CUKUROVA UNIVERSITY

INSTITUTE OF NATURAL AND APPLIED SCIENCES

M.Sc. Thesis

Mohamed Izzat GHANNOUM

Sources of Resistance in Durum Wheat (*Triticum durum*) and its Wild Relatives *Aegilops* spp. to Russian Wheat Aphid [(*Diuraphis noxia*(Mordvilko)(Homoptera:Aphididae)] and Use of Random Amplified Polymorphic DNA(RAPD) analysis to detect genetic variability in RWA

Plant Protection Department

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This study has been accepted as a Master Thesis in the field of Plant Protection on March 06, 1998, by the Jury members listed below:

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YÜKSEK LİSANS TEZİ

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Mohammed Izzat GHANNOUM

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Bu çalışma Durum buğdayı ve bunun yabani akrabalarından *Aegilops* türlerinin, Dünyanın bir çok ülkesinde hububatın önemli zararlısı olan Rus Buğday Afidi [(*Diuraphis noxia* (Mordvilko) (Homoptera:Aphididae)]'ne karşı dayanıklılığının belirlenmesi amacı ile gerçekleştirilmiştir. Aynı zamanda farklı ülkelerden getirtilen Rus Buğday Afidi'nin RAPD DNA analizi ile genetik varyasyonları belirlenmiştir.

Çalışma sonucunda dayanıklılığın antibiosis, antixenosis ve tolerans mekanizmalarında genotipler arasında önemli farklılıkların olduğu ortaya konulmuştur. Rus Buğday Afidi RAPD DNA analizi ile % 75 benzerlik serisinde 9 genetik gruba ayrılabileceği bulunmuştur.

Anahtar Kelimeler : Buğday, *Diuraphis noxia* ,dayanıklılık, genetik varyasyon

ABSTRACT

M.Sc. THESIS

SOURCES OF RESISTANCE IN DURUM WHEAT (*Triticum durum*) AND ITS WILD RELATIVES *Aegilops* spp. TO RUSSIAN WHEAT APHID [*Diuraphis noxia* (Mordvilko)(Homoptera:Aphididae)] AND USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS TO DETECT GENETIC VARIABILITY IN RWA

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This study was carried out to identify sources of resistance and mode of resistance in durum wheat and its wild relatives *Aegilops* spp. to Russian wheat aphid [*Diuraphis noxia* (Mordvilko) (Homoptera:Aphididae)], one of the most serious pest of cereals in several parts of the world, and to study the genetic variability in RWA populations from different countries using Random Amplified Polymorphic DNA (RAPD) analysis to detect genetic variability in RWA.

The results of the study showed significant differences among genotypes at the three mechanisms of resistance measurement antibiosis, antixenosis, and tolerance. Based on RAPD analysis of RWA, all the populations could be classified in to nine genetic groups at 75 percent similarity levels.

Key words : Durum wheat, Diuraphis noxia, resistance, genetic variation.

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APPREVIATION

°C : Centegrade

CTAB : Cetyltrimethylammonium bromide

CIMMYT: International Maize and Wheat Improvement Center

cm : Centemeter

DNA : Oxy Ribose Nuclic Acid

EDTA : Disodium Ethylenediamimine Tetraacetate

GS : Growth Stage

h : Hour

ICARDA: International Center for Agricultural Research in the Dry Areas

INRA : National Institute of Agricultural Research

IPM : Integrated pest Managment

(L:D) : Light : Dark

LSD : Least Significant Different

mM: m Molar

OP: Operon Primer

PCR : Polemerase Chain Reaction

pH : The Negative Logarithm of Hydrogen

RAPD: Random Amplified Polymorphic DNA

RCB : Randomized Comple Block

RFLP : Restriction Fragment Length Polymorphism

rpm : Revolution Per Minute

RWA : Russian Wheat Aphid

µl : Microlitre

µM: Micromole

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1. INTRODUCTION

Durum wheat covers 10% of the total wheat area in the world. About 50% of this area is in the developing countries. Ninety percent of the world's durum wheat area is found in the Mediterranean region (Nachit, 1992). Durum is a traditional crop in the region; its grain is used for several end products, e.g., pasta, couscous, burgul, and frike (roasted green wheat), and in some areas is preferred for home bread-making. High and stable yielding germplasm with stress resistance and good grain quality is the main objective of the CIMMYTand ICARDA joint durum wheat breeding program. Several biotic stresses limit durum wheat production in the region, of which Russian Wheat Aphid, (RWA) *Diuraphis noxia* Mordvilko, is an important one. This insect is one of the most important insect pest of Cereals in many parts of the world particularly in the dry areas. RWA originally collected from the Caucusus region, and then has been reported in USA, Chile, Iran, Canada, Ethiopia, Australia, China and most countries bordering the Mediterranean.

RWA feeds on new growth especially at the base of the new leaves, causes longitudinal chlorotic streaks, spike deformation, convolute leaf rolling and stunting in the host plant (Gilchrist et al., 1984). This results in lower grain yield and poor grain quality. RWA injects phytotoxins during feeding. These phytotoxins causes breakdowns of chloroplast (Fouche et al., 1984). RWA has been reported as a vector of viruses in South Africa, spreading barley yellow dwarf, brome mosaic, and barley stripe mosaic (Von Wechmar et al., 1991).

RWA often causes economic losses in cereals in the absence of control measures. Miller and Haile (1988) reported 41 to 71% yield loss in barley and 60% yield loss in wheat from small plots in Ethiopia. Yield losses between 35 and 60% have been reported in South African wheat tests (Du Toit and Walters, 1984). Aalbersberg et al (1989) reported 21 to 92% yield loss in wheat in South Africa. Economic losses from the RWA in the United States were more than 200 million US$ (Burd et al., 1993). Insecticides are not generally effective in controlling RWA, because of the insect's habit of secluding itself within the rolled leaves.

Host plant resistance offers one of the few economical control methods that could reduce yield losses due to feeding by RWA. Since 1984, increasing efforts have been made to find sources of resistance to this pest in wheat and barley germplasm. Butts & Pakendorf (1984) and Du Toit & Van Niekerk (1985) demonstrated that potential RWA resistance exists in the ancestral wheat species *Triticum monococcum*, *T. timopheevi*, *T. dicoccoides*, and *Aegilops squarrosa*. Resistance to RWA has also been identified in *T. aestivum* lines in the ex USSR. Three resistant bread wheat lines PI 137739, PI 262660, and PI 294994 were identified in South Africa in 1986 (Du Toit, 1987 ; 1988). Webster .et al. (1993) and Mornhinweg et al. (1995) identified RWA resistant barley genotypes. Kindler & Springer (1991) reported resistance among wild *hordeum* species. So far, no resistance to RWA has been identified in durum wheat (Nachit, Pers. Comm.).

Evolution of new biotypes in RWA is one of the major concern when developing host plant

resistance. Populations within an insect species that have the ability to damage plant entries normally resistant to that insect are considered as a new biotype (Puterka and Burton, 1991). This phenomenon commonly occurs when aphids are managed with host plant resistance. Differences among the individual insects in survival caused by the host plant resistance result in natural selection. If the insect populations harbor genetic variability that circumvent control measures, then evolution of virulent biotypes is virtually inevitable. Therefore, an understanding of the genetic variability among populations is an essential step in identification and deployment of suitable resistance genes. Although a variety of genetic markers can be used to study variability in insect populations (Via, 1990; Munstermann and Conn, 1997), few have been definitive for RWA. For example, Puterka et al. (1993) found that isozyme profiles of RWA populations differ little among populations. On the other hand, they found that PCR-based technique namely, Random Amplified Polymorphic DNA (RAPD) can be efficiently used to detect genetic variability among geographically diverse populations of RWA. The objectives of this study were to:

1 -Identify sources of resistance in durum wheat and their wild relatives and determine the mechanisms in these identified sources.

2 -study genetic variability in RWA populations from Lebanon, Syria, Morocco, and USA, using a PCR-based method namely, Random Amplified Polymorphic DNA (RAPD) analysis.

2. LITERATURE REVIEW

2.1. Description

RWA is a small (less than 2.3 mm), spindle-shaped aphid, pale yellow-green to grey-green in color with a dusting of fine mealy wax (Stoetzel, 1987). The antennae are short and the cornicles are vestigial. A supra caudal process on the eighth abdominal tergite in combination with the cauda gives a characteristic "forked tail" appearance when viewed from the side. RWA is probably the most easily identified aphid in small grains because of its unique shape as well as the symptoms it produces on the plants. Scouting for plant damage symptoms is the easiest method to detect RWA in the field. Damage appears as white to reddish streaks running the length of the leaves, which may be tightly rolled. Plants are usually stunted. The aphid most easily confused with RWA is the greenbug, *Schizaphis graminum* (Rondani). This species is similar in color to RWA, but has cornicles present and may have a dark green stripe running the length of its back. However, this characteristic is lacking in some biotypes of the greenbug. Greenbug damage appears as generalized red or yellow areas on the leaf where its feeding has destroyed the chlorophyll. Greenbug tends to feed on the upper surface of the leaf anywhere along its length, and doesn't cause leaf rolling, whereas RWA tend to feed at the base of the youngest leaf or within curled leaves prior to heading.

2.2. Distribution

Reports of a serious aphid pest of field crops in the southern Caucasus are found in Russian publications dated before 1900. This aphid was identified by Mordvilko and named *Brachycolus korrotnewi* in 1900. In 1912, Kurdjumov recognized that the "barley aphid" was a different species, which he named *B. noxius*. Subsequently, the genus was renamed *Diuraphis* (Aizenberg). Several other synonyms exist (Stoetzel, 1987).

Kovalev et al. (1991) detailed the history of D. noxia's spread in Russia beyond its primary range within the Iran-Turkestan mountains. One of the first reports on the pest status of RWA outside this range was Alfaro (1947), who documented it as a pest of wheat *(Triticum aestivum* L.), and barley *(Hordeum vulgare* L.), in Spain. There were few reports from thereon until the aphid suddenly appeared as a serious pest of wheat in South Africa in 1978 (Walters et al., 1980). and reached United State in 1987 (Webster et al., 1987). It reached Chile in 1987 (Zerene et al., 1988) and Canada in 1988 (Morrison, 1988). Its distribution also includes Ethiopia (Haile and Megenasa, 1987), the Middle East, Central Asia, North Africa, southern Europe (Blackman and Eastop, 1984), and areas as far east as Xinjiang Autonomous Region in northwestern China (Zhang, 1991). Hughes and Maywad (1990) using the CLIMEX computer model showed the potential areas of spread by this pest and the already infested areas.

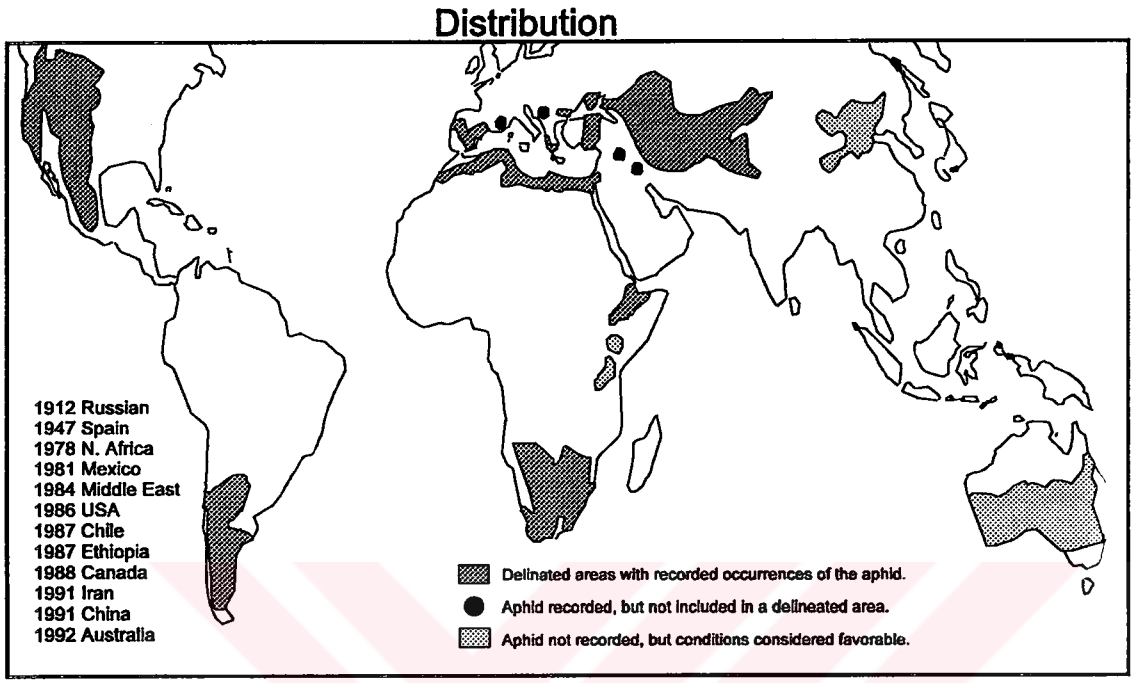

Figure 1. Areas of the world delinated by the CLIMEX model as favorable for *D. noxia*.

2.3. Environment

Grossheim (1914) studied the influence of temperature and humidity on the aphid. The aphid favors dry, warm conditions, but is able to survive heavy rains(Araya and Fereres, 1991), High temperatures such as those prevailing in Yemen (Erdelen, 1981), and temperature as low as -20°C recorded in Alberta (Butts, 1992). The aphids life span was shown to decrease with increasing temperature from 5 to 30°C, but nymph production was shown to increase from 5 to 20°C (Michels and Behle 1988). Nymph production peaks at about four per day, and may exceed 70 in a typical lifetime. Aalbersberg et al.(1987) found that high temperatures (around

20°C) induced rapid development and early production of offsprings. Webster and Starks (1987) studied RWA fecundity at three constant temperature regimes, and Kieckhefer and Elliott(1989) at fluctuating temperatures.Their conclusions were that RWA was capable of rapid population growth under a range of temperatures likely to be encountered during the small-grain growing season in states of the western USA. Webster et al. (1993a), comparing RWA from Stillwater, Oklahoma and Bethlehem, South Africa, established no difference in fecundity between aphids from the two origins at 15°C, but at 25°C aphids from Stillwater had lower fecundity and their rate of reproduction peaked sooner than for the aphids from Bethlehem. Alternating temperatures were suggested to be better for rearing the aphid than constant temperatures. Aalbersberg et al. (1989) reported logarithmic population build-up of RWA in the field, in South Africa, and its deleterious effect on wheat yield.

Girma et al. (1990) showed that reproduction on wheat was optimal at the jointing stage. Girma et al. (1993) found that wheat plants are particularly sensitive to RWA at any growth stage before heading, even at initial infestations as low as three aphids per plant. Susceptibility is increased if the plants are also drought stressed (Riedell 1989) or under nitrogen stress (Riedell 1990). Burd el al. (1993) found that RWA caused a reduction in leaf turgor, which was closely related to the degree of chlorosis of the leaves. Burd and Burton (1992) reported that wheat plants rapidly recovered from the effects of infestation after removal of the aphids, and that duration of infestation was more important than the level of infestation. Baugh and

Phillips (1991) established a curvilinear relationship between water potential in the host-plant and alate production. Vernalization was not shown to affect the performance of RWA, but Thomas and Butts (1990) showed that, in Canada, infestation of winter wheat in the autumn reduced the crop's freezing tolerance, and increased the likelihood of winter-killing by severe cold. Hammon and Peairs (1992) found that RWA overwintered in Colorado on south-facing slopes of furrow-irrigated fields.

2.4. Feeding Habits

Typically, the aphid feeds at the bases of newly formed leaves and floral parts of barley and wheat. It also infests rye, *Scale cereals* L.; triticale and a range of grasses (Kindler and Springer, 1989).

The new leaves of susceptible plants do not unroll and the spike becomes deformed and the plant itself stunted (Robinson, 1992b ; Burd and Burton 1992). It was suggested that a toxin is introduced into the plant when RWA feeds, which appears to destroy the chloroplast membrance and be assocated with development of longitudinal chlorotic streaks (Fouche et al., 1984), and ensuing reduction in photosynthetic capacity. Von Wechmar and Rybicki (1981) reported that RWA transmits three plant viruses. On depletion of its food source, and under adverse environmental conditions, viviparous winged females are produced in the colonies, and are then borne by the wind to initiate new colonies.

2.5. Damage and yield losses

RWA is devastating because of its direct injury to cereal plants and the effect of the phytotoxin it injects during feeding, which causes breakdowns of chloroplasts. Fouche et al. (1984), found that a reduction of the chlorophyll in infested leaves of up to 85% results in yield reductions of 25-50%. Kruger and Hewitt (1984), found that if RWA feeding occurs in the flag leaf, a contorted grain head interfering with head extension and self-pollination results. The physiological effects of RWA feeding in many ways mimic drought stress, even in the presence of ample soil moisture, and limit the ability of plants to adjust to soil moisture deficit (Riedell, 1989). Hewitt (1988) reported a 21 to 92% yield loss of wheat in South Africa. In 1988 wheat production losses in the United States due to RWA damage were 130 million US$ (Anonymous, 1989). By 1989, economic losses from RWA in the United States were more than 200 million US$ (Burton,1989). In the U.S the cumulative economic loss from 1987 through 1990 attributed to RWA exceeds 657 million US$ with over 70 million US$ being spent on control, 250 million US$ in lost production, and 325 million US$ in additional lost economic activity to local communities (Massey and Amosson., 1991).

In 1990, 100 U.S. scientists were working on the aphid and 3 million US$ were invested in their research (Kessler, 1991). Archer and Bynum (1992) reported on economic injury levels in wheat in Texas, concluding that a yield loss of 0.48% ensued from each 1% of infested tillers.

2.6. Control Methods

2.6.1. Chemical Control

Option for chemical management of RWA has been relatively restricted due to the aphid's habit of feeding deep within the leaf whorl and rolled leaves. Contact insecticides have not generally been effective in controlling the aphid, although chlorpyrifos vaporizes is able to penetrate rolled leaves(Hill and Butts, 1991, Hill et al., 1993). There was an interest in developing insecticide, Imidaclorprid(NTN), a low-toxicity, broad spectrum, systemic insecticide with good potential for seed and soil application (Anderson, 1990; Brenchley, 1990). There is the inevitable risk, however, that in using insecticides to control this aphid, resistance may develop in the aphid. This will increase the difficulty of effective management in the future.

2.6.2. Biological Control

RWA has become established in countries far from its primary range, without its naturally occurring biological enemies (Table 1) . There have been collecting expedition to areas where the aphid appears to be satisfactorily controlled in nature, to collect predatory and parasitoids (Gonzalez et al.,1990;Mckinnon et al., 1992). Hymenopterous parasitoids, aphidophagous coccinellids, predatory bugs, and predacious fly and midge larvae have been sought. Robinson (1992a) documented the principle predators such as the lacewing and parasitoids occur in the

High Valley of Mexico. Naranjo et al. (1990) researched the biology of *Scymnus frontalis* (F.) for its use in the USA to control RWA. Although able to feed within rolled leaves. Many of the predators and parasitoids native to the area of origin of RWA are also found in countries where the aphid has become newly established. Stary and Gonzalez (1991) investigated the possibility of using the parasitoid wasp *Diaeretiella rapae* (m'Intosh) for RWA control, and Reed et al. (1992) provided life table for this wasp. In addition to aphidophagous insects, there is an interest in the use of entomopathogenic fungi for the control of RWA. Feng and Johnson (1991) and Feng et al.(1991) found that Zoophthora, Conidiobolus, and Pandora, species had a significant effect on the population build-up of cereal aphids, including RWA. Wang and Knudsen (1993) studied the effect of *Beauveria bassiana* (Bals), on the reproduction of RWA and found that the fungus increased mortality. Wilson et al. (1991) and Clement et al. (1992) reported that endophytic fungi adversely affect RWA.

Table 1. List of natural enemies of RWA from Syria and other Mediterranean rim countries.

Family	Genus / Species	Location
Aphidiidae	Aphidius colemani **Viereck**	Syria
Braconidae	*Diaeretiella rapae* **M'Intosh**	Syria
"	*Aphidius ervi* **Haliday**	Greece
"	Praon sp.	Syria, Greece, France
"	Lysiphlebus ambiguus **(Haliday)**	Greece

Braconidae	Aphidius matricariae **Haliday**	Turkey, Greece
"	Aphidius rhopalosiphi **D.Perez**	Turkey, Spain
"	Aphidius uzbekistanicus **Luzhetski**	Greece
"	Aphidius spp.	France, Spain
"	Ephedrus plagiator **(Nees)**	Turkey, France
"	Praon gallicum **Stary**	France
"	Praon volucre **(Haliday)**	Turkey, France
Coccinellidae	*Coccinella sp.*	Syria
"	*Coccinella septempunctata* **L.**	Syria
Chamaemyiidae	*Leucopis sp.*	*Syria*
Encyrtidae	Aphelinus asychis **Walker**	Turkey
Encyrtidae	Aphelinus nr. Varipes	Turkey, France, Greece
Encyrtidae	Aphelinus spp.	France
Syrphidae	Syrphus sp.	Syria

2.6.3. Host Plant Resistance

Wheat , barley and triticale are the preferred small-grain hosts (Walters et al., 1980). Kindler and Springer (1989) found that jointed goatgrass (*Triticum cylindricum*) was the most suitable host, followed by barley, European dunegrass (*Elymus arenarius* L.) and little barley (*Hordeum pusillum* Nutt.). Webster et al. (1987) found that Russian wheat aphids could damage other cereal grains including oats (*Avena sativa* L.) and rye (*Secale cereale* L.) as well as kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.). They also reported that Russian wheat aphids caused little damage to corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum americanum* K.) , tall fescue (*Festuca arundinacea* Schreber.), and bermuda grass (*Cynodon dactylon* L.).

Host plant resistance offers one of the few economical control methods that could reduce yield losses due to feeding by RWA. Since 1984, increasing efforts have been made to find sources of resistance to this pest in wheat and barley germplasm. Butts and Pakendorf (1984); Du Toit and Van Niekerk (1985) demonstrated that potential RWA resistance exists in the ancestral wheat species *Triticum monococcum*, *T. timopheevi*, *T. dicoccoides*, and *Aegilops squarrosa*. Resistance to RWA has also been identified in *T. aestivum* lines from Soviet Union. Two resistant bread wheat lines PI 137739, PI 262660 and PI 294994 were identified in South Africa in 1986 (Du Toit, 1987 ; 1988). Webster .et al., (1993b) and Mornhinweg et al. (1995)

identified RWA resistant barley genotypes. Kindler and Springer (1991) reported resistance among wild *Hordeum* species. So far, no resistance to RWA has been identified in durum wheat.

2.7. Genetic variability in insect population

Genetic variation in insect population provides a means of identifying the origin of newly introduced populations (Tomiuk et al., 1979; Singh and Cunningham, 1981; Loxdale et al., 1983). Identifying the breeding structure of the introduced insect species is necessary to understand and monitor genetic changes that occur as populations adapt to new regions (Puterka et al., 1993). The use of allozymes as genetic markers to study the breeding structure of insect populations began in the late 1950s and continues to be widely applied.

However, there are a number of insect groups for which surveys have found little or no electrophoretically detectable genetic variation. Low allozyme variability is notable throughout the Hymenoptera (Graur, 1985). and in the Aphididae (Homoptera). Apparent low genetic variability in the Hymenoptera has been attributed to deleterious and lethal recessive being exposed directly to selection in the haploid male. Very few polymorphic loci have been detected in the pea aphid, *Acyrthosiphon pisum* (Harris) (Suomalainen et al., 1980; Simon et al., 1982); the rose aphid, *Macrosiphum rosae* (Linnaeus) (Tommiuk & Wohrmann, 1984); the English

grain aphid, *Sitobion avenae* (Fabricus) (Loxdale et al., 1985a,b); the birdcherry-oat aphid, *Rhopalosiphum padi* (Linnaeus) (Loxadale & Brookes, 1988); the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Steiner et al., 1985a); the green peach aphid *Myzus persicae* (Sulzer) (May & Holbrook, 1978; Wool et al., 1978; Brookes & Loxadale, 1978); or in the greenbug, *Schizaphis graminum* (Rondani) (Steiner et al., 1985b; Beregovy & Starks, 1986; Abid et al., 1989). The low level of allozyme variation found in the Aphididae have not been explained , although low variability is found generally in cyclical thelytokous species (Nevo et al., 1984).

Very little genetic variability revealed by allozyme electrophoresis limit the use of this technique in studying genetic variability of aphid population. During last decade, several molecular marker systems have been developed which can detect genetic variation directly at DNA level (Via, 1990; Munstermamn and Conn, 1997). One such technique is Restriction Fragment Length Polymorphism (RFLP). Substantially more genetic variation has been detected with this technique in mitochondrial DNA and genomic DNA with multicopy probes (Carvalho et al., 1991; Shufran et al., 1991). Since middle of last decade, techniques using the polymerase chain reaction (PCR) to amplify specific genomic regions have become widespread (Saiki et al., 1985, 1988; Mullis and Faloona, 1987). Most of these techniques involve using primers that flank and amplify specific regions of a genome. Recently, Williams et al. (1991) described the development of a technique that uses a single 10bp primer to amplify arbitrary

regions of a genome PCR. The polymorphism at these arbitrary regions is termed as Random Amplified Polymorphic DNAs (RAPDs). RAPD analysis is technically simple, fast, large number of samples can be handled in a short period of time and requires only small quantities of DNA. Using this analysis, they demonstrated that the RAPD process reveals polymorphisms in the genomes of wide variety of different species. Some of these regions are repetitive, whereas others arise from unique. Using RAPD analysis, Black et al. (1992) showed substantially high level of polymorphism in several aphids and their parasitoids. Subsequently, in RWA Puterka et al. (1993) showed that RAPDs are much more efficient than isozymes in detecting genetic polymorphism among populations of USA, South Africa, Mexico, France and Turkey.

3. MATERIALS AND METHODS

3.1. Russian Wheat Aphid Rearing

Russian wheat aphid is reared in a greenhouse maintained at a temperature between 15 and 25 °C, relative humidity of 70% ±5% and a photoperiod of 14:10 (L.D). The greenhouse is located at Tel Hadya ICARDA's headquarters in Syria. Haurani, a local susceptible wheat cultivar, was used as the host for rearing the aphid. It was grown in plastic pots 17 cm diameters in a soil mixture (soil: peat:sand, 2:1:1). Seeds of wheat were sown in hills around the edge of each pot at the rate of approximately 15 seeds per pot, and kept on a metal table (1m x 3m) which is caged (1mx1mx1m) with fine mesh on a metal frame. Pots were watered twice per week. The Russian wheat aphid was harvested from stock colonies and used to infest plants in both the greenhouse and the field experiments. RWA colonies were allowed to develop for three weeks until they started to reproduce. The aphids were shaken off the leaves onto large paper sheets with talcum powder. When needed, they were transferred to the field in a cooling box.

3.2. Initial Evaluation

3.2.1. Field evaluation

Plant materials for testing were sown at Tel Hadya farm, on late November. Seeds were planted in hill plots five seeds per hill and 50 cm apart. 144 Advanced durum genotypes with

crosses made between durum and its wild relatives developed in the CIMMYT/ICARDA durum breeding program (Nachit, 1994) were planted using a Lattice design, and the 72 lines of advanced durum wheat lines were planted using RCB experimental design. Plants were infested at GS 11 with 50 to 60 insects per hill in two applications, using an aphid's pazoka, corresponding to about 10 aphids per plant. Scoring was done three times during the season, at tillering (GS 20), jointing (GS 30), and post-heading (GS 60), using an ascending damage rating scale described by Du Toit (1987), from one to six, where 1 = Small isolated chlorotic spots on the leaves; 2 = Larger chlorotic spots on the leaves; 3 = Chlorotic spots tend to become streaky; 4 = Mild streaks visible and leaves tend to roll lengthwise; 5 = Prominent white / yellow streaks present, leaves tightly rolled; 6 = Severe white / yellow streaks, leaves tightly rolled and start to die.

3.2.2. Greenhouse evaluation

The same germplasm was planted in a plastic house at ICARDA, and the same infestation and evaluation procedure for the field test was used, except that only two aphids per plant were used.

3.2.3. Advanced evaluation

After the initial evaluation, those accessions that showed low level of infestation were selected for advanced evaluation. Ten replications of each entry, including the susceptible

control were seeded in flats, using a randomized complete-block design. Five seeds were planted per hill, and upon emergence the number of plants was thinned to three. The evaluation procedure was the same as described above. All data from previous experiments were analyzed using MSTAT-C(Michigan State University Version 1.42).

3.3. Mechanisms of resistance

The entries that showed good level of resistance (score≤3 in Du toit scale), were studied for mechanisms of resistance. These lines are the following:

1- *Aegilops biuncialis* 400004.

2- Haucan/Aeg. 400020//omtel-1/3/omlahn DPT96 314.

3- RSP car DPT96 204.

4- *Aegilops ovata* 401650.

5- *Aegilops ovata* 401650.

6- Susceptible check (Korifla).

3.3.1 Antibiosis

Five seeds of each genotype were sown in a standard greenhouse soil mixture in plastic pots (17 cm in diameter). An RCB design was used with 10 replications. After emergence, plants were thinned to one seedling per pot. At one leaf stage, each individual leaf seedling was infested by five adult aphids from the plastic house culture. The plants were covered with a

plastic cage (15 cm in diameter by 30 cm high) with a cloth screen top and screened ventilation holes on sides. Plants and aphids were observed daily, and the adults removed shortly after five nymphs were present. The nymphs were held on the test plant until they matured and began to reproduce. At this time all the aphids, but one were removed from the plant, and the number of nymphs recorded daily until the adults stopped reproducing.

3.3.2. Antixenosis

In this test, the genotypes were planted in standard greenhouse soil mixture in plastic pots (17 cm in diameter). Three seeds of each of the six genotypes were planted on the edge of pots, and thinned to one plant per genotype. The experimental design used was an RCB with 10 replications. At one leaf stage when plants were 5-6 cm high, 60 adult apterous aphids were released in the center of each pot. The number of aphids per plant was recorded at 24, 48 and 72h intervals.

3.3.3. Tolerance

Three seeds from each entry were planted in a plastic pot. Upon emergence, seedlings were thinned to one per pot. Plant height from the soil surface of a seedling was recorded before infestation, and plants with similar heights were paired together. For each pair, each plant was infested with ten apterous adult aphids of various ages, and the other plant served

as a control. All plants were covered with ventilated plastic cages (15 cm in diameter and 35 cm long). The experimental design was a randomized complete block with three replications. Plants were monitored at 48 h intervals. Aphids were removed or added to maintain the level of 10 adults per plant, and the experiment was terminated 21 days after infestation. Plant height was recorded for both infested and control plants. Damage rates were assigned to infested plants using DuToit scale. The infested and control plants were cut at soil level, oven-dried (70-75°C) for three days, and then weighed.

3.4. Genetic variability

3.4.1. Sampling of populations

The RWA used for this study were collected from wheat and barley from 4 countries. Six populations were collected from 6 locations in Lebanon, Ryak, Ayat, Kfar Dan, Tal Amara, Shams Tar and Ghazza, and one population of Rose wheat aphid *Metopolophium dirhodum* was collected from Haush Harim, Lebanon. Two populations were collected from Ein Sharqi and Mosiaf in Syria, and one population was sent from INRA (National Institute of Agricultural Research, Settat, Morocco). The fourth collection used in this study was from USA, collected and shipped by Ms. Aparna Telang from Nebraska, USA. All RWA populations were shipped in 100 % ethyl alcohol. Twenty five aphids from each collection were collected and stored in deep freezer at -82° C for DNA extraction.

Table 2. Populations of *D.noxia* and *M.dirhodum* collected for RAPD analysis

Sample No.	Species	Origin/site
1-	*D.noxia*	Nebraska, USA
2-	*D.noxia*	Settat, Morocco
3-	*D.noxia*	Ryak, Lebanon
4-	*D.noxia*	Ayat, Lebanon
5-	*M.dirhodum*	Haush Harim, Lebanon
6-	*D.noxia*	Kfar Dan, Lebanon
7-	*D.noxia*	Tal Amara, Lebanon
8-	*D.noxia*	Shams Tar, Lebanon
9-	*D.noxia*	Ghazza, Lebanon
10-	*D.noxia*	Ein Sharqi, Syria
11-	*D.noxia*	Mosiaf, Syria

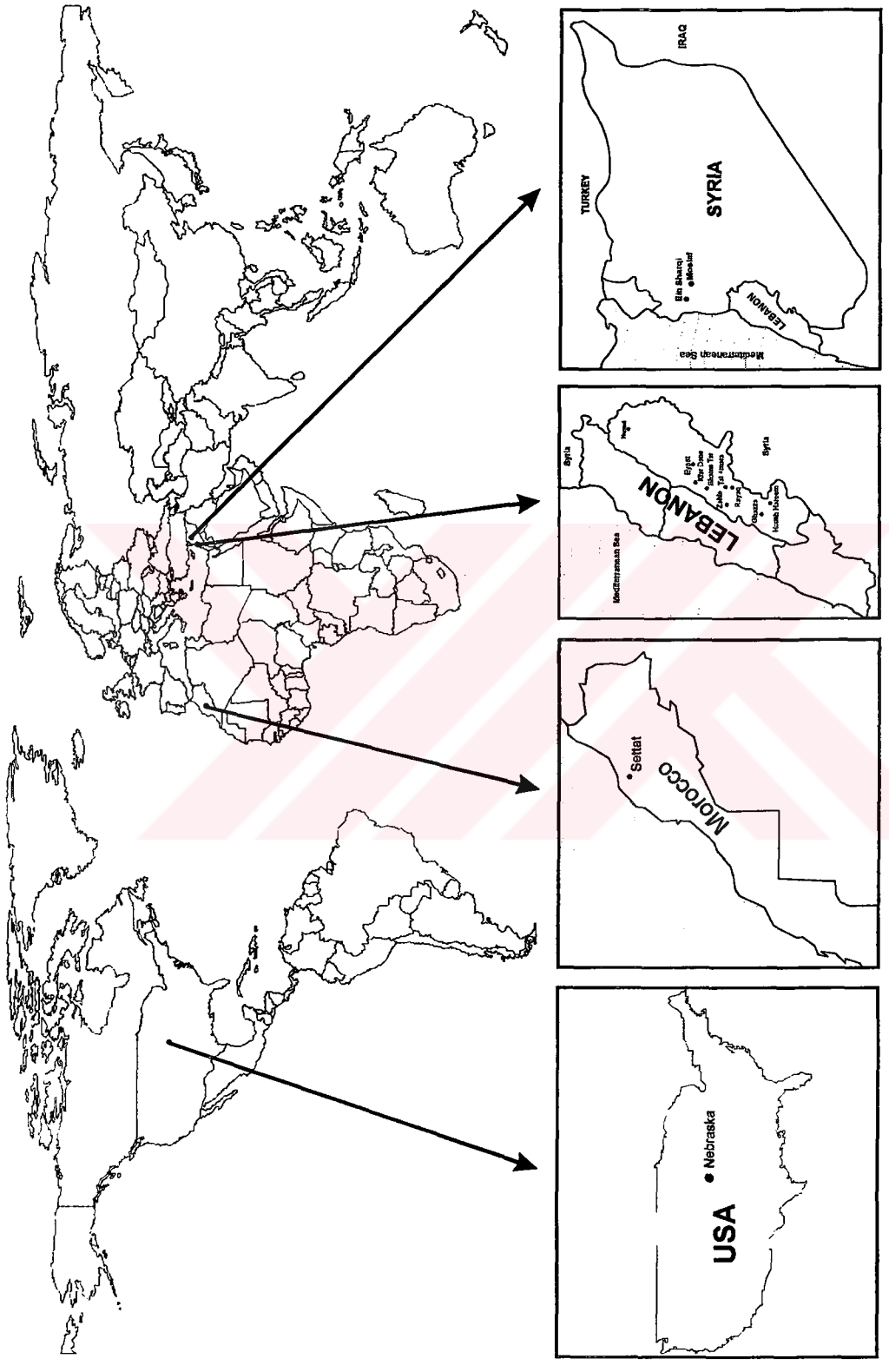

Figure 2. Map showing sampling sites of different geographical population of RWA and *M.dirhodum* were collected for RAPD-PCR

3.4.2. Extraction of DNA

DNA extraction protocol adapted from Weigand et al.(1993), with few modifications. 25 insects per population were bulked to obtain a balanced of conservation and moderately variable characters for detection by RAPD analysis. The insects, randomly selected from each population, were ground with 500 µl of ice cold extraction buffer (5 nM ascorbic acid, 1.4 M NaCl, 20 mM EDTA, 2% cetyltrimethylammonium bromide [CTAB] and 100mM Tris [pH 7.5]) in a microfuge tube using a miniature pestle. Samples were incubated at 57° C for 1 hour. After the incubation the samples were extracted with one-third volume of chloroform: isoamyl alcohol mix and centrifuged at 12000 rpm (Effendorf centrifuge) for 15 min to separate phases. The aqueous phase was collected and 0.1 volume of 5M potassium acetate and 0.66 volumes of cold, 2-propanol were added. After holding at -20 °C overnight, DNA pellets were obtained by centrifugation at 14,000 rpm (Effendorf centrifuge) for 15min, rinsed in 70% ethanol, dried briefly, and subsequently dissolved in 75 µl of 25mM Tris (pH 7.5) buffer overnight at 4 °C. The isolated DNA was quantified by spectrophotometer (A260nm). The quality of isolated DNA was tested by agarose gel electrophoresis (Weigand et al., 1993).

3.4.3. RAPD-PCR analysis

Forty two primers (kits C, E and F) obtained from Operon Technologies (Alameda, California, USA), were tested on 11 bulk population. Sequence of these primers are presented

in Table 3. Only the results of 21 primers which produced well separated and reproducible banding pattern were scored for polymorphism and analyzed further. DNA amplifications were performed in 20 µl reaction containing 1x PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$ and 0.001% gelatin), 200 µM each of dATP, dTTP, dGTP and dCTP, 5 picomoles of a single 10-base primer, 25 ng of genomic DNA, and 0.75 unit of *Taq* DNA polymerase (Boehringer Mannheim, Germany). Amplifications were performed in a thermocycler (Perkin Elmer, 9600) programmed for 4 min at 94° C; for 40 cycles of 30 sec each at 94°C, 1 min at 36°C and 2 min at 72°C; and a final extension of 10 min at 72°C. The reaction mix without template DNA served as control and was electrophoresed along with the samples. Amplified DNA products were loaded onto 1.5% agarose gels and electrophoresed in 1x TAE (0.04M Tris-acetate, 0.001M EDTA). Gels were stained with ethidium bromide (0.5 µg/ml of distilled water) and photographed under UV light using Image analyzer (Egal eye, Stratagene, USA).

Table 3. Operon primer number and primer sequence used for RAPD analysis of aphid populations:

Primer no.	Sequence (5'- 3')	Primer no.	Sequence (5'- 3')	Primer no.	Sequence (5'- 3')
OPC-01	TTCGAGCCAG	OPE-06	AAGACCCCTC	OPF-01	ACGGATCCTG
OPC-02	GTGAGGCGTC	OPE-07	AGATGCAGCC	OPF-02	GAGGATCCCT
OPC-03	GGGGGTCTTT	OPE-08	TCACCACGGT	OPF-03	CCTGATCACC
OPC-05	GATGACCGCC	OPE-11	GAGTCTCAGG	OPF-04	GGTGATCAGG
OPC-06	GAACGGACTC	OPE-12	TTATCGCCCC	OPF-06	GGGAATTCGG
OPC-07	GTCCCGACGA	OPE-13	CCCGATTCGG	OPF-07	CCGATATCCC
OPC-08	TGGACCGGTG	OPE-14	TGCGGCTGAG	OPF-08	GGGATATCGG
OPC-09	CTCACCGTCC	OPE-15	ACGCACAACC	OPF-10	GGAAGCTTGG
OPC-10	TGTCTGGGTG	OPE-17	CTACTGCCGT	OPF-11	TTGGTACCCC
OPC-11	AAAGCTGCGG	OPE-18	GGACTGCAGA	OPF-14	TGCTGCAGGT
OPC-12	TGTCATCCCC	OPE-19	ACGGCGTATG	OPF-15	CCAGTACTCC
OPC-13	AAGCCTCGTC			OPF-16	GGAGTACTGG
OPC-14	TGCGTGCTTG			OPF-18	TTCCCGGGTT
OPC-17	TTCCCCCCAG			OPF-20	GGTCTAGAGG
OPC-18	TGAGTGGGTG				
OPC-20	ACTTCGCCAC				

3.4.4. Data analysis

The fragment pattern of each sample derived from RAPD analysis was coded in binary form, i.e., as 1's and 0's, representing the presence and absence of each fragment, respectively. Nei's genetic distance (a dissimilarity index ; Nei, 1972) was computed from the binary data for all pair-wise combinations of samples. The unweighted pair-group method for the arithmetic average (UPGMA; Sneath and Sokal, 1973) and neighbor joining (both mid-point and out-group) method (NJM; Saitou and Nei, 1987) were used to estimate phenogram and phylogram, respectively, on the basis of the distance matrix data. All the data analysis was performed using the software package NTSYS-pc (Rohlf, 1993).

4. RESULTS AND DISCUSSION

4.1. Mechanisms of Resistance

4.1.1. Initial and Advanced Screening Tests

Of the 144 accessions of *Aegilops* and 72 lines of durum wheat screened in the initial tests, five *Aegilops* and 14 durum wheat showed resistance to RWA. These five accessions and 14 durum wheat showed various levels of resistance in the advanced screening test based on the DuToit Scale, the average damage ranged from 2.25 to 3.0. The highest levels of resistance were found in *Aegilops biuncialis*, *Aegilops ovata* and durum wheat lines Haucan/Aegilops Columnaris, RSP Car (Table 4&5). Most of the resistance germplasm has been found in barley, wheat, and its wild relatives using the same evaluation and damage score (Du Toit 1987, 1989; Webster et al. 1987; Formusoh et al. 1992, 1994). All these sources of resistance are being used by CIMMYT/ICARDA durum wheat program to develop RWA resistant varieties.

Table 4. Reaction of selected accessions of *Aegilops spp.* for resistance to RWA.

SPECIES/GENE	ORIGIN	DAMAGE SCORE
Aegilops biuncialis	SYR	2.25
Aegilops ovata	MAR	2.75
Aegilops ovata	Tifelt	2.75
Aegilops ovata	Sefrou	3
Aegilops biuncialis	SYR	3
Cham 5	APCB 95-10	5

Table 5. Reaction of selected genotypes of durum wheat for resistance to RWA.

NAME	PEDIGREE	DAMAGE SCORE
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	ICD91-0604-WABL-0AP-10AP-0AP-8AP-0TR	2.5
RSP car	RSP93-0006-2AP-0AP-2AP-0TR	2.75
Sbl1/Aeg.	IC400007ICD92-0437-WABL-0AP-1AP-0TR	3
Sbl1/Aeg.	IC400007ICD92-0437-WABL-0AP-9AP-0TR	3
Krf/BaladiaHamra//Krf/3/T.mon5566/4/Carzio	ICD92-0744-WABL-0AP-10AP-0TR	3
Rufom-5/T.Araraticum 500140//Carzio	ICD92-0764-WABL-0AP-1AP-0TR	3
RSP mono	RSP93-0004-4AP-0AP-2AP-0TR	3
RSP mono	RSP93-0004-4AP-0AP-3AP-0TR	3
Waha	(Check)	3
Mrb5/T.mon5221//Chah88/3/Omguer-1	ICD91-0579-WABL-0AP-2AP-0AP-4AP-0TR	3
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	ICD91-0604-WABL-0AP-10AP-0AP-7AP-0TR	3
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	ICD91-0604-WABL-0AP-11AP-0AP-2AP-0TR	3
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	ICD91-0604-WABL-0AP-11AP-0AP-3AP-0TR	3
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	ICD91-0604-WABL-0AP-11AP-0AP-6AP-0TR	3
Korifla	Susceptible check	3.5

4.1.2. Mode of resistance

Antibiosis :

Significant differences ($P<0.01$) were found among genotypes in antibiosis as measured by the number of nymphs produced per adult, which ranged from 4.2 nymphs from adults feeding on the *Aegilops biuncialis* 400004, to 54.1 nymphs on the Susceptible control Korifla (Table 6). The life span of RWA on *A. biuncialis 400004* was also significantly shorter (13.7 days) than that on the susceptible check (30.6 days). RWA resistance is most commonly expressed through antibiosis (Du Toit 1987,1989;Webster 1990;Formoush et al. 1992,1994). *A. biuncialis 400004* was the highly antibiotic entry to RWA, with a fecundity of 4.2 nymphs. Aphids feeding on antibiotic plants can experience reduced body size, delayed development (Panda 1979). Significant correlation between fecundity and life span was found among all entries in the antibiosis test.

Table 6. Fecundity, life span of RWA on durum wheat genotypes and *Aegilops* accessions.

Entry	Fecundity	Life span (days)
A. biuncialis 400004	4.2 a	13.7
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	26.6 b	24.6
RSP car	33.0 bc	26.6
A. ovata 401650	41.0 bc	29.9
A. ovata 401650	29.5 b	25.5
Susceptible check (Korifla)	54.1 c	30.6
LSD (0.01)	21.72	

Antixenosis:

There was a significant difference ($P<0.01$) among genotypes of the number of aphids/plant at 24h, 48h and 72h after aphid release. The least preferred line is Haucan/Aeg.400020//Omtel -1/3/Omlahn-3, which had about 4 aphids/plant across all three period tests, which is about 2/3 less than that of the susceptible check (Table 7.). Apparently, the aphid selected nearby plants soon after released and had not moved enough to show a behaviorally based preference at 48h. After 72h, some aphids had left the plants, resulting in a decrease in aphids per plant. The decrease in aphids on some entries was substantial and clearly illustrated in the entry *A. biuncialis* 400004; at 72h, the number of aphids decreased from 9.0 to 4.9.

Table 7. Antixenosis of RWA on durum wheat genotypes and *Aegilops* accessions.

Entry	Interval after infestation		
	24h	**48h**	**72h**
A. biuncialis 400004	11.7 b	9.0 b	4.9 ab
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	4.6 a	4.2 a	4.3 a
RSP car	10.3 b	9.7 b	8.9 bc
A. ovata 401650	10.0 b	10.7 b	10.1 c
A. ovata 401650	7.4 ab	7.6 ab	7.5 abc
Susceptible check (Korifla)	11.2 b	10.7 b	11.8 c
LSD value at (0.05)	4.34	4.65	4.34

Tolerance:

Significant differences ($p<0.01$) in tolerance to RWA were found among genotypes based on plant damage, % plant height and dry weight. Significant differences were detected between infested and noninfested tolerance test entries in plant growth and dry weight at the end of the test. Growth of infested accessions ranged from 9 cm with the *Aegilops ovata* 401650 to 25 cm with the durum line RSP car, with an average of 16.16, while that of the non-infested was 23.33.Similarly, dry weight of infested entries ranged from 0.20 g with *A.ovata* 401650 to 0.50 in the Korifla with an overall test average of 0.36 g. Damage rating in tolerance ranged from 1.4 in *A.biuncialis* and *A.ovata* 401650 to 2.9 in susceptible check based on D.toit score (Table 8.).The most tolerant entries were *A.biuncialis* and *A.ovata* 401650.Du Toit 1987, reported that PI 262660 also showed tolerance based on plant height and dry weight measurements. Tolerance as mechanism of resistance may provide resistance that is more stable than antibiosis and antixenosis (Smith 1989).

Table 8. Damage score and percent loss in plant height and dry weight of durum wheat genotypes and *Aegilops* accessions, due to RWA feeding.

Entry	Plant height			Dry weight			Damage score
	I	NI	%loss	I	NI	%loss	
A. biuncialis 400004	12	16	25.0	0.28	0.38	26.3	1.4
Haucan/Aeg.400020//Omtel-1/3/Omlahn-3	21	30	30.0	0.47	0.71	33.8	2.2
RSP car	25	37	32.4	0.43	0.85	49.4	2.5
A. ovata 401650	09	16	43.7	0.20	0.32	37.5	1.8
A. ovata 401650	10	13	23.0	0.33	0.42	21.4	1.4
Susceptible check (Korifla)	20	28	28.5	0.50	0.69	27.5	2.9
CV (%)	17.06			31.20			40.47

I = Infested plants, NI = Noninfested plants

4.2. Genetic Variability

4.2.1.Genetic variation among geographical populations of RWA

Previous studies in aphids have revealed that RAPD-PCR method is the most efficient method for assessing genetic polymorphism in insect populations than allozymes (Black et al., 1992; Puterka et al., 1993). Therefore in the present study we used only RAPD-PCR method to assess genetic variation prevailing among populations of RWA from Syria, Lebanon, Morocco and USA.

Initially, 42 different primers of arbitrary nucleotide sequence were used to amplify DNA segments (genetic fingerprints) from the genomic DNA (extracted from bulk of 25 individuals each) of 11 aphid (10 populations of RWA and one population of *M.dirhodum*) of the populations. Figures (3-4,7-24) show the amplification products of the 21 primers, which were resolved by agarose-gel electrophoresis and visualized by staining with ethidium bromide. Out of 42 primers, only 21 (50%) primers gave reproducible and satisfactory amplification of the PCR products among the 11 populations. Polymorphism revealed by different primers also varied greatly (Table 9). For instance, the primer OPC-09 amplified the highest number (8) of discrete polymorphic RAPD bands in 11 populations (Figure 3), while the primer OPC18 amplified only one polymorphic RAPD band (Figure 4). A total of 107 loci (bands) were amplified and all of them showed polymorphism at least among two populations.

Table 9. Operon Primer number, primer sequence and the number of polymorphic RAPD bands resolved by the aphids population.

Operon no.	Sequence (5'- 3')	No. of polymorphic RAPD bands (Loci)
OPE-08	TCACCACGGT	5
OPE-11	GAGTCTCAGG	7
OPE-12	TTATCGCCCC	7
OPE-14	TGCGGCTGAG	6
OPE-18	GGACTGCAGA	6
OPE-19	ACGGCGTATG	4
OPF-02	GAGGATCCCT	6
OPF-03	CCTGATCACC	6
OPF-04	GGTGATCAGG	5
OPF-06	GGGAATTCGG	4
OPF-08	GGGATATCGG	7
OPF-14	TGCTGCAGGT	5
OPC-01	TTCGAGCCAG	4
OPC-05	GATGACCGCC	7
OPC-06	GAACGGACTC	7
OPC-09	CTCACCGTCC	8
OPC-11	AAAGCTGCGG	4
OPC-13	AAGCCTCGTC	3
OPC-14	TGCGTGCTTG	3
OPC-17	TTCCCCCCAG	2
OPC-18	TGAGTGGGTG	1
Total		**107**

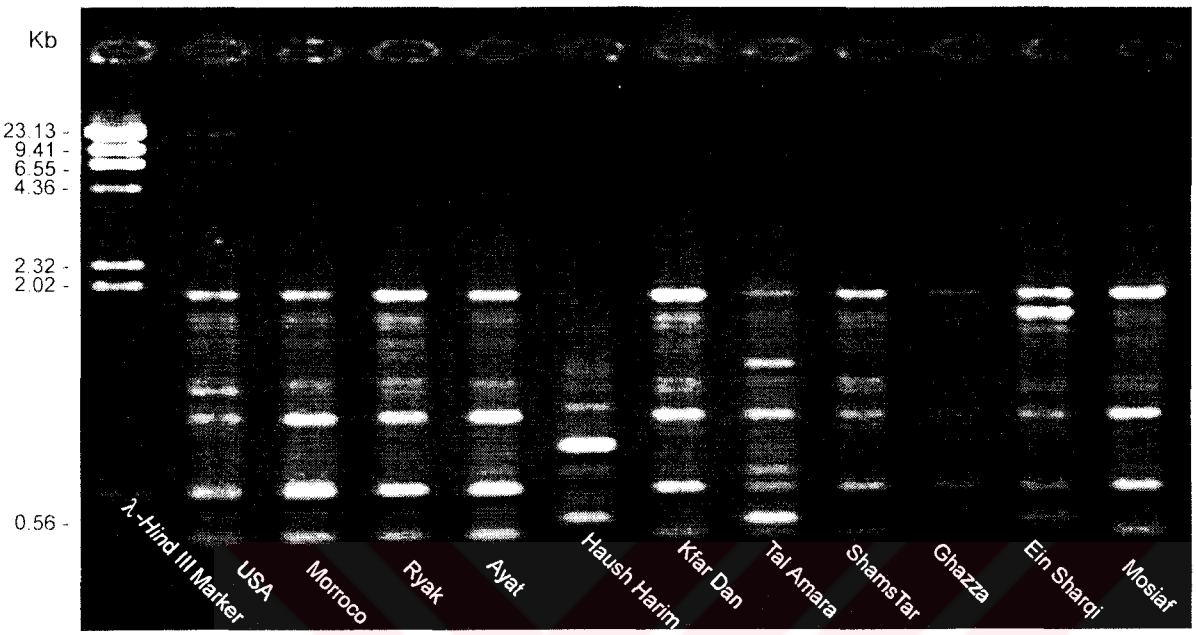

Figure 3. RAPD-PCR analysis of the aphid populations with primer, OPC-9.

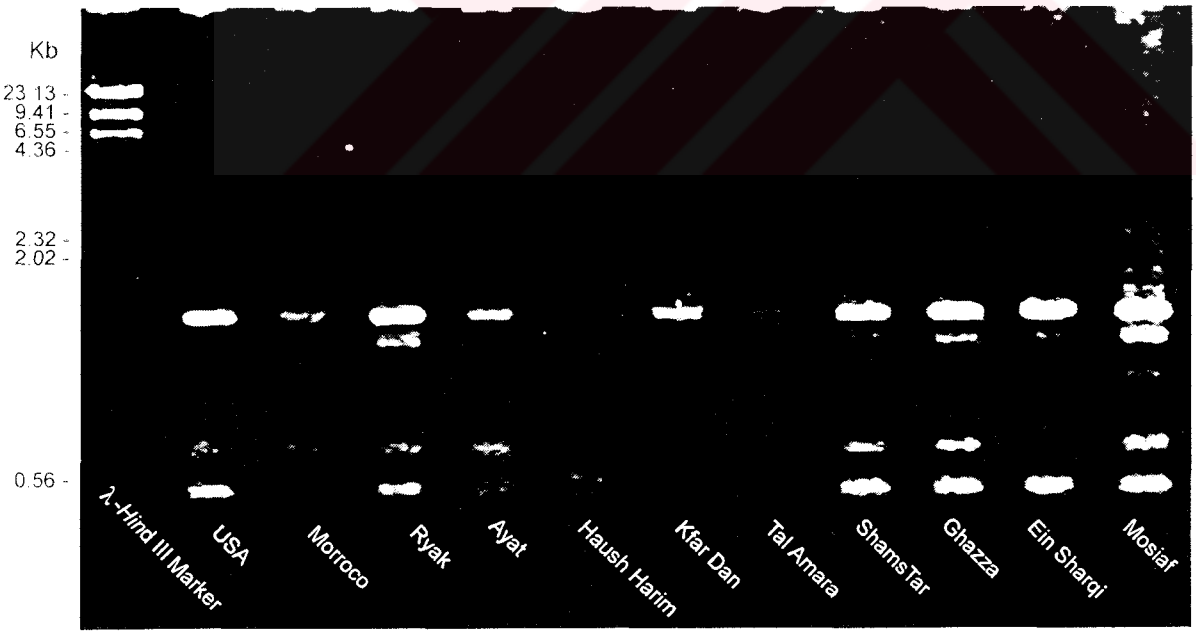

Figure 4. RAPD-PCR analysis of the aphid populations with primer, OPC-18.

Bulk DNA approach used in this study is a powerful means to study genetic variability of natural populations (Carter et al., 1996). With this method, a consensus fingerprint of a RWA population for each randomly primed PCR reaction was obtained. By this bulking strategy, uninformative data from individual-specific variation was minimized and polymorphisms representing approximately 20% or more of the population were available for scoring (Michelmore et al., 1991). Further more, it has been reported that genetic variation among individuals of an aphid population is very low, because of its pathenogenetic mode of reproduction in nature (Puterka et al., 1993). Therefore, bulking strategy followed in the present study is most appropriate to study genetic variation among different populations of RWA. Additionally, the bulking method was more efficient in time and resources than if it had designed the study by using DNA from one individual per reaction (Carter et al. 1996). For example, in the present study, to screen and replicate (two times) 25 individuals from 11 populations with 21 primers require 11,550 PCR reactions; whereas, use of population bulking requires only 462 PCR reactions. By using this method, primers can be efficiently surveyed for informative characters (loci). Twenty-one primers with 107 informative characters (loci) used in the present study allowed greater confidence that estimates of the genetic relatedness are broadly based. Presence and absence of a specific band scored at all the amplified polymorphic fragments. A computer analysis based on Nei's genetic distance (Nei, 1972) was performed. Table10. shows the pair-wise genetic distance and geographic distance (kilometers) for the11 populations.

Table 10. Estimates of Nei's genetic distance (Lower) and Geographical distance (Upper) among 11 populations of aphids based on RAPD-PCR analysis.

Population	1	2	3	4	5	6	7	8	9	10	11
1-Nebraska, USA	**0.000**	5790	9017	9000	9007	8985	9002	8975	9010	9100	9200
2-Setat, Morocco	0.016	**0.000**	3870	3870	3870	3870	3870	3870	3870	4000	4100
3-Ryak, Lebanon	0.049	0.065	**0.000**	30	55	25	10	40	60	300	200
4-Ayat, Lebanon	0.068	0.067	0.066	**0.000**	65	15	30	30	85	400	300
5-Haush Harim,Lebanon	1.438	1.454	1.470	1.518	**0.000**	80	85	80	35	320	280
6-Kfar Dan, Lebanon	0.050	0.049	0.048	0.033	1.621	**0.000**	35	15	75	350	250
7-Tal Amara, Lebanon	0.088	0.104	0.084	0.070	1.588	0.068	**0.000**	35	65	290	190
8-Shams Tar, Lebanon	0.058	0.074	0.056	0.041	1.462	0.040	0.058	**0.000**	80	410	310
9-Ghazza, Lebanon	0.042	0.058	0.040	0.042	1.446	0.041	0.060	0.032	**0.000**	420	320
10-Ein Sharqi, Syria	0.041	0.057	0.040	0.058	1.462	0.040	0.058	0.048	0.032	**0.000**	100
11-Mosiaf, Syria	0.040	0.056	0.039	0.056	1.404	0.039	0.074	0.015	0.031	0.031	**0.000**

The genetic distance ranged from 0.016 (between USA and Morocco, geographical distance 5790 Km) to 1.621 (between Haush Harim, and Kfar Dan, Lebanon, geographical distance 80 Km). In general, higher genetic distance was estimated between the population from (*M.dirhodum*) and rest of the RWA populations (the genetic distance ranged from 1.404 to 1.621). This observation of higher genetic distance between these two aphid species is obvious and comparable to the observations of Black et al., 1992. in their study with RAPD analysis, they observed higher genetic polymorphism between the aphid species than within the species and were able to identify different species based on RAPD profiles. Within RWA species, the genetic distance ranged from 0.016 to 0.088 indicating narrow genetic variability among the geographical populations of RWA. These results of narrow genetic variability contradicts the observations of Black et al. ,1992. in which they observed greater genetic differences among the population from Syria and South Africa. Among the Lebanese populations of RWA, the least genetic distance, 0.032 was observed between Shams Tar and Ghazza. Among the two Syrian populations (Ein Sharqi and Mosiaf) the genetic distance was 0.031. The narrow genetic variability observed among the geographical populations of RWA in the present study, indicates that the populations are under process of differention. Narrow genetic variability among the populations of RWA could be attributed to directional selection acting on the parthenogenetic clones (Blackman, 1985; Wool et al., 1978; Soumalainen et al., 1980; Simon et al., 1982) and founder effects (May and Holbrook, 1978; Steiner et al.,1985b). The few surviving genotypes

rapidly dominated niches within a geographic region through their well-developed migration mechanism (flight) and parthenogenetic reproduction.

Correlation coefficient was estimated between genetic distance as estimated by RAPD-PCR analysis and corresponding geographic distance. The correlation coefficient was -0.1336, indicating no relationship between the genetic distance and the geographic distance; population separation is independent of geographical distance. This result gives an indication that the RWA populations are accidental introduction as reported in South Africa (Walters et al., 1980), Mexico (Gilchrist et al., 1984) and USA (Stoetzel, 1987) rather than migration along the land routs. This assumption is further supported by cluster analysis (see below) where populations from Morocco and USA clustered very closely together. Further more these two populations closely clustered with population from Ryak, Lebanon.

Genetic relatedness among the populations was determined by cluster analysis (UPGMA, Figure 5. and NJM, figure 6.) based on the genetic distances. The analysis grouped the populations into 11 distinct clusters (genotypes). In both the methods the population of Haush Harim, Lebanon (unidentified species) clearly out-lied from the populations from other RWA populations. Since NJM is most accurate (Rolf, 1993) than UPGMA, only result of the NJM is described in detail. Among RWA populations, the population from Mosiaf, Syria out-lied from the other populations. The population from USA and Morocco genetically more related than other populations. At 75% similarity levels, all the populations could be classified

into 9 groups; the populations from Lebanon, formed 4 groups, from that of Syria two groups, and the population from USA and Morocco formed two groups. Of course, the *M.dirhodum* species formed a separate cluster as well. The populations from Ayat and Kfar Dan, and populations from Shamstar and Tal Amara, Lebanon merged into single group. Other populations from Lebanon formed separate groups.

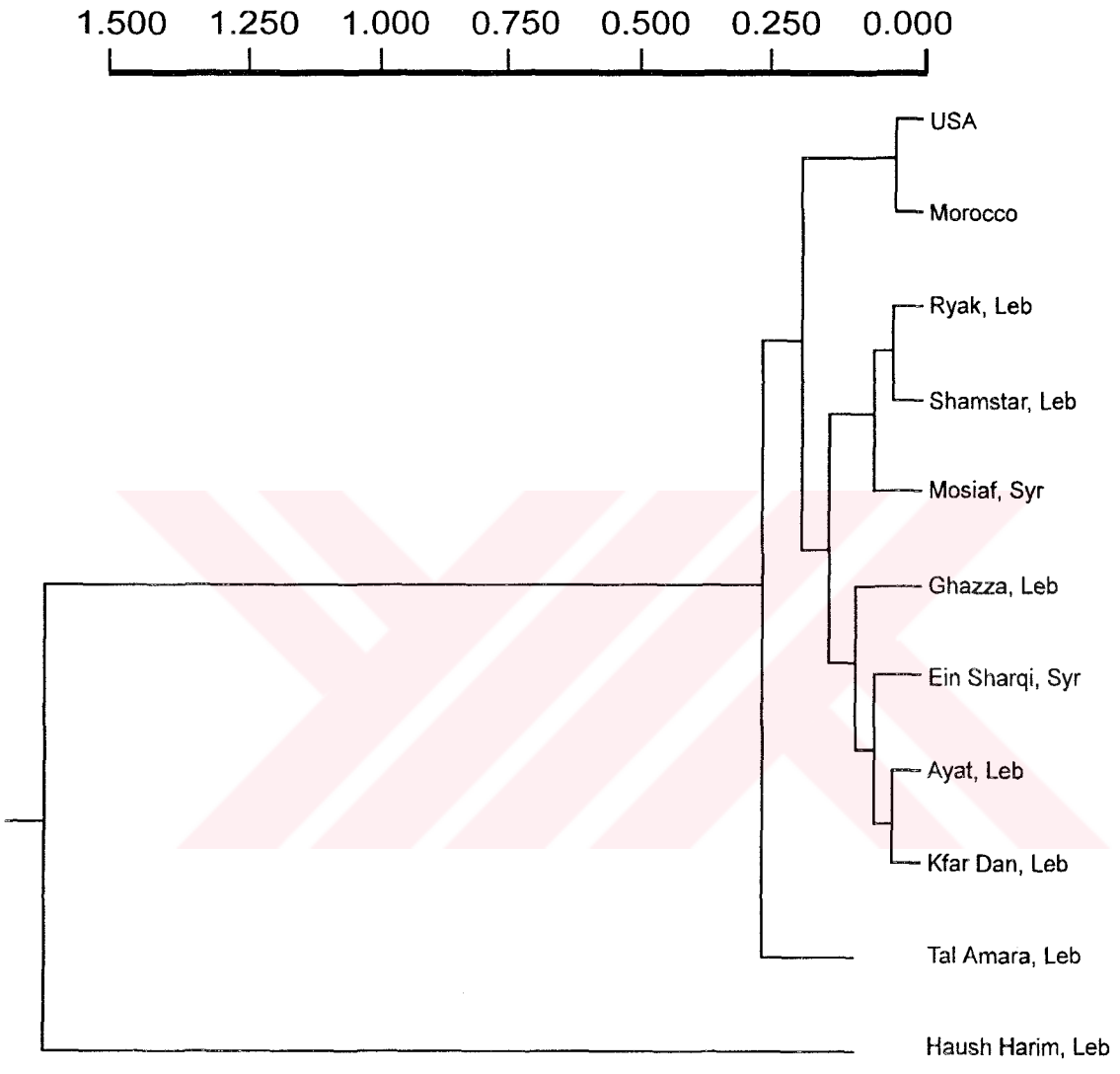

Figure 5. Dendrogram of genetic relationships among 10 populations of RWA and *M.dirhodum* species of aphid, based on genetic distances (Nei, 1072) of RAPD-PCR data using UPGMA method.

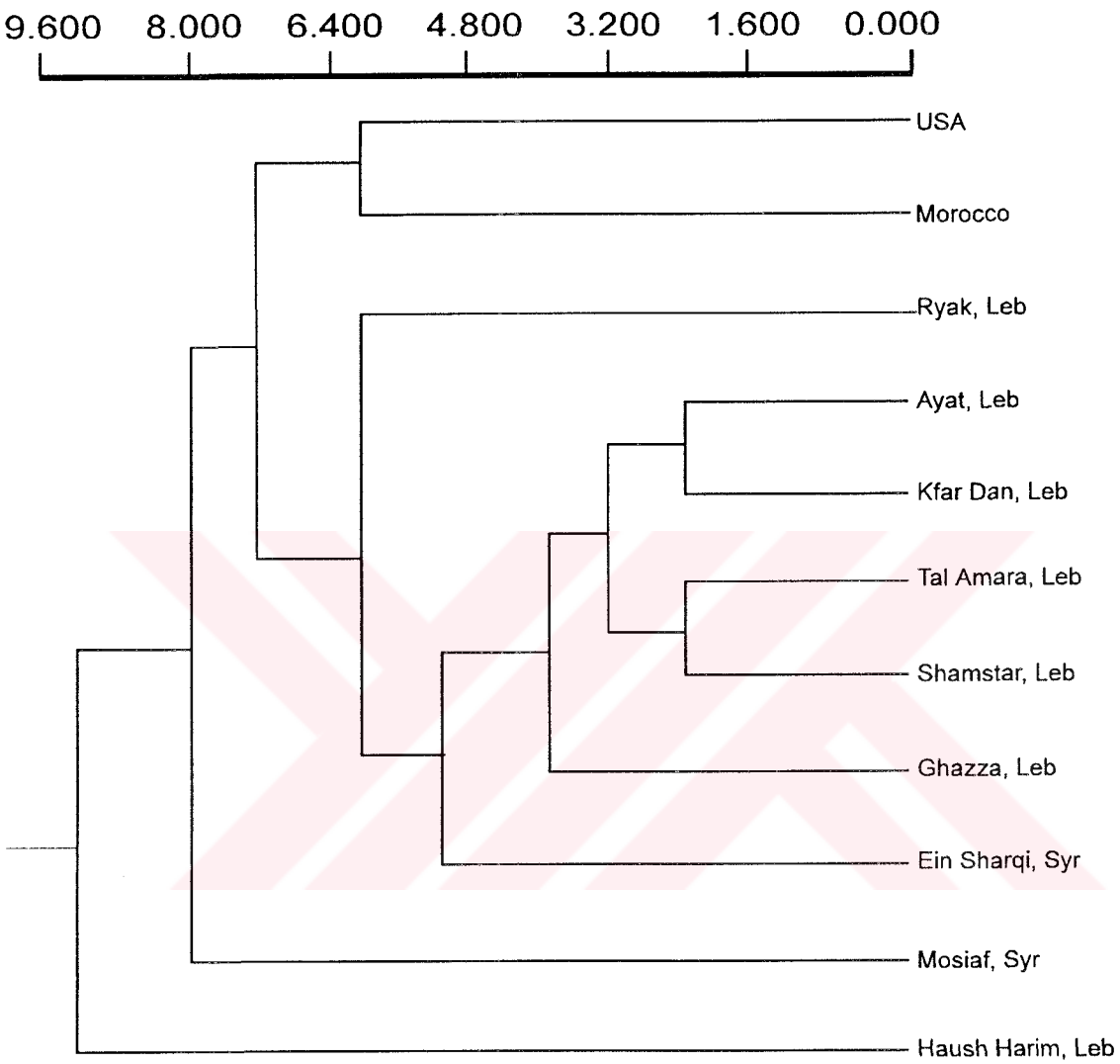

Figure 6. Dendrogram of genetic relationships among 10 populations of RWA and *M.dirhodum* species of aphid, based on genetic distances (Nei, 1072) of RAPD-PCR data using NJM method.

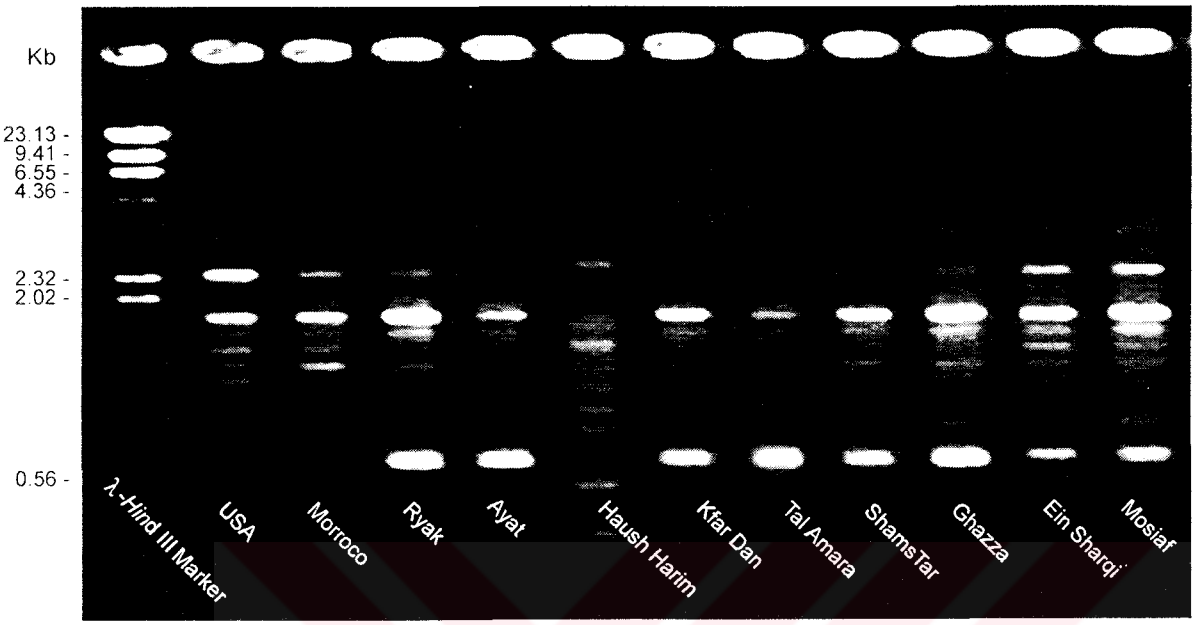

Figure 7. RAPD-PCR analysis of the aphid populations with primer, OPE-8.

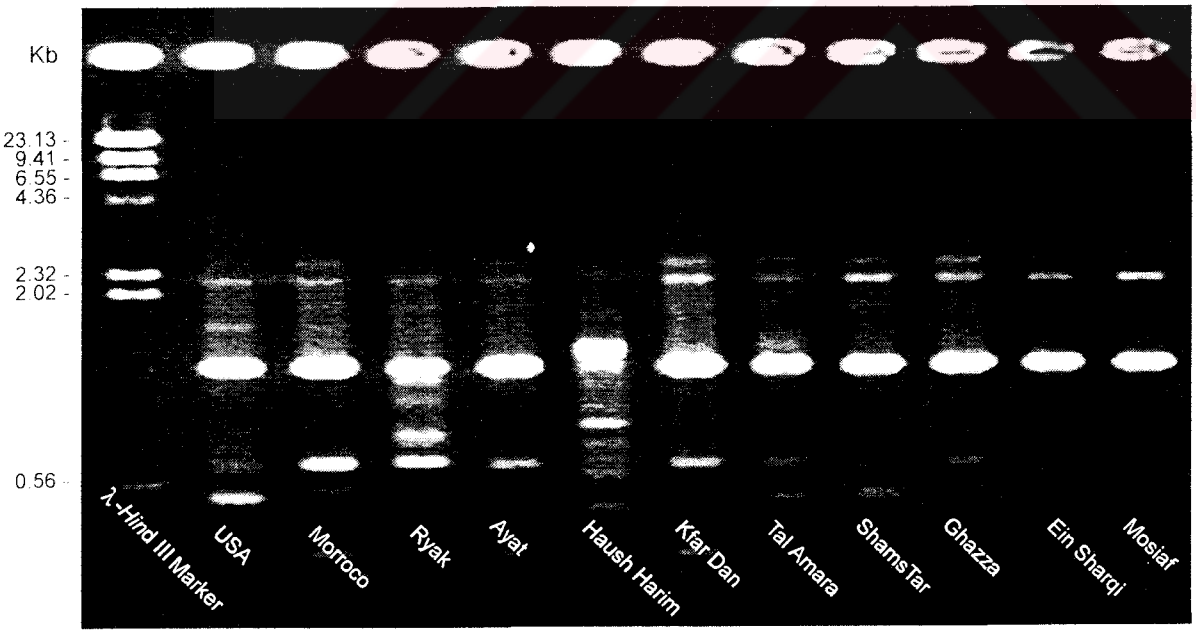

Figure 8. RAPD-PCR analysis of the aphid populations with primer, OPE-11.

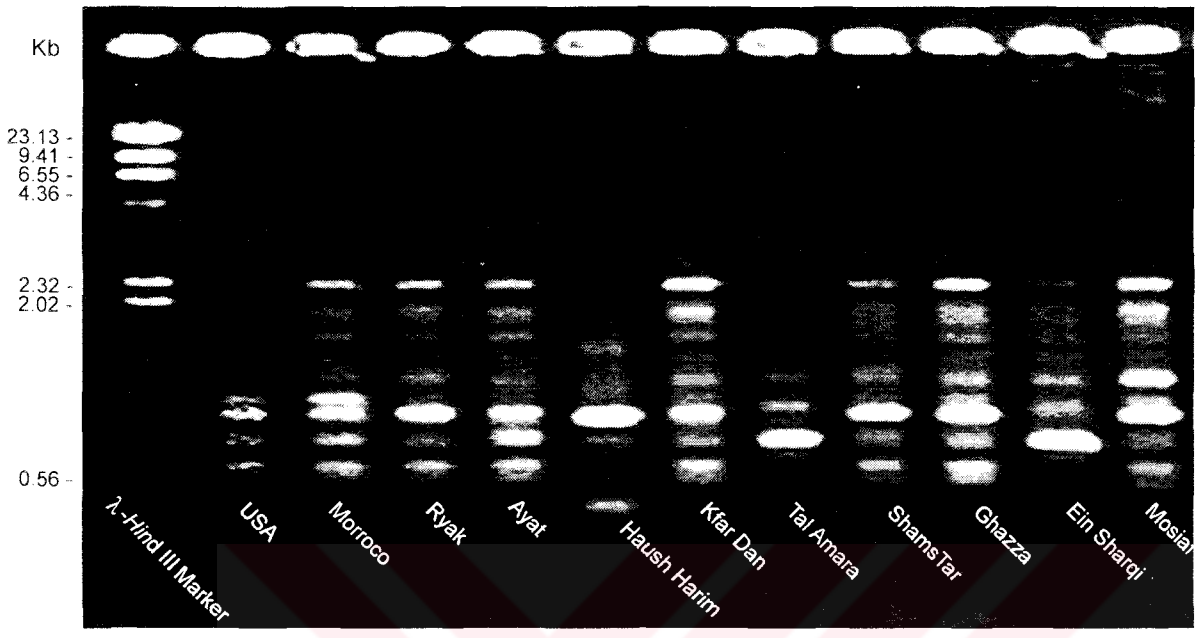

Figure 9. RAPD-PCR analysis of the aphid populations with primer, OPE-12.

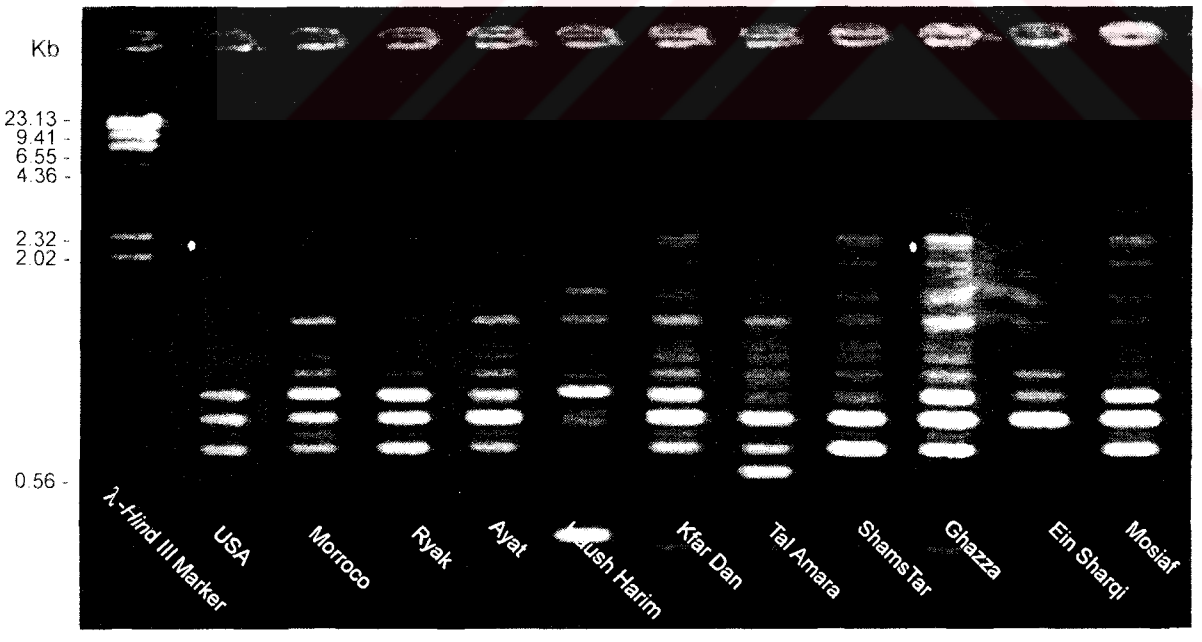

Figure 10. RAPD-PCR analysis of the aphid populations with primer, OPE-14.

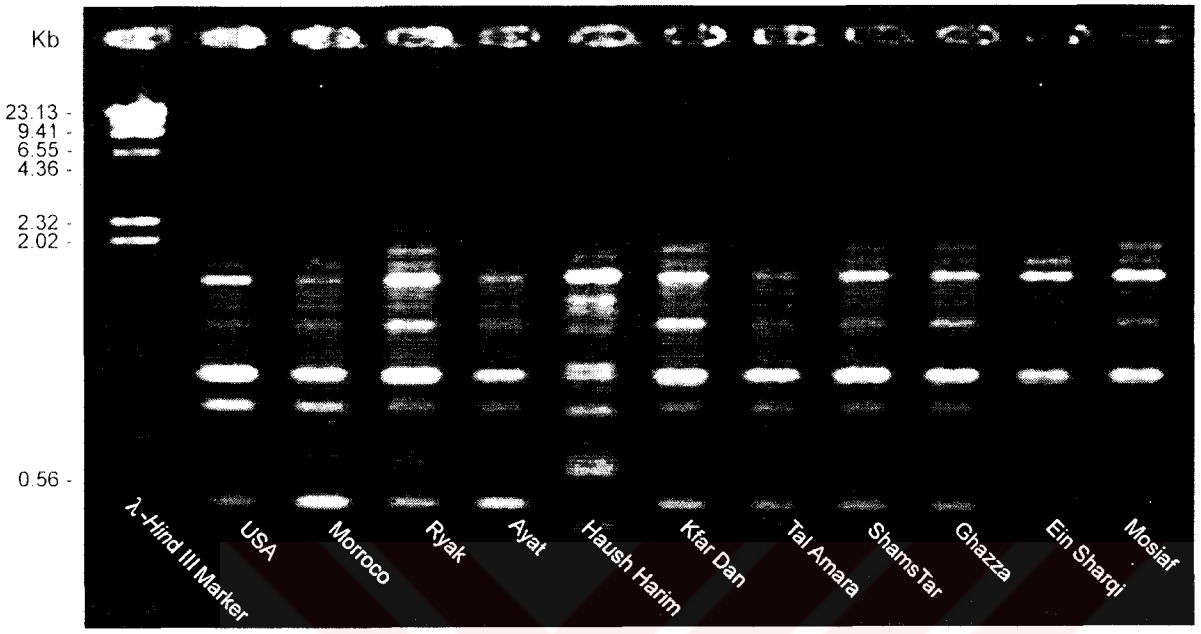

Figure 11. RAPD-PCR analysis of the aphid populations with primer, OPE-18.

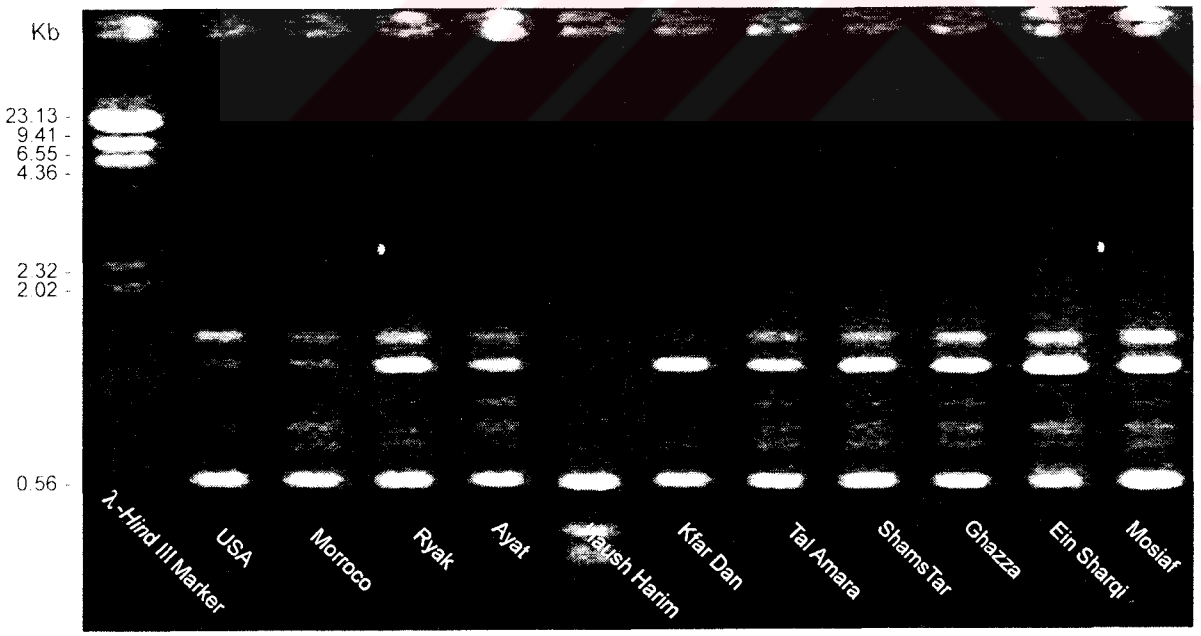

Figure 12. RAPD-PCR analysis of the aphid populations with primer, OPE-19.

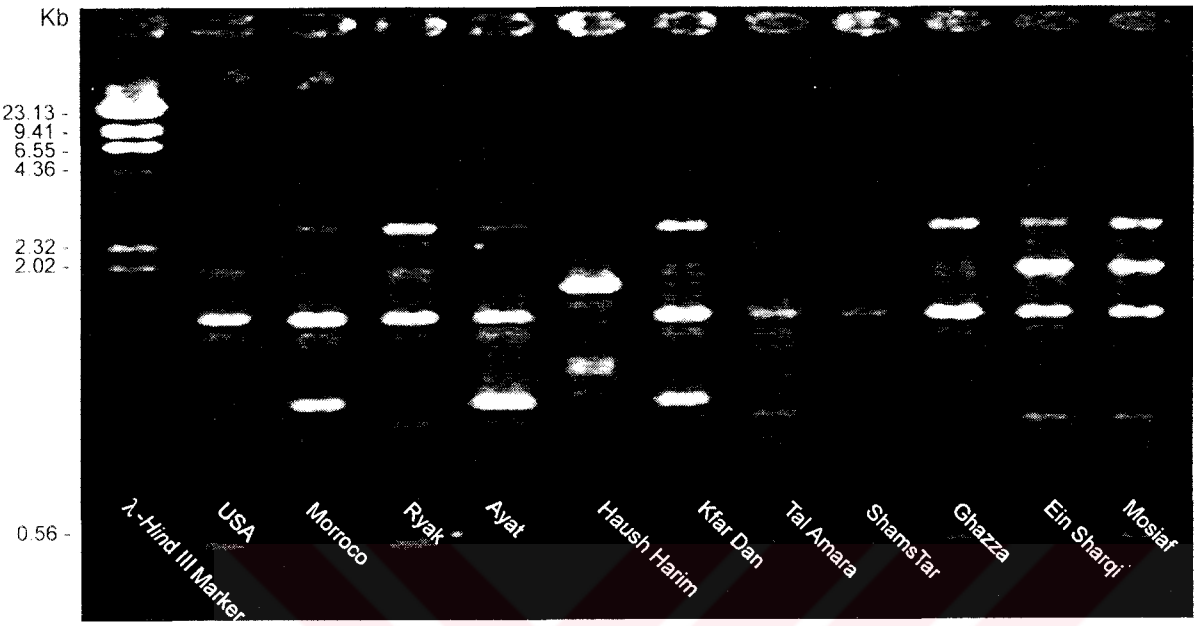

Figure 13. RAPD-PCR analysis of the aphid populations with primer, OPF-3.

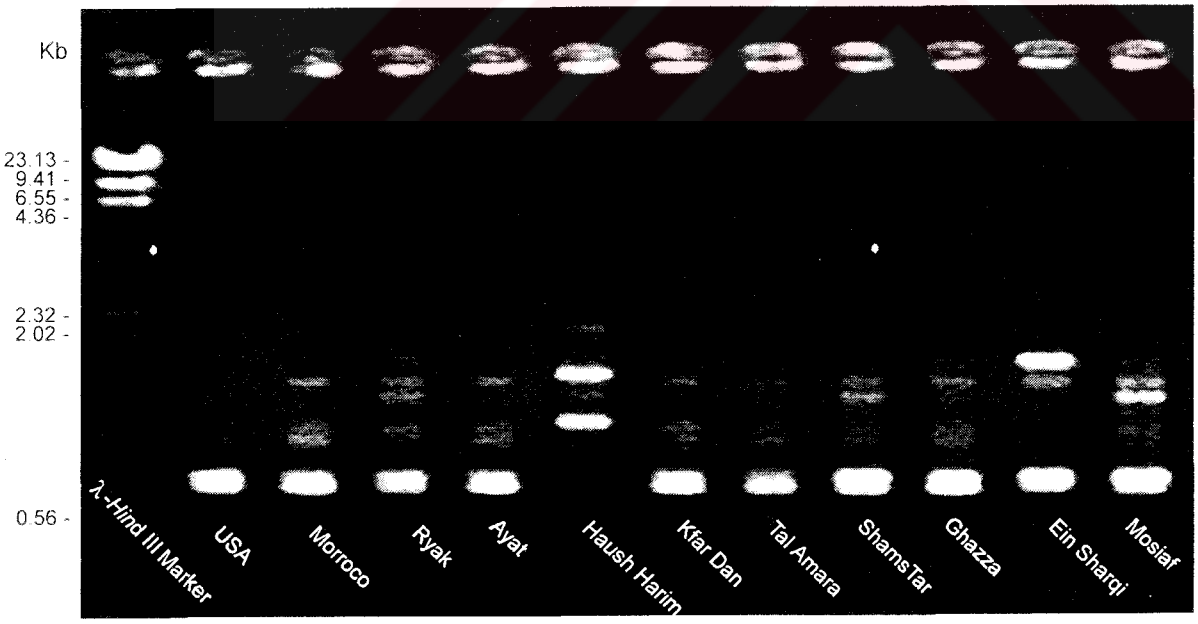

Figure 14. RAPD-PCR analysis of the aphid populations with primer, OPF-4.

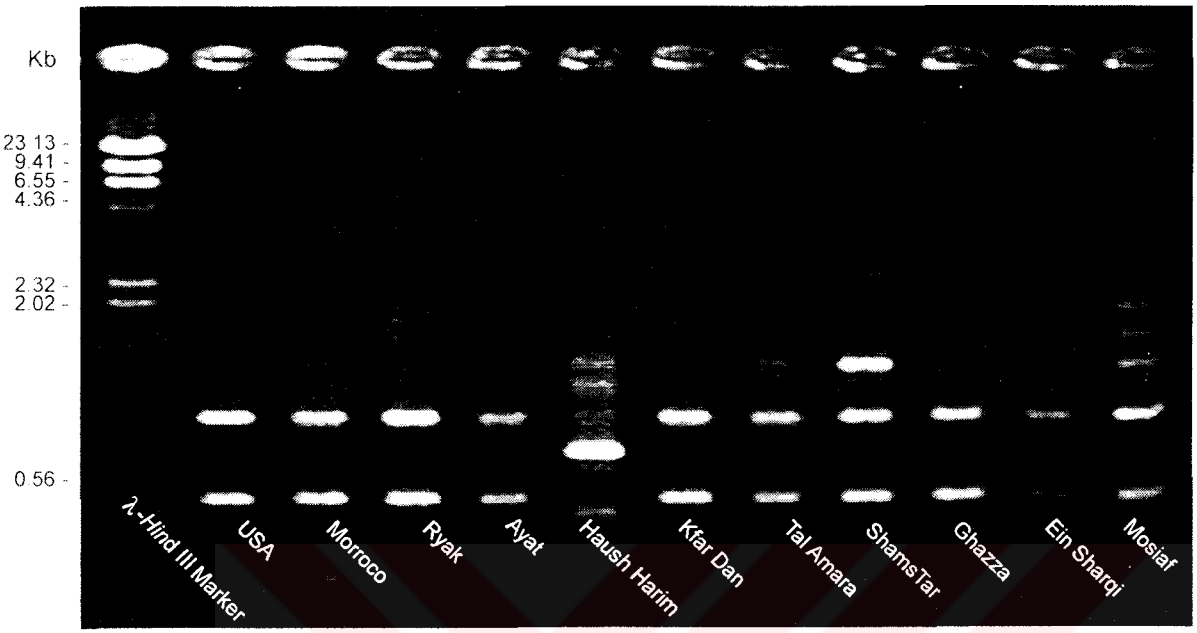

Figure 15. RAPD-PCR analysis of the aphid populations with primer, OPF-6.

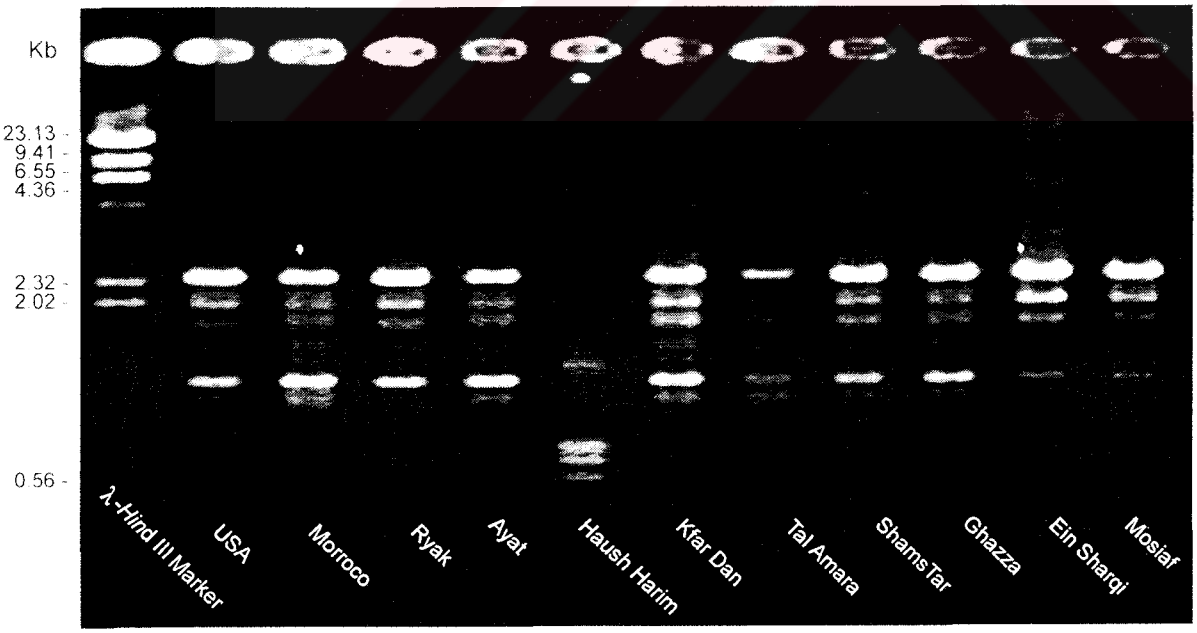

Figure 16. RAPD-PCR analysis of the aphid populations with primer, OPF-8.

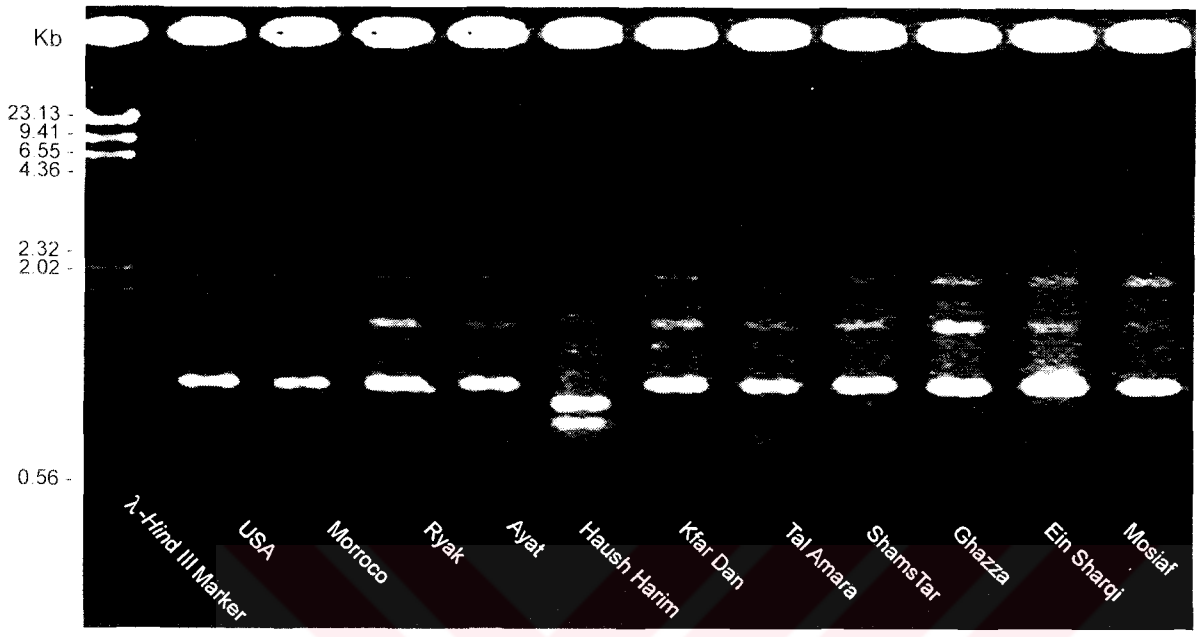

Figure 17. RAPD-PCR analysis of the aphid populations with primer, OPF-14.

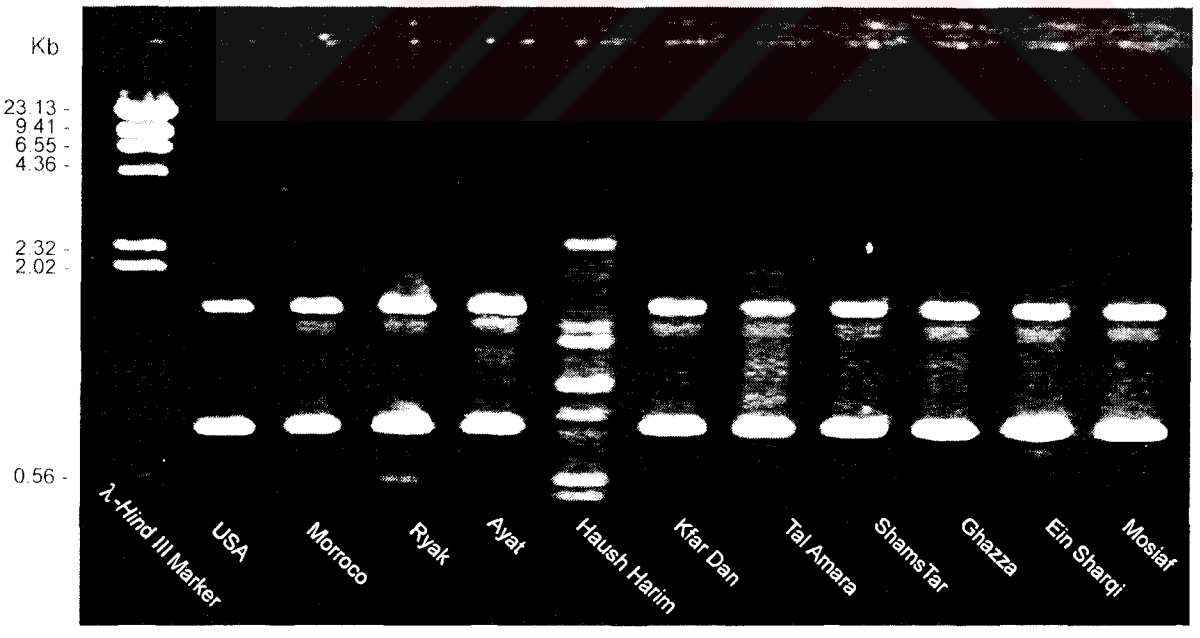

Figure 18. RAPD-PCR analysis of the aphid populations with primer, OPC-1.

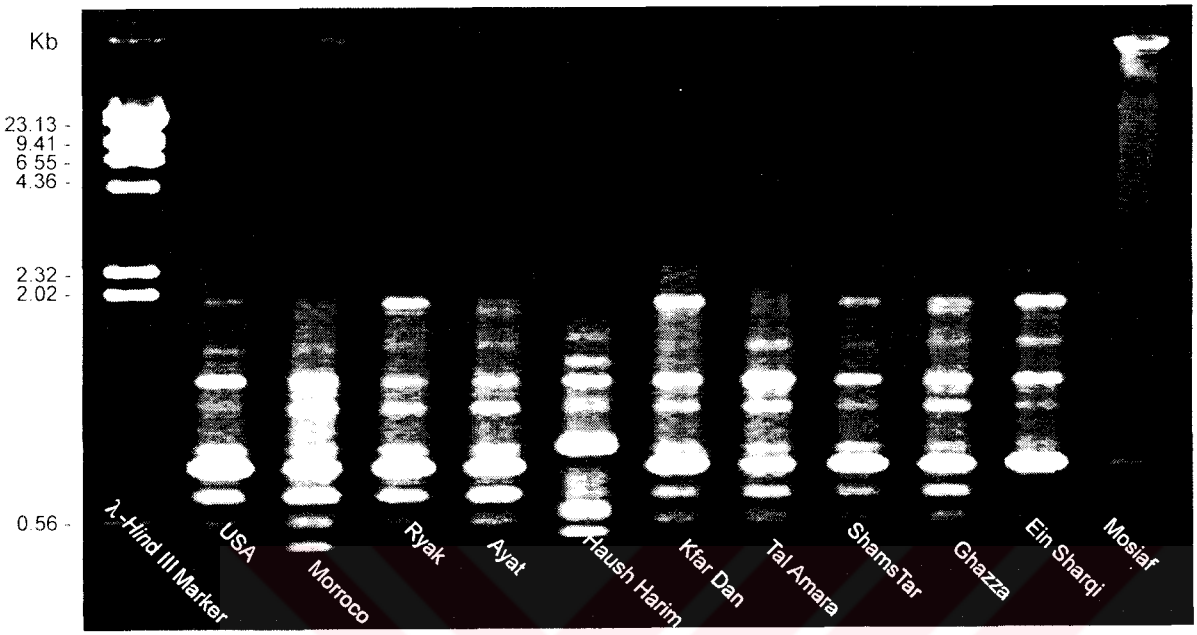

Figure 19. RAPD-PCR analysis of the aphid populations with primer, OPC-5.

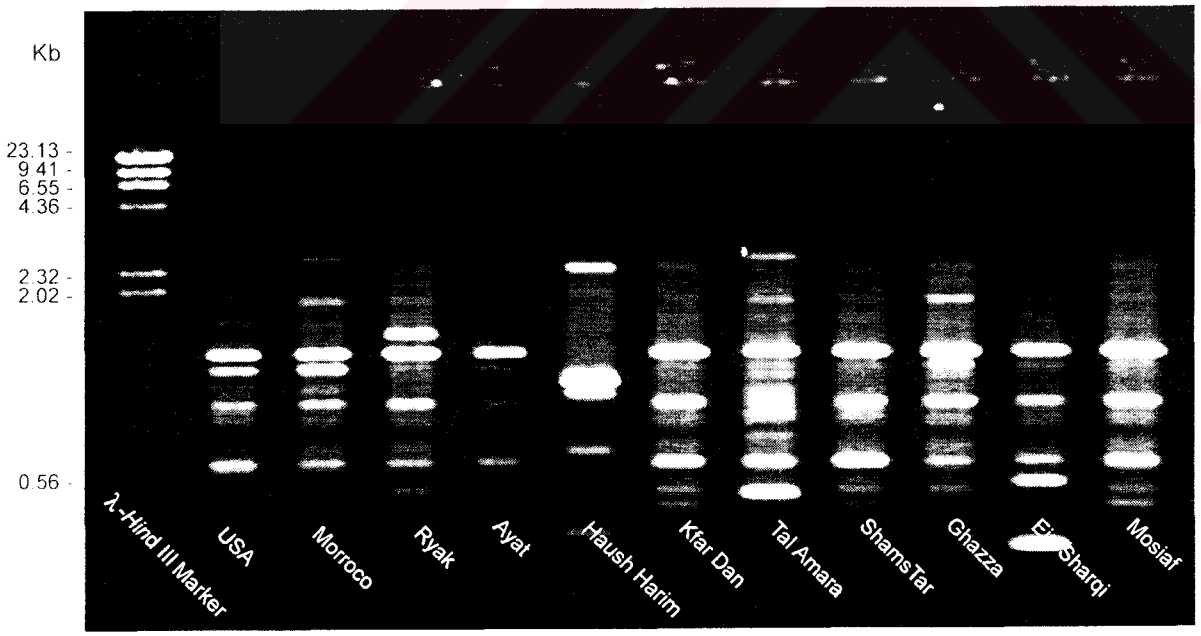

Figure 20. RAPD-PCR analysis of the aphid populations with primer, OPC-6.

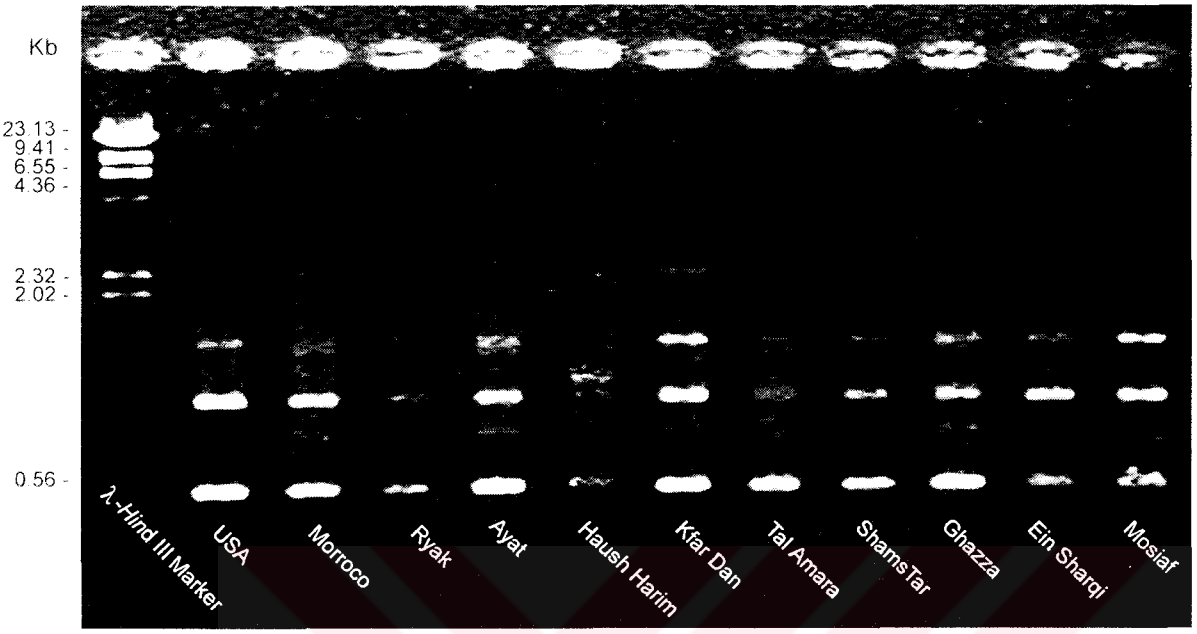

Figure 21. RAPD-PCR analysis of the aphid populations with primer, OPC-11.

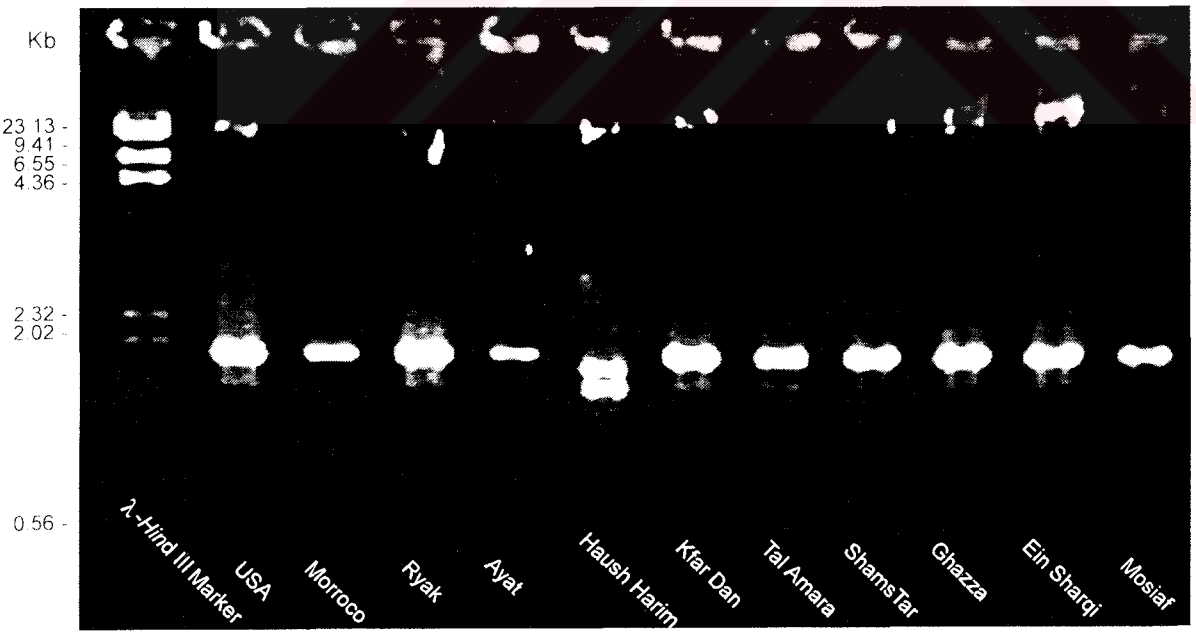

Figure 22. RAPD-PCR analysis of the aphid populations with primer, OPC-13.

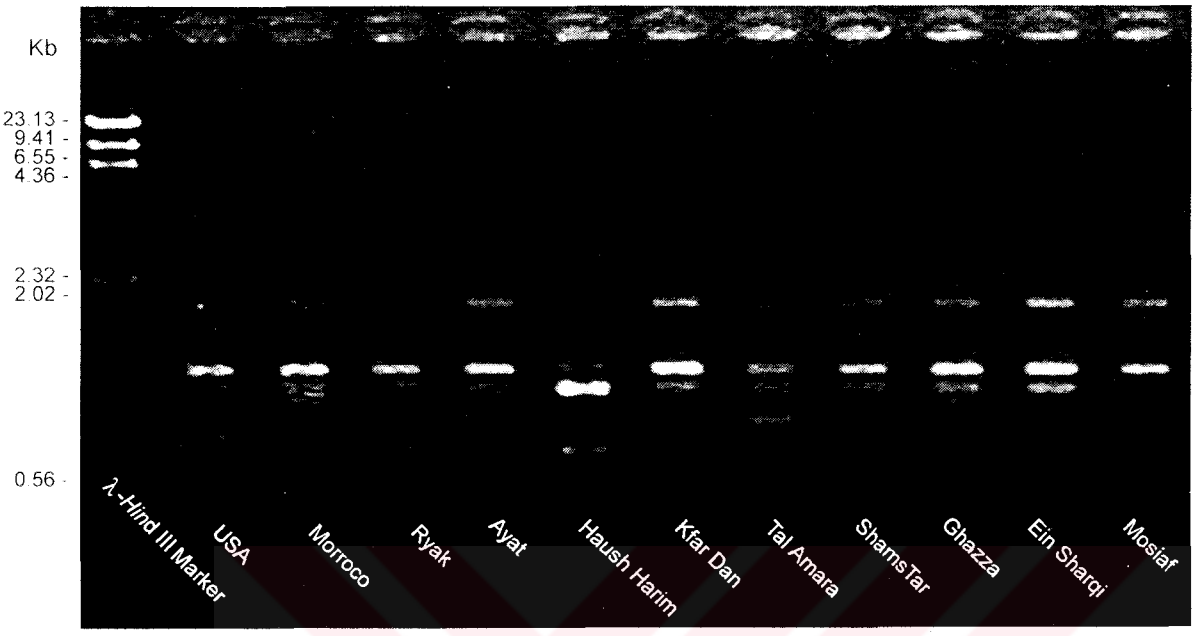

Figure 23. RAPD-PCR analysis of the aphid populations with primer, OPC-14.

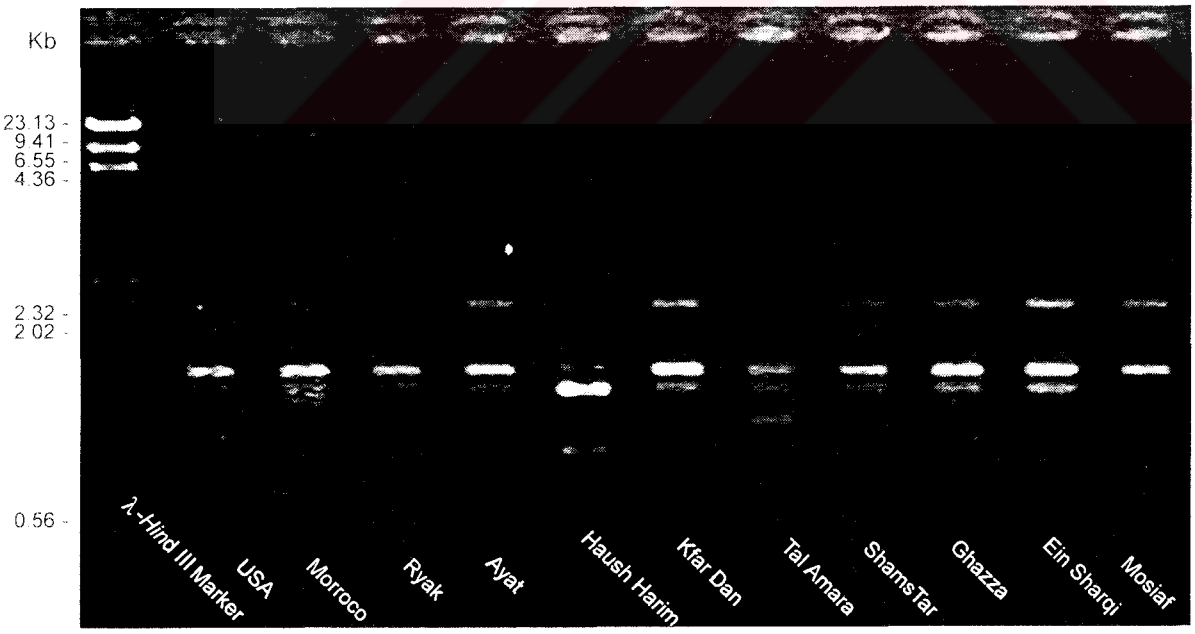

Figure 24. RAPD-PCR analysis of the aphid populations with primer, OPC-17.

5. CONCLUSIONS AND RECOMMENDATION

5.1. Conclusions

5.1.1. Mechanisms of Resistance:

This study shows that the durum genotypes and accessions tested have different mechanisms of resistance to RWA. The data also show that antibiosis and antixenosis are the two most important mechanisms in the resistant accessions. It is also interesting to note that genotype like Haucan/Aeg.400020//Omtel-1/3/Omlahn-3 has high level of antibiosis but at the same time has a moderate level of antixenosis.

Combining different mechanisms should be sought in breeding for resistance to insects, as this should slow down biotype development. The inheritance study is underway to determine the number of genes and their mode of action.

5.1.2. Genetic variability among geographical populations of RWA

The present study clearly demonstrated that RAPD markers are useful in detecting both inter and intra specific polymorphism in the aphids. Bulking strategy followed in the present study is most appropriate to study genetic variation among different populations of RWA. The banding pattern revealed through RAPD-PCR are often species specific and offers the promise of rapid species diagnostics. The genetic distance within the *D. noxia* ranged from 0.016 to 0.088 indicating narrow genetic variability among the geographical populations. The lack of correspondence between geographical distance and genetic distance may suggest random establishment of clones through commerce rather than through migration. This view was further supported by cluster analysis.

More extensive sampling of populations from North America and Mediterranean regions coupled with classical biotyping will be needed to eludiciate the evolutionary forces (like selection, hybridization, migration and mutation) acting on the RWA populations. Knowledge on evolutionary forces acting on the RWA population will help in formulating integrated management strategies, to manage this important pest of cereals. Distribution map of different genotypes of RWA along with their biotype will help in deployment of suitable resistant cultivars.

5.2. RECOMMENDATIONS

5.2.1. Mechanisms of resistance

More effort should be put on screening durum wheat and its wild relatives to identify additional sources of resistance to RWA. Biotype development should be monitored using a set of differentials that will be planted in hot spot areas. All the new sources of resistance should be studied for their mechanisms of resistance. Material with combined mechanisms of resistance should be used in the breeding programs, as this should slow down biotype development. The inheritance of resistance should be studied. Polygenic resistance should be used in the breeding programs, as this may be durable compared to monogenic resistance.

5.2.2. Genetic variation among geographical populations of RWA

More geographical populations of RWA from different countries in West Asia and North Africa should be periodically sampled and RAPD-PCR analysis coupled with biotype studies should be performed. This will give a very clear picture on population structure of RWA and evolutionary forces such as migration, selection, sexual hybridization, etc. acting on the population. Information on these aspects may help in designing suitable IPM strategies to manage this pest.

Further studies on Genetics of intra population variation as revealed by RAPD-PCR analysis along with studies on ecologically important traits such as host alternation and polyphenism could illuminate the nature of selective pressure on life-cycle expressions in RWA.

REFERENCES

AALBERSBERG, Y.K.; M.C. VAN DER WESTHUIZEN, and P.H. HEWITT., 1987. A simple key to the diagnosis of the instars of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Homoptera:Aphididae). Bull. Entomol. Res. 77:637-640.

AALBERSBERG, Y.K.; M.C. VAN-DER WESTHUIZEN and P.H. HEWITT., 1989. Characteristics of the population build-up of the Russian wheat aphid and the effect on wheat yield In the eastern Orange Free State. Ann. App. Biolo. 114(2): 231-242.

ABID,H.S.,KINDLER,S.D.,JENSEN,S.G.,THOMAS-COMPTON,M.A. and SPOMER,S.M., (1989). Isozyme characterization of sorghum aphid species and biotypes(Homoptera :Aphididae). Ann. Entomol. Soc. Ame. 82,303-306.

ALFARO, A., 1947. Notas sobre *Brachycolus noxuius* Mordw., nueva plaga paranuestros trigos cebadas. Boletin de Patologia Vegetal y Entomologia Agricola. 15:125-130.

ANDERSON, J.E., 1990. RWA control in Colorado with NTN-33893. In pages 132-136, G.D. Johnson,ed., Proceeding of the Fourth Russian wheat aphid Workshop, Oct. 10-12, 1990. Montana State University, Bozeman, Montana.

ANONYMOUS., 1989. Economic impact of the Russian wheat aphid in the western United States. 1987-1988. Great plains Agricultural Council, Publication No. 129, Ft. Collins, Co.

ARAYA, J.E. and A. FERERES., 1991. Cereal aphid survival under flooding conditions. Zeitschrift-fuer-Pflanzenkrankheiten-und-Pflanzenschutz (Germany, F.R.). 98(2): 168-173.

ARCHER, T.L. and BYNUM, E.D. JR., 1992. Economic injury level for the Russian wheat aphid (Homoptera : Aphididae) on dryland winter wheat. J. Econom. Entomol. 85(3) :987-992.

BAUGH, B.A. and PHILLIPS, S.A. JR., 1991. Influence of population density and plant water potential on Russian wheat aphid (Homoptera: Aphididae) alate production. Environ. Entomol. 20(5):1344-1348.

BEREGOVOY,V.H. and STARKS,K.J., (1986). Enzyme patterns in bio-types of the greenbug, *Schizaphiis graminum* (Rondani) (Ho-moptera:Aphididae). Journal of the Kansas Entomological Society 59,517-523.

BLACK, W.C.IV.DUTEAU; N.M.PUTERKA; G.J.NECHOLS.J.R. and PETTORINI,J.M., 1992. Use of the random amplified poly-morphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids. Bull.Entomol. Res.,82,151-159.

BLACKMAN.R.L., 1985. Aphid cytology and genetics.In:Szelegiewicz, H. (ed). Evolution and biosystematics of aphids. Proceedings of the International Aphid Symposium.Jabolona, Poland,pp. 171-237.

BLACKMAN, R.L., and V.F. EASTOP., 1984. Aphid on the world's Crop: an Identification and Information Guide. Wiley, Chichester, UK.

BRENCHLY, R.G., 1990. Aphid control in wheat and barley with NTN-33893 seed treatments. In pages 130-131, G.D. Johnson, ed., Proceedings of the Fourth Russian Wheat Aphid Workshop, Oct. 10-12, 1990. Montana State University, Bozeman, Montana.

BURD, J.D. and BURTON, R.L., 1992. Characterization of plant damage caused by Russian wheat aphid (Homoptera:Aphididae). J. Econom. Entomol. 85(5): 2017-2022.

BURD, J.D.; BURTON, R.L. and WEBSTER, J.A., 1993. Evaluation of Russian wheat aphid (Homoptera: Aphididae) damage on resistant and susceptible hosts with comparisons of damage ratings to quantitative plant measurements. J. Econom. Entomol. 86 (3) : 974-980.

BURTON, R.L., 1989. Second annual report on the Russian wheat aphid. USDA-ARS, Washington, D.C.

BUTTS, R.A., 1992. The influence of seeding dates on the impact of fall infestations of Russian wheat aphid in winter wheat. Great Plains Agricultural Council publication 142 : 120-122.

BUTTS, P.A. and K.W. PAKENDORF., 1984. The utility of the embryo count method in characterizing cereal crops for resistance to *Diuraphis noxia*, pp. 53-57. In M.C. Walters [ed.], Progress in Russian wheat aphid (*Diuraphis noxia* Mordw.) Research in the Republic of South Africa. Technical Communication No. 191, Department of Agriculture, Republic of South Africa, Pretoria.

BURD,J. D.R.L. BURTON, and J. A. WEBSTER., 1989. Plant damage caused by Russian wheat aphid feeding. P. 144-145. In proc. Russian Wheat Aphid Conf., Albuquerque, NM.25-27 Oct. 1989. Coop. Ext. Serv., New Mexico State Univ., Las Cruces.

CARTER, M.C.A., ROBERTSON, J.L., HAACK, R.A., LAWRENCE, R.K. and HAYES, J.L., 1996. Genetic relatedness of North American populations of Tomicus piniperda (Coleoptera: Scolytidae). J. Econ. Entomol., 89: 1345-1353.

CARVALHO,G.R.MACLEAN, N.WRATTEN, S.D.CARTER,R.E. and THURSTON,J.P., 1991. Differentiation of aphid clones using DNA fingerprints from individual aphids.Proc.R. Soc. London.B,243,109-114

CLEMENT, S.L.; LESTER, D.G.; WILSON, A.D. and PIKE, K.S., 1992. Behavior and performance of *Diuraphis noxia* (Homoptera:Aphididae) on fungal endophyte-infected and uninfected perenni alrye grass. J. Econom. Entomol. 85(2) : 583-588.

DU TOIT, F., 1987. Resistance in wheat (*Triticum aestivum*) to *Diuraphis noxia* (Homoptera :Aphididae). *Cereal Res. Commun.* 16:105-106.

DU TOIT, F., 1988. Another source of Russian wheat aphid (*Diuraphis noxia*) resistance in *Triticum aestivum.* Cereal Res. Commun 16 : 105-106.

DU TOIT, F., 1989. Components of resistance in three bread wheat lines to Russian wheat aphid (Homoptera:Aphididae) in South Africa. J.Econ.Entomol. 82:1779-1781.

DU TOIT, F. & VAN NIEKERK, H.A., 1985. Resistance in *Triticum* species to the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Homoptera:Aphididae). Cereal Res. Commun. 13(4): 371-378.

DU TOIT, F. & M.C. WALTERS., 1984. Damage assessment and economic threshold values for chemical control of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) on winter wheat, pp. 58-62. In Department of Agriculture, Republic of South Africa, Technical Communication 191. Environ. Entomol. 19(5):1438-1442.

ERDELEN, C., 1981. Die Blattlaus fauna der Arabischen Republik Jemen unter besonderer Berucksichtigung der wirtschaftlich bedeutsamen Arten an Getreide. Pp. 264 Ph.D. thesis, Univ. Bonn.

FENG, M.G., J.B. JOHNSON, and S.E. HALBERT., 1991. Natural control of cereal aphis (Homoptera:Aphididae) by entomopathogenic fungi (Zygomycetes: Entomophtorales) and parasitoids (Hymenoptera : Braconidae and Encyrtidae) on irrigated spring wheat in southwestern Idaho. Environ. Entomol. 20:1699-1710.

FENG, M.G. and JOHNSON, J.B., 1991. Bioassay of four entomophthoralean fungi (Entomophthorales) against *Diuraphis noxia* and *Metopolophium dirhodum* (Homoptera: Aphididae). Environ. Entomol. 20(1) : 338-345.

FORMUSOH, E.S. 1994. Resistance to Russian wheat aphid (Homoptera:Aphididae) in wheat and wheat related hybirds. J. Econ. Entomol. 87:241-244.

FORMUSOH, E.S.;G.E.WILDE;J.H.HATCHETT and R.D.COLLINS., 1992. Resistance to Russian wheat aphid (Homoptera:Aphididae) in Tunisian wheats. J. Econ. Entomol. 85:2505-2509

FOUCHE, D.; R.L. VERHOEVEN; P.H. HEWITT; M.C. WALTERS; C.F. KRIEL and J.DE JUGER., 1984. Russian wheat aphid (*Diuraphis noxia*) feeding damage on wheat, related cereals and a Brumus grass species. PP.22-23 In M.C. Walters (ed.), Progress in Russian wheat aphid. Research in the Republic of South Africa. Technical communication 191. Dep. of Agr. Pretoria, Republic of South Africa

GIRMA, M.; WILDE, G. and REESE, J.C., 1990. Influence of temperature and plant growth stage on development, reproduction, life span, and intrinsic rate of increase of the Russian wheat aphid (Homoptera:Aphididae). Environ. Entomol. 19(5) : 1438-1442.

GIRMA, M.; WILDE, G.E. and HARVEY, T.L., 1993. Russian wheat aphid (Homoptera: Aphididae) affects yield and quality of wheat. J. Econom. Entomol. 86(2) : 594-601.

GILCHRIST, L. I., R.RODRIGUEZ & P. A. BURNETT., 1984. The extent of Freestate streak and *Diuraphis noxia* in Mexico, pp. 157-163. In Barley yellow dwarf, a p r o c e e d i n g s of the workshop. Centro International de Mejoramiento de Maiz y Trigo, Mexico City, Mexico.

GONZALEZ, D., F. GILSTRAP, N. ZAREH, L. MCKINNON, and P. STARY., 1990. Foreign exploration for natural enemies of RWA in China, Iran, Turkey and Holland. In pages 154-165, G.D. Johnson,ed., Proceedings of the Fourth Russian Wheat Aphid Workshop, Oct. 10-12, 1990. Montana State University, Bozeman, Montana.

GRAUR, D., 1985. Gene diversity in Hymenoptera. Evolution 39, 190-199.

GROSSHEIM, N.A., 1914. The aphid *Brachycolus noxius*, Mordvilko. Memoirs of the Natural History Museum of the Zemstvo of the Government of Tavrida, Simferopol 3:35-78 (in Russian).

HAILE, A., and T. MEGENASA., 1987. Survey of the aphids in parte of Shewa,Welo and Tigrai, Ethiopia. Ethio. J. Agric. Sci. 9:39-53.

HAMMON, R.W. and PEAIRS, F.B., 1992. Distribution of overwintering Russian wheat aphid (Homoptera: Aphididae) in furrow-irrigated small grains in western Colorado. J. Econom. Entomol. 85(6) : 2452-2458.

HEWITT, P.H., 1988. The South African experience with the Russian wheat aphid. In pages 1-3, F.B. Peairs. and S.D. Pilcher,eds., Proceedings of the Second Russian Wheat Aphid Workshop, Oct. 11-12, 1988, Denver, Colorado.

HILL, B.D., and R.A. BUTTS., 1991. Efficancy and mode of contact of chlospyrifos against RWA. In pages 139-142, G.D. Johnson,ed., Proceedings of the Fourth Russian Wheat Aphid Workshop, Oct. 10-12, 1990. Montana State University, Bozeman, Montana.

HILL, B.D.; BUTTS, R.A. and SCHAALJE, G.B.,1993. Reduced rates of foliar insecticides for control of Russian wheat aphid (Homoptera: Aphididae) in western Canada. J. Econom. Entomol. 86(4) : 1259-1265.

HUGHES, R.D. and MAYWALD, G.F.,1990. Forecasting the favourableness of the Australian environment for the Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae), and its potential impact on Australian wheat yields. Bull. Entomol. Res. 80(2) : 165-175.

KESSLER,K., 1991. RWA, the fight goes on. The furrow, Western Edition., Nov.-Dec. :41-42.

KIECKHEFER, R.W.; ELLIOTT, N.C.,1989. Effect of fluctuating temperatures on development of immature Russian wheat aphid (Homoptera:Aphididae) and demographic statistics. J. Econom. Entomol. 82(1) : 119-122.

KINDLER, S.D. and SPRINGER, T.L., 1989. Alternate hosts of Russian wheat aphid (Homoptera: Aphididae). J. Econom. Entomol. 82(5) :1358-1362.

KINDLER, S.D. and SPRINGER, T.L., 1991. Resistance to Russian wheat aphid in wild *Hordeum* species. Crop-science 31(1): 94-97.

KOVALEV, O.V.; POPRAWSKI, T.J.; STEKOLSHCHIKOV, A.V.; VERESHCHAGINA, A.B.;GANDRABUR, S.A., 1991. *Diuraphis Aizenberg* (Homoptera, Aphididae):key to apterous viviparous females, and review of Russian language literature on the natural history of *Diuraphis noxia* (Kurdjumov, 1913). J. App. Entomol. 112 (5) : 425-436.

KRUGER, G.H., and P.H. HEWITT., 1984. The effect of Russian wheat aphid (*Diuraphis noxia*) extract on photosynthesis of isolated chloroplasts: preliminary studies, pp. 34-37. In M.C. Walters (ed.), progress in Russian wheat aphid (*Diuraphis noxia*) research in the Republic of South Africa. Tech. Commun. 191, Department of Agric., Republic of South Africa.

LOXDALE, H.D. and BROOKES,C.P., 1988. Electrophoretic study of enzymes from cereal aphid populations.V.Spatial and temporal genetic similarity of holocyclic populations of the bird-cherry oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae),in Britain. Bull. of Entomol. Res. 78,214-249.

LOXADALE, H.D., CASTANERA, P. and BROOKES, C.P., 1983. Electrophoretic study of enzymes from cereal aphid populations. 1. Electrophoretic techniques and staining systems for characterizing isoenzymes from six species of cereal aphids. *Bull. Entomol.* Res., 73, 645-657.

LOXDALE, H.D.; RHODES,J.A. and FOX,J.S., (1985a). Electrophoretic study of enzymes from cereal aphid populations.IV.Detection of hidden genetic variation within populations of the grain aphid *Sitobion avenae*(F.) (Hemiptera:Aphididae). Theoretical and Applied Genetics 70,407-412.

LOXDALE,H.D.;TARR,I.J.;WEBER,C.P.;BROOKES,C.P.;DIGBY,P.G.N. and CASTANER A,P., (1985b). Electrophoretic study of enzymes from cereal aphid populations. III. Spatial and temporal genetic variation of populations of *Sitobion avenae*(F)(Homoptera:Aphididae). Bull. Entomol. Res. 75,121-141.

MASSEY, B. & S. AMOSSON., 1991. Economic impact of the Russian wheat aphid in the western United States: 1989- 1990 Great Plains Agric. Council pub. No. 139.

MAY, B. and HOLBROOK,F.R., 1978. Absence of genetic variability in the green peach aphid, Myzus persicae (Hemiptera: Aphididae). Annals of the Entomological Society of America 71,809-812.

MCKINNON, L.K.; GILSTRAP, F.E.; GONZALEZ, D.; WOOLLEY, J.B.; STARY, P. and WHARTON, R.A., 1992. Importations of natural enemies for biological control of Russian wheat aphid, 1988-1991. Great Plains Agricultural Council publication 142 : 136-145.

MICHELMORE, R.W., I. PARAM, and R.V.KESSELI., 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl.Acad.Sci U.A.A. 88: 9828-9832.

MICHELS, G.J., and R.W. BEHLE., 1988. Reproduction and development of *Diuraphis noxia* (Homoptera:Aphididae) at constant temperatures. J. Econ. Entomol. 81:1097-1101.

MILLER, R.H., and HAILE, A., 1988. Russian wheat aphids on barley in Ethiopia. SO: RACHIS(ICARDA). Barley and Wheat Newsletter. 7(1-2) : 52-53 .

MORNHINWEG, D.H.; PORTER, D.R. and WEBSTER, J.A., 1995. Inheritance of Russian wheat aphid resistance in spring barley.Crop-science 35(5) : 1368-1371.

MORRISON, P., 1988. Current distribution and economic impact. In page 5,F.B. Peairs. and S.D. Pilcher,eds., Proceedings of the Second Russian Wheat Aphid Workshop, Oct. 11-12, 1988, Denver, Colorado.

MULLIS, K.B. and FALOONA, F.A., 1987. Specific synthesis of DNA in vitro via a polymerase chain reaction. Methods in Enzymology 155, 335-350.

MUNSTERMANN, L.E. and CONN, J.E., 1997. Systematics of mosquito disease vectors (Diptera, Culicidae): Impact of molecular biology and cladistic analysis. Ann. Rev. Entomol., 42: 351-369.

NACHIT, M.M., 1994. ICARDA Annual report 1994.

NARANJO, S.E.; GIBSON, R.L.; WALGENBACH, D.D., 1990. Development, survival, and reproduction of Scymnus frontalis (Coleoptera: Coccinellidae), an imported predator of Russian wheat aphid, at four fluctuating temperatures .Ann. Entomol.Soci.Ame. 83(3) : 527-531.

NEI, M., 1972. Genetic distance between populations. Amer. Naturalist, 106: 283-292.

NEVO, E.; BEILES, A. and BEN-SHLOMO, R., 1984. The evolutionary significance of genetic diversity: ecolgical, demographic and life history correlates.PP. 13-213 in Mani, G.S.(Ed.) Evolutionary dymamics of genetic diversity. Berlin, Springer Verlag.

PANDA, N. 1979. Principles of host plant resistance to insect pests. Allan/Universe,New York

PUTERKA, G.J. and BURTON, R. L.,1991. Aphid genetic in relation to host plant resistance. In:Peters. D.C., Webster.J. and Chlouber, C.S.(eds) Proceedings, Aphid-plant interactions: Populations to molecules, 12-17 August. 1990 MP-132. Oklahoma Agricultural Experimental Station Stillwater. Pp. 59-69.

PUTERKA, G.J., BLACK, W.C., STEINER, W.M. and BURTON, R.L., 1993. Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers. Heredity, 70: 604-618.

REED, H.C.; REED, D.K. and ELLIOTT, N.C., 1992. Comparative life table statistics of *Diaeretiella rapae* and Aphidius matricariae on the Russian wheat aphid. The Southwestern entomologist 17 (4) : 307-312.

RIEDELL, W.E., 1989. Effects of Russian wheat aphid infestation on barley plant response to drought stress [*Diuraphis noxia*, glycine betaine]. Physiologia Plantarum (Denmark). 77(4) : 587-592.

RIEDELL, W.E., 1990. Tolerance of wheat to Russian wheat aphids: nitrogen fertilization reduces yield loss. Journal-of-plant-nutrition 13 (5) : 579-584.

ROBINSON, J., (1992a). Predators and parasitoids of Russian wheat aphid in central Mexico. The-Southwestern-entomologist 17 (12) : 185-186.

ROBINSON, J., (1992b). Russian wheat aphid:agrowing problem for small-grain farmes. Outlook on Agric. 21:57-62.

ROBINSON, J., and B. SKOVMAND., 1992. Evaluation of Summer wheat and other Triticeae for resistance to Russian wheat aphid.Gen. Res. Crop Evol. 39:159-163.

ROHLF, F.J., 1993. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Applied Biostatistical Inc. New York.

SAIKI, R.K.; SCHARF, S.; FALOONA, F.; MULLIS, K.B.; HORN, G.T.; ERLICH, H.A. & ARNHEIM, N.,1985. Enzymatic amplification of ß-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230, 1350-1354.

SAIKI, R.K.; GYLLENSTEN, U.B. and ERLICH, H.A., 1988. The polymerase chain reaction. Davies, K.E. (Ed.). Genome analysis: a practical approach. Oxford (UK):141-152.

SAITOU, N. and NEI, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406-425.

SHUFRAN.K.A.BLACK,W.C.IV and MARGOLIES, D.C., 1991. DNA fingerprinting to study spatial and temporal distributions of an aphid, *Schizaphis raminum* (Homoptera : Aphididae). Bull.Entomol.Res.,81,303-313.

SMITH, C.M., 1989. Plant resistance to insects a fundamental approach. Wiley. New York.

SIMON,J.P.; PARENT, M.A, & AUCLAIR,J.L., 1982. Isozyme analysis of biotypes and field populations of the pea aphid, *Acyrthosiphon pisum.* Entomologia Experimentalis et Applicata 32,186-192.

SINGH, S.M. and CUNNINGHAM, T.K., 1981. Morphological and genetic differentiation in aphids (Aphididae). Can. Entomolo., 113, 539-550.

SNEATH, P.H.A. and SOKAL R.R., 1973. Numerical taxonomy. Freeman, San Francisco.

STARY, P. and GONZALEZ, D., 1991. The Chenopodium aphid, *Hayhurstia atriplicis* (L.) (Homoptera,Aphididae), a parasitoid reservoir and a source of biocontrol agents in pest management. J. App. Entomol. 111(3) : 243-248.

STEINER,W.W.M.; VOEGTLIN,D.J. and IRWIN, M.E., (1985a). Genetic differentiation and its bearing on migration in North American populations of the corn leaf aphid, *Rhopalosiphum maidis*(Fitch) (Homoptera: Aphididae). Annals of the Entomological Society of America 78,518-525.

STEINER,W.W.M.; VOEGTLIN,D.J.; IRWIN, M.E.& KAMPMEIER,G., (1985b).Electrophoretic comparison of aphid species: detecting differences based on taxonomic statuus and hostplant. Journal of Comparative Biochemistry and Physiology B.81,295-299.

STOETZEL M. R., 1987. Information on and identification of *Diuraphis noxia* (Homoptera:Aphididae) and other aphid species colonizing leaves of wheat and barley in the United States. J. Econ. Entomol. 80, 696-704.

SUOMALAINEN,E.; SAURA,A.; LOKKI,J. and TEERI,T., (1980). Genetic polymorphism and evolution in parthenogenetic animals. IX,Absence of variation within parthenogenetic aphid clones. Theoretical and Applied Genetics 57,129-132.

THOMAS, J.B., and R.A. BUTTS., 1990. Effect of Russian wheat aphid on cold hardiness and winterkill of overwintering winter wheat. Can. J. Plant Sci. 70:1033-1041.

TOMIUK, J., WORHMANN.K.and EGGERS-SCHUMACHER. H. A.,1979. Enzyme patterns characteristic for the identification of aphid species.Z.ang.Entomol.,88:440-446.

TOMIUK, J.& WOHRMANN, K., 1984. Genotypic variability in natural populations of *Macrosiphum rosae* (L.) In Europe. Biologisches Zentralblatt 103,113-122.

VIA, S., 1990. Ecological genetics and host adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. Ann. Rev. Entomol. 35: 421-446.

VON WECHMAR, M.B., and E.P. RYBICKI., 1981. Aphid transmossion of three viruses causes Freestate streak.S. Afr.J.Sci. 77:488-492.

VON WECHMAR, M.B.; LAUBSCHER, J.M. and WILLIAMSON, C., 1991. The pathogenesis of aphid lethal paralysis virus in *Diuraphis noxia* and other small grain aphids. Misc.-public. Agricultural Experiment Station, Oklahoma State University (no.132).

WALTERS, M.C.; F. PENN; F. DU TOIT; T.C. BOTHA; K. AALBERSBERG, P.H.HEWITT, and W. BROODTYK. , 1980. The Russian wheat aphid. Farming in South Africa, Leaflet Series, Wheat C3, pp. 1-6 Government Printer, Pretoria.

WANG, Z.G. and KNUDSEN, G.R., 1993. Effect of *Beauveria bassiana* (Fungi: Hyphomycetes) on fecundity of the Russian wheat aphid (Homoptera:Aphididae). Environ. Entomol. 22(4) : 874-878.

WEBSTER, J.A.; STARKS, K.J. and BURTON, R.L., 1987. Plant resistance studies with *Diuraphis noxia* (Homoptera: Aphididae), a new United States wheat pest. J. Econom. Entomol. 80 (4) : 944-949.

WEBSTER, J.A. and STARKS, K.J., 1987. Fecundity of *Schizaphis graminum* and *Diuraphis noxia* (Homoptera: Aphididae) at three temperature regimes. Journal of the K a n s a s Entomological-Society 60 (4) : 580-582.

WEBSTER, J.A.; DU TOIT, F. and POPHAM, T.W., (1993a). Fecundity comparisons of the Russian wheat aphid (Homoptera:Aphididae) in Bethlehem, South Africa, and in Stillwater, Oklahoma. J. Econom. Entomol. 86 (2) : 544-548.

WEBSTER, J.A.; PORTER, D.R.; BAKER, C.A. and MORNHINWEG, D.W., (1993b). Resistanceto Russian wheat aphid (Homoptera: Aphididae) in barley: effects on aphid feeding. J. Econom. Entomol. 86 (5) : 1603-1608.

WEIGAND F., M. BAUM, S. UDUPA., 1993. DNA Molecular Marker Techniques,Technical Manual No. 20 .

WILLIAMS, J.G.K., KUBELIK, A.R., LIVAK, K.J., RAFALSKI, J.A. and TINGERY, S.V., (1991). DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Research 18,6531-6535.

WILSON, A.D.; CLEMENT, S.L. and KAISER, W.J., 1991. Survey and detection of endophytic fungi in *Lolium* germplasm by direct staining and aphid assays. Plant-disease 75(2): 169-173.

WOOL, D., BUNTING,S. and VAN EMDEN, H.F., 1978. Electrophoretic study of genetic variation in British *Myzus persicae* (Sulzer) (Hemiptera:Aphididae). Biochemical Genetics 16,987-1006.

ZERENE, Z. MIREYA; CAGLEVIC. D,MILAN; RAMIREZ, and A. IGNACIO., 1988. A new cereal aphid detected in Chile [*Diuraphis*]. Agricultura-Tecnica (Chile). 48(1) : 60-61.

ZHANG, G., 1991.Russian wheat aphid (RWA) in China Miscellaneous publication Agricultural Experiment Station,-Oklahoma-State-University 132, 327-328.

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Appendix 1. List of solutions used for RAPD-PCR analysis of aphids

Solutions

2x CTAB	2% CTAB (cetyltrimethy lammonium bromide) 1.4 M Na Cl 0.1 M Tris -HCL, pH 7.5 20 mM EDTA 5 nM Ascorbic Acid
Washing buffer	76% ethanol /10 mM ammonium acctate
TE Buffer	10 mM Tris-base 1 mM EDTA,pH 8.0
Chloroform/isoamylalcohol (24:1)	24 ml/1 ml
1X TAE buffer	40 mM Tris-acetate 1 mM EDTA,pH 8.0
Loading buffer (10X)	0.25% Bromophenol blue 50% Glycerol 60 mM EDTA, pH 8.0

Note: All the chemicals were procured from Sigma chemical company, St-Laris, USA.