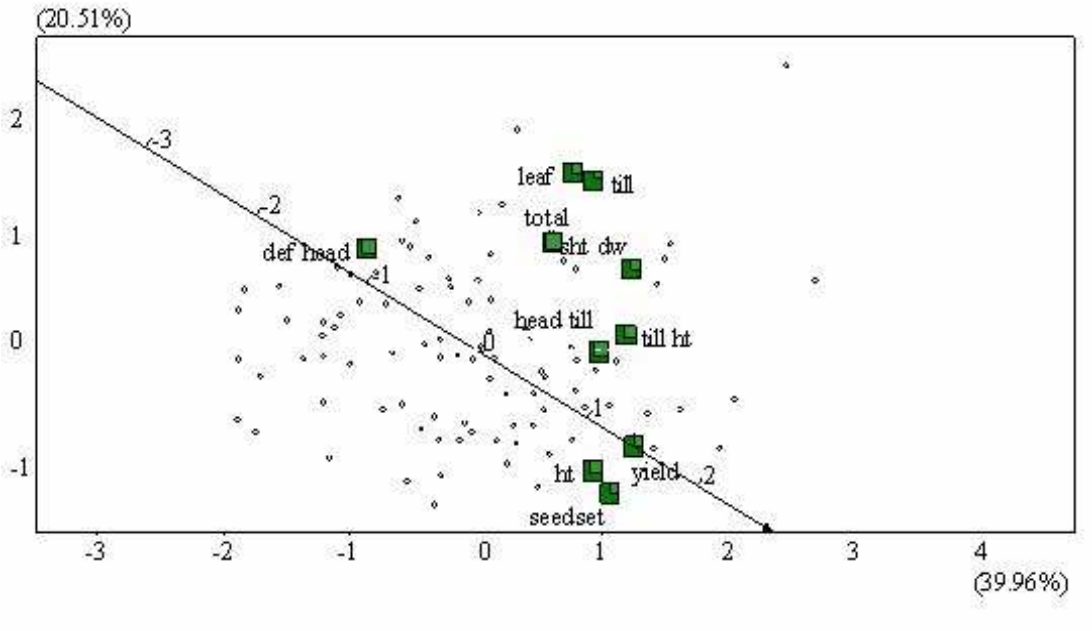

Figure 1. Biplot showing the correlations between grain yield (yield) with other growth and development traits *i.e.* percentage of deformed ears (def head), number of leaves per plant (leaf), number of tillers per plant (till), total leaf length (total), mean tiller height (till ht), percentage of headed tillers (head till), plant height (ht) and % seed set (seedset). The dots show the individual values for each genotype in each rep. Due to their failure to set seed, the winter wheats were excluded from the analysis.

DISCUSSION

The higher scores for chlorosis and leaf rolling in the drought-stressed plots as compared to the well-watered plots during the first observation suggest that the Kenyan varieties are more vulnerable to RWA damage under dry conditions. The effects of RWA infestation show up earlier in the drought-stressed plots. This is also seen in plant height in which the effect of infestation is delayed in the well-watered plots. In the early observations, the differences in plant height between infested and

non-infested plants were greater in the drought-stressed plots, indicating that the plants in these plots were more sensitive. In the last observation, however, the differences in plant height were greater in the well-watered plots.

The significant interaction between the watering interval and infestation with respect to leaf rolling is due to the fact that infestation results in a higher degree of leaf rolling under drought stress. Leaf rolling usually leads to a reduced photosynthetic area resulting in a reduction in biomass production and subsequently in lower yields.

The higher number of aphids per plant in the drought-stressed plots could be due to increased leaf rolling in these plots. RWA reproduces faster inside rolled leaves. However, as the conditions of the drought-stressed plants deteriorate faster than those of the well-watered ones, the aphids in the well-watered plants eventually have a reproductive advantage and become more in number.

RWA infestation appeared to induce the plants, particularly the well-watered ones, to develop more tillers and more leaves.

The greatest loss from RWA attacks appears to be a reduction in seed set (Table 8). Regardless of the watering interval, the reduction in seed set was very high, approaching 50% in many cases. The reduction in seed set is partly a result of head trapping. The trapping delays ear emergence and interferes with pollination and hence seed set. Once trapped ears finally emerge, they usually have deformed shapes. This leads to a higher percentage of deformed ears in RWA infested plots.

The ability to select varieties that yield better than others under RWA infestation is important in reducing losses caused by this aphid. In the absence of real yield data, traits that are highly correlated with grain yield, such as percentage of deformed ears, seed set and plant height, may be used for selection.

CONCLUSION

Leaf damage symptoms, such as chlorosis, leaf rolling and leaf folding, which are commonly used to rate RWA damage in wheat seedlings, become less conspicuous

and more difficult to score when plants advance beyond the early booting stage. Although infestation resulted in reduced plant height in most of the varieties, the reduction was usually small and non-significant, especially in the well-watered plots.

Infestation and drought had little effect on the number of leaves and number of tillers per plant in all the varieties. These traits may thus not be useful parameters for estimating RWA damage if infestation only takes place when plants have already reached the tillering stage. Shoot and root fresh and dry weight were generally reduced by infestation, with the reduction being greater under dry conditions.

Seed set per plant was greatly reduced by infestation and this is taken to be the main cause of yield reduction in the field when RWA infestation occurs after the seedling stage.

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CHAPTER 4

Effect of the Russian wheat aphid on seed quality of Kenyan wheat varieties under well-watered and dry conditions

ABSTRACT

Many studies on the effect of the Russian wheat aphid (RWA) (*Diuraphis noxia* Mordvilko) on wheat have focused on how the infestation affects the infested plants in terms of growth and development, and finally grain yield. Where the next crop is sown from the harvested seed, the RWA infestation may affect the performance of the progeny of the infested plants because of poor seed quality. The effect of RWA infestation on the quality of wheat seeds of some Kenyan varieties under well-watered and dry conditions was studied by observing 1000-seed weight, seedling vigour, percentage of normal seedlings, seedling dry weight and rate of seed quality deterioration under accelerated ageing conditions. Infestation resulted in significant reductions in 1000-seed weight, seedling vigour, percentage of normal seedlings and percentage of viable seeds. The damages due to infestation were significantly greater under dry conditions than under well-watered conditions for all the measured traits, except 1000-seed weight.

Key words: Russian wheat aphid, seed quality, seedling vigour, wheat

INTRODUCTION

Aphids may affect plants directly during feeding or indirectly through transmission of diseases. Among the forms of direct damage by aphids are nutrient drain (when aphids occur in large numbers), damage related to a sensitivity reaction of the hosting plants, chlorosis due to degeneration and disappearance of chloroplasts in the vicinity of the feeding puncture and localized effects due to aphid toxins (Fouche *et al*. 1984; Kruger and Hewitt, 1984; Miles, 1990; Smith *et al*. 1991). The Russian wheat aphid (RWA) (*Diuraphis noxia* Mordvilko) has established itself as a serious pest of wheat and barley worldwide. It causes localized chlorotic spots that begin to coalesce to form characteristic streaks as the attack gets severe (Du Toit, 1987; Souza *et al*., 1991).

Numerous studies have been conducted to determine the effect of RWA on wheat (Webster *et al*. 1987; Smith *et al*. 1991; Archer and Bynum, 1992; Porter *et al*. 1993; Miller *et al*. 1994; Zwer *et al*. 1994; Nkongolo, 1996). Most of these studies focus only on the effect of the aphid attack on seedling or plant development. The damage resulting from RWA attack is manifested through leaf chlorosis, leaf rolling, leaf folding and plant stunting.

Severe RWA infestation of adult wheat plants results in stunted plants with poorly emerged ears and poorly formed seeds (Peairs, 1998). Yield losses ranging from 25 to 90% have been reported in Kenya (Macharia *et al*., 1999). Late feeding of RWA on wheat ears may result in smaller grains with reduced test weight (Hein *et al*., 1998).

The damage on adult wheat plants due to RWA is likely to reduce the quality of seeds produced by these plants. The relationship between seed size and seed quality with respect to seed germination and seedling vigour has been studied by many seed scientists. The rate of seedling growth or seedling vigour in cereals was found to be influenced by seed size among other factors. Evans and Bhatt, (1977) observed a positive correlation between seed size and early seedling vigour in wheat, while Nayeem and Deshpande (1987) reported that seed test weight had significantly positive correlations with root length, shoot length, fresh weight and dry weight of

seedling in wheat. The same positive relationship between seed size and seed quality had earlier been reported in wheat and barley (Kaufmann and McFadden, 1963; Kaufmann and Guitard, 1967; Ries and Everson, 1973).

A similar relationship between seed size and seedling vigour has been reported in potatoes with respect to plants established from true potato seeds. It has been shown that seed size affects germination, seedling vigour and final yield of a seedling-transplanted crop (Bhatt *et al*., 1988, 1989; Upadhya and Cabello, 2000). Large seeds gave higher germination, produced more vigorous seedlings and gave significantly higher tuber yield than small ones.

The attainment and maintenance of high viability and vigour are important goals in wheat seed production. Due to financial constraints, many Kenyan wheat farmers sow their fields with farm-saved seed, with many going for certified seed only after more than three years. Since many of the farmers who plant farm-saved seed are unable to effectively control RWA, and since nearly all unsprayed fields suffer from RWA attacks, the possibility exists that the quality of the farmers' seeds are reduced by RWA. This may lower the yields of the subsequent crop due to poor germination and reduced seedling vigour.

The standard way of determining the quality of seeds is testing for purity and germination in the laboratory. Germination tests give information on whether the seed can germinate under optimal conditions, but provide little information on seedling development thereafter. Seed lots with equal germination but different vigour may give very different stand establishment in the field. Vigour indicates the ability of seed to germinate and grow rapidly under sub-optimal conditions.

Vigour refers to the rate of development of seedlings. It may be measured as the gain in dry weight with time (ISTA 1993). It may also be determined by monitoring the germination of seeds under conditions that are stressful to the seeds, e.g. the cold germination test (TeKrony, 1983), or by the accelerated ageing test, which involves subjecting the seeds for a period to unfavourable conditions, followed by germination under recommended conditions (Jianhua and McDonald, 1996, Wang *et al*., 2004).

The accelerated ageing test is one of the most popular seed vigour tests. Under conditions of high temperature and high relative humidity, low quality seeds (with low vigour) deteriorate more rapidly than high quality seeds (Jianhua and McDonald, 1996). The viability of seeds that have undergone accelerated ageing may be determined directly through a laboratory germination test or indirectly through a biochemical test such as the tetrazolium test (AOSA 1983; Matthews and Powell, 1987).

The tetrazolium test differentiates live from dead tissues of seed embryos on the basis of presence or absence of dehydrogenase enzyme activity. Upon hydration of viable seeds, the activity of the dehydrogenase enzyme increases, resulting in the release of hydrogen ions. These ions reduce the colourless tetrazolium salt solution (2,3,5-triphenyl tetrazolium chloride) into formazan, a red chemical compound. Living cells thus become red, while dead cells remain colourless. Seed viability is interpreted according to the staining pattern of the embryo and the intensity of the staining.

In this study the effect of RWA on the seed quality of some Kenyan wheat varieties under well-watered and dry conditions was investigated. The objective was to determine the effect of RWA infestation in the field on the quality of seeds produced and whether dry conditions, which are often experienced in the field, magnify this effect.

MATERIALS AND METHODS

Source of seeds

Seeds of eight Kenyan wheat varieties (91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi and Mbega) were used in the study. These seeds were obtained from an earlier study of the effect of RWA infestation on adult plants of these varieties under well-watered and dry conditions.

In the experiment from which the seeds were obtained, the varieties were sown in the greenhouse in flats measuring $30 \times 20 \times 10$ cm and containing a mixture of forest soil and sand in a volume ratio of 2:1.

The flats were arranged in a split-split plot design with three replicates and were watered daily to field capacity under natural lighting and temperature conditions. One week after emergence, the seedlings were thinned to leave 20 plants per flat. Each flat coincided with a sub-sub-plot

Each replication consisted of two main plots of which one was well-watered and the other drought-stressed. The well-watered flats received water to field capacity daily, whereas the drought-stressed flats were watered to field capacity at two-day intervals. The varieties were assigned randomly to the sub-plots and each sub-plot was split into two sub-sub-plots of which one was infested and the other was not.

Aphid infestation was done when the plants were attaining the stem elongation stage (Zadoks 30-35). The plants in one sub-sub-plot were infested with five adult aphids, while those in the adjacent sub-sub-plot were non-infested. The aphids were transferred onto the plants using a paintbrush and immediately after infestation all the flats were caged separately to prevent aphid movement from one flat to another. The cages, measuring $20 \times 30 \times 120$ cm, were made from clear polythene paper on two sides and a fine net on the other two sides and the top.

The watering interval treatment was started immediately after infestation: one main plot continued with daily watering while the other plot was watered at two-day intervals to induce drought stress on the plants. These treatments continued till the plants were at the grain filling stage. The treatments were then terminated and the plants were allowed to grow to harvest maturity.

Determination of 1000-seed weight

Plants from each flat were harvested in bulk, threshed and the clean seeds were thoroughly mixed. A sample of about 1000 seeds was then drawn from each bulk. The samples were weighed and the exact number of grains in each sample was determined by counting. The weight of 1000 seeds (in g) was then calculated for each sample.

Germination test and seedling development

The seeds of each variety were bulked per treatment. There were four treatments. These were watered/infested, watered/non-infested, stressed/infested and stressed/non-infested. Seeds in each bulk were well mixed before drawing samples for the germination test. Germination was done following the ISTA (1993) protocol in which each sample was planted in four replicates of 50 seeds each. Planting was done in sand. To break seed dormancy, the germination boxes were chilled by putting them in a room at 10^{o}C for three days. After chilling, the boxes were moved to a germination room with a temperature of 30^{o}C. The day the boxes were moved to the 30^{o}C room was taken as the beginning of the germination test (day 1). At day 5, the heights (cm) of the first 10 seedlings from one end of each germination box were measured. The seedlings were then returned to the germination room. At day 9, heights of the same 10 seedlings were measured again, and the seedlings were returned to the germination room for another three days before a final germination evaluation was done on the day 12. During this final evaluation, the seedlings were carefully removed from the boxes and the roots were washed. By observing both shoot and roots, the seedlings were classified into normal, abnormal and dead seeds for the ones that failed to germinate. The 10 seedlings from each box whose heights had been measured were used to determine seedling dry weight. Immediately after the evaluation of the germination on day 12, these 10 seedlings were put in a paper bag and dried in an oven at 60^{o}C for 24 hours before determining their dry weight (g).

Accelerated ageing

The accelerated ageing test was performed using seeds of two varieties, Mbuni and Kongoni. The choice of the two varieties was based on availability of seed after the laboratory germination test and the tediousness of the tetrazolium test, which makes it difficult to work with many varieties. Seeds from plants of Mbuni and Kwale that had received one of the four different treatments, i.e. watered/infested, watered/non-infested, stressed/infested and stressed/non-infested, and had been bulked separately across the three replications, were used. The seeds were aged following the water added method described by Matthews and Powell, (1987). The initial moisture content of the seeds was determined using a grain moisture meter. A sample of one hundred

seeds was taken from each seed bulk and divided into four lots (replications) of 25 seeds each. The seeds were then placed in aluminium foil bags. By using a micropipette the precise amount of water, required to bring the seeds to a moisture content of 20%, was added to each bag. The amount of water was calculated on the basis of the formula:

$$W2 = \frac{100 - A}{100 - B} \times W1$$

Where: A = initial seed moisture content

B = required seed moisture content (20%)

$W1$ = initial weight of seed (in g)

$W2$ = final weight of seed (in g)

The amount of water (in g) to be added is thus $W2 - W1$

After adding the water, the bags were heat-sealed and shaken for about 30 seconds and left to lie on a lab bench for 24 hours. After the 24-hour moisture equilibration period, the bags were placed side down in the deterioration chamber set at 42^{o}C. Three sets of four samples of each variety were deteriorated for 48, 72 and 96 hours respectively, before they were removed for the tetrazolium test. In total there were 48 bags for each of the two varieties (4 treatments × 3 deterioration periods × 4 reps).

Tetrazolium test

The tetrazolium test was performed on seeds that had undergone accelerated ageing. All the 25 seeds in each bag were cut longitudinally along two-thirds of their lengths from the embryo end. Immediately after cutting, the seeds were immersed in a 0.5% solution of 2,3,5-triphenyl tetrazolium chloride for four hours. The seeds were then washed with distilled water before evaluation for viability. Evaluation was done by observing the staining pattern at the embryo end of the seed and comparing with the tetrazolium staining chart by ISTA (1993) and classifying each seed as either viable or non-viable. The percentage of viable seeds in each lot was then calculated.

Data analysis

The data was subjected to ANOVA using GLM analysis in SPSS release 10.0. For thousand seed weight, the data were analysed as split-split plot using repeated measures. For the other traits, since the seeds had already been bulked per treatment, GLM univariate analysis was used and the data were analysed as randomized complete block design. Data of seedlings derived from infested plants and those of seedlings derived from corresponding non-infested plants were compared on the basis of a paired t-test.

RESULTS

ANOVA results

Table 1 shows the ANOVA results for the seed and seedling traits scored. The varieties (V) exhibited significant ($P < 0.05$) differences for 1000-seed weight and highly significant differences for all the measured seedling traits. The effect of infestation (I) was highly significant in all the traits, whereas the watering interval (W) significantly affected all the traits except seedling height at day 5. The W × V interaction was significant for 1000-seed weight, percentage of normal seedlings and seedling dry weight. The V × I interaction was significant for 1000-seed weight, seedling height at day 9 and seedling dry weight, while the 3-way interaction W × V × I was significant for percentage of normal seedlings and seedling dry weight. The W × I interaction was not significant for any of the traits, suggesting that the effect of infestation was not influenced by the soil moisture of the flats in which the parental plants were raised.

Comparison of varieties for the measured traits

Significant differences were observed between the varieties in all the seed classes with respect to the studied seedling characteristics (Table 2). For seedling heights both on day 5 and day 9, Mbega seedlings were among the shortest in all classes, whereas Fahari had the tallest. Apart from Fahari, which was always much taller than the rest

of the varieties, the differences in seedling height among the other varieties at day 5 appear to be due to drought and infestation. In the well-watered, non-infested class these varieties showed no significant differences in seedling height.

Seedling height differences between varieties were greater at day 9 than at day 5 for most of the seed classes. Although significant varietal differences were evident in all the seed classes, the differences in the well-watered non-infested class were smaller compared to the other classes.

All the seed classes had very high germination percentages as shown by percentage of normal seedlings. The lowest germination was observed in the drought-stressed infested class of Kwale, which had a germination of 96.5%. This was way above the minimum germination set in the ISTA standards, which is 85% for wheat. Significant differences in germination among the varieties were observed in all the seed classes except the well-watered non-infested class.

Table 1. ANOVA results for 1000-seed weight, seedling height, percentage of normal seedlings and dry weight of 10 seedlings (- = non-significant, *, **, *** = significant at P = 0.05, 0.01 and 0.001, respectively).

Source of variation	1000-seed weight	Seedling height at 4 days	Seedling height at 8 days	Percentage of normal seedlings	Dry weight of 10 seedlings
Variety (V)	**	***	***	**	***
Infestation (I)	***	**	***	***	***
Watering interval (W)	***	-	*	***	***
V × W	**	-	-	***	**
V × I	*	-	**	-	***
W × I	-	-	-	-	-
W × I × V	-	-	-	*	*

Table 2. Means for seedling height at day 5 and day 9, percentage of normal seedlings and dry weight of 10 seedlings. The seedlings were derived from well-watered, infested plants (WI), well-watered, non-infested plants (WNI), drought-stressed, infested plants (SI) and drought-stressed, non-infested plants (SNI).

	Seedling height at day 5 (cm)				Seedling height at day 9 (cm)				Percentage of normal seedlings				Dry weight of 10 seedlings (g)			
Variety	WI	WNI	SI	SNI	WI	WNI	SI	SNI	WI	WNI	SI	SNI	WI	WNI	SI	SNI
91B33	2.95ab	4.15a	3.65bc	3.95abc	6.50ab	10.75ab	9.05bc	10.40b	100.0c	99.3	97.0ab	99.0ab	0.058a	0.135ab	0.087bc	0.146bc
Fahari	5.98c	6.15b	6.10d	6.23d	16.28d	18.18d	16.23d	16.78d	97.8abc	99.8	99.5b	99.8b	0.126de	0.183e	0.116c	0.150bc
Kwale	3.80ab	4.05a	4.03c	3.65abc	9.78bc	11.25b	9.70bc	8.88a	97.5ab	99.5	96.5a	97.3ab	0.089abc	0.112a	0.081bc	0.072a
Mbuni	4.35b	3.93a	3.83bc	4.50c	11.08c	11.05b	9.43bc	10.83b	99.8bc	100.0	97.0ab	98.3ab	0.142e	0.168de	0.100bc	0.139bc
Chiriku	3.98ab	4.23a	3.50bc	3.08a	10.65c	12.58c	11.33c	11.25b	97.3a	99.5	99.5b	99.5b	0.079ab	0.145bcd	0.097bc	0.133b
Kongoni	2.98ab	3.60a	2.80b	3.55ab	9.80bc	11.20b	8.13b	12.33c	99.3a	99.5	96.5a	99.5b	0.116cde	0.140bc	0.066ab	0.152bc
Nyangumi	3.98ab	4.00a	3.55bc	4.13bc	9.70bc	10.53ab	8.58bc	10.80b	99.3a	99.3	99.0ab	98.8ab	0.102bcd	0.166cde	0.078bc	0.174c
Mbega	2.60a	3.53a	1.45a	3.13a	6.30a	10.13a	4.03a	8.85a	97.3a	99.3	98.0ab	96.8a	0.067ab	0.154bcd	0.038a	0.098a

*Means in the same column followed by the same letter are not significantly different at P = 0.05.

1000-seed weight

The effect of the watering interval on 1000-seed weight was surprising since the drought-stressed plants produced seeds with significantly higher seed weights than the well-watered plants. On average, the 1000-seed weight for seeds from watered non-infested plants was 29.2 g, whereas for seeds from stressed non-infested plants it was 31.7 g. Similarly, the 1000-seed weight for seeds from well-watered infested plants was 15.9 g compared with 17.9 g for the seeds from the drought-stressed infested plants (Figure 1). Infestation resulted in reduction in 1000-seed weight in all the varieties under well-watered and dry conditions. On average, this reduction was of similar magnitude in both the seeds from well-watered and drought-stressed plants, with reductions of 28.5% and 31.1%, respectively. Variation was however observed among varieties, i.e. low interaction with some, like Chiriku, showing a small reduction in well-watered plants but a large reduction in drought-stressed plants.

Germination and seedling development

Drought-stress and infestation of wheat plants did not affect the speed of emergence of seedlings obtained from their seeds. Seedling emergence occurred three days after the germination boxes were moved to the 30^{o}C chamber for all the seed classes.
According to observations made at day 5, seedlings from seeds of well-watered, non-infested plants were taller than those from seeds of well-watered, infested plants for all the varieties except Mbuni (Figure 2). At this time the height differences between seedlings from infested and non-infested parent plants were small and non-significant for all varieties except Kongoni.

Seedlings from drought-stressed plants showed similar trends, although they were generally shorter than the ones from well-watered plants. The seedlings from non-infested plants were significantly taller than those from the infested ones in Mbuni, Kongoni and Nyangumi.

Differences in height between seedlings from infested and non-infested plants were more evident at day 9, particularly in the seedlings from well-watered plants (Figure

3). Among the seedlings from well-watered plants, those from non-infested plants were always taller than those from infested plants, with the differences being significant for 91B33, Mbuni, Kongoni, Nyangumi and Mbega.

The seedlings from seeds produced by non-infested plants generally grew more between day 5 and day 9 than those from seeds produced by infested plants. This difference was more pronounced in the seedlings derived from well-watered plants than in those from drought-stressed ones.

The effects of both drought stress and infestation of parental plants on seedlings in the next generation were further manifested in their dry weights. Except for the seedlings derived from drought-stressed Kwale, the seedlings from infested parent plants always had significantly lower dry weights than those from non-infested parent plants (Figure 4). Infestation of parental plants resulted in a greater reduction in dry weights of seedlings from well-watered than drought-stressed parental plants in 91B33, Fahari, Chiriku and Mbega. However, in Mbuni, Kongoni and Nyangumi, such infestation caused a greater reduction in the weights of seedlings after drought stress of the parental plants.

Generally, seeds produced by well-watered parental plants had a higher percentage of normal seedlings compared to seeds from drought-stressed parental plants (Figure 5). In nearly all cases, seeds from non-infested parental plants had a higher percentage of normal seedlings than seeds from infested parental plants. This was true for seeds from both well-watered and drought-stressed parental plants.

Accelerated ageing resulted in reduced viability in each of the four seed classes of Mbuni and Kwale (Figure 6). The percentage of viable seeds was always higher in seeds derived from well-watered parental plants than in seeds derived from drought-stressed parental plants. In all cases, seeds that were aged for 48 hours had the highest percentage of viable seeds, whereas those aged for 96 hours had the lowest. The deterioration was higher in the seeds derived from drought-stressed parental plants than in seeds derived from well-watered ones. Deterioration was also higher in seeds from infested parental plants than in seeds from non-infested ones.

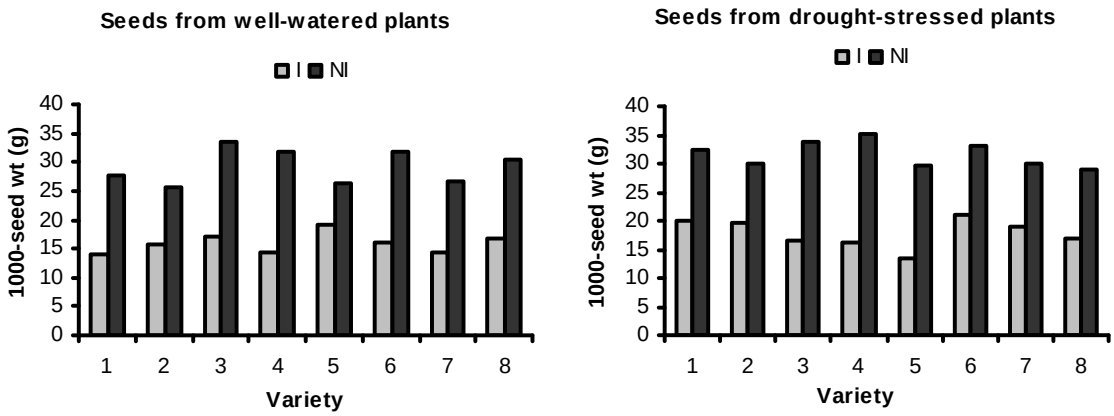

Figure 1. Thousand seed weight for wheat seeds harvested from infested (I) and non-infested (NI) plants that were well-watered or drought-stressed. The varieties are 1 = 91B33; 2 = Fahari; 3 = Kwale; 4 = Mbuni; 5 = Chiriku; 6 = Kongoni; 7 = Nyangumi and 8 = Mbega.

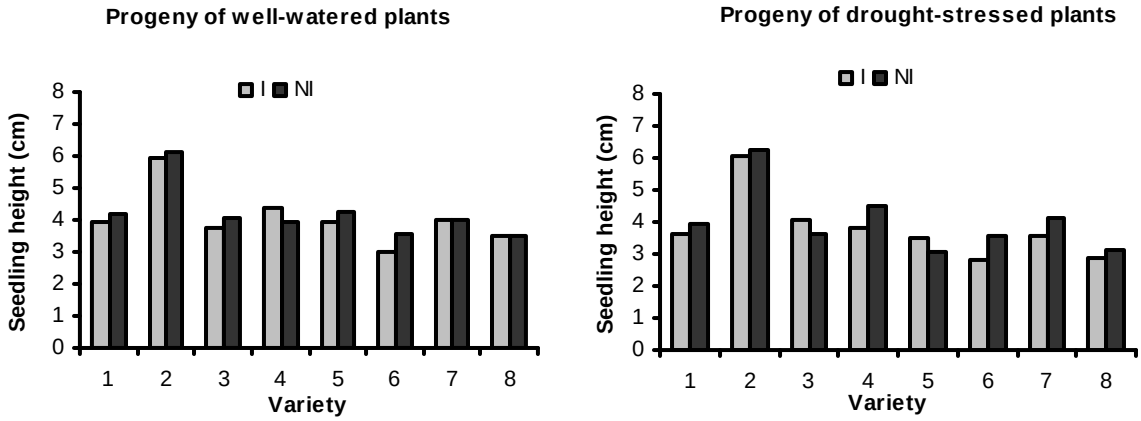

Figure 2. Height, at day 5, of seedlings from seeds of infested (I) and non-infested (NI) wheat plants that were well-watered or drought-stressed. The varieties are 1 = 91B33; 2 = Fahari; 3 = Kwale; 4 = Mbuni; 5 = Chiriku; 6 = Kongoni; 7 = Nyangumi and 8 = Mbega.

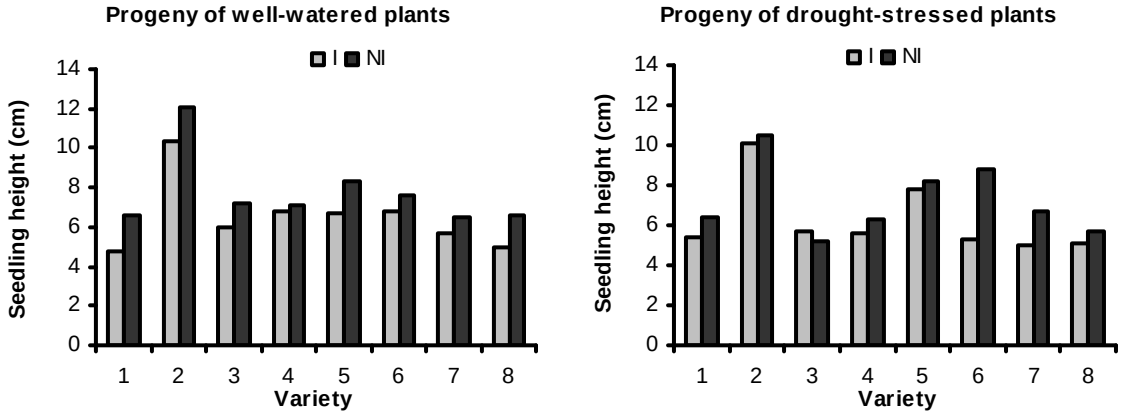

Figure 3. Height, at day 9, of seedlings from seeds of infested (I) and non-infested (NI) wheat plants that were well-watered or drought-stressed. The varieties are 1 = 91B33; 2 = Fahari; 3 = Kwale; 4 = Mbuni; 5 = Chiriku; 6 = Kongoni; 7 = Nyangumi and 8 = Mbega.

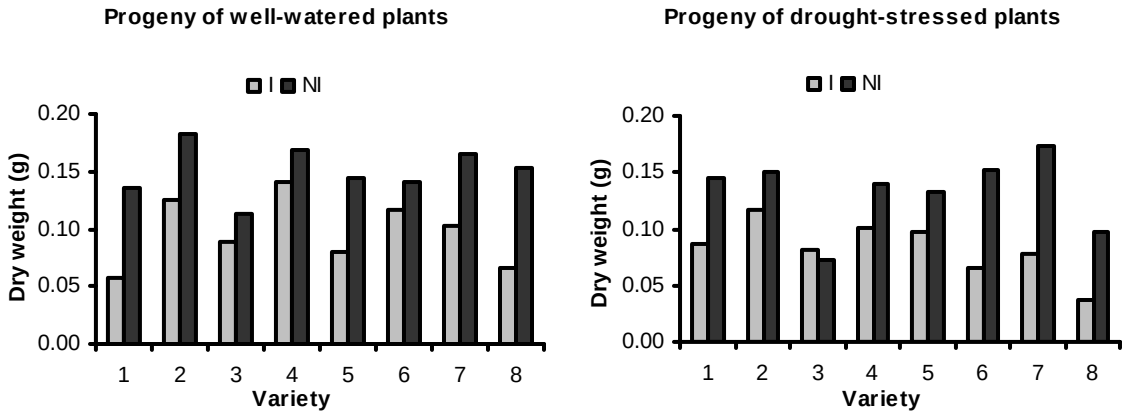

Figure 4. Dry weights of 10 seedlings derived from seeds of infested (I) and non-infested (NI) plants that were well-watered or drought-stressed. The varieties are 1 = 91B33; 2 = Fahari; 3 = Kwale; 4 = Mbuni; 5 = Chiriku; 6 = Kongoni; 7 = Nyangumi and 8 = Mbega.

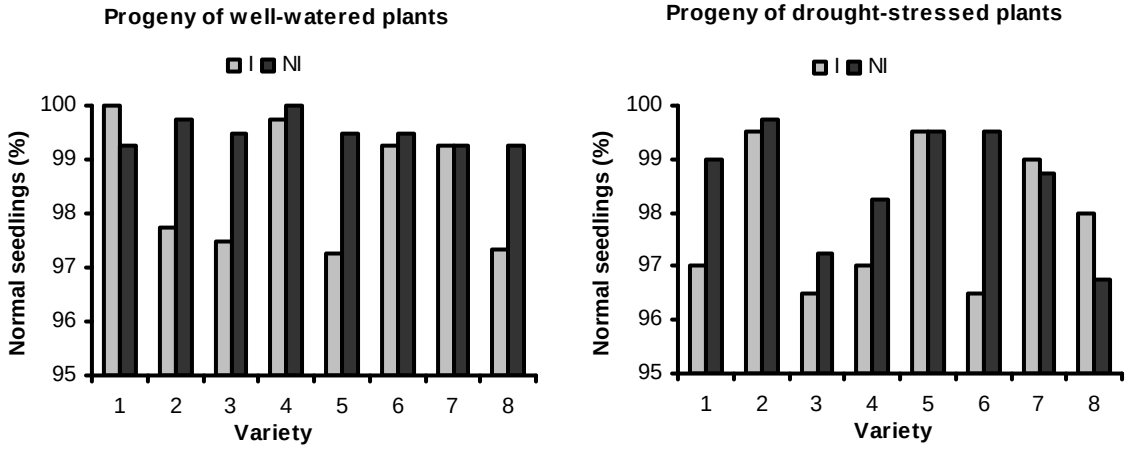

Figure 5. Percentage of normal growing seedlings derived from seeds of infested (I) and non-infested (NI) plants that were well-watered or drought-stressed. The varieties are 1 = 91B33; 2 = Fahari; 3 = Kwale; 4 = Mbuni; 5 = Chiriku; 6 = Kongoni; 7 = Nyangumi and 8 = Mbega.

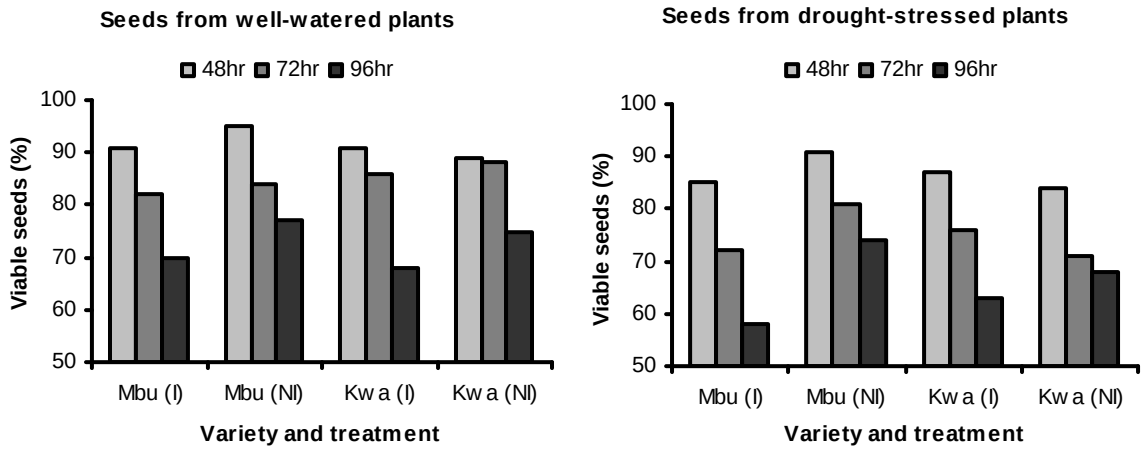

Figure 6. Percentage of viable seeds from infested (I) and non-infested (NI) plants of Mbuni (Mbu) and Kwale (Kwa), that were well-watered or drought-stressed. Viability was tested using the Tetrazolium chloride test, after accelerated ageing for 48, 72 and 96 h.

Table 3. Damage due to infestation of parental plants as observed in 1000-seed weight, seedling height, percentage of normal seedlings and seedling dry weight for seeds derived from well-watered (W) and drought-stressed (D) parental plants. The damage was calculated by subtracting the means for infested plants from those of non-infested plants.

	1000-seed weight (g)		Seedling height at day 5 (cm)		Seedling height at day 9 (cm)		Normal seedlings (%)		Dry weight of 10 seedlings (g)	
Variety	W	D	W	D	W	D	W	D	W	D
91B33	13.48*	12.64*	0.26	0.31	1.80*	1.02*	-0.75*	2.00*	0.078*	0.137*
Fahari	10.00*	10.10*	0.17	0.15	1.75*	0.36	2.00*	0.25	0.057*	0.138*
Kwale	16.55*	17.12*	0.27	-0.39	1.18*	-0.46*	2.00*	0.75	0.023*	-0.009
Mbuni	17.26*	18.88*	-0.41	0.68*	0.37	0.73*	0.25	1.25	0.027*	0.129*
Chiriku	7.12*	16.19*	0.28	-0.40	1.63*	0.35	2.25*	0.00	0.066*	0.123*
Kongoni	15.76*	12.11	0.60*	0.72*	0.84*	3.49*	0.25	3.00*	0.024*	0.146*
Nyangumi	12.50*	11.12*	0.01	0.57*	0.81*	1.67*	0.00	-0.25	0.064*	0.165*
Mbega	13.47*	12.20*	0.02	0.23	1.68*	0.60*	1.92*	-1.25*	0.087*	0.076*

* Difference is significant at P = 0.05.

The damage on seed quality due to infestation may be measured by subtracting the mean for seeds or seedlings from infested parental plants from the mean of seeds or seedlings from corresponding non-infested parental plants. It was expressed most clearly in 1000-seed weight, seedling height and seedling dry weight (Table 3). Except in the case of drought-stressed Kongoni, significant damage was always observed with respect to 1000-seed weight. Similarly, it was only in the case of drought-stressed Kwale that the damage of seedlings with respect to dry weight was not significant. Most of the damages in seedling height at day 5 were not significant. However, at day 9 most of the damages were significant. This indicates that seedlings derived from non-infested parental plants grew more than the ones from infested parental plants, hence the greater damage at day 9.

DISCUSSION

Both infestation and watering interval had significant effects on the measured seed quality traits. The significant interaction between variety and infestation for most of the traits implies that varieties responded differently to infestation. Similarly, the varieties were differently affected by watering interval as shown by the significant interaction between variety and watering interval. The watering interval, however, did not differently influence the effect of infestation as shown by the non-significance of their interactions.

Reductions in seed size and test weight have been associated with reduction in seed quality in wheat (Evans and Bhatt, 1977; Nelson, 1997). RWA infestation caused significant reductions in 1000-seed weight in both the well-watered and drought-stressed plants. This implies that wheat fields experiencing any of these conditions are likely to produce seeds of lower quality than non-infested fields. Contrary to our expectation, the drought-stress treatment did not lead to a reduction in 1000-seed weight. In fact, on average, the drought-stressed plants produced significantly heavier seeds than the well-watered plants. This could be due to a higher number of aphids on the well-watered/infested plants than in the drought-stressed/infested plants, especially in the later stages of development (Chapter 3). It is also possible that the

lower seed set in the drought-stressed plants compared with the seed set of well-watered plants reduced the impact of drought in the former by reducing the sink size and enabling a better filling of the fewer seeds.

The rate of seedling development has often been used as an indicator of seed vigour. Seeds with higher vigour give rise to stronger and faster growing seedlings (Sharma and Anderson, 2003; TeKrony and Egli, 1991). The fact that in most of the varieties seedlings from non-infested plants grew (in height) more than those from infested plants between day 5 and the day 8 indicates that RWA infestation in wheat seed fields could result in reduced seed vigour in the harvested seed. With respect to seedling height, drought stress resulted in an increased damage due to infestation in some varieties, such as Kongoni and Nyangumi. However, for some varieties, such as Fahari, Chiriku and Mbega, infestation resulted in a greater damage, with respect to seedling height, in seedlings of well-watered than in those of drought-stressed plants. The different reactions shown by the varieties could be due to the different levels of drought stress that the different varieties were exposed to. Though watered at similar intervals, the varieties could be utilizing water at different rates resulting in different stress levels. Due to different growth rates, the varieties were not exactly at the same growth stage at the time of infestation and commencement of the stress treatment. This could also have contributed to the differences in seedling vigour exhibited by the different varieties.

Although drought stress generally did not lead to a reduction in seed size, it led to a significant reduction in percentage of normal seedlings and a greater deterioration of the seeds as shown by a lower percentage of viable seeds following the accelerated ageing test. This implies that drought stress could have interfered with some physiological and/or biochemical process during seed development, leading to reduced seed quality. These findings need further investigation.

The results indicate that RWA infestation reduces the quality of the seeds produced by infested plants. This implies that in a system where the harvested grain is used as the seed for the next crop, the effect of infestation is carried forward to the next generation. This effect is even more considerable if the infestation of the parental crop is accompanied by dry conditions. As generally seeds from infested plants lose

viability faster than seeds from non-infested plants, the poor seed storage conditions often found in farms will further increase losses for farmers who use farm-saved seed. If farmers are unable to spray against RWA in their fields, they can reduce losses in the next crop by spraying only the part of the field from which the seed crop will be harvested.

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CHAPTER 5

Genetic variation in the Russian wheat aphid resistant wheat line PI 294994 and inheritance of resistance in crosses with Kenyan wheat varieties

ABSTRACT

Morphological and molecular variations within the Russian wheat aphid (RWA) resistant winter wheat line PI 294994 were studied. Also studied were the effectiveness of its resistance against four Kenyan RWA isolates and the genetics of the resistance. Among 40 PI 294994 plants, two plants headed much earlier than the rest. These two plants also had AFLP fingerprints different from the others and did not require vernalization. Altogether this indicates that PI 294994 consists of different lines. Regardless of the observed differences, all the PI 294994 plants were resistant to all four Kenyan RWA isolates, whereas Halt, another winter wheat reported to be resistant to RWA in the USA, was susceptible. Intercrosses of three PI 294994 derived lines, which differed with regard to morphological and/or AFLP markers, did not show segregation for RWA resistance in the F_2. Crosses between the three lines and two Kenyan wheat varieties produced F_2 populations with different segregation ratios for RWA resistance. While segregation ratios in some F_2 populations fitted the model in which resistance is controlled by one dominant and one recessive gene, the segregation ratio in one population fitted only the one dominant gene model.

Key words: AFLP markers, Breeding for resistance, Russian wheat aphid, Variations in PI 294994.

INTRODUCTION

Significant yield and quality losses due to the Russian wheat aphid (RWA) (*Diuraphis noxia* Mordvilko) have been documented around the world (Du Toit and Walters, 1984; Du Toit, 1988; Miller and Haile, 1988; Peairs and Pilcher, 1988; Pike and Allison, 1991; Elsidaig and Zwer, 1993; Robinson, 1993; Saidi and Quick, 1996; Kinyua *et al*., 2001). The aphid causes characteristic longitudinal leaf chlorosis, leaf rolling and stunted growth (Hewitt *et al*. 1984; Kiriac *et al*., 1990; Miller *et al*. 1994; Zwer *et al*. 1994). Extensive chlorosis leads to death of plants, while leaf rolling retards plant development. Rolling of the flag leaf, for example, delays ear emergence, leading to sterility of florets. The aphid is devastating because of its direct injury to the cereal plant and the effect of the phytotoxin it injects during feeding (Smith *et al*. 1991).

In South Africa, where the aphid was first reported to be a serious pest of wheat and barley, yield losses of between 35 and 60% were recorded (Du Toit and Walters, 1984). The Russian wheat aphid is a relatively new pest of wheat in Kenya. It was first identified in farmers' fields in 1995 (Macharia *et al*., 1999). It then spread quickly to all the wheat growing areas of the country and it became evident that all the commercial wheat varieties in Kenya were susceptible to RWA (Malinga *et al*., 2001). In Kenya, the damage usually appears when crops have attained the tillering stage. Yield losses ranging from 25 to 90% have been reported (Macharia *et al*., 1999).

Insecticide application is normally the first step taken to control RWA. In South Africa, annual large-scale aphicide applications were initially used to protect crops from RWA (Du Toit and Walters, 1984; Du Toit, 1989). The characteristic habit of RWA of rolling cereal leaves, however, makes its control difficult since it secludes itself within the rolled leaves. Aphids secluded in the rolled leaves are partially protected from natural enemies and from contact insecticides, thereby necessitating farmers to use systemic insecticides. Systemic aphicides, however, are very

expensive. Altogether, the most effective, economical and environmentally safe option of controlling RWA is the use of resistant cultivars (Elsidaig and Zwer, 1993; Zhang *et al*. 1998).

Several wheat introductions, most of them from the *D. noxia* area of origin, have been found to possess resistance to RWA. These include PI 137739 and PI 262660 with genes *Dn1* and *Dn2*, respectively (Du Toit, 1987), PI 372129 with *Dn4* (Nkongolo *et al*., 1991b), PI 294994 with *Dn5* (Marais and Du Toit, 1993) and PI 243781 with *Dn6* (Saidi and Quick, 1996). These lines have some disadvantages that render them useless as commercial varieties and the resistance genes have to be transferred to adapted cultivars. Scientists at Colorado State University (USA) have developed several RWA resistant cultivars carrying the *Dn4* gene (Quick *et al*., 1996; Peairs *et al*., 1999; 2003).

Two RWA-resistant wheats, PI 294994 and Halt, were received from Dr. J. Quick of Colorado State University to be evaluated for possible use in the Kenyan breeding programme. PI 294994 is a winter wheat line, originating from Bulgaria, which has been found to have excellent resistance to the Russian wheat aphid (Du Toit, 1990; Elsidaig and Zwer, 1993; Zhang *et al*., 1998). Halt contains the resistance gene *Dn4* derived from the resistant line PI 372129 from the former Soviet Union (Nkongolo *et al*., 1991a; Quick *et al*., 1996).

Different researchers have come up with different results as regarding the number and types of resistance gene(s) present in PI 294994. Marais and Du Toit (1993) reported that resistance in PI 294994 was controlled by one dominant gene, while Saidi and Quick (1996) reported that the resistance was controlled by two dominant genes. Elsidaig and Zwer (1993) reported that resistance in PI 294994 was controlled by one dominant and one recessive gene. Dong and Quick (1995) obtained F_2 segregation data which strongly supported the latter hypothesis.

Apart from the number of genes controlling RWA resistance in PI 294994, there have also been conflicting results concerning the allelism of the resistance gene(s) in this line with resistance genes in other lines. Marais and Du Toit (1993) found that the resistance gene in PI 294994 is not allelic to the resistance genes *Dn1*, *Dn2*, *dn3* (a

recessive gene) and *Dn4*, and they designated it *Dn5*. However, Saidi and Quick (1996) suggested that PI 294994 has at least one RWA resistance gene in common with each of the lines PI 137739 (*Dn1*), PI 262660 (*Dn2*), PI 372129 (*Dn4*), and PI 243781 (*Dn6)* since no susceptible plants were observed in F_2 populations of their crosses with PI 294994. Zhang *et al*. (1998) concluded that the different results reported by the different researchers on the inheritance and allelism of the resistance genes in PI 294994 were due to the presence of different RWA-resistant selections within PI 294994. If variations exist within PI 294994, then it is understandable that different scientists arrived at different conclusions concerning the control of RWA resistance.

The objectives of this study were to:

1. study morphological variations among PI 294994 plants during growth in a greenhouse
2. study variations within PI 294994 by means of AFLP fingerprinting
3. investigate the effectiveness of RWA-resistance genes of PI 294994 and Halt against Kenyan isolates of RWA by infesting seedlings of the two wheats with RWA and observing the damage
4. determine the allelism and inheritance of RWA-resistance genes in three PI 294994 plants differing with regard to morphological and/or AFLP fingerprints
5. initiate a programme to transfer RWA resistance to Kenyan wheat varieties.

MATERIALS AND METHODS

Morphological variation within PI 294994

Seeds of PI 294994 and Halt were kindly obtained from Dr. J. Quick of Colorado State University, USA in February 1999. For the Kenyan varieties the seeds were obtained from the Kenya Plant Health Inspectorate Service (KEPHIS) Seed Quality Control Centre, Nakuru and Kenya Seed Company Ltd. Nakuru, Kenya. Based on reports (Zhang *et al*., 1998) that there is non-uniformity in the RWA-resistant line PI

294994, we decided to work with seeds from individual PI 294994 plants. Forty seeds of PI 294994 were planted singly in pots in the greenhouse. Four plants of Halt were planted similarly to enable the comparison of number of days to flowering and enable comparison of AFLP markers. Planting was done in February, 1999 in Wageningen University, The Netherlands. At the 2-leaf stage, the seedlings were transferred to a vernalization chamber maintained at a temperature of 4^{o}C and a photoperiod of 8 h daily. Vernalization was done for 45 days. After vernalization, the seedlings were returned to the greenhouse at 20^{o}C, and morphological observations were made to detect any differences among the PI 294994 plants. Among the traits observed were number of days to heading and number of days to anthesis. When the plants were at the tillering stage, about 200 mg of fresh leaf samples were taken from each plant for DNA analysis.

DNA extraction

The frozen leaf samples from the 40 PI 294994 (designated PI 1 to PI 40) and 4 Halt seedlings (total of 44 samples) were crushed into powder in 2 ml tubes and 1 ml CTAB (65^{o}C) was added and mixed on a vortex. The tubes were then incubated in a shaking water bath at 65^{o}C for 90 minutes. During the 90 minutes, the tube contents were mixed every 15 minutes by inversion. After 90 minutes, the tubes were removed from the water bath and allowed to cool for 5 minutes before adding 0.8 ml chloroform/isoamyl alcohol (24:1). The tubes were then shaken by inversions for 10 minutes before centrifuging at 1300 rpm for 5 minutes. DNA was precipitated by pipetting the aqueous layer (supernatant) into a new 2-ml tube, adding an equal volume of iso-propanol (2-propanol), shaking by inversions and centrifuging at 1300 rpm for 5 minutes. The DNA pellets were then rinsed with 76% ethanol and dried by leaving the tubes to stand for 1 h. The pellets were dissolved in 200 µl TE buffer before adding 10 µl RNAse and incubating for 30 minutes. The DNA was then precipitated by adding 10 µl 2.5 M NaCl and 0.6 ml 96% ethanol, leaving to stand for 10 minutes, mixing gently and then centrifuging at 1300 rpm for 5 minutes. The aqueous layer was poured out and the DNA pellet was washed with 0.1ml 76% ethanol, dried for 30 minutes in vacuum and dissolved in 50 µl TE buffer before storing at –20^{o}C.

DNA restriction and primer selection

Two sets of restriction enzymes were tried. In one set, the rare cutter was *Eco*RI and the frequent cutter was *Mse*I, while in the other set, the rare cutter was *Pst*I and the frequent cutter was *Mse*I. 0.5 µg of DNA from each of the 44 samples was digested by preparing a 40 µl digestion reaction mixture for each sample (5 µl DNA, 0.5 µl 5U *Eco*RI/PstI, 1.0 µl 5U *Mse*I, 8 µl 5×RL buffer and 25.5 µl deionized water). The mixtures were incubated for 2 h at 37^{o}C.

Adaptors were ligated to the restricted DNA by adding 10 µl of a mixture containing 1.0 µl *Eco*RI/*Pst*I adaptor, 1.0 µl *Mse*I adaptor, 1.0 µl 10mM ATP, 2.0 µl 5× RL buffer, 1.0 µl 1U T_4 DNA ligase and 4.0 µl deionized water. The mixture was incubated for 4 h to obtain the primary template.

During the primer selection stage only 12 out of the 44 DNA samples were used, with several primer combinations being used for each sample. 15 µl of the primary template was diluted 10 times and used in pre-amplification to generate the secondary template. The adapters and primers used in the AFLP protocols are listed in Table 1.

The primer combinations used for pre-amplification were:

*Eco*RI/ *Mse*I primers: A = $E01^{+1}$/ $M02^{+1}$ (template A)
B = $E02^{+1}$/ $M22^{+2}$ (template B)
*Pst*I/ *Mse*I primers: C = $P00^{+0}$/ $M02^{+1}$ (template C)
D = $P00^{+0}$/ $M22^{+2}$ (template D)

N.B. The superscripts represent the number of selective nucleotides

For the radioactive PCR, the two rare cutter primers, *Eco*RI and *Pst*I were labelled with ^{33}P. The E-primers labelled were $E36^{+3}$ and $E36^{+4}$, while the P-primer labelled was $P11^{+2}$. In the active PCR the following primer combinations were tried for the above templates:

Template A: $E36^{+3}$/ $M54^{+3}$

Template B: $E36^{+3}$/ M54-G^{+4}, E36-A^{+4}/ M54-G^{+4}, E36-A^{+4}/ M54-GC^{+5}

Template C: $P11^{+2}$/ $M54^{+3}$

Template D: $P11^{+2}$/ M54-G^{+4}, $P11^{+2}$/ M54-GC^{+5}, $P11^{+2}$/ $M50^{+3}$

The primer combinations that gave the best patterns were used to perform AFLP analysis on all the 44 DNA samples.

Table 1. List of adapters and primers used

Adapter or Primer	Sequences
*Eco*RI adapter	CTC GTA GAC TGC GTA CC
	CTG ACG CAT GGT TAA
Universal E-primer ($E00^{+0}$)	5- GAC TGC GTA CCA ATT C -3
$E01^{+1}$	5- GAC TGC GTA CCA ATT CA -3
$E02^{+1}$	5- GAC TGC GTA CCA ATT CC -3
$E36^{+3}$	5- GAC TGC GTA CCA ATT CAC C -3
E36-A^{+4}	5- GAC TGC GTA CCA ATT CAC CA -3
*Mse*I adapter	GAC GAT GAG TCC TGA G
	TA CTC AGG ACT CAT
Universal M-primer ($M00^{+0}$)	5- GAT GAG TCC TGA GTA A -3
$M02^{+1}$	5- GAT GAG TCC TGA GTA AC -3
$M22^{+2}$	5- GAT GAG TCC TGA GTA ACC -3
$M50^{+3}$	5- GAT GAG TCC TGA GTA ACA T -3
$M54^{+3}$	5- GAT GAG TCC TGA GTA ACC T -3
M54-G^{+4}	5- GAT GAG TCC TGA GTA ACC TG -3
M54-GC^{+5}	5- GAT GAG TCC TGA GTA ACC TGC -3
*Pst*I adapter	CTC GTA GAC TCG GTA CAT GCA
	CAT CTG ACG CAT GT
Universal P-primer ($P00^{+0}$)	5- GAC TGC GTA CAT GCA G -3
$P11^{+2}$	5- GAC TGC GTA CAT GCA GAA -3

Testing for resistance to Kenyan RWA isolates

It had earlier (Chapters 2 and 3) been observed that PI 294994 was resistant to the Kenyan RWAs found in the Eldoret area, while Halt was susceptible. In this study we checked whether the two wheats reacted similarly to RWA isolates from four regions of the country. Aphids were collected from wheat fields in four major wheat-growing regions of Kenya, namely Nakuru, Eldoret, Laikipia and Narok districts in February 2001. The aphids were transferred onto young wheat plants and taken to Moi University where they were raised in isolation on young plants of a mixture of Kenyan wheat varieties in the greenhouse. These aphids were used in a three replicate Randomised Complete Block Design experiment in which two Kenyan varieties, Mbuni and Kongoni, together with the winter wheat varieties Halt and PI 294994 were used. In all, there were 60 flats (4 aphid isolates and 1 placebo × 4 wheat varieties × 3 reps). Fifteen seedlings of each variety were established per flat. They were infested with three adult aphids at the two-leaf stage. Observation for damage was done two weeks later. The placebo was the non-infested control flat for each variety and replication. Assessment was done for leaf chlorosis and leaf rolling using the methods described by Nkongolo *et al*. (1989). The seedlings were also observed for plant height (length (cm) from the base of the seedling to the tip of the uppermost fully emerged leaf), number of leaves per plant and total leaf length (cm). Each plant was scored/ measured separately and the mean value per flat was determined for each character.

Allelism and inheritance studies

Results of the morphological observations and the AFLP analysis of the 40 PI 294994 plants (PI 1 to PI 40) enabled the classification of the plants into three groups of closely resembling plants. Morphologically, two groups (early and late maturing) were identified. AFLP analysis also separated between the two groups but also divided the late maturing group into two. The two groups of late maturing plants were designated group 1 and 2, respectively, while the group of early maturing plants was designated group 3. Three plants, designated P1, P2 and P3, were selected from group

1, group 2 and group 3, respectively, for use in the allelism and inheritance studies of the RWA-resistance gene(s) in PI 294994. Lines obtained from P1, P2 and P3 were intercrossed and crossed with two Kenyan varieties (Mbuni and Kongoni) to study allelism of their resistance gene(s) and the inheritance patterns of the gene(s). This was done in the following way:

During a period of five weeks in January and February 2000, weekly planting of 20 seeds harvested from each of the three PI 294994 plants was done in the greenhouse. The 20 seeds were planted in 2 flats (10 seeds per flat) measuring 30 cm × 20 cm × 10 cm, containing a mixture of forest soil and sand at a volume ratio of 2:1. In total there were 30 flats (3 lines × 5 planting dates × 2 flats). One week after emergence, the seedlings were transferred to a vernalization chamber maintained at 4^{o}C and a photoperiod of 8 h daily for a period of seven weeks. One week before the end of vernalization, weekly planting of Mbuni and Kongoni started in the greenhouse. Like in the case of the P1, P2 and P3 derived lines, 5 weekly planting dates were used for the Kenyan varieties, resulting in 20 flats being planted (2 varieties × 5 planting dates × 2 flats).

When the plants had attained the heading stage, some flats of each of the three lines and the two Kenyan varieties were isolated from the rest. At anthesis all the plants in these flats were emasculated. P1, P2 and P3 were then intercrossed and each was crossed with both Kenyan varieties. The crosses made were: P1 × Mbuni, P1 × Kongoni, P2 × Mbuni, P2 × Kongoni, P3 × Mbuni, P3 × Kongoni, P1 × P2, P1 × P3, and P2 × P3. After pollination, the ears were bagged and the plants were left to grow to maturity. Finally, seeds were bulked per cross.

a) Allelism studies

Sixty F_1 seeds resulting from each of the crosses between P1, P2 and P3 were planted in the greenhouse, in three flats as described above (20 seeds per flat). The seedlings were vernalized for seven weeks and returned to the greenhouse where they grew to maturity. The F_2 seeds from each cross were bulked and 200 F_2 seeds from each cross were planted in 10 flats (20 seeds per flat). At the 2-leaf stage, the F_2 seedlings were infested with 3 adult aphids. Two weeks after infestation, the seedlings were observed

for expression of leaf damage symptoms. The allelic relationships between the RWA resistance gene(s) in P1, P2 and P3 was determined by studying the segregation in the F_2 populations from the crosses. Any segregation for RWA susceptibility and resistance in an F_2 population would indicate that the genes controlling resistance in its two parents were non-allelic.

b) Inheritance studies of the RWA resistance gene(s) in the three PI 294994 derived lines

The F_1 seeds harvested from the 6 crosses between P1, P2 and P3 with the Kenyan varieties Mbuni and Kongoni were used to study the inheritance patterns and hence the number of genes controlling resistance in the three lines.

Sixty F_1 seeds from each cross were planted in 3 flats in the greenhouse. At the time of planting the F_1 seeds, one flat (20 seeds) for each of the resistant parental lines P1, P2 and P3 was also planted. The seedlings were vernalized at the 2-leaf stage and one week before the end of vernalization, the Kenyan parents, Kongoni and Mbuni were also planted in the greenhouse, in a staggered manner, such that they would flower simultaneously with the F_1 plants. Upon their transfer back to the greenhouse, the F_1 seedlings, together with their PI 294994 and Kenyan parents, were infested with 3 adult RWA per plant. Observations for resistance in the F_1 plants were made two weeks after infestation. Further, the number of aphids per plant in the F_1 plants of P3 × Mbuni and P3 × Kongoni were counted and compared to those on the three parents. The decision to pay more attention to the crosses involving P3 was due to the discovery that P3 has the spring wheat growth type, making it more suitable for use in Kenyan breeding programmes. The plants were then allowed to develop to the anthesis stage when the Kenyan parents were emasculated and backcrossing was done with the F_1 to produce the BC_1 seeds. Some of the F_1 plants from each of the 6 crosses were left to produce F_2 seeds.

The number and type of resistance genes present in P1, P2 and P3 was determined by studying the segregation for RWA resistance in the F_2 populations. From each F_2 population, 200 seeds were planted in flats in the greenhouse. Twenty seeds were

planted in each flat as described above. In total there were 6 F_2s x 10 flats = 60 flats. At the two-leaf stage, the seedlings were tested for RWA resistance by infesting each seedling with three adult aphids. Leaf symptoms were scored 2 weeks after infestation to determine the numbers of resistant and susceptible plants. These numbers were determined for each F_2 and used to determine the number of resistance genes in the PI 294994 lines by means of the goodness of fit test.

Few BC_1 seeds were obtained and segregation ratios were not studied. Upon discovering that the P3 line did not require vernalization to develop to the reproductive stage, the BCF_1 from the P3 × Mbuni and P3 × Kongoni were used in further backcrosses in an ongoing programme to develop RWA resistant varieties.

Data analysis

Data for leaf chlorosis, leaf rolling, plant height, number of leaves per plant were analysed using the SPSS programme release 10.0 and means were separated using the Duncan Multiple Range Test. The Chi-square test was used to determine the number of resistance genes in the PI 294994 lines. The observed numbers of resistant and susceptible F_2 plants were tested against the expected numbers at segregation ratios of 3:1, 13:3 and 15:1, corresponding to one dominant gene, one dominant and one recessive gene and two dominant gene models, respectively.

RESULTS

Variation in PI 294994

Morphological observations

In the greenhouse studies, two plant types were detected in terms of earliness. Out of the 40 PI 294994 plants, two plants grew faster and reached the heading stage much earlier than the rest (Figure 1). The two early maturing plants were morphologically

similar to each other, while the plants in the late maturing group exhibited only small differences in number of days to heading.

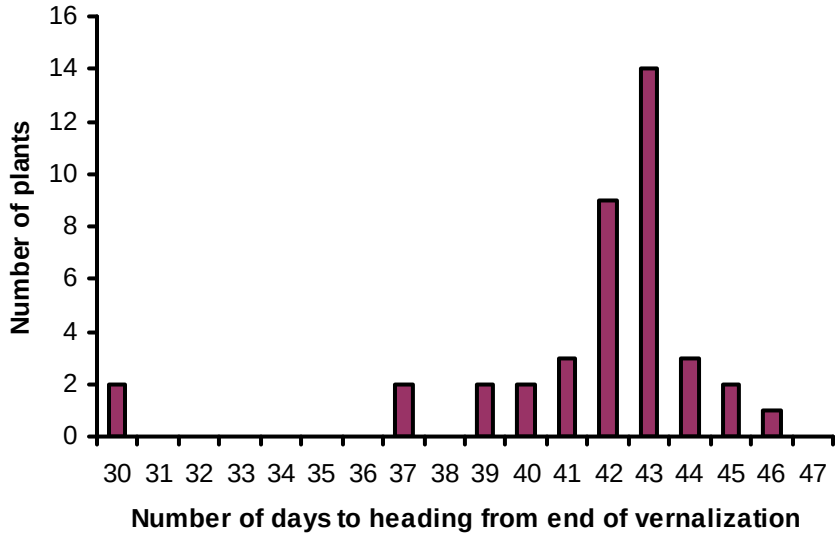

Figure 1. Number of days to heading for the 40 PI 294994 plants after they were removed from the vernalization chamber. Two plants were much earlier than the rest.

AFLP studies

The autoradiogram obtained with the primer combination of $E36^{+3}$/ $M54\text{-}G^{+4}$ showed 10 polymorphisms among the 40 PI 294994 plants and one (315 bp) between the PI 294994 plants and Halt (Figure 2). The 10 polymorphic bands among the PI 294994 plants had sizes 338, 323, 310, 269, 263, 251, 223, 153, 142 and 103 bp. Although Halt is supposedly a uniform variety, some polymorphisms were detected among the 4 plants included in the test. These were markers with 281 and 103 bp.

Based on the polymorphisms at the 10 band positions among the PI 294994 plants, the plants could be classified into three groups of closely resembling plants (Table 2). Generally, group 1 plants showed all the polymorphic bands except the 338, 269 and 251 bp bands. Group 2, in which about 75 % of the plants belonged, had plants that showed only the 323, 310, 223, 153 and 103 bp bands. The third group was the smallest and had only two plants (PI 17 and PI 38). These two plants had marker profiles that were very different from those of the plants in the other two groups. The

group expressed polymorphic bands with 338, 269, 263, 251, 223 and 142 bp and shared only three bands with plants in group 1 and one band with plants in group 2.

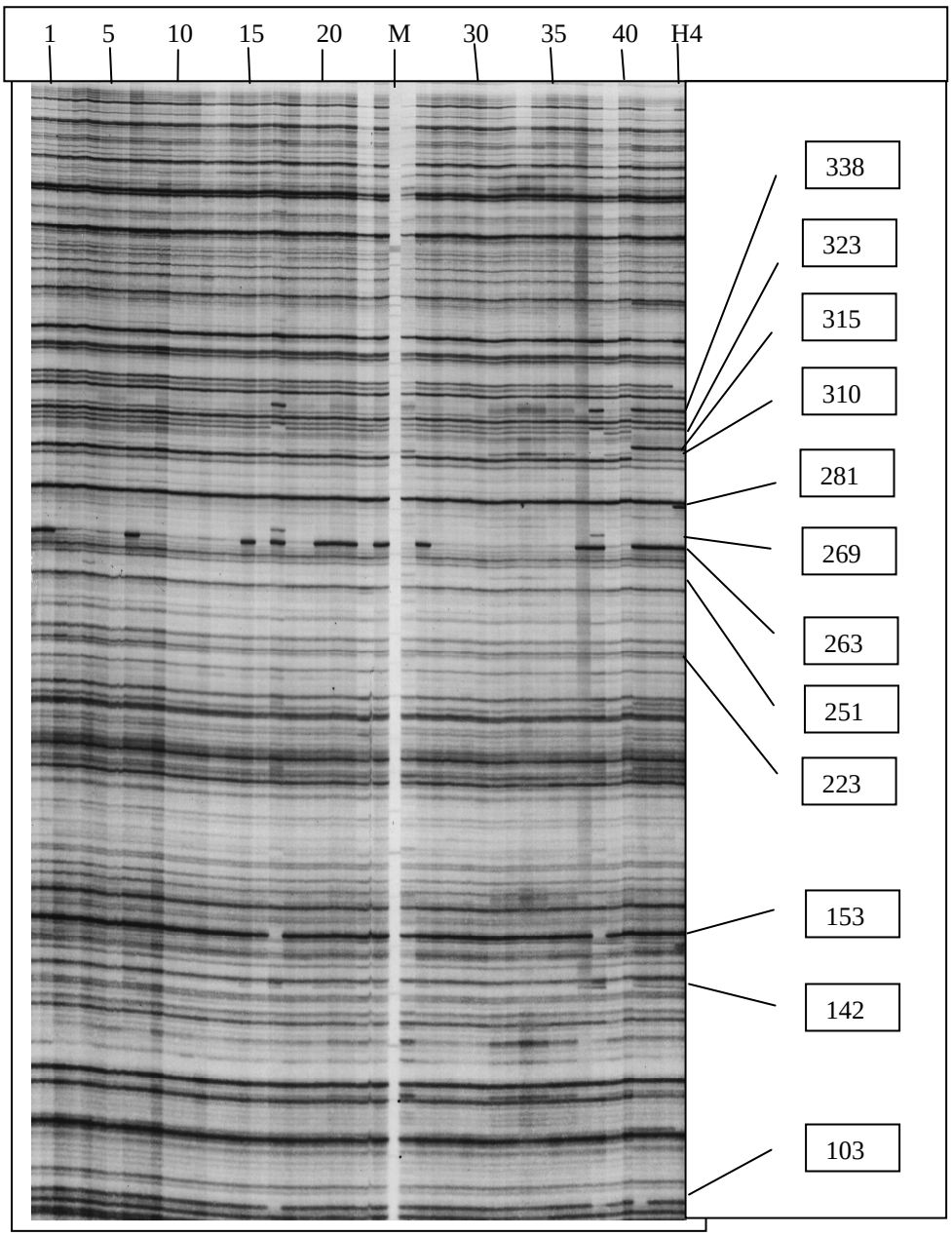

Figure 2. AFLP polymorphic markers generated by primer combination E36^{+3}/ M54-G^{+4} among 40 plants of PI 294994 (PI 1 to PI 40) and 4 plants of Halt. PI 1 is in lanes 1 and 2 followed by PI 2 to PI 24. The molecular size marker (M) is between PI 24 and PI 25. The 4 lanes of Halt come after PI 40.

Table 2. Polymorphisms observed among 40 PI 294994 plants (PI 1 to PI 40) when using the primer combination E36^{+3} / M54-G^{+4}. (+) represents presence of the band while (–) represents its absence.

Group	Plant No.	AFLP polymorphic markers (bp)									
		338	323	310	269	263	251	223	153	142	103
1	PI 1	-	+	-	-	+	-	+	+	+	+
1	PI 7	-	+	-	-	+	-	+	+	+	+
1	PI 15	-	+	+	-	+	-	+	+	+	+
1	PI 20	-	+	+	-	+	-	+	+	+	+
1	PI 21	-	+	+	-	+	-	+	+	+	+
1	PI 22	-	+	+	-	+	-	+	+	+	+
1	PI 24	-	+	+	-	+	-	+	+	+	+
1	PI 26	-	+	+	-	+	-	+	+	+	+
1	PI 37	-	+	-	-	+	-	+	+	+	+
2	PI 2	-	+	+	-	-	-	+	+	-	+
2	PI 3	-	+	+	-	-	-	+	+	-	+
2	PI 4	-	+	+	-	-	+	+	+	-	+
2	PI 5	-	+	+	-	-	-	+	+	-	+
2	PI 6	-	+	+	-	-	-	+	+	-	+
2	PI 8	-	+	+	-	-	-	+	+	-	+
2	PI 9	-	+	+	-	-	-	+	+	-	+
2	PI 10	-	+	+	-	-	-	+	+	-	+
2	PI 11	-	+	+	-	-	-	+	+	-	+
2	PI 12	-	+	+	-	-	-	+	+	-	+
2	PI 13	-	+	+	-	-	-	+	+	-	+
2	PI 14	-	+	+	-	-	-	+	+	-	+
2	PI 16	-	+	+	-	-	-	+	+	-	+
2	PI 18	-	+	+	-	-	-	+	+	-	+
2	PI 19	-	+	+	-	-	-	+	+	-	+
2	PI 23	-	+	+	-	-	-	-	+	-	+
2	PI 25	-	+	+	-	-	-	+	+	-	+
2	PI 27	-	+	+	-	-	-	+	+	-	+
2	PI 28	-	+	+	-	-	-	+	+	-	+
2	PI 29	-	+	+	-	-	-	+	+	-	+
2	PI 30	-	+	+	-	-	-	+	+	-	+
2	PI 31	-	+	+	-	-	-	+	+	-	+
2	PI 32	-	+	+	-	-	-	+	+	-	+
2	PI 33	-	+	+	-	-	-	+	+	-	+
2	PI 34	-	+	+	-	-	-	+	+	-	+
2	PI 35	-	+	+	-	-	-	+	+	-	+
2	PI 36	-	+	+	-	-	-	+	+	-	+
2	PI 39	-	+	+	-	-	-	+	+	-	+
2	PI 40	-	+	+	-	-	-	+	+	-	+
3	PI 17	+	-	-	+	+	+	+	-	+	-
3	PI 38	+	-	-	+	+	+	+	-	+	-

Screening for resistance to Kenyan RWA isolates

Aphids from the four locations showed significant damage levels on susceptible Kenyan varieties (P = 0.05) when compared with the control (Table 3). Infestation with aphids from all the locations resulted in significant levels of leaf chlorosis and leaf rolling. Plant height, number of leaves per plant and total leaf length were also significantly reduced. Observations on leaf chlorosis indicated that aphids from Nakuru caused significantly more damage than those from Eldoret. However, for the other characters, the damages caused by aphids from different locations were not significantly different.

Table 3. The mean effects of infestation with different RWA isolates on susceptible varieties (Mbuni and Kongoni) with respect to leaf chlorosis, leaf rolling, plant height, number of leaves per plant and total leaf length per plant. The means for non-infested plants are included for comparison.

Source of RWA isolate	Chlorosis	Leaf rolling	Plant height (cm)	No. of leaves	Total leaf length (cm)
Non-infested	1.00a	1.00a	26.35a	6.20a	113.37a
Eldoret	3.91b	2.34b	23.52b	5.25b	81.60b
Nakuru	5.04c	2.36b	23.32b	4.90b	71.86b
Laikipia	4.48b	2.44b	22.85b	4.98b	77.09b
Narok	4.31b	2.46b	21.98b	5.03b	71.74b

Values followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test (P = 0.05).

Based on leaf chlorosis and leaf rolling scores, PI 294994 was resistant to aphids from all the locations. The damage on PI 294994 was significantly lower (P = 0.01) than for Mbuni, Kongoni and Halt (Table 4). Mbuni had the highest level of chlorosis (4.81). It was significantly higher than for Halt (4.19). The score for Kongoni was intermediate between the two. For leaf rolling, Halt had the highest score: 2.7. It was significantly higher than the scores for Mbuni (2.37) and Kongoni (2.36).

Table 4. Average chlorosis and leaf rolling scores for Mbuni, Kongoni, PI 294994 and Halt across aphid isolates from different locations.

Variety	Chlorosis score	Leaf rolling score
Mbuni	4.81c	2.37b
Kongoni	4.39bc	2.36b
PI 294994	1.60a	1.05a
Halt	4.19b	2.70c

Means followed by the same letter are not significantly different
from each other according to Duncan's Multiple Range Test (P = 0.05).

Since Mbuni, Kongoni and Halt were susceptible to aphids from the four locations based on chlorosis and leaf rolling, and PI 294994 was highly resistant to aphids from all the locations, it is unlikely that there were different biotypes of the aphid in the 4 wheat growing areas. Due to its high resistance, PI 294994 was considered to be a useful source of RWA resistance in Kenyan wheat breeding programmes.

Putative allelism of RWA resistance gene(s) in PI 294994-derived lines

The F_2 populations obtained from intercrosses between the three PI 294994-derived lines (P1, P2 and P3) did not segregate for RWA resistance. The 200 F_2 seedlings from each of the crosses were all resistant to RWA, indicating that the three parental plants carried the same resistance gene or tightly linked ones (in case different genes are involved).

RWA resistance in F_1 and segregation for resistance in F_2

All the F_1 plants from all six crosses between the PI 294994 derived lines and the Kenyan varieties were resistant to RWA: there were no symptoms of chlorosis and leaf rolling. The resistance was as high as in their PI 294994 derived parental lines. In the F_1s the numbers of aphids per plant were also much lower than in their Kenyan parents, but they were slightly higher than in P3, a resistant parent (Figure 3).

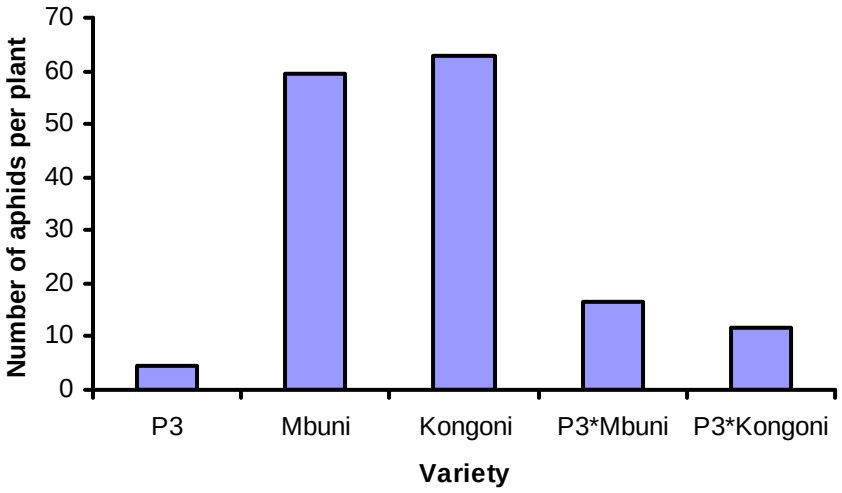

Figure 3. Number of aphids per plant in two F_1s and their parents. P3 is one of the RWA resistant single seed derived lines from PI 294994.

Table 5 shows the observed frequencies of RWA resistant and susceptible F_2 seedlings from the crosses between PI 294994 derived lines P1, P2 and P3, and two Kenyan varieties Mbuni and Kongoni. The observed frequencies were tested against the frequencies expected for a one dominant gene model, a two gene model in which resistance is conferred by a dominant allele at one locus and a recessive allele, when homozygous, at the second locus, and a two dominant genes resistance model. For the two gene models, independent segregation for these genes was assumed.

The Chi-square goodness-of-fit test for the F_2 populations indicated that two genetic models could fit the observed segregation data. The models that fit the observed segregation are indicated by non-significant χ^2 values. At the 0.05 probability level, the numbers of resistant to susceptible plants in the F_2 populations fitted the 3:1 ratio for P2 × Mbuni and P3 × Kongoni crosses. The F_2 segregation in the four other crosses did not fit the 3:1 model as indicated by significant (P = 0.05) χ^2 values.

The ratios of resistant to susceptible plants in the F_2 populations of P1 × Mbuni, P1 × Kongoni, P2 × Mbuni, P2 × Kongoni and P3 × Mbuni fit the 13:3 ratio, indicating that RWA resistance is controlled by two genes, one dominant and one recessive. Among the 6 crosses, only P3 × Kongoni produced F_2 plants with a segregation ratio that did not fit the 13:3 ratio.

Table 5. Observed and expected frequencies of resistant and susceptible plants in the F_2 populations, and χ^2 values for the expected ratios. The ratios of 3:1, 13:3 and 15:1 are those expected for 1 dominant resistance gene, 1 dominant and 1 recessive resistance gene and 2 dominant resistance genes, respectively.

Cross	Category	Observed frequency	Frequency for expected ratios			χ^2 for expected ratios		
			3:1	13:3	15:1	3:1	13:3	15:1
P1 × Mbuni	Resistant	158	144.8	156.9	179.2	4.85*	0.043	35.312***
	Susceptible	35	48.3	36.1	13.8			
P1 × Kongoni	Resistant	162	144.8	156.9	179.2	8.22**	0.895	23.265***
	Susceptible	31	48.3	36.1	13.8			
P2 × Mbuni	Resistant	155	148.5	160.9	183.9	1.14	1.170	63.649***
	Susceptible	43	49.5	37.1	14.1			
P2 × Kongoni	Resistant	165	147.0	159.3	182.0	8.82**	1.085	22.344***
	Susceptible	31	49.0	36.7	14.0			
P3 × Mbuni	Resistant	154	141.8	153.6	175.5	4.24*	0.005	37.034***
	Susceptible	35	47.3	35.4	13.5			
P3 × Kongoni	Resistant	137	142.5	154.4	176.5	0.85	10.515**	123.756***
	Susceptible	53	47.5	35.6	13.5			

*, **, *** significant at P = 0.05, 0.01 and 0.001, respectively

None of the F_2 populations fitted the 15:1 ratio for the two dominant resistance genes model as indicated by highly significant ($P < 0.001$) χ^2 values for all crosses.

DISCUSSION

Results from previous studies (Zhang *et al*. 1998) suggested that PI 294994 is composed of different lines. The results from this study give further support to this suggestion. The two early maturing plants differed from the rest in many respects, including vernalization requirement and AFLP markers. Despite the expressed differences, the variant plants showed equally strong resistance to Kenyan RWA. The absence of segregation for resistance to RWA in the F_2 populations of the intercrosses of the PI 294994 variants indicates that they share at least one resistance gene, or that the genes controlling resistance in these variants are tightly linked. The small number of aphids in line P3 and in its F_1s compared with the susceptible Kenyan parent suggest that the mechanism of resistance in this line is either antibiosis or antixenosis as opposed to tolerance.

In different studies, the number of genes conferring resistance to RWA in PI 294994 has been reported to be one dominant gene, one dominant and one recessive gene and, at times, two dominant genes. In this study, no evidence was found that resistance in any of the three PI 294994 derived lines was controlled by two dominant genes. In both crosses involving P1, the segregation in resistant and susceptible F_2 plants fitted only the 13:3 ratio, indicating that in this line resistance is conferred by two genes, one dominant and one recessive gene.

It is also likely that RWA resistance in P2 is controlled by one dominant and one recessive gene. The segregation in resistant and susceptible F_2 plants from P2 × Mbuni, however, fitted both the 3:1 and 13:3 ratios for the one dominant gene model and the one dominant and one recessive gene model, respectively. A study involving a larger number of plants would determine whether the segregation in resistant and susceptible F_2 plants fits the 3:1 or 13:3 ratio. The segregation for the F_2 from the P2 × Kongoni cross, however, fitted only the 13:3 ratio, indicating that resistance is due to one dominant and one recessive gene.

The two F_2 populations from crosses involving P3 fitted different segregation ratios. P3 × Mbuni produced F_2 plants fitting the 13:3 ratio, while the F_2 from P3 × Kongoni fitted the 3:1 ratio. A test for homogeneity showed that the overall proportions of resistant and susceptible plants fit a 13:3 ratio, with a pooled χ^2 equal to 0.646 (1 d.f., P = 0.25-0.5). There was, however, a large heterogeneity χ^2 equal to 13.036, P = 0.01-0.025 among the proportions in the 6 F_2 populations. This shows that the segregation ratio in the F_2 population of P3 × Kongoni is significantly different from the ratios in the other F_2 populations. There is no clear explanation for this discrepancy as it would suggest that RWA resistance in P3 is expressed differently in different backgrounds. Since it was not possible in this particular study to check the segregation ratios in the backcross populations, further investigations of these segregations are needed.

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CHAPTER 6

Possible AFLP marker(s) for Russian wheat aphid resistance gene(s) in a line selected from PI 294994

ABSTRACT

The wheat accession PI 294994 was found to possess high resistance to Kenyan isolates of the Russian wheat aphid (*Diuraphis noxia* Mord.) A line, P3, derived from PI 294994 was selected for use in our breeding programme since it does not require vernalization and hence is more suitable to Kenyan conditions. The emergence of new aphid biotypes necessitates combining more than one resistance genes in future varieties. This will be done more efficiently with the use of marker assisted selection. Two backcross selfed (BC_1S_1) populations from crosses between P3 and two Kenyan varieties, Mbuni and Kongoni, were used in AFLP analysis to identify markers associated with the RWA resistance. Out of 224 primer combinations that were used in bulked segregant analysis, only five combinations produced polymorphisms that could be related to resistance. AFLP analysis of individual plants from the bulks showed the association of the polymorphic markers with the resistance to be rather weak since some susceptible plants also exhibited the bands specific to the resistant bulk. More primer combinations should be tested to identify markers more closely associated with the resistance gene(s) in this line.

INTRODUCTION

Significant economic losses due to the Russian wheat aphid (RWA) (*Diuraphis noxia* Mordvilko) have been reported in many parts of the world (Du Toit and Walters, 1984; Du Toit, 1988; Stoetzel, 1987; Morrison, 1988; Miller and Haile, 1988; Robinson, 1993). Among the damage symptoms caused by RWA are longitudinal leaf chlorosis and leaf rolling. By secluding itself in the rolled leaves, the aphid is partially or completely protected against contact insecticides and natural enemies, making its control difficult (Hewitt *et al*. 1984; Kiriac, 1990; Miller *et al*. 1994; Zwer *et al*. 1994). The need to apply expensive systemic insecticides, coupled with the environmental concerns on the use of insecticides necessitated the development of RWA resistant cultivars (Webster *et al*. 1987).

Several RWA resistance genes have been discovered. They include *Dn1*, *Dn2* (Du Toit, 1987), *Dn4* (Nkongolo *et al*., 1991), *Dn5* (Marais and Du Toit, 1993) and *Dn6* (Saidi and Quick, 1996). Developing RWA resistant cultivars requires a reliable method of selecting plants containing a resistance gene. Although selection based on phenotypic expression of leaf damage symptoms has been used successfully in breeding for RWA-resistant wheat, the method has some limitations. These include the inability to perform screening in the absence of RWA and having to screen only under conditions which favour survival of the aphid (Michels and Behle, 1988). Environmental influence on symptom expression may result in inaccurate classification of phenotypes. Miller *et al*. (2001) reported that an average misclassification rate of 10% for the greenhouse screening method is possible. It is highly desirable to employ a screening technique that is based on molecular markers linked to the resistance gene (Ma *et al*., 1993; 1994).

Genetic variation among Russian wheat aphids has been demonstrated. Preliminary evidence presented by Bush *et al*. (1989) suggested that there was significant genetic variation among RWA collections obtained in Texas in 1968. Puterka *et al*. (1992) also reported biotype differences in RWA collected from different global locations.

This raises the possibility that a RWA resistance source detected in one location may not be effective against RWA from other locations. The high level of resistance exhibited by PI 262660 (Du Toit, 1989), for instance, was not expressed when using RWA isolates collected in the United States (Nkongolo *et al*., 1989). Recently, the emergence of a new RWA biotype (Biotype B), which attacks previously resistant varieties containing the *Dn2* or *Dn4* resistance genes, has been reported (Peairs *et al*., 2003; Peng *et al*., 2003). The presence of RWA biotypes necessitates incorporation of more than one resistance gene in future varieties. This calls for the use of marker assisted selection (MAS), which allows more efficient selection than phenotypic screening.

The identification of RWA resistance genes and the development of resistant cultivars may be accelerated through the use of molecular markers. Molecular marker techniques have been used to identify and map genes for RWA resistance in wheat (Melchinger, 1990; Ma *et al*., 1998; Myburg *et al*., 1998). In recent years, several molecular marker systems have been developed and applied to a number of crop species, including cereals. These include Restriction Fragment Length Polymorphism (RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) or microsatellites and Single Nucleotide Polymorphisms (SNPs). However, RFLP requires large amounts of DNA and is less amenable to automation, making AFLPs and SSRs more popular markers in cereal breeding (Korzun, 2003).

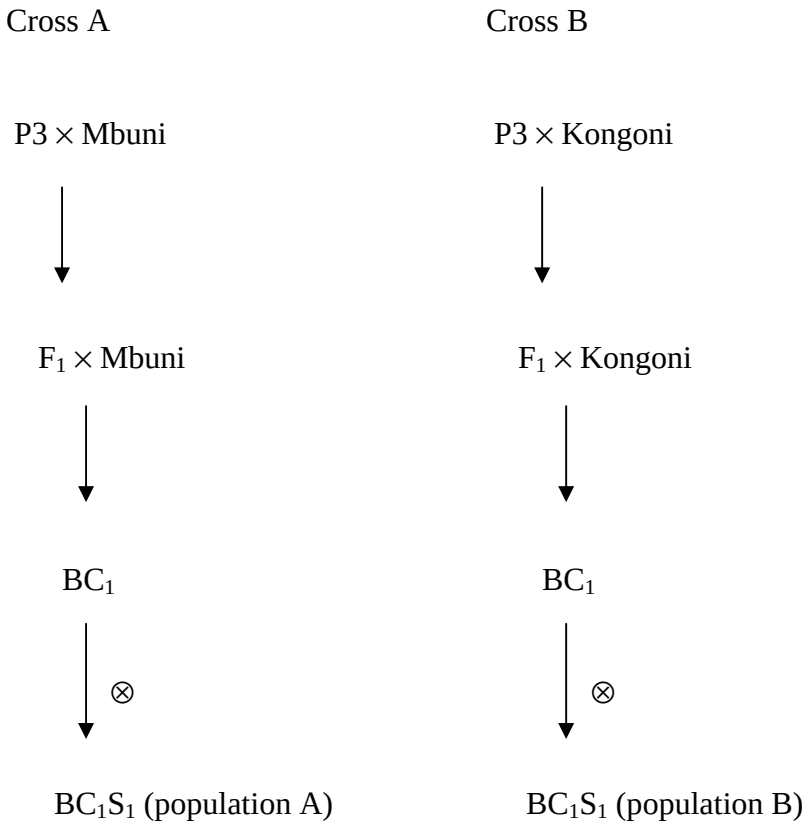
PI 294994 is one of the accessions that has been found to possess a high level of resistance to RWA. When tested in Kenya, PI 294994 was resistant to isolates of RWA from all the wheat growing regions, whereas Halt, a RWA resistant cultivar from USA, was susceptible. While Halt is known to possess a single dominant RWA-resistance gene (*Dn4*), there is yet no agreement on the number of RWA-resistance genes in PI 294994: different researchers report between one and three resistance genes (Marais and Du Toit, 1993; Elsidaig and Zwer, 1993; Saidi and Quick, 1996; Zhang *et al*., 1998).

In our studies, three distict lines (P1, P2 and P3) derived from PI 294994 were all equally resistant to Kenyan isolates of RWA. P3 had the added advantage of not

requiring vernalization, and thus was chosen for use in the breeding programme started at Moi University, Eldoret, Kenya. The objective of the present study was to identify AFLP markers linked to the RWA resistance gene(s) of P3.

MATERIALS AND METHODS

The PI 294994 derived line designated P3 was crossed to Mbuni and Kongoni. Backcrosses of resistant F1 plants and selfings were done following the scheme below:



140 seeds from each BC_1S_1 were planted in flats in a greenhouse at Moi University, Eldoret, Kenya. At the two-leaf stage, each seedling was infested with two adult RWA. The seedlings were scored for RWA damage two weeks after infestation. The seedlings were classified as either resistant or susceptible based on expression of leaf chlorosis, leaf rolling and leaf folding. (These are common symptoms associated with RWA attack on susceptible wheat). 96 seedlings, which could clearly be classified as resistant or susceptible, were selected based on their phenotypic appearance from the

BC_1S_1 of each cross for DNA analysis. Leaf samples for DNA analysis were harvested from individual seedlings by cutting about 2 cm leaf pieces, putting in paper bags and drying in an oven at 65^oC for three hours.

The dried leaf samples were used at the Laboratory of Plant Breeding at Wageningen University, the Netherlands to search for AFLP markers associated with RWA resistance. In total, DNA from 81 resistant and 15 susceptible seedlings was analyzed from population A, while from population B DNA was analyzed from 76 resistant and 20 susceptible seedlings. In addition, DNA from the three parents (P3, Mbuni and Kongoni) was also analysed.

Small pieces of dried leaves (about 5 mm^2) were used for DNA extraction. The leaf samples were ground on a multi-96 grinder and DNA was isolated using the CTAB method, following the protocol described by Stewart and Via (1993). AFLP was performed following the method of Vos *et al*., (1995). DNA was digested with *Eco*RI and *Mse*I as the rare and frequent cutters, respectively. Upon ligation of adapters, DNA templates were prepared by performing PCR using E and M primers with one selective base each. In the second PCR the E primers had three selective bases, whereas the M primers had four selective bases.

Bulked segregant analysis (BSA) (Michelmore *et al*. 1991) was performed on DNA bulks of resistant and susceptible BC_1S_1 plants from both populations (A and B). Each pool was composed of DNA from eight plants. There were eight resistant and two susceptible pools for each population. For each primer combination, AFLP was performed using a resistant pool for population A, a susceptible pool for population A, a resistant pool for population B, and a susceptible pool for population B. AFLP analysis was also performed using DNA from the parents P3, Mbuni and Kongoni.

The E primers were labelled with either 700 or 800 nm infra-red dye (IRD 700 and IRD 800, respectively) for detection with a Licor automated laser sequencer (Li-cor inc, Lincoln, NE, USA). A total of 16 *Eco*RI primers (E31-E46) and 14 *Mse*I primers were used (Table 1). The odd numbered E-primers were labelled with IRD 800, while the even numbered primers were labelled with IRD 700. In total, BSA was conducted with 224, *i.e*. (16 × 14) primer combinations. For any primer combination that showed

a polymorphic band discriminating resistant and susceptible pools, AFLP was run using DNA from 16 individual resistant and 16 individual susceptible plants (8 plants from the pool and 8 additional plants from other pools) to determine if the polymorphism is associated with RWA resistance.

Table 1. List of primers used.

Primer type	Primer No.	Primer sequence
*Eco*RI universal primer	E00	5′-GACTGCGTACCAATTC-3′
*Eco*RI + 1 primer	E01	E00 + A
*Eco*RI + 3 primers	E31	E00 + AAA
	E32	E00 + AAC
	E33	E00 + AAG
	E34	E00 + AAT
	E35	E00 + ACA
	E36	E00 + ACC
	E37	E00 + ACG
	E38	E00 + ACT
	E39	E00 + AGA
	E40	E00 + AGC
	E41	E00 + AGG
	E42	E00 + AGT
	E43	E00 + ATA
	E44	E00 + ATC
	E45	E00 + ATG
	E46	E00 + ATT
*Mse*I universal primer	M00	5′-GATGAGTCCATGAGTAA-3′
*Mse*I + 1 primer	M02	M00 + C
*Mse*I + 4 primers	M48-A	M00 + CAC A
	M48-C	M00 + CAC C
	M48-G	M00 + CAC G
	M48-T	M00 + CAC T
	M52-A	M00 + CCC A
	M52-C	M00 + CCC C
	M52-G	M00 + CCC G
	M52-T	M00 + CCC T
	M54-A	M00 + CCT A
	M54-C	M00 + CCT C
	M54-G	M00 + CCT G
	M54-T	M00 + CCT T
	M55-A	M00 + CGA A
	M56-A	M00 + CGC A

Nomenclature and sequences according to Keygenehttp:/www.keygene.nl/html/nomenclature.htm

RESULTS AND DISCUSSION

In our studies, though we ran BSA for as many as 224 primer combinations, a very low number of polymorphisms was observed. Out of the 224 primer combinations used only five combinations generated polymorphisms that could be related to RWA resistance. These were E32M55-A, E38M55-A, E40M55-A, E41M52-A and E34M52-G (Table 2). Primer combination E34M52-G produced a polymorphic band in population A, while the other four combinations produced polymorphisms in population B. These bands were observed in the DNA bulks of resistant plants but were absent in the bulks of susceptible plants. One polymorphic band, generated by primer combination E33M52-T, was observed in the susceptible bulk in population B but not in the resistant bulk.

Table 2. Polymorphisms discriminating between resistant and susceptible bulks in populations A and B. Presence of band is indicated by +, while absence is indicated by -.

Primer combination	Band size	Bulks for population A		Bulks for population B	
		Resistant	Susceptible	Resistant	Susceptible
E32M55-A	690	-	-	+	-
E33M52-T	305	-	-	-	+
E34M52-G	85	+	-	-	-
E38M55-A	660	-	-	+	-
E40M55-A	210	-	-	+	-
E41M52-A	124	-	-	+	-

When AFLP was run with DNA from individual plants from the five bulks showing a polymorphic band, the association of the polymorphisms with RWA resistance was observed to be weak. The bands observed in the resistant bulks were not shown by all the individual plants from those bulks. Each of these bands also appeared in some of the susceptible plants (Table 3). None of the primer combinations tested so far produced polymorphic bands that co-segregated with the resistance gene. Some of the polymorphic bands, such as the one produced by primer combination E40M55-A in the bulk of resistant plants in population B, had no association with the RWA resistance when individual plants were tested. The most promising marker was the one produced by primer combination E41M52-A, although the band was also expressed in 4 out of 14 susceptible plants. The fact that some individual plants did

not show the polymorphic bands observed in the resistant pool, while some susceptible plants showed the bands, indicates that the resistance gene and the polymorphisms are not close enough, allowing some recombination to occur between them. It is also possible that a few plants were misclassified during the resistance screening. Earlier results (Chapter 2) had indicated that even among susceptible varieties variations occurred between individual seedlings in the expression of leaf symptoms.

Table 3. Number of plants showing AFLP polymorphisms among the individual plants.

Primer combination	Plant group		Fraction of plants with polymorphic band		Total
			Bulked plants (in the pool)	Added plants* (not in pool)	
E32M55-A	Population (resistant)	B	5/8	4/7	9/15
	Population (susceptible)	B	2/8	3/8	5/16
E34M52-G	Population (resistant)	A	4/8	4/8	8/16
	Population (susceptible)	A	2/8	3/6	5/14
E38M55A	Population (resistant)	B	6/8	6/8	12/16
	Population (susceptible)	B	2/8	4/8	6/16
E40M55-A	Population (resistant)	B	4/8	3/8	7/16
	Population (susceptible)	B	1/8	5/8	6/16
E41M52-A	Population (resistant)	B	6/8	5/8	11/16
	Population (susceptible)	B	2/8	2/6	4/14

*Plants that were not in the pool of 8 that gave the polymorphic band with the specific primer combination

The low number of polymorphisms may be explained by the nature of the wheat genome. Molecular genetics developments have been relatively slow in wheat as compared to other crops, such as maize, due to the polyploidy, the genome size, the very high percentage of repetitive sequences and the low level of polymorphism (Hoisington *et al.*, 2002). The line P3 was derived from a single plant of PI 294994 that differed morphologically and genetically with respect to AFLP markers from other PI 294994 plants. It was later observed that the AFLP banding patterns of P3

were generally more similar to those of the two Kenyan varieties than the other PI 294994 derived lines. This could have contributed to the low number of polymorphisms observed between the resistant and susceptible bulks.

The AFLP polymorphisms observed so far are not tightly linked to the resistance gene and may not be useful in a marker assisted selection programme. More primer combinations need to be tested to obtain more useful markers.

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CHAPTER 7

General Discussions

The Russian wheat aphid problem

Unlike many problematic cereal aphids, the Russian wheat aphid (RWA) is not a known transmitter of diseases. Since the mid 1980s, however, it has been ranked as one of the most important aphid pests of wheat and barley in an increasing number of countries (Blackman and Eastop, 1984; Miller and Haile, 1988; Saidi and Quick, 1996). The devastating effect of RWA in wheat is believed to be caused by a toxin that is injected into the plants during feeding, rather than transmission of diseases (Smith *et al*. 1991). Killing of the aphids usually leads to recovery of the plants and disappearance of the symptoms. The symptoms associated with RWA attack are leaf chlorosis, leaf rolling and leaf folding, which are accompanied by plant stunting. In extreme cases plants are killed, especially if they are attacked at the seedling stage. Leaf rolling in adult plants leads to ear trapping and floret sterility.

Infestations that occur after the tillering stage affect the plants differently than those that occur at the seedling stage. Apart from causing chlorosis, leaf rolling and reducing plant height, RWA infestation of adult wheat plants also causes ear deformation, accompanied by a reduction in seed set, and subsequently a reduction in grain yield.

The control of RWA using insecticides is possible, though not usually successful since the aphid secludes itself in the rolled leaves and is protected from contact insecticides (Du Toit and Walters, 1984). Effective systemic insecticides are expensive, making chemical control of RWA economically unattractive. Due to economic and environmental concerns, the control of RWA by means of resistant varieties has been favoured over the use of insecticides. Several resistance genes have been found in wheat lines from the area of origin of the aphid in central Asia. In the USA and South Africa, where the aphid has been a problem since the 1980s, large

areas are now planted with RWA resistant wheat varieties (Thomas *et al*., 2002; Tolmay and Mar'e, 2000).

Biotypes of RWA

The existence of different biotypes of RWA is gaining prominence as a phenomenon that needs to be addressed in resistance breeding programmes. Although the presence of biotypes of RWA had been reported more than 10 years ago (Bush *et al.* 1989; Puterka *et al*. 1992), RWA resistant cultivars with a single dominant resistance gene (*Dn4*) developed at Colorado State University were effective in controlling the aphid in the USA. Since 2003, however, varieties with the *Dn4* gene have succumbed to a new RWA biotype that has been designated biotype B (Peairs *et al*., 2003).

The two RWA resistant winter wheats (Halt and PI 294994) tested with the Kenyan varieties differed in their reactions to RWA infestation. Halt gave a susceptible reaction, whereas PI 294994 was highly resistant. Halt is a resistant variety developed in the USA and has the resistance gene *Dn4*, originally from PI 372129, while PI 294994 has been reported to have the *Dn5* resistance gene. In our tests, Halt was just as susceptible as the Kenyan varieties with respect to most traits contrasting between resistance and susceptibility. In fact Halt had the highest score for leaf rolling among the varieties tested. Similar results were obtained when infestation was done with aphids from different wheat growing regions of Kenya. The observation that Halt was susceptible to the Kenyan RWA, whereas it was resistant in the USA, indicated that the Kenyan RWA was different from the USA one and hence it is further evidence that different biotypes of RWA exist.

The emergence of the resistance breaking RWA biotype in the USA suggests that extra measures are needed upon development of RWA resistant varieties to minimize chances of development of new biotypes. The extensive use of resistant varieties may enhance the development of resistance breaking biotypes by exerting selection pressure on the aphid (Kindler and Hays, 1999; Naber *et al*., 2000). The emergence of resistance breaking biotypes is dependent on the mode of resistance (Baenziger, 2001). Antibiosis exerts the highest pressure and is the most likely to lead to biotype development. Antixenosis exerts little pressure, whereas tolerance exerts no pressure,

resulting in more durable resistance. RWA resistance conferred by the *Dn4* gene is mainly through tolerance combined with a low level of antixenosis (Quick, 1989; Nkongolo *et al*., 1989). The emergence of the biotype B aphid in the USA indicates that even with this mode of resistance additional measures need to be taken to reduce chances of emergence of resistance breaking biotypes. These measures include the use of different resistance genes, such as through pyramiding or gene deployment, and combining the use of resistance genes with the use of chemical sprays (Van der Arend, 2003). Another strategy, which may be effective against the aphid but which is difficult to apply is to ensure that part of the crop area is planted with susceptible varieties to sustain the main avirulent aphid population (Sloderbeck, 1997).

RWA effect on seedlings of Kenyan wheat varieties

In Kenya, the RWA problem started in 1995 when the aphid was first reported. The symptoms of RWA attack in the field are usually observed after the crop has attained the tillering stage. The delay in the time of serious outbreaks may be due to the fact that there is only one cropping season per year, leaving a dry spell of more than six months between crops. This dry spell ensures that there is little substrate the aphids can live on. These conditions drastically reduce aphid numbers prior to the start of the next season.

Results of RWA infestation on Kenyan varieties indicated that most of the current commercial varieties are susceptible to the aphid. When infested at the two leaf stage, leaf chlorosis and leaf rolling scores on the Kenyan varieties increased steadily until seven weeks after infestation, when some of the plants were beginning to die. Leaf folding scores initially increased but dropped three weeks after infestation. By the seventh week after infestation, the infested plants were virtually showing no leaf folding at all. The Kenyan varieties showed small variations in the speed of expression of RWA damage such that, during the early observations, some varieties were significantly more susceptible than others. These differences however disappeared, with all the varieties exhibiting high levels of susceptibility. Plant to plant differences in the expression of leaf chlorosis, leaf rolling and leaf folding existed such that, even within a variety, some plants had high scores for chlorosis but

low scores for leaf rolling and vice versa. Mean scores per plot, however showed a high correlation between leaf chlorosis and leaf rolling.

Although the Kenyan varieties were all susceptible to RWA, significant variations occasionally occurred between them with respect to damage scores. The variety Fahari, for example, exhibited a significantly higher level of chlorosis than all the other varieties. While this is a result that warrants further verification, the existence of such differences among the Kenyan varieties can be used to recommend the less affected varieties to the farmers who are unable to control the RWA. This, however, offers little consolation since the bottom line is that all the varieties are almost equally susceptible. The solution to the Kenyan problem does not lie in selecting more tolerant/resistant varieties among the existing ones, but in introducing resistance from truly resistant sources.

RWA effect on adult plants of Kenyan varieties

When the eight Kenyan wheat varieties screened in this study were infested at the early tillering stage, significant varietal differences with respect to leaf chlorosis and leaf rolling were observed at the early booting stage. Drought-stressed plants had higher scores for chlorosis and leaf rolling than well-watered plants. This corresponds to observations in farmers' fields in Kenya where RWA infestations are reported to be more severe during dry spells. The leaf damage symptoms, especially chlorosis, became more difficult to assess as the plants developed beyond the booting stage.

Although varietal differences were observed, the effect of infestation on number of tillers and number of leaves per plant was not evident at the early booting stage under both well-watered and drought-stressed conditions. In later stages the effect of infestation on these two traits began to show: at the anthesis stage, infestation had affected the well-watered and the drought-stressed plants differently. Infestation resulted in increased numbers of leaves and tillers in the well-watered plots, whereas in the drought stressed plots the numbers of leaves and tillers were reduced. The fact that infestation reduced the growth of existing tillers, as shown by reduced plant height, could induce the plants to produce more tillers. Production of more tillers,

however, is only possible when there is sufficient soil moisture and hence the higher number of leaves and tillers in well-watered plants.

The most devastating effect of RWA infestation of adult plants of the Kenyan varieties was the reduction in seed set. The tight rolling of flag leaves caused by the aphid delays ear emergence, leading to floret sterility. Within the rolled flag leaves, rapid aphid multiplication occurs with some of the aphids residing on the ears after emergence. The ear trapping associated with RWA apparently interferes with pollen development as most of the involved florets had anthers without pollen grains. During the delayed emergence of these ears, the awns remain trapped much longer, causing the ears to bend and become deformed.

Seed quality

Apart from affecting plant development, infestation also reduces the quality of the seeds produced by the infested plants. Infested plants had significantly lower 1000-seed weight and seedling vigour than non-infested ones, indicating that the effects of infestation occur both in the infested crop and in the subsequent crop if sown with the harvested seed.

For both well-watered and drought-stressed plants, infestation significantly reduced 1000-seed weight with reductions of 29 and 32 %, respectively. However, contrary to expectation, drought-stressed plants produced seeds with higher 1000-seed weight than the well-watered plants, whether infested or non-infested. Apparently, since the treatments were discontinued during the grain filling stage, drought stress affected seed set but did not last long enough to negatively influence seed weight. The higher seed weight for the drought-stressed plants could be attributed to the reduction in seed set, which results in reduced sink size and hence a better grain filling. The negative correlation between seed set and individual seed weight has been observed by many researchers (Slafer *et al*., 1996). The most widely accepted explanation for this negative correlation is that the lower the number of seeds m^{-2} the greater the availability of photoassimilates for each seed. This leads to increased individual seed weight. Drought stress may also contribute to higher average seed weights by discouraging the positioning of seeds in more distal positions, which tend to produce small sized seeds.

Seed size did not influence the speed of seedling emergence in a laboratory germination test. Although the seeds from infested plants were smaller and / or lighter than those from non-infested plants, all the seeds emerged after 3 days at 30^{o}C. The effect of seed size was, however, evident with respect to seedling vigour. The seedlings derived from non-infested plants grew faster than those derived from infested plants, resulting in greater plant height and seedling dry weight five days after emergence. Infestation also resulted in a higher rate of deterioration when seeds were exposed to accelerated ageing conditions. Apparently, when parental plants are exposed to drought stress, the vigour of the seeds produced is reduced more than the germination ability. Viera *et al*. (1992) observed that extreme drought stress, that gave rise to very small, shrivelled and misshaped soya bean seeds, negatively affected seed vigour, but not germination ability.

Although drought stress did not reduce seed weight, seedlings derived from drought-stressed plants were generally slower in growth and had a lower percentage of normal seedlings than seedlings derived from well-watered plants. Similarly, when the seeds were exposed to accelerated ageing conditions, it was observed that the viability of seeds derived from drought stressed plants deteriorated faster than those from well-watered plants. Apparently drought stress affecting parental plants leads to reduction in seed quality with respect to aspects that can't be visually assessed. Consequently, germination ability and vigour tests of seed quality are progressively more discriminating estimates of seed quality than visual estimates (AOSA, 1983). This may be a result of elemental deficiencies and imbalances associated with seed development in stressful conditions (Dornbos Jr., 1995). In soybean, for instance, drought stress resulted in reduced calcium content in seeds, leading to impaired membrane integrity. This resulted in reduced germination percentage and reduction in seedling dry weight (Powell, 1986; Hecht-Buchholz, 1979).

Variation within PI 294994

Although PI 294994 can be a good source of resistance for a Kenyan wheat breeding programme, there is still a strong debate on the genetics of the resistance. Different researchers have come up with different results as regarding the number and types of

resistance gene(s) present in PI 294994. Marais and Du Toit (1993) reported that the resistance of PI 294994 was controlled by one dominant gene, while Saidi and Quick (1996) reported that it was controlled by two dominant genes. Elsidaig and Zwer (1993) reported that resistance of PI 294994 was controlled by one dominant and one recessive gene. Dong and Quick (1995) obtained F_2 segregation data which strongly supported the latter hypothesis. Zhang *et al*. (1998) concluded that the different results reported by the different researchers on the inheritance of resistance and the allelism of the resistance genes of PI 294994 were due to the presence of different RWA-resistant selections within PI 294994.

Because of the conflicting reports on the number of genes in PI 294994 and the suggested possibility that the accession is composed of different lines, morphological and molecular marker analysis by means of Amplified Fragment Length Polymorphisms (AFLP) of single plants were conducted to study variation within the accession. Morphological observations on 40 plants of PI 294994 in the greenhouse clearly identified two types of plants based on earliness. Two plants were distinctly earlier maturing than the rest. This distinction was confirmed when AFLP analysis showed the two plants to have similar banding patterns, which were distinctly different from those of the late maturing plants. The early maturing plants were further distinguished from the late maturing ones when it was observed that they were of the spring type (requiring no vernalization) as opposed to the latter group, which were of the winter type. Apart from distinguishing between the early and late maturing plants, AFLP analysis also showed that the late maturing plants separate into two groups based on their banding patterns. It thus appears that among the 40 closely observed PI 294994 plants, there are at least 3 different plant types.

Previously, variation within PI 294994 was reported only with respect to the genetic basis of RWA resistance. This is the first report of developmental and morphological differences among PI 294994 plants. Results from this study, especially with respect to developmental and morphological observations, provides strong evidence that PI 294994 is a composite of different lines. In this study, the two groups of late maturing plants that only differed with respect to AFLP fingerprints were designated 1 and 2 , while the group of early maturing plants was designated 3. Plants from the three groups were equally resistant to Kenyan RWA. Single plant derived lines P1, P2 and

P3, were obtained from the three groups, respectively. Crosses between P1, P2 and P3 with two Kenyan varieties (Mbuni and Kongoni) produced F_1 plants that were as resistant as the PI 294994 parents based on observation of chlorosis and leaf rolling. Aphid counts on the P3 plants and the F_1 plants resulting from the crosses P3 × Mbuni and P3 × Kongoni were much lower compared with those on the Kenyan parents, suggesting that the resistance is due to either antibiosis or antixenosis as opposed to tolerance.

It was necessary to investigate whether the observed morphological and molecular differences within PI 294994 may explain the reported discrepancies on the number and type of RWA resistance genes in the line. Intercrosses between PI 294994 plants from the three groups did not segregate for RWA resistance in the F_2 generation. This indicates that the three groups share at least one resistance gene or that the resistance genes are in tight linkage. The segregation for RWA resistance in the F_2 populations from crosses involving P1, P2 and P3 with Kenyan varieties Mbuni and Kongoni fitted two genetic models in Chi-square tests. In crosses with both Mbuni and Kongoni, P1 and P2 produced F_2 plants fitting a 13 resistant: 3 susceptible ratio, indicating the presence of one dominant and one recessive resistance gene. This agrees with the results of Elsidaig and Zwer (1993). The crosses involving P3, however, produced F_2 plants with different segregation ratios. While P3 × Mbuni produced F_2 plants fitting the 13:3 ratio, the F_2 plants from P3 × Kongoni fitted the 3:1 ratio. There is no clear explanation for this discrepancy as it would suggest that RWA resistance in P3 is expressed differently in different backgrounds. This needs further investigation using a larger number of F_2 plants and further confirmation by study of the segregation in the BC_1 populations.

The emergence of resistance breaking RWA biotypes indicates that breeding programmes should not rely on one resistance gene but should look for ways of combining resistance genes. This can be done more efficiently if molecular markers for resistance genes are available. Our efforts to identify Amplified Fragment Length (AFLP) polymorphisms associated to RWA resistance in P3, the spring type wheat line, have so far not been very successful as the polymorphisms observed are only loosely associated with the resistance.

Implications of our results

Leaf symptoms of chlorosis, leaf rolling and leaf folding are useful in separating RWA resistant and susceptible genotypes. Chlorosis and leaf rolling scores for susceptible varieties increase with time and hence they become easier to score in later observations. Scoring of leaf folding needs closer monitoring as it is expressed over a short time before the scores fall again. Leaf damage symptoms are less obvious and more difficult to score in adult plants. A more useful trait for identification of susceptible varieties at the adult stage is the reduction in seed set, which is caused mainly by the trapping of the ear, leading to floret sterility. This problem can easily be noticed in the field by the appearance of deformed ears and a breeder may select against lines/plants with a high percentage of deformed ears.

Although all the Kenyan varieties tested were susceptible to RWA, the small differences that were observed among them may be useful in selecting a variety to be used use as the recurrent parent in a backcross programme.

The fact that Halt was susceptible to the Kenyan biotype underscores the fact that breeders should first test resistance sources with the local RWA biotypes before utilizing them in their breeding programmes.

The RWA resistance in line P3 was effective against the Kenyan RWA isolates and has been chosen as the source of resistance in the breeding programme started in Eldoret, Kenya. Due to the low number of aphids observed in this line, its resistance is probably based on antibiosis or antixenosis. This type of resistance has been reported as being more likely to be broken by new biotypes as compared with resistance based on tolerance. There is therefore need to search for other sources of resistance that can be incorporated to make the resistance more durable. The work on the study of genetics of resistance in P3 and the development of molecular markers for the resistance gene(s) should continue to facilitate future introgressive RWA resistance breeding.

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Summary

The Russian wheat aphid (RWA) (*Diuraphis noxia* Mord.) is a major pest of wheat and barley in many parts of the world. In South Africa, where it was first reported as a pest of wheat, yield losses ranging from 35 to 60% have been reported. In the USA, cumulative losses of about $1 billion were attributed to the RWA between 1986 and 1991. Unlike many important cereal aphids, RWA is not a known transmitter of diseases, but causes damage by injecting a toxin into the plants during feeding. This toxin causes longitudinal leaf chlorosis, leaf rolling and leaf folding. Beyond the seedling stage, the aphid causes plant stunting, ear trapping and floret sterility.

Wheat (*Triticum aestivum* L.) is the most important cereal crop in Kenya after maize. It is mainly used for bread making. The country currently produces less than 50 % of the required wheat and aims at improving production to meet the rising demand. The RWA has been a problem in Kenya since 1995, when it was first detected. It has spread to all the wheat producing areas and since many farmers are ill equipped to control the aphid, significant yield losses have been reported. All the current Kenyan wheat varieties are susceptible, with attacks becoming noticeable mainly during the tillering stage. The rolling of leaves caused by the aphid ensures that the aphids are protected from contact insecticides. This necessitates the use of more expensive systemic insecticides. Notwithstanding the possibility to control the RWA by chemical spraying, many Kenyan farmers fail to spray or spray late due to financial constraints. Due to both economic and environmental concerns, the development of RWA resistant wheat varieties is seen as the most desirable option for controlling the aphid.

In our studies, we compared eight popular Kenyan wheat varieties for their reaction to RWA infestation at the seedling and adult plant stages. The varieties used were 91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi and Mbega. The objectives were to determine whether there were varietal differences in the levels of susceptibility and whether the varieties reacted differently at the seedling and adult plant stages. The tests for adult plants were done under well-watered and dry

conditions to study whether drought stress, which is frequently experienced in Kenyan wheat fields, influences the severity of damage due to RWA attack. Most Kenyan farmers use the seed harvested from their fields to plant the next crop. Since most of the farmers' crops suffer from various levels of RWA infestation there is a chance that the quality of the harvested seed is affected. This was tested by determining the quality of seeds produced by infested plants under well-watered and drought-stressed conditions. Two RWA resistance sources, Halt and PI 294994, were tested against Kenyan RWA isolates. The number and inheritance patterns of the RWA resistance genes in three PI 294994 derived lines were studied. Further, Amplified Fragment Length Polymorphism (AFLP) markers for RWA resistance in one PI 294994 derived line were studied in two selfed backcross populations from crosses between the line and the Kenyan wheat varieties, Mbuni and Kongoni.

Based on the leaf symptoms of chlorosis, leaf rolling and leaf folding, all the Kenyan varieties tested were found to be susceptible to RWA when compared with the resistant line PI 294994. Halt, which is a resistant variety developed in the USA, was susceptible to Kenyan isolates of RWA. This indicates that the Kenyan RWA isolates are different from the USA ones. Generally, the chlorosis and leaf rolling scores in the Kenyan varieties increased with time, although leaf folding began to drop five weeks after infestation. Differences among the Kenyan varieties in the extent of leaf chlorosis emerged as early as one week after infestation. These differences, however, appeared to be due to the differences in the time of onset of the expression of chlorosis among the varieties. Fahari was always among the varieties with the highest chlorosis score. Seven weeks after infestation, Fahari had a significantly higher score for chlorosis than all the other varieties. Significant phenotypic correlations existed between chlorosis, leaf rolling, leaf folding and number of aphids per plant. Apart from the leaf symptoms, infestation reduced plant growth and development in the Kenyan varieties as shown by reduction in plant height, number of leaves per plant, total leaf length per plant, number of tillers per plant and shoot and root fresh and dry weights. Significant varietal differences occurred among infested plants with respect to plant height, number of leaves per plant, total leaf length and number of tillers per plant. The varieties also differed with respect to shoot and root dry weight.

In the adult plants, drought-stressed plants had higher scores for chlorosis and leaf rolling than well-watered plants. This corresponds to observations in farmers' fields in Kenya where RWA infestations are reported to be more severe during dry spells. The effect of infestation in the well-watered and drought-stressed plants with respect to number of leaves and number of tillers per plant was not immediately evident. By the time the plants attained the milk development stage, however, infestation had produced different effects in the well-watered and drought-stressed plants. Infestation resulted in increased numbers of leaves and tillers in the well-watered plots, whereas in the drought stressed plots the numbers of leaves and tillers were reduced. The fact that infestation reduced the growth of existing tillers, as shown by reduced plant height, could induce the plants to produce more tillers. Production of more tillers, however, is only possible when there is sufficient soil moisture and hence the occurrence of a higher number of leaves and tillers in well-watered plants only.

The most devastating effect of RWA infestation of adult plants of the Kenyan varieties was the reduction in seed set. The tight rolling of flag leaves caused by the aphid delays ear emergence, leading to floret sterility. The ear trapping associated with RWA apparently interferes with pollen development as most of the involved florets had anthers without pollen grains.

RWA infestation reduced the quality of the seeds produced as shown by increased rate of seed deterioration under accelerated ageing conditions. Infestation also resulted in reduced seedling vigour. This implies that in a system where the harvested grain is used as the seed for the next crop, the effect of infestation is carried forward to the next generation. The effect of infestation on seed quality was more pronounced under dry conditions, resulting in significant reduction in percentage of normal seedlings and a greater deterioration of the seeds giving a lower percentage of viable seeds following the accelerated ageing test.

Variations within PI 294994 were identified during morphological observations in the greenhouse and by AFLP analysis in the lab. The PI 294994 plants tested could be separated into three distinct groups, all of which had equally high resistance to Kenyan RWA. A single line was extracted from each of the three groups to obtain three lines P1, P2 and P3. The line P3 was discovered to require no vernalization and

therefore to be suitable for use in a Kenyan breeding programme. Low numbers of aphids were counted in P3 plants and in the F_1 plants produced in crosses between P3 and Kenyan varieties, suggesting that the resistance in P3 was based on either antibiosis or antixenosis as opposed to tolerance. Segregation in the F_2 populations indicated that resistance in P1 and P2 was controlled by two genes (one dominant and one recessive). For P3, the results were inconclusive since in one F_2 population the segregation indicated that the resistance was controlled by one dominant gene, whereas in another population the segregation indicated that resistance was due to one dominant and one recessive gene.

To date, AFLP analysis has not generated AFLP markers that are closely associated with the RWA resistance gene(s) in the PI 294994 derived line P3. Out of 224 primer combinations used in Bulked Segregant Analysis, only five combinations generated bands that were related to resistance. On running AFLP analysis for individual plants it was found that none of the polymorphic bands generated by the five primer combinations co-segregated with the resistance gene(s) since the bands were also present in some susceptible plants. This suggests that the resistance gene(s) and the polymorphic bands observed so far are not tightly linked, resulting in some recombination between them.

Samenvatting

De Russische tarweluis (in het Engels: “Russian wheat aphid” en daarom hier afgekort als: RWA), *Diuraphis noxia* Mord., is in vele delen van de wereld een belangrijke plaag van tarwe en gerst. In Zuid-Afrika, waar RWA als eerste als een tarweplaag beschreven werd, zijn opbrengstverliezen van 35 tot 60% gerapporteerd. In de Verenigde Staten werd tussen 1986 en 1991 een cumulatieve schade ter grootte van ongeveer $ 1 miljard toegeschreven aan RWA. In tegenstelling tot vele belangrijke graanluizen staat RWA niet bekend als een vector van ziekten: de schade wordt veroorzaakt doordat tijdens het zuigen een giftige stof wordt ingespoten. Dit toxine veroorzaakt streepvormige chlorose van het blad, opgerold blijven van het blad en opstropen van het blad. Na het kiemplantstadium veroorzaakt de luis groeibelemmeringen van de plant, insluiting van de aar in de schijnhalm en steriliteit van het bloempje.

Tarwe (*Triticum aestivum* L.) is, na maïs, het belangrijkste graangewas in Kenia. Het wordt vooral gebruikt voor het bakken van brood. Het land produceert momenteel minder dan 50% van de vereiste hoeveelheid tarwe en streeft er naar de productie te verhogen ten einde te voorzien in de toenemende vraag. Sinds 1955, toen RWA voor het eerst werd aangetroffen, is de plaag een probleem geweest in Kenia. De plaag heeft zich naar alle gebieden waar tarwe geproduceerd wordt verspreid en aangezien veel boeren slecht uitgerust zijn om de luis onder de duim te houden, zijn aanzienlijke opbrengstverliezen gerapporteerd. Alle gangbare Keniaanse tarwerassen zijn vatbaar. De aantasting treedt vooral tijdens de uitstoeling aan het licht. Het door de luis veroorzaakte oprollen van het blad verzekert de luis van bescherming tegen contactinsecticiden. Dit vergt gebruik van duurdere systemisch werkende insecticiden. Ondanks de mogelijkheid RWA door chemische bestrijding te beheersen komen veel Keniaanse boeren er niet toe te spuiten, of ze spuiten wegens financiële beperkingen te laat. Uit zowel economische als milieu-overwegingen wordt de ontwikkeling van RWA-resistente tarwerassen gezien als de aantrekkelijkste keuze voor beheersing van de luis.

In ons onderzoek vergeleken we acht populaire Keniaanse tarwerassen ten aanzien van hun reactie op inoculatie met RWA tijdens het zaailingstadium en in volwassen

plantstadia. De gebruikte rassen waren 91B33, Fahari, Kwale, Mbuni, Chiriku, Kongoni, Nyangumi en Mbega. De doeleinden waren vast te stellen of er rasverschillen zijn in de mate van vatbaarheid en of de rassen verschillend reageren in het zaailingstadium en in volwassen plantstadia. De toetsen voor volwassen plantstadia werden uitgevoerd bij goede watervoorziening (zeg: vochtig) en onder droge omstandigheden (zeg: droog) teneinde na te gaan of droogte, hetgeen vaak voorkomt in Keniaanse tarwevelden, de ernst van de schade ten gevolge van een RWA aantasting beïnvloedt. De meeste Keniaanse boeren gebruiken het zaad dat ze van hun velden oogsten voor uitzaai van het volgende gewas. Omdat de meeste tarwegewassen te lijden hebben van RWA besmetting is er een kans dat de kwaliteit van het geoogste zaad beïnvloed is. Dit werd getoetst door de kwaliteit van de zaden die door besmette planten bij vochtige en bij droge omstandigheden geproduceerd waren vast te stellen. Twee RWA-resistente herkomsten, Halt en PI 294994, werden beproefd ten aanzien van Keniaanse RWA-isolaten. Het aantal en het overervingspatroon van de RWA-resistentiegenen in drie uit PI 294994 verkregen lijnen werden bestudeerd. Een uit PI 294994 verkregen RWA-resistente lijn werd gebruikt als donor in een terugkruisingsprogramma met de Keniaanse rassen Mbuni en Kongoni. Aan de twee populaties die door zelfbevruchting van terugkruisingsfamilies waren verkregen werden “Amplified Fragment Length Polymorphism (AFLP)” analyses uitgevoerd teneinde merkers voor RWA-resistentie op te sporen.

Gebaseerd op de bladsymptomen betreffende chlorose, oprollen en opstropen bleken alle onderzochte Keniaanse rassen vatbaar te zijn in vergelijking met de resistente lijn PI 294944. Halt, een resistent ras dat ontwikkeld is in de Verenigde Staten, bleek vatbaar te zijn voor de Keniaanse isolaten van RWA. Dit geeft aan dat de Keniaanse isolaten verschillen van de isolaten in de VS. In het algemeen namen de scores voor chlorose en bladoprolling van de Keniaanse rassen in de loop van de tijd toe, hoewel de bladopstroping vanaf vijf weken na de inoculatie afnam. Verschillen tussen de Keniaanse rassen ten aanzien van de mate van bladchlorose werden al vanaf één week na de inoculatie zichtbaar. Deze verschillen waren echter toe te schrijven aan de verschillen tussen de rassen in het tijdstip van aanvang van de expressie van de chlorose. Fahari was steeds één van de rassen met de hoogste score voor chlorose. Fahari had zeven weken na de inoculatie een significant hogere score voor chlorose

dan elk van de andere rassen. Er bestonden significante correlaties tussen chlorose, bladoprolling, bladopstroping en aantal luizen per plant. De inoculatie leidde, behalve tot bladsymptomen, ook tot verminderde plantgroei en -ontwikkeling van de Keniaanse rassen (dit bleek uit verminderde plantlengte, een lager aantal bladeren per plant, een gereduceerde totale bladlengte, een geringere uitstoeling en een lager vers en droog spruit- en wortelgewicht). Voor plantlengte, aantal bladeren per plant, totale bladlengte en aantal halmen per plant vertoonden de geïnoculeerde planten significante rasverschillen. De rassen verschilden ook ten aanzien van droog gewicht van spruiten en wortels.

Bij de volwassen planten hadden de droog opgekweekte planten hogere scores voor chlorose en bladoprolling dan de vochtig opgekweekte planten. Dat stemt overeen met waarnemingen van praktijkvelden in Kenia, waarvan bekend is dat RWA-aantastingen gedurende droge perioden ernstiger zijn. Het effect van inoculatie van de 'droge' planten en van de 'vochtige' planten ten aanzien van het aantal bladeren en het aantal halmen per plant werd niet onmiddellijk duidelijk. Tegen de tijd dat de korrels het melkstadium bereikten bleek echter dat inoculatie bij de 'vochtige' planten en bij de 'droge' planten tot verschillende effecten leidde. Inoculatie leidde in de vochtige veldjes tot een hoger aantal bladeren en halmen, terwijl bij de droge veldjes het aantal bladeren en halmen afnam. Daar de groei van de bestaande halmen bij inoculatie verminderde, hetgeen uit de geringere plantlengte bleek, induceerde de inoculatie wellicht de productie van een groter aantal halmen. Productie van meer halmen is echter alleen mogelijk wanneer er voldoende bodemvocht is en daarom deed deze toename van het aantal bladeren en halmen zich alleen voor bij de 'vochtige' planten.

Het meest verwoestende effect van RWA-inoculatie van volwassen planten van de Keniaanse rassen was de verminderde zaadzetting. Het door de luizen veroorzaakte strakke oprollen van de vlagbladeren vertraagt het uitaren, hetgeen tot steriliteit van de bloempjes leidt. De bij RWA optredende insluiting van de aar heeft effect op de pollengenese hetgeen blijkt uit het feit dat de meeste van de betrokken helmdraden geen pollenkorrels bevatten.

RWA-inoculatie verminderde de kwaliteit van de geproduceerde zaden. Dit bleek uit de toegenomen snelheid van zaadverslechtering bij kunstmatig versnelde

veroudering. Inoculatie verminderde ook de kiemplantvitaliteit. Voor een systeem waarbij het geoogste graan gebruikt wordt als zaaizaad voor het volgende gewas betekent dit dat het effect van aantasting overgedragen wordt op de volgende generatie. Het effect op de zaadkwaliteit van de inoculatie was meer uitgesproken onder droge omstandigheden en dat resulteerde in een significante afname van het percentage normale kiemplanten alsmede in een sterkere zaadverslechtering bij kunstmatig versnelde veroudering, hetgeen leidt tot een lager percentage vitale zaden.

Binnen PI 294994 werden variaties vastgesteld, zowel bij het doen van morfologische waarnemingen als bij AFLP analyse in het laboratorium. De onderzochte PI 294994 planten konden in drie disjuncte groepen, die niet verschilden in hun hoge resistentie tegen Keniaanse RWA, worden ondergebracht. Uit elk van de drie groepen werd een lijn getrokken. Hiermee werden de drie lijnen P1, P2 en P3 verkregen. Lijn P3 bleek geen vernalisatie-behoefte te hebben en was daardoor geschikt voor gebruik in een Keniaans veredelingsprogramma. Op zowel P3-planten als op F1-planten, die verkregen werden uit kruisingen tussen P3-planten en Keniaanse rassen, werden lage aantallen luizen werden geteld. Dit suggereert dat de resistentie van P3 op antibiose dan wel antixenose berustte, en niet op tolerantie. De splitsing in de F2 populaties wees uit dat de resistentie van P1 en P2 berustte op twee genen (één dominant en één recessief). Voor P3 waren de resultaten niet overtuigend omdat de splitsing in één F2-populatie erop wees dat de resistentie gereguleerd werd door één dominant gen; terwijl de splitsing van een andere populatie aangaf dat de resistentie berustte op een dominant en een recessief gen.

Tot op heden heeft AFLP-analyse geen AFLP merkers opgeleverd die sterk gekoppeld zijn met het/de RWA-resistentie gen(en) in de uit PI 294994 verkregen lijn P3. Op een totaal van 224 'primer' combinaties die gebruikt zijn in “Bulked Segregant Analyses” waren er slechts vijf combinaties die bandjes genereerden welke te relateren waren aan resistentie. Bij AFLP-analyse van individuele planten bleek dat geen enkele van de polymorfe banden die door die vijf 'primer' combinaties werden voortgebracht co-segregeerde met de resistentie gen(en) omdat de bandjes ook in enkele vatbare planten aanwezig waren. Dit suggereert dat de resistentie gen(en) en de tot dusverre waargenomen polymorfe bandjes niet sterk gekoppeld zijn, waardoor er recombinatie optreedt.

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Oliver Kiplagat was born on 22nd October 1961 in Iten, Keiyo District, Kenya. He began his education at Singore Primary School in 1970 and did his secondary and high school education at St Patrick's High School, Iten between 1977 and 1982. In 1984, he joined the University of Nairobi to study for B.Sc. degree in Agriculture. Upon graduation in 1987, he was employed as an Agricultural Officer at the National Seed Quality Control Station within the Scientific Research Division of the Ministry of Agriculture. This Division became the Kenya Agricultural Research Institute (KARI) in 1989. Through a scholarship from the Canadian International Development Agency (CIDA), Oliver did his MSc. degree in plant breeding at the University of Alberta, Canada between 1992 and 1995 and wrote a thesis entitled 'Moisture-stress induced sterility and outcrossing in wheat'. In 1997, he joined Moi University as a Junior Research Fellow at the Department of Crop Science and Seed Technology in the Faculty of Agriculture. It is at Moi University that he got a scholarship to study for a Ph.D. degree in plant breeding at Wageningen University in 1998 under a sandwich programme. The scholarship was offered by NUFFIC through the MHO-Moi University Seed Technology Project, a collaborative project between Moi University in Kenya and Larenstein and Wageningen Universities in the Netherlands. The Ph.D. research topic was inspired by the need to address the Russian wheat aphid problem currently facing the Kenyan wheat farmers. After the Ph.D. studies, Oliver will return to Moi University to teach and continue with research.

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