**An-Najah National University**
**Faculty of Graduate Studies**

# Identification of Resistant Sources to Leaf Rust and Powdery Mildew Disease in Oats

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# Dedication

This Project is dedicated to my mother, father, sisters, brother and friends.

# Acknowledgments

I would like to express my deepest respect and most sincere gratitude to my god and to my supervisor Dr. Munqez Shtaya, for his guidance and encouragement at all stages of my work.

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I would like to express my sincere thanks and appreciation to my mother, father, sisters and brother for their support.

## الإقرار

أنا الموقع أدناه, مقدم هذه الرسالة التي تحمل العنوان:

# Identification of Resistant Sources to Leaf Rust and Powdery Mildew Disease in Oats

# تحديد مصادر مقاومة لمرضي البياض الدقيقي وصدأ الأوراق في محصول الشوفان

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص, باستثناء مـــا تمـــت الإشارة إليه حيثما ورد, وأن هذه الرسالة ككل, أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

## Declaration

The work provided in this thesis , unless otherwise referenced , is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

**Student's Name:** **اسم الطالب:**

**Signature:** **التوقيع:**

**Date:** **التاريخ:**

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# List of Abbreviations

| No | | |
|---|---|---|
| 1 | DS | Disease severity |
| 2 | SSV | substomatal vesicle |
| 3 | IH | infection hyphae |
| 4 | HMC | haustorial mother cell |
| 5 | PGT | primary germ tube |
| 6 | AGT | appressorial germtube |
| 7 | CWA | cell wall apposition |
| 8 | ECM | extra-cellular material |
| 9 | PAL | phenylalanine ammonia lyase |
| 10 | NBS | N-terminal nucleotide-binding site |
| 11 | LRR | leucine-rich repeat |
| 12 | HR | hypersensitive response |
| 13 | PR | partial resistance |
| 14 | NSGC | National Small Grains Collection |
| 15 | AUDPC | Area under Disease **P**rogress **C**urve |
| 16 | LP | Latent period |
| 17 | IF | infection frequency |
| 18 | RLP50 S | relative latent period of seedlings |
| 19 | ANOVA | Analysis of variance |
| 20 | RLP | longer relative latency period |
| 21 | RIF | relative infection frequency |
| 22 | IT | Infection type |

**Identification of Resistant Sources to Leaf Rust and Powdery Mildew Disease in Oats**

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## Abstract

A collection of 120 different accessions of oats from different countries in the region were kindly provided by the National Small Grains Collection, Idaho (NSGC), USA. A local wild accession collected from the experimental farm of the Faculty of Agriculture at Tulkarm, Palestine was used as a susceptible control. The main objectives of this study are to find new sources of resistance to oat leaf rust, and powdery mildew and to characterize the resistance to rust in oats under controlled conditions to study the components of resistance to the macroscopic level.

During the 2008-2009 growing season the susceptible local accession (control accession) showed 56% DS (100% AUDPC) of powdery mildew. DS ranged from very high to very low, and the frequently distribution was markedly skewed towards low DS. During the same growing season the susceptible local accession (control accession) showed 48% DS (100% AUDPC) of rust. DS of rust ranged from very high to very low, and the frequently distribution was markedly skewed towards low DS. Nearly half of the collection displayed AUDPC < 50%. Thirteen of them, with AUDPC ≤ 20% (10.8% of the collection), were selected to study their reaction to leaf rust at seedling stage. These resistant accessions were selected and grown in the field to obtain seeds for further studies.

# Chapter One

# Introduction

## 1.1. History of oats

Knowledge of oats cultivated before the Christian era are ungenerous, it seems that it was not been accepted in early times unlike wheat and barley. Probably that oat was considered as a weed between these cereals. The first seeds found in archaeological remains belonging to Egypt, to the Twelfth Dynasty (2000 - 1788 BC). These oats were identified as *Avena strigosa* and may also be *Avena fatua* o *Avena sterilis* (López-Bellido, 1991). Cultivated oats belong to the order Poales, family Poaceae, genus *Avena* which contains many species from which *sativa* and *byzantina* are the most cultivated.

## 1.2. Uses and Properties.

The grain has been used as food for livestock (horses and mules, and cattle and sheep) and humans (on a smaller scale) in dietetic products, since ancient times. The plant itself is used as fodder, grazing, hay or silage alone or with legumes (Gibson and Benson, 2002). It also presents important cosmetic qualities, so it is one of the main active ingredients in many cosmetic products. Oatmeal is a cereal rich in protein, fats, carbohydrates and vitamin B1 or thiamine. It also contains minerals like phosphorus, potassium, magnesium, calcium and iron. 80% of total fats in oats are abundant unsaturated and essential fatty acid linoleic acid.

## 1.3. Economic Importance.

Oats was within the top five in world cereal production. Oat is the most important winter cereal in cold climates of the northern hemisphere (López-Bellido, 1991). Today production has fallen to sixth place found in cereal crops worldwide (FAO, 2009).

## 1.4. Botanical Features

Oat is an annual grass with pseudo fasciculate root system, which allows the plant to make better use of land in poor regions (Clement and Prast, 1969). Its stem is thick, straight and quite soft, with little resistance to lodging. Its leaves are flat, elongated. The inflorescence of oat is panicle, spikelets are a cluster of two or three flowers placed on long stems, producing a conical shape (Guerrero, 1977).

It is a self-pollinating plant, and anther dehiscence occurs while opening the flowers. However, there is a certain proportion of flowers that open their glumes before the maturation of stamens and pistils (Guerrero, 1977). The grain consists of a caryopsis hairy and elongated, closely surrounded by a lemma and palea.

## 1.5. Ecology of the Crop.

In time of flowering and grain formation, oat is very sensitive to warm temperatures, causing sterility, poor grain filling and lower yields, so in these conditions, early maturing varieties grown. The minimum and maximum temperature for the cultivation of oats is 4.8 º and 37 º C, respectively, while the optimum range varies from 25-30 ºC (López-Bellido, 1991)

Oat has a high transpiration rate, but excess water will damage it. (Guerrero, 1977). Soil moisture has different effects on the seed: if there is no water the seed will not germinate, but excess water can induce seed dormancy (Banting, 1966).

# Chapter Two

# Literature review

## 2.1. Important Diseases affecting oat

The most destructive diseases, harmful and therefore, most important, affecting oats all over the world, are the rust (*Puccinia coronata*) and powdery mildew (*Blumeria graminis* f. sp. *avenae*) (Harder and Haber, 1992; Yu and Herrmann, 2006). These diseases does cause severe losses in production of oats and grain quality in terms of seed weight (Hammami et al 2007; Yu and Herrmann, 2006). An estimate loss of up to 20% and 32% in yield due to rust and powdery mildew respectively has been reported (Long and Hughes, 2003).

### 2.1.1. *Puccinia coronata,* the leaf brown rust.

After a uredospore of rust has landed on a plant leaf, the spore germinates from the germ pores that are thinner areas in the wall of uredospores (Lewis and Day, 1972; Webster, 1980; Clifford, 1985). The germ tube grows perpendicular to the long axis of the leaf towards leaf stomata (Lewis and Day, 1972; Clifford, 1985). Free moisture and darkness are essential factors for the germination and penetration of the leaf. Germination occurs at a temperature range of 5 to 25 C, and is high at 10-20 C (Clifford, 1985, Smith et al., 1986). Colonisation is limited by temperature and increases to a maximum in the temperature range 5-25 C (Smith et al., 1986).

After reaching leaf stomata, the germ tube induces formation of appressorium, subsequently, a penetration peg grows through the stomatal opening and after reaching the substomatal cavity, a cigar-shaped

substomatal vesicle (SSV) is formed. The SSV germinates from either end and infection hyphae (IH) grow between cells that reach the mesophyll, where they attack cells. A penetration peg forms from the haustorial mother cell (HMC) and, if the penetration is successful, a haustorium is formed within the cell. As in B. graminis, the haustorium is separated from the plant cytoplasm by an extension of the plant plasma membrane and is not truly intracellular but functions as the feeding organ of the fungus (Heath, 1997; Heath and Skalamera, 1997). Under optimum conditions, the sporulation commences 6-8 days after infection (Smith et al., 1986).

Leaf rust has a complicated macrocyclic lifecycle, producing five distinct spore forms (Clifford, 1985; Webster, 1980), and is heteroecious, needing two unrelated hosts to complete the life cycle; the primary host and the alternate host (Webster, 1980). A single uredinium may contain 50.000 to 400.000 uredospores and, in a growing season, four-five generations of uredospores might be formed. This can result in a rapid build-up of infection in a crop, making this spore state the most destructive (Webster, 1980)

The first symptom of infection with leaf rust is the appearance of chlorotic halos on the leaf, which occur primarily on the upper leaf surface. Orange-brown pustules of uredial spores appear soon afterwards in the chlorotic halos. Infection is normally confined to the leaf, and leaf-sheaths but with severe infections late in the season, some stem, glume and awn infections can occur. At this time, grey telia may be formed in stripes on leaf-sheaths covered by the epidermis.

**Picture (1): Oat leaves with pustules of rust (*Puccina coronata*).**

### 2.1.2. *Blumeria graminis,* (powdery mildew of cereals.)

*B. graminis* differs from all other powdery mildews by forming two germ tubes (Kunoh et al., 1979). A part of the asexual life cycle of *B. gramin*is is illustrated in Fig. 5. The primary germ tube (PGT) emerges from the conidia within 0.5-1.5 h after contact with the epidermal cell surface of the leaf (Staub et al., 1974; Kunoh, 2002). The PGT remains short (5-10 μm) and aseptate, and is believed to play several important roles as a prerequisite to appressorium formation (Aist and Bushnell, 1991; Green et al., 2002).

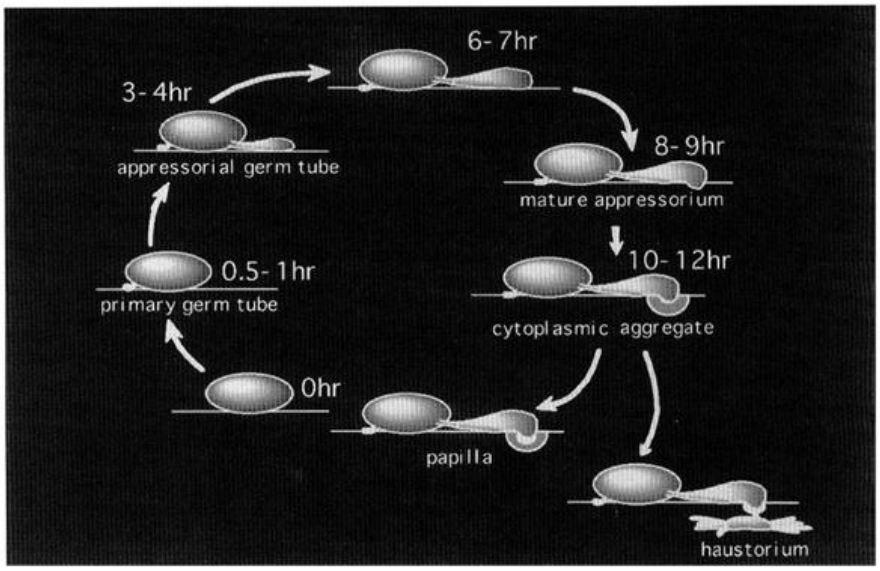


**Figure (1): Infection process of powdery mildew (*B. graminis*) (Kunoh, 2002).**

The appressorial germtube (AGT) emerge 3-3.5 h after the PGT. This germtube elongates, ultimately to 30-40 μm, becomes septate, and swells at the tip to form a single, hook-shaped appressorial lobe by about 8-10 h (Aist and Bushnell, 1991; Kunoh, 2002). Beneath the appressorial lobe a penetration peg is formed, which tries to penetrate the underlying host epidermal cell cuticle and cell wall about 12-15 h after contact.

The attempted penetration of the cell wall and cuticle will cause localised deposition of material by the epidermal cell into the inside surface of the cell wall, directly beneath the appressorium and penetration peg (Kunoh et al., 1996; Zeyen et al., 2002). This cell wall apposition (CWA) is known as the papilla response, and will arrest the growth of the fungus, if effective. If the penetration attempt fails, another lobe and penetration attempt might form, usually opposite the first lobe though nearer to the conidium. In this way, one AGT can form 3-4 lobes in the attempt to penetrate the cell. If the fungus succeeds in penetrating the cell wall cuticle

and ineffective papilla, the feeding organ of the fungus, the haustorium, will be formed (Hirata, 1967)

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Within 1-1.5 h after penetration, the haustorial primordium becomes visible. This develops to form the characteristic haustorium with finger-shaped projections (Hirata, 1967). The haustorium is not in direct contact with the host cell cytoplasm, but is surrounded by the extrahaustorial membrane, which is an invagination of the host plasma membrane forming the interface between the haustorial apparatus and the host cytoplasm (Hirata, 1967; Green et al., 2002). The haustorium presumably takes up nutrients from the host for continuous growth on the aerial epidermal leaf surface. The growing hyphae branch, producing a hyphal colony with a large number of haustoria in epidermal cells. The colony begins to form conidiophores and conidia after the tertiary haustorial generation. Five to eight days after inoculation, heavy sporulation occur. At this time, the asexual lifecycle is completed (Hirata, 1967; Aist and Bushnell, 1991).

Powdery mildew on cereals is seen as spots or patches of a white to greyish powdery mildew growth on entire leaves and stems of plants. In heavily diseased leaves, the tissue can be completely covered by the mildew. During the growing season, the fungus exists as mycelium and reproduces and causes most infections with its asexual conidial stage. Only towards the end of the season, or when the food supply is diminished will the sexual or perfect stage, the cleistothecium with ascospores be produced

on infected leaves if both mating types are present, and this can be observed as black spots in the white to greyish mycelium (Agrios, 1997).

## 2.2. Types of resistance.

Despite the large number of microorganisms capable of causing disease, most plant species are resistant to any given pathogen. Plants utilize a range of defense mechanisms, ranging from passive mechanical or preformed chemical barriers, which provide non-specific protection against a wide range of organisms, to more active host-specific responses that provide host- or varietal-specific resistance. Research elucidating the mechanisms by which plants react to an attack by fungal pathogens and successfully defend themselves, and fungal pathogens successfully invade the host plant, is important in the attempt to cope with and reduce the damage caused by fungal attack.

### *2.2.1. The importance of extra-cellular material in pathogenesis.*

The establishment of a pathogenic relationship is dependent upon contact between the cell surfaces of the pathogen and host. Any encounter between a fungal pathogen and a potential host plant, will immediately cause interactions between the two individuals. The role of these very early interactions in pathogenesis is still unknown, and is subject of much interest and investigation.

Many fungal pathogens release extra-cellular material (ECM) during the initial phases of the infection process (Nicholson and Epstein, 1991; Epstein and Nicholson, 1997). The most obvious functions of ECM is involvement in adhesion of spores, germlings and appressoria to host surfaces, a prerequisite for successful infection by fungal pathogens. The importance in disease establishment of ECM, released by *B. graminis* and *P. hordei* to the surface of their cereal hosts is discussed briefly, since this is important in relation to this thesis.

The release of ECM occurs rapidly after pathogen - host contact. For example, conidia of *B. graminis* release ECM to the leaf surface within 2 min after contact, accordingly prior to germination (Nicholson *et al.,* 1988; Kunoh *et al.*, 1988). ECM secretion from rust fungi has been most intensively studied in the species *Uromyces viciae-fabae* infecting faba bean. The uredospores of *U. viciae-fabae* secretes a considerable amount of material to the uredospore - plant cell interface prior to germination, which is termed the adhesion pad (Deising *et al.*, 1992).

The very fast release from the *B. graminis* conidia and *U. viciae-fabae* uredospores could indicate, that the ECM is preformed in the fungal spores. Carver *et al.*, (1999) suggests that the ECM of *B. graminis* is released from the conidial wall projections, where they touch the leaf surface. In addition to the secretion from fungal spores, the growing rust germ tube and the primary and appressorial germ tube of powdery mildew

also secrete ECM (Beckett *et al.*, 1990; Clement *et al.,* 1993; Carver *et al.*, 1999). On artificial substrates, the ECM released from *B. graminis* has been shown to contain highly active esterase enzymes, including a cutinase (Nicholson *et al.*, 1988; Pascholati *et al.*, 1992).

This conidial exudate has been shown to be able to degrade a component of the barley cuticle, during the first 30 min after the contact stimulus (Kunoh *et al.*, 1990). However, since only very small amounts of ECM are released to the leaf surface by *B. graminis,* it is impossible to visualise and analyse ECM on undisturbed intact leaf surfaces (Carver *et al.*, 1999). Therefore, the importance and role of ECM in germination, penetration and growth of *B. graminis* is still unknown.

In the interaction between *U. viciae-fabae* and faba bean, esterases including cutinase have also been detected in the adhesion pad, and have now been shown conclusively to be involved in attachment of uredospores of the rust pathogen to the cuticle of the host (Deising *et al.*, 1992). The adhesion pad beneath the uredospores with its content of enzymes including cutinase, could spread onto the leaf surface with the growing rust germ tube, the cutinase eroding the surface while the tube is growing, causing proper germ tube adherence.

However, also the additional ECM secreted by rust germ tubes, with its content of proteins, is known to be involved in germ tube adhesion

(Chaubal *et al.*, 1991; Clement *et al.*, 1993). Moreover, fungal proteases capable of breaching structural plant cell wall proteins, have been isolated from extracellular washing fluids of infection hypha of *U. viciae-fabae* (Rauscher *et al.*, 1995). These proteases are involved especially in the degradation of fibrous, hydroxyproline-rich proteins. Since such proteins are important in plants for plant cell wall stability and play a role in defence against fungal pathogens, it could be argued that the extracellular proteases of *U. viciae- fabae* may be involved in localised breaching of the host cell wall (Rauscher *et al.*, 1995).

From all these more or less well-documented pre-penetration events it can be concluded, that contact between a fungal spore and the host surface, and germination and growth across the epidermal tissue, is definitely not just a passive process (Clement *et al.*, 1993). If the ECM released to leaf surfaces is not only involved in adhesion, but also has enzymatic activity causing some degree of degradation of the leaf surface wax and cuticle (Nicholson *et al.*, 1988; Pascholati *et al.*, 1992), this erosion of the surface cuticle could cause recognition of the pathogen by the host plant cell in the pre-penetration stage.

This recognition could result in early cell responses, involved in defence against pathogens. Several lines of evidence have demonstrated that plant cells recognise the presence of microbes on their surface and initiate defence associated responses against attacking microbes, in advance of attempted penetration (Cho and Smedegaard-Petersen, 1986).

### *2.2.2. Papilla based penetration resistance.*

Many penetration attempts, by directly penetrating pathogenic fungi are stopped at the penetration stage, regardless of compatible or incompatible plant - fungal interactions (Zeyen *et al.,* 2002). Penetration failure is very often caused by encasement of the penetration peg in a very localised deposition of material between the cell wall and the plasmalemma, termed as the papilla (Kunoh *et al.,* 1996). Papilla deposition is one of the most common morphological changes in plant cells at the site of attempted penetration by fungal pathogens, and is a plant cell response shared by diverse types of resistance (Heath, 1980).

Even in compatible interactions, the general background resistance of the attacked cells cause a number of unsuccessful attacks, due to papilla deposition (Bushnell, 2002). Moreover, even in the case of successful penetration of the plant cell, an ineffective papilla can often be observed at the penetration site. Thus, it is plausible that the rate of papilla deposition, the amount of deposited material in the papilla and the composition of the deposited material, are important for the effectiveness of the papilla and thus for the outcome of attack (Kunoh *et al.,* 1996).

Probably the most important constituent of the papilla is callose, a carbohydrate containing β-1-3, linked glucan (Heath and Skalamera, 1997; Zeyen *et al.*, 2002). However, localized autofluorescence is also observed in papilla, as a common epidermal cell response to *B. graminis* appressoria in cereals (Kita *et al.*, 1980; Carver *et al.*, 1994). This autofluorescence is due to autofluorogenic phenolic compounds, being another important

constituent of the formed papilla (Zeyen *et al.*, 2002). The phenolics are synthesised as a part of the lignin biosynthetic pathway, and has been shown to be involved in resistance to attempted penetration from appressoria of *B. graminis* (Carver *et al.*, 1994).

The importance of these phenolic compounds in penetration resistance has been shown, by inhibition of PAL (phenylalanine ammonia lyase), the enzyme that catalyses the first committed step in phenylpropanoid biosynthesis. Inhibition of PAL decreased the percentage of fungal attacks associated with localised autofluorescence, and at the same time increased the susceptibility of the epidermal cells to successful penetration (Carver *et al.*, 1994; Carver *et al.*, 1998). However, Carver *et al.* (1992) showed that the inhibition of PAL, causing reduction in autofluorogenic phenolic compounds, only increased the susceptibility of epidermal cells to the appropriate ff. spp. of *B. graminis*, but not to inappropriate ff. spp. of this pathogen in barley, oat and wheat. Furthermore, leaf treatment with the sugar D-mannose, suppresses penetration resistance to inappropriate ff. spp. without having effect on localised autofluorescent responses (Zeyen *et al.*, 2002).

These results indicate, that the reduced content of phenolics in the papillae caused by PAL inhibition is only one factor involved in the papillae based resistance. Another important component accumulating at the papillae is hydrogen peroxide ($H_2O_2$) a reactive oxygen species

(Thordal-Christensen *et al.,* 1997; Olesen *et al* 2003). The oxidative power of $H_2O_2$ is, beyond other biochemical processes, involved in maturation of the papillae by its involvement in protein cross-linking and phenolic polymerisation (Zeyen et al., 2002).

### *2.2.3. The* forma specialis *based non-host resistance.*

*B. graminis* exhibits specialised physiologic forms (formae speciales; ff. spp.), capable of infecting specific genera of the Gramineae (Tosa, 1996). This means that specific cereals are only natural hosts for one specialised form of *B. graminis*, the appropriate *forma specialis* (f. sp.) and are resistant against all other ff. spp. (Tosa, 1996). Specificity at the f. sp. – genus level is believed to follow a gene-for-gene relationship (Tosa, 1989; Tosa and Sakai, 1990).

*Blumeria graminis* f. sp. *hordei* (*Bgh),* f. sp. *tritici* (*Bgt*) and f. sp. *avena* (*Bga*) are the appropriate powdery mildews of barley, wheat and oat, respectively. Attack by appropriate f. sp. of *B. graminis* in these cereals, will cause a high percentage of penetration attempts to be successful, causing haustorium formation and establishment of disease. However, when attacked by either of the inappropriate ff. spp., penetration attempts fail in association with papilla deposition by epidermal cells, death of the attacked cells, or if penetration succeeds and haustoria is formed the plant cell die soon afterwards (Olesen *et al.*, 2003).

### *2.2.4. Race-specific resistance.*

Race specificity is controlled by gene-for-gene interactions in which corresponding genes in the host and parasite determine whether the two organisms are compatible (Flor, 1971). Gene-for-gene interactions are common in plant - pathogen interactions, and reflect the ability of a plant to recognize an intruding pathogen. When any one of the many genes for resistance (*R* genes) in the host, is matched by a specific, corresponding gene for avirulence (*Avr* genes) in the parasite, host and parasite are incompatible (Scholtens-Toma *et al.*, 1991). A very simplified model for the gene-for-gene interaction, is that any specific *Avr* gene of a pathogen encodes an molecule, called a specific elicitor that, after recognition by a matching receptor in a particular plant genotype encoded by a corresponding *R* gene, initiates defense responses (De Wit, 1997).

However, this model of direct interaction between plant receptor and pathogen elicitors is probably more the exception than the rule (De Wit, 1997; Panstruga and Schulze-Lefert, 2002). Most likely, a cascade of events is induced by the *Avr* encoded elicitor, this cascade results in an "indirect" recognition by the corresponding R protein of the plant, resulting in induction of resistance mechanisms (De Wit, 1997). Moreover, each R protein probably recognizes more than one pathogen elicitor and thereby more than one pathogen (Panstruga and Schulze-Lefert, 2002). This is supported by the observations in Arabidopsis of only approximately 100 *R* loci contained in the genome. This is a rather low number, considering all

the pathogen elicitors the plant has to respond to (Panstruga and Schulze-Lefert, 2002).

The response to race-specific resistance, conferred by gene-for-gene interaction, in the cereal - powdery mildew system, is the localized and rapid cell death at attempted infection sites (De Wit, 1997; Lamb and Dixon, 1997). This activation of host cell death, known as the hypersensitive response (HR), will finally result in resistance since the dead cell is no longer able to supply the intruder with nutrients, necessary for the establishment of disease by biotrophic fungi (Freialdenhoven *et al.*, 1994; Schulze-Lefert and Vogel, 2000).

### *2.2.5. Partial resistance against leaf rust*

Two types of resistance against leaf rust are distinguished. One is of the hypersensitive nature, the other is known as partial resistance (PR) and is characterized by a reduced rate of epidemic, in spite of a susceptible infection type (Parlevliet, 1978). PR results from reduced infection frequency, a longer latent period, and a reduced spore production (Jacobs and Kiriswa, 1993). The HR kind of resistance is governed by major genes, designated Pa-genes, and is race-specific and not durable at all. PR are of poly-genic nature, appear race non-specific and durable (Clifford, 1985). The known genes involved in the resistance in barley against *P. hordei,* are effective only to certain races of the pathogen and not to any other pathogens (Parlevliet, 1993). Breeding efforts, to develop varieties resistant

to the *P. hordei*, have been done primarily by introducing new resistance genes, conditioning HR or PR resistance.

However a limited number of known resistance genes for use, and a quick adaptation of the pathogen to the new resistance have complicated this work (Walther, 1987). Because of the durability of PR compared with the short-lived HR kind of resistance, breeders have special interest in using PR in their breeding programs. Histological studies to gain insight into the nature of PR, using the barley – *P. hordei* interaction as the model system has been made (Niks, 1981; Niks, 1982).

By examining different lines of barley with different degree of PR towards *P. hordei* it was found, that the infection was arrested after growth of the primary infection hypha from the substomatal vesicle and production of haustorial mother cells designated early abortion. There was no significant difference in germination, appressorium formation, stomata penetration and SSV formation, between the different lines of barley. These experiments did not indicate whether haustoria were produced.

However, later experiments did show that PR towards *P. hordei* in barley often is prehaustorial (Niks, 1986). After inoculation of barley varieties with high levels of PR with *P. hordei* a proportion of the colonies failed to form haustoria, the proportion failing being related to the degree of PR. Moreover, after the formation of the first haustorium, the formation of further haustoria was delayed for some time in varieties with high level of PR. This is in contrast to the HR. In almost all host - rust interactions

studied so far, the HR has been demonstrated to be post-haustorial, being elicited after the formation of the first haustorium (Heath, 1982).

# Chapter Three

# Objectives

The main objectives of this study are:

1. Find new sources of resistance to oat leaf rust, *Puccinia coronate* f.sp. *avenae* and powdery mildew *Erysiphe graminis* f. sp. *avenae*.
2. Characterize the resistance to rust in oats under controlled conditions to study the components of resistance to the macroscopic level.

# Chapter Four

# Material and Methods

### 4.1. Plant Material.

A collection of 120 different accessions of oats from different countries in the region were provided by the National Small Grains Collection, Idaho (NSGC), USA (Table 1). A local wild accession collected from the experimental farm of the Faculty of Agriculture at Tulkarm, Palestine was used as a susceptible control.

**Table (1): Origin and source of the 121 oat accessions used in this study.**

| Origin | Number of accessions |
|---|---|
| Cyprus | 6 |
| Israel | 5 |
| Syria | 1 |
| Turkey | 108 |
| Palestine | 1 |

### 4.2. Field experiment:

Field testing was performed at the experimental farm of the Faculty of Agriculture at Tulkarm, Palestine during the growing season 2008–2009. Accessions were sown in November 2008 in three complete randomized blocks. Each accession was represented by 25–30 seeds in a single row, 1 m long per replicate. A spreader row, of the local accession, was sown every five accessions of the collection as a spreader and control.

**Picture (2): Field preparation and planting**

### 4.2.1. Inoculation method

No artificial inoculation was performed since both powdery mildew and rust are common to our region.

### 4.2.2. Scoring assessment

Disease severity (DS) was estimated three times during the growing season as the percentage of leaves covered by the pathogens. These 3 evaluations were used to calculate Area under Disease **P**rogress **C**urve (AUDPC) using the formula:

$$AUDPC = \sum ½[(S_i+E_{i+1})\ (t_{i+1}-t_i)]$$

Where Si is the rust severity at assessment date i, ti is the number of days after the first observation on assessment date i and k is the number of successive observations. The means of the observed AUDPC values were converted into relative values and expressed as a percentage of the susceptible local line (Sillero et al 2000).

## 4.3. Confirmation of Resistance under Controlled Conditions.

Components of resistance of selected accessions were determined. Selected accessions were those that showed a AUDPC <25% in the field and compatible interaction (IT ≥ 7). Seeds of the selected accessions were sown in soil in plastic trays (35 x 20 x 8 cm) with three replicates of three plants each

**Picture (3): Preparation of plants for inoculation with leaf rust**

In each tray, eight to ten accessions were included. Eleven days after sowing, the first leaf of each plant was placed in a horizontal position with the help of metal staples and inoculated with isolate TU-09 of *Puccinia coronate* f.sp. *avenae* (collected from the field where the collection was sown during the 2008-2009 growing season).

The inoculation was carried out in by dusting a mixture of freshly collected spores and wheat flour (1:10, v/v). Each tray was inoculated with 3 mg of spores that resulted in about 200 spores/$cm^2$ deposition (Niks and Rubiales, 1994). The inoculated plants were kept in an inoculation chamber for 12 h at 20 ºC with a relative humidity of about 100% and in darkness. Plants were then transferred to a growth chamber at 18–22 ºC and white fluorescent light (12 h light/12 h dark).

**4.4. Disease scoring for seedling disease test**

The most important component to determine partial resistance is latency period that is the period between incubation and sporulation. Latent period (LP) of each plant was evaluated by estimating the number of hours after inoculation at which 50% of ultimate numbers of pustules were matured. The colonies were seen by eyes as small light green flecks on the leaves, which formed halo's, later on it was seen as orange pustules.

When the halo becomes visible two transverse lines were drawn by marker, which enclosed 50 – 60 infection units. LP50s were calculated by using the formula given below, on daily pustules counts with a 10 X pocket lens. At the time of the final count infection frequency (IF) was measured using a metal strip with 2 x 0.5 $cm^2$ window (Parlevliet and Kuiper, 1977). Experiment consisted of three replicates and RPL50S and Infection Frequency were averaged over three.

The relative latent period of seedlings (RLP50 S) were calculated relative to the LP of the local control in the seedlings, where RLP50S, described by (Parlevliet, 1975). The period between incubation and sporulation lasts 4-6 days in seedling susceptible genotype and 6 – 8 days in resistant genotypes.

Formula 1 : $LP\ 50 = T_1 + (T_2 - T_1) * (N_{100}/2 - N_1)/ (N_2 - N_1)$

With

$T_1$ the time just before 50% of the final number of postulates sporulates,

$T_2$ the time just after the 50% point,

$N_1$ number of sporulating postulates at $T_1$,

$N_2$ number of sporulating postulates at $T_2$,

$N_{100}$ half the number of postulates sporulating at the moment of the final count

## 4.5. Data analysis:

Analysis of variance (ANOVA) was conducted by using PROC GLM in an SAS program (SAS Institute, 1988). Comparisons between lines were made by the Duncan-test.

# Chapter Five

# Results

## 5.1. Reaction in the field

### 5.1.1. Powdery mildew:

During the 2008-2009 growing season the susceptible local accession (control accession) showed high susceptibility to powdery mildew with 56% DS (100% AUDPC) (Fig. 2). DS in the collection ranged from very high to very low, and the frequently distribution was markedly shifted towards high DS. More than 50% of the collection showed high DS meanwhile 10.7% of the collection showed low DS with Relative AUDPC from 0-20%. These accessions could be used as a good source for partial resistance to powdery mildew.

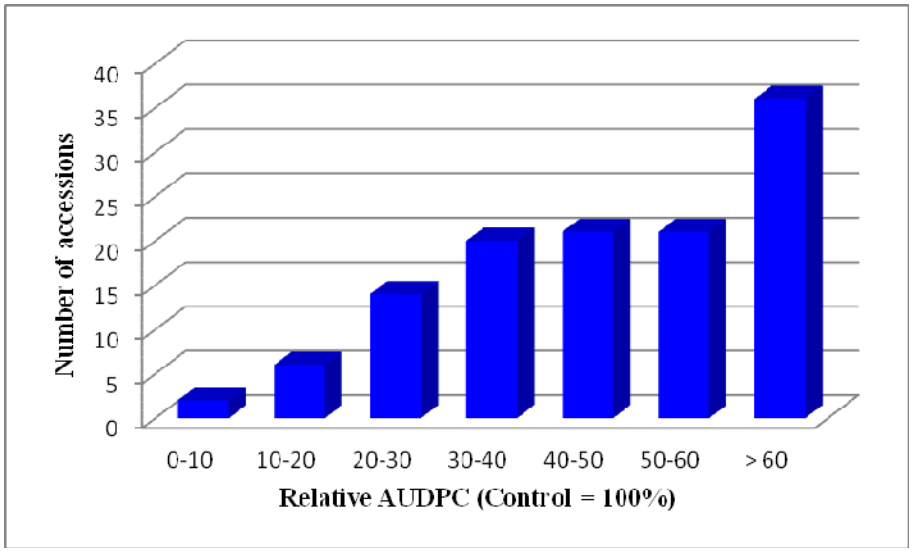


**Figure (2): Distribution of the 121 oat accessions according to the relative AUDPC of *Erysiphe graminis* f.sp. *avenue***

### 5.1.2. Leaf rust

During the 2008-2009 growing season the susceptible local accession (control accession) showed 48% DS (100% AUDPC) of rust (Fig. 3). DS of rust ranged from very high to very low, and the frequently distribution was markedly shifted towards low DS. Nearly half of the collection displayed AUDPC < 50%. Thirteen accessions showed AUDPC ≤ 20% (10.8% of the collection), were selected to study their reaction to leaf rust at seedling stage (Table 2).

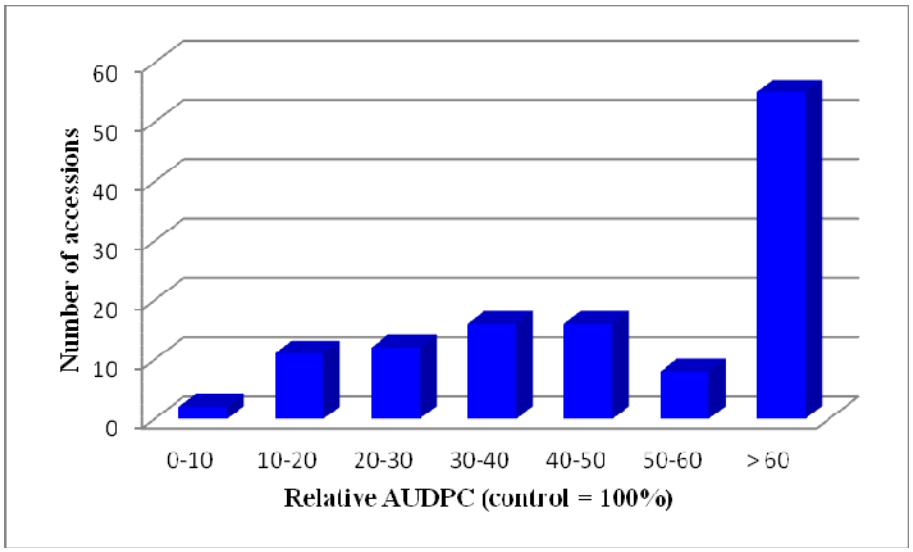


**Figure (3): Distribution of the 121 oat accessions according to the relative AUDPC of *Puccinia coronata* f.sp. *avenae***

## 5.2. Reaction in the growth chamber

Table 2 shows the macroscopic components of resistance to leaf rust in the selected accessions. All the 13 tested accessions showed a compatible interaction (IT = 9). The RLP in all accessions was significantly longer than the control (wild). RLP ranged from 27.3% to 47.6% longer than the susceptible control. The high RLP was noticed in three accessions (PI 295954, PI 293344 and CIav 4788) (Table 2). The relative infection frequency (RIF) of the selected accessions was significantly lower than on the control (wild). The low RIF were noticed in accessions Clav5234 and PI293347 (51.6% and 54.3% of the susceptible control)

**Table (2): Macroscopic components of resistance to leaf rust (*Puccinia coronata* f.sp. *avenae*) in selected oat accessions.**

| | Seedlings in growth chamber | | | Adult plants in the field |
|---|---|---|---|---|
| Accession | IT[1] | (DS%)[3] | RLP[2] | RIF[2] |
| PI 295954 | 9 | 1.5[b4] | 147.6[a,4] | 60.3[def4] |
| PI 293344 | 9 | 5.3[b] | 145.3[ab] | 78[c] |
| CIav 4788 | 9 | 5.3[b] | 144[abc] | 67.6[cd] |
| PI 293348 | 9 | 3.6[b] | 141.6[bcd] | 61[def] |
| PI 341007 | 9 | 7.1[b] | 141.6[bcd] | 75.6[c] |
| PI 293347 | 9 | 3.7[b] | 140.6[cd] | 54.3[ef] |
| PI 298126 | 9 | 4.2[b] | 140.3[cd] | 88[b] |
| PI 295953 | 9 | 3.7[b] | 139.6[d] | 69.6[cd] |
| PI 293345 | 9 | 4.5[b] | 139.3[d] | 63.6[de] |
| CIav 5235 | 9 | 4.8[b] | 133.3[e] | 75.3[c] |
| PI 295955 | 9 | 2.8[b] | 131[ef] | 74.6[c] |
| CIav 5234 | 9 | 4.5[b] | 129.3[f] | 51.6[f] |
| PI 177837 | 9 | 2.5[b] | 127.3[f] | 71.3[cd] |
| Local (wild) | 9 | 22.9[a] | 100[g] | 100[a] |

(1) Infection type (IT) according to the 0-9 scale of McNeal et al. (1971)

(2) Relative latency period (RLP) and relative infection frequency (RIF) referred to Local (wild) = 100 %. The actual values for local (wild) were 120 h (latency period) and 66 pustules per $cm^2$ (infection frequency).

(3) Estimated as the percentage of leaf area covered by uredia.

(4) Data with the same letter per column are not statistically different (Duncan, $P < 0.05$).

# Chapter Six
# Discussion and Conclusions

## 6.1 Discussion

For a long time, the genetic heritage of landrace oat species in the Mediterranean region was investigated by many oat researchers. Zillinsky and Murphy (1967) showed that some landrace oat accessions collected in Tunisia possess a high oat crown rust resistance level. Loskutov (2002), after evaluation of many landrace oat accessions collected from different regions in the world, reported that resistance to oat crown rust came mostly from North Africa such as Tunisia, Algeria and Morocco.

In the present study we found several accessions with good levels of partial resistance, some of them were chosen for the analysis of macroscopic components. A long latency period (LP) is a good indicator of partial resistance to leaf rust in oats (Brake and Irwin, 1992), barley (Parlevliet, 1979) and wheat (Ohm and Shaner, 1976). The mean latent period of the thirteen selected accessions with partial resistance ranged from 6.3 - 7.3, (1.3 – 2.3 days longer than the latent period of susceptible check).

These results are in agreement with the results reported by Brière and Kushalappa (1995). Luke et al (1984) also reported a 7-day difference in latent period between inoculated adult plants of the slow-rusting cultivar Red Rustproof and the susceptible cultivar Fulghum. Differences in latent period between barley lines with high partial resistance and susceptible checks varied between 3.7 and 7.8 days (Johnson and Wilcoxson, 1979). These differences ranged from 1.5 to 4.6 days for wheat slow-rusting lines (Kuhn et al, 1978).

The results obtained by studying the infection frequency (IF) showed us that the same lines that had submitted a higher LP had an IF lower than the susceptible control. Something similar occurs in plants of other species such as legumes (Sillero et al., 2000) and barley (Shtaya et al., 2006). The observed resistance to rust in oats is incomplete, and is expressed as an increase of LP and a decrease in the number of colonies. This is shown as a reduction in final disease severity (Parlevliet, 1979). This is consistent with our results because the lines had a higher LP and a lower IF.

In a study of resistance to oat rust resistance levels were obtained in part in a series of experimental oat lines showed a reduction in the severity of rust after 30 years exposure to diverse populations of the pathogen (Leonard, 2002). In the last decade working with QTLs in oat to search for rust resistance (Zhu and Kaeppler, 2003; Zhu et al., 2003; Portyanko et al., 2005). The measure of the latency period and infection frequency was performed in the first expanded leaf, according to Brake and Irwin (1992) the response of the 1$^{st}$ and 4$^{th}$ leaf reflect the response of plants when grown in the field.

The obligate fungus *Blumeria graminis* f. sp. *avenae* can infect plants from the first leaf stage until senescence. Resistance to the infection of powdery mildew fungus may be based on a race-specific gene-for-gene interaction of resistance gene(s) in oat and avirulence gene(s) in the infecting fungus isolate. This type of plant–pathogen interaction is associated with the hypersensitive response and may not be durable. Most

of the powdery mildew resistance genes identified in different oat genotypes confer complete resistance to different sets of fungus isolates in the seedling stage of the host plant (Leonard, 2002).

In the present study, 120 oat accessions from our region were screened for resistance to powdery mildew. From 120 accessions only 14 (11.7% of the collection) showed high level of partial resistance to powdery mildew under field condition. No artificial inoculation was done since powdery mildew infection is common in the zone (personal experience). Hsam et al. (1997) used twelve powdery mildew isolates collected in Germany and Denmark to test a collection of common oat cultivars and breeding lines grown in Western Europe and North America. These isolates were selected for their ability to produce differential response patterns permitting characterization of five oat mildew resistance groups.

From a total of 259 cultivars and lines tested 48 accessions were characterized by susceptible or intermediate responses and 38 accessions (14.5% of oat cultivars and lines tested) revealed isolate-specific resistance response patterns. Finkner et al. (1953) found 59 resistant oat accessions out of about 4000 cultivars and breeding lines grown in the USA (1.5% of the collection).

## 6.2 Conclusions

1. The collection used in the present study is an important source for partial resistance to oat leaf rust and powdery mildew.
2. The level of resistance to both diseases in the collection is very low
3. The selected accessions could be used as a valuable source for partial resistance against oat leaf rust and powdery mildew.
4. The latency period (LP) is an important components of resistance that could be used to distinguish susceptible accessions from accessions with partial resistance.
5. Further studies are needed to identify the number of genes/QTLs present in each selected accessions and the relation between these genes and other known genes for resistance against oat leaf rust and powdery mildew

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جامعة النجاح الوطنية
كلية الدراسات العليا

# تحديد مصادر مقاومة لمرضي البياض الدقيقي وصدأ الأوراق في محصول الشوفان

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قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في الإنتاج النباتي بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

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## الملخص

تم إجراء هذة التجربة بهدف البحث عن مصادر مقاومة لمرضي البياض الدقيقي و صدأ الأوراق في محصول الشوفان و كذلك بهدف دراسة المكونات الدقيقة لمقاومة مرض صدا الأوراق تحت الظروف المخبرية. استخدمت في الدراسة مجموعة من أصناف الشوفان مكونة من 120 صنف من دول مختلفة إضافة الى صنف بري محلي وذلك خلال الموسم الزراعي 2008–2009. زرعت الأصناف في ثلاثة مكررات. خلال موسم النمو تم تقييم نسبة اصابة النباتات بمرضي البياض الدقيقي وصدأ الأوراق ثلاث مرات بواقع مرة كل اسبوعين واستخدمت النتائج في تحديد منحنى تطور المرض (AUDPC).

أظهرت النتائج تباين بين اصناف المجموعة في شدة الاصابة بالمرضين حيث بلغت شدة الاصابة على الشاهد 56% للبياض الدقيقي و48% لصدأ الاوراق. بشكل عام، كان هنالك ميل واضح نحو شدة الحساسية للمرضين بينما اظهر عدد قليل من الاصناف درجة عالية من المقاومة الجزئية للمرضين (تقريباً 11% من النباتات). ترافقت المقاومة العالية لبعض الاصناف لمرض صدأ الاوراق مع زيادة ملحوظة في فترة الحضانة لمسبب المرض تراوحت بين 27.3–46.7% اطول من الشاهد. تم انتخاب الاصناف التي اضهرت مقاومة عالية للمرضين بهدف اجراء مزيد من الدراسة عليها.