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Evaluation of Local Wheat Cultivars Susceptibility to Infection with Black Stem Rust (*Puccinia graminis* f. sp. *tritici*)

تقييم حساسية أصناف قمح محلية للإصابة بمرض صدأ الساق الأسود المُتَسَبب عن فطر (*Puccinia graminis* f. sp. *tritici*)

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ABSTRACT: The present study was conducted to assess the susceptibility of seven local wheat cultivars from Palestine to infection with black stem rust caused by *Puccinia graminis* f. sp. *tritici*. Two techniques of disease inoculation were applied during bioassays: global inoculation of entire wheat plants with urediospores and localized inoculation with urediospores on wheat leaf-pieces incubated under humid conditions. Susceptibility of tested cultivars was evaluated according to disease scale based on number and size of typical uredial pustules that appeared after inoculation on entire plants or leaf-pieces. Results obtained on bioassay of susceptibility and disease rating on entire plants indicated that Anbar, Kamatat and Hetiya safra cultivars were the least susceptible to *P. g. tritici* infection, whereas Debiya beda cultivar was the most susceptible. The other tested cultivars such as Nab-El-Jamal, Debiya sawda and Senf 870 were moderately susceptible. On leaf-pieces, Anbar and Kamatat were the least susceptible cultivars, whereas Debiya beda and Nab-El-Jamal were the most susceptible cultivars. The other tested cultivars such as Debiya sawda, Senf 870 and Hetiya safra were moderately susceptible. Significant reductions were obtained for the size of uredial pustules formed on leaf-pieces when inoculated in an unwounded state compared to the wounded, indicating the importance of wounds during inoculation. The global results indicated the possibility of using the above method of disease inoculating, scaling and rating for evaluation of wheat cultivars susceptibility for the eventual use in breeding program for resistant varieties in Palestine.
Keywords: *cultivars, susceptibility, black stem rust, Puccinia graminis* f. sp. *tritici, inoculation method, evaluation method, Palestine.*

المستخلص: تم إجراء هذا البحث لدراسة حساسية سبعة من أصناف القمح المحلية في فلسطين للإصابة بمرض صدأ الساق الأسود المتسبب عن فطر: *Puccinia graminis f. sp. tritici* تم استعمال طريقتين للعدوى بالمرض خلال إجراء التجارب: طريقة العدوى الكلية لنباتات كاملة بجراثيم المرض وطريقة العدوى الموضعية على أجزاء من الأوراق المحفوظة في أطباق بتري تحت ظروف رطبة. تم تقييم الإصابة بعد حصول العدوى على النباتات الكاملة وأجزاء الأوراق باستعمال تدريج خاص بالمرض (Disease scale) مبني على عدد و حجم بثرات المرض (Uredial pustules) التي تكونت على النباتات الكاملة و الأجزاء الورقية. أظهرت النتائج التي تم الحصول عليها بعد تقييم الإصابة على النباتات الكاملة أن الأصناف التالية: عنبر، كماتات و هيتية صفراء كانت الأقل حساسية للإصابة بالمرض، بينما كان الصنف دبية بيضاء الأكثر حساسية، أما الأصناف الاخرى مثل ناب الجمل، دبية سوداء وصنف 870 فكانت متوسطة الحساسية. أما على الأجزاء الورقية، فقد كانت الأصناف عنبر و كماتات الأقل حساسية، بينما الأصناف دبية بيضاء و ناب الجمل الأكثر حساسية، أما الأصناف الاخرى مثل دبية سوداء، صنف 870 و هيتية صفراء فكانت متوسطة الحساسية. كان هناك نقص معنوي في حجم بثرات المرض المتكونة

على الأجزاء الورقية عندما تمت العدوى على أجزاء غير مجروحة بالمقارنة مع الأجزاء المجروحة. تشير النتائج في مجملها إلى إمكانية استعمال الطريقة المذكورة في العدوى و تقييم الإصابة وحساسية الأصناف في برنامج تهجين مستقبلي لإنتاج أصناف مقاومة للمرض في فلسطين باستخدام الأصناف المحلية المقاومة أو غير الحساسة.

كلمات مدخلية : *الأصناف، الحساسية، صدا الساق الأسود، طريقة العدوى، طريقة التقييم، المسبب الفطري، Puccinia graminis* f. sp. *tritic*.فلسطين.

INTRODUCTION

Black stem rust caused by *Puccinia graminis* f. sp. *tritici* is one of the most serious diseases that may affect wheat plants especially under humid conditions (Roelfs and Groth, 1988). Severe infections with this fungus reduces nutrient flow to the heads, resulting in small and shriveled grains. In addition, infected plants have usually weak stems that will be prone to lodging leading to further grain losses (Leonard and Szabo, 2005; Loughman, *et al.* 2005). The disease may be controlled by the use of fungicides or by the use of resistant varieties. However, because of environmental and health hazards, in addition to the need for reducing production costs, there is a tendency to reduce the use of fungicides and increase the genetic resistance (Loughman, *et al.* 2005). More than 40 major genes for resistance against *P. g. tritici* have been identified and mapped so far in bread wheat and its relatives (McIntosh, 1988; Knott, *et al.* 2005; Leonard and Szabo, 2005). The resistance to *P. g. tritici* was reported to be by R-genes and all these genes have a short effective life with exception of *Sr2* resistance gene, which have long stable partial resistance in adult plant stage (McIntosh, *et al.* 1995; Hayden, *et al.* 2004; Spieimeyer, *et al.* 2003). This ephemeral effectiveness of resistance has caused breeders to look for other types of resistance such as partial resistance and induced resistance which appear to be more durable and race-non-specific (Van Asch, *et al.* 1992; Adhikari and McIntosh, 1998; Niks, *et al.* 2000; Bhardwaj, *et al.* 2003).

The present study aimed at evaluating the susceptibility of 7 wheat cultivars used in the production of wheat flour in Palestine to infection with black stem rust causal agent. This evaluation was performed by disease scaling and rating based on the number and size of the rust pustules that appeared on inoculated leaf-pieces and entire plants of wheat cultivars.

MATERIALS AND METHODS

Source of Inoculum for Rust Infection

A strain of *Puccinia graminis* f. sp. *tritici* named PG1 was obtained from naturally infected wheat plants (cv: Nab-El-Jamal) grown in Tulkarm area, Palestine. Uredospores that were obtained from one uredial pustule of this strain infecting plant of the same cultivar were used to inoculate several plants of the same cultivar growing on a mixture of soil and peatmoss (1:1 ratio) in black polyethylene bags (cylindrical shape with 3.5 L capacity). These plants were maintained as a culture of this strain in a growth chamber at 25±2° C, 80±5% relative humidity, and 16 hours of illumination/day used during the whole period of study. Urediospores that were produced in pustules on the above-mentioned plants were used during the experiments for evaluation of wheat cultivars susceptibility to rust infection.

Wheat Cultivars used in the Experiments

The following local wheat cultivars were used in evaluation of the susceptibility to stem rust infection: Anbar, Nab-El-Jamal, Kamatat, Debiya beda, Debiya sawda, Senf 870, and Hetiya safra. Healthy kernels of each cultivar were sown in black polyethylene bags (cylindrical shape with 3.5 L capacity) containing growing medium of soil and peatmoss (1:1 ratio). All bags were kept under growth chamber conditions (25±2°C, 80±5% relative humidity, and 16 hours of illumination/day) and growing wheat plants were used for rust infection bioassays when reached 5-week old so that they were at fourth leaf stage.

Preparation of Spore Suspension for Rust Infection

A spore suspension of *P. g. tritici* urediospores (strain PG1) was used for the inoculation during bioassays. The suspension was prepared by direct picking of the freshly harvested urediospores from pustules of this strain appeared on wheat infected plants grown under growth chamber conditions. Sterile needles were used for picking urediospores from their pustules. Capped vials containing sterile distilled water were used for preparation of the suspension. The urediospores were washed with sterile 0.1 % dodecyl soleplate and sterile distilled water prior to preparation of the suspension according to the technique developed by Fasters, *et al.* (1993). A vigorous shaking of the vials after transferring of urediospores was necessary for obtaining a homogenous distribution of the spores in the suspension before counting them using hemocytometer. Concentration of the spore suspension was adjusted at $1.5x10^5$ urediospores/mL, the sole concentration that was used in all bioassays.

Techniques of Rust Infection used in Bioassays

Two techniques of rust inoculation were applied in the present study, the first is the inoculation of entire wheat plants by spraying them with a spore suspension. This was achieved by spraying a standardized volume (3 mL/ one polyethylene bag containing 5 wheat plants) of spore suspension containing $1.5x10^5$ urediospores/mL using one-liter calibrated hand sprayer. The sprayed plants were then covered with transparent plastic bags for 3 days to maintain 100% RH needed for germination and penetration of the inoculated urediospores.

The plants were then kept for additional 18 days under growth chamber conditions (25±2°C, 80±5% RH, and 16 h of illumination/day) before being evaluated for uredial pustule development. The total time from inoculation to evaluation was thus three weeks. Five replicates were used and each replicate was represented by a set of 5 wheat plants with 5-week old (4^{th} leaf stage) in one polyethylene bag; the second is the inoculation of wheat leaf-pieces by laying 25-μL droplet of the spore suspension containing $1.5x10^5$ urediospores/mL on each leaf-piece (6 cm long). The leaf-piece was cut from 4th leaf-stage of each plant and then disinfected with 0.025% sodium hypochlorite for 30 sec and rinsed with sterile distilled water 3 times before being inoculated. Incubation of inoculated leaf-pieces was carried out under humid conditions using Petri dishes (8.5 cm diameter) containing moistened filter paper. Each inoculated leaf-piece was placed on a glass slide mounted on a glass cavalier to avoid the direct contact of the leaf-piece with the moistened filter paper to prevent rotting. Wounded and unwounded leaf-pieces were used in the inoculation for comparison. Wounding of leaf-pieces was done by scratching the upper surface with a sterile needle before loading the droplet of spore suspension in the same site of wounding. Ten replicates representing 10 Petri dishes with 10 wounded or unwounded leaf-pieces were used for each cultivar. All Petri dishes were incubated under the same conditions as the entire plants (growth chamber conditions) for three weeks before being evaluated for uredial pustule development.

Evaluation of Cultivars Susceptibility to Rust Infection

The development of uredial pustules on inoculated entire plants and leaf-pieces of wheat cultivars was evaluated after 3 weeks of incubation under growth chamber conditions according to the following criteria: i) counting the number of pustules that could be observed on the inoculated entire plants, so the number of pustules per leaf per plant could be calculated for each cultivar. The range and mean number on each cultivar was calculated; ii) measuring the size or diameter of pustules appeared on the inoculated leaf-pieces and entire plants. The range and mean of pustules number and size on each cultivar were calculated and presented for the purpose of comparison of cultivars susceptibility; and iii) rating susceptibility of tested cultivars using disease scaling. This scaling is based either on the number of pustules/leaf/plant and consisted of the following scales: 0 – 1 pustules/leaf/plant as least susceptible or resistant, 1 – 2 pustules/leaf/plant as moderately susceptible, and > 2 pustules/leaf/plant as highly susceptible, or on the pustule size and consisted of the following scales: 0 – 1 mm as least susceptible or resistant, 1 – 2 mm as moderately susceptible, and > 2 mm as highly susceptible.

Statistical Analyses

The data were subjected to ANOVA analysis. Moreover, Duncan's multiple range test (DMRT) was used to test differences between means of uredial pustules size and number on inoculated entire plants and leaf-pieces.

RESULTS AND DISCUSSION

Evaluation of Uredial Pustules Development on Entire Plants

Significant differences ($P<0.05$) were obtained between mean number of typical uredial pustules of *P. g. tritici* (strain PG1) formed on entire plants of seven wheat cultivars after global spraying with urediospore suspension (Table 1). Rating susceptibility of tested cultivars based on disease scaling according to the number of pustules/leaf/plant has resulted in the following classification: Anbar, Kamatat, and Hetiya safra were demonstrated as least susceptible cultivars (mean number of pustules/leaf/plant 0.4 - 0.6 with disease scaling 0 - 1); Nab-EL-Jamal, Debiya sawda, and Senf 870 as moderately susceptible cultivars (mean number of pustules/ leaf/plant 1.4 - 1.6 with disease scaling 1 – 2);

Table 1. Development of Uredial Rust Pustules Caused by *Puccinia graminis tritici* (strain PG1) on Entire Plants of 7 Wheat Cultivars 3 Weeks after Inoculation and Incubation at 25 ± 2°c, 80 ± 5% RH and 16 h of illumination/ day.

Wheat cultivars	Number of uredial pustules (per leaf per plant) [1]		Uredial pustule size (mm) [1]	
	Range	Mean	Range	Mean
Anbar	0-1	0.4 a [2]	0.5-1.0	0.6 a [2]
Nab-EL-Jamal	1-3	1.6 b	2.0-3.0	2.4 b
Kamatat	0-1	0.4 a	0.5-1.0	0.8 a
Debiya sawda	1-2	1.6 b	2.0-3.0	2.4 b
Senf 870	0-3	1.4 b	0.5-1.0	0.6 a
Hetiya safra	0-1	0.6 a	0.5-1.0	0.8 a
Debiya beda	1-4	2.6 c	2.0-3.0	8.8 b

[1] *Values are the average of 5 replicates. Each replicate contained 5 wheat plants grown in one polyethylene bag.*

[2] *Means within each column followed by different letters are significantly different at P<0.05 using Duncan's multiple range test (DMRT).*

and Debiya beda as highly susceptible cultivar (mean number of pustules/leaf/plant 2.6 with disease scaling > 2). Also, significant differences ($P<0.05$) were obtained between means of uredial pustule size of *P. g. tritici* (strain PG1) formed on entire plants of the seven cultivars after global spraying with urediospore suspension (Table 1). Rating susceptibility of the same cultivars based on disease scaling according to the pustule size has resulted in the following classification: Anbar, Kamatat, Senf 870, and Hetiya safra were demonstrated as the least susceptible cultivars (mean pustule size 0.6 - 0.8 mm with disease scaling 0 – 1); Nab-El-Jamal, Debiya sawda, and Debiya beda as the highly susceptible cultivars (mean pustule size 2.4 - 2.8 mm with disease scaling > 2) (Table 1).

Evaluation of Uredial Pustules Development on Leaf Pieces

Significant differences ($P<0.05$) were obtained between the mean size of typical uredial pustules of *P. g. tritici* (strain PG1) formed on unwounded leaf-pieces of seven local wheat cultivars after urediospores localized inoculation (Table 2). Rating susceptibility of tested cultivars based on disease scaling according to the pustule size has resulted in the following classification: Anbar, Kamatat, Senf 870, and Hetiya safra showed the least (zero) susceptibility (mean pustule size 0 mm with disease scaling 0 – 1); Debiya sawda demonstrated moderate susceptibility (mean size of pustules 1.3 mm with disease scaling 1 – 2), and Nab-El-Jamal and Debiya beda showed highest susceptibility (mean size of pustules 2.1 - 2.3 mm with disease scaling > 2). Furthermore, significant differences ($P<0.05$) were obtained between the mean size of typical uredial pustules of *P. g. tritici* (strain PG1) formed on wounded leaf-pieces of the same wheat cultivars after urediospores localized inoculation (Table 2). Rating susceptibility based on the disease scaling according to the pustule size has resulted in the following classification: Anbar and Kamatat were demonstrated as least susceptible (mean size of pustules 0.3 – 0.4 mm with disease scaling 0 – 1); Debiya sawda, Senf 870, and Hetiya safra as moderately susceptible (mean size of pustules 1.3 – 1.8 mm with disease scaling 1 – 2); Nab-

EL-Jamal and Debiya beda as highly susceptible cultivars (mean size of pustules 2.3 – 3.3 mm with disease scaling > 2) (Table 2).

Table 2. Development of Uredial Rust Pustules Caused by *Puccinia graminis tritici* (strain PG1) on detached Leaf Pieces of 7 Wheat Cultivars 3 Weeks after Inoculation and Incubation in Petri Dishes at 25 ± 2°C, 80 ± 5% RH and 16 h of illumination/ day.

Wheat cultivars	Uredial pustule size on unwounded leaf-pieces (mm) [1]		Uredial pustule size on wounded leaf-pieces (mm) [1]	
	Range	Mean	Range	Mean
Anbar	0	0 a [2]	0-1	0.3 a [2]
Nab-EL-Jamal	2-3	2.1 c	2-3	2.3 d
Kamatat	0	0 a	0-1	0.4 a
Debiya sawda	1-2	1.3 b	1-2	1.5 bc
Senf 870	0	0 a	1-2	0.8 c
Hetiya safra	0	0 a	1-2	1.3 b
Debiya beda	2-3	2.3 c	3-4	3.3 e

1) *Values are the average of 10 replicates. Each replicate contained one detached leaf-piece kept in Petri dish under humid conditions.*

2) *Means within each column followed by different letters are significantly different at P<0.05 using Duncan's multiple range test (DMRT).*

The inoculation techniques used in the present study are characterized by good sensitivity because the stem rust fungus, *P. g. tritici*, is functionally an obligate biotroph, and although it can be cultured with great difficulty on artificial media, cultures grow slowly and upon subculturing, they develop abnormal ploidy levels and lose their ability to infect host plants (Leonard and Szabo, 2005). Also, the rust spores that were applied in the above-mentioned techniques of inoculation in form of spore suspension were freshly harvested and precisely counted using hemocytometer. In addition, the infectivity of inoculated tissues during inoculation was enhanced by wounding of the tissues because results obtained have indicated that significant reductions were obtained for the size of uredial pustules formed on unwounded inoculated leaf-pieces compared to the wounded.

It was observed that "zero results" designated for the least susceptibility of some cultivars to the strain PG1 of *P. g. tritici* could be attributed to the highly resistant unwounded tissues of these cultivars inoculated by localized inoculation technique. It seems that the lack of wounding and/or stomata opening at the site of inoculation may explain the lack of penetration thus no infection was obtained. Many investigators have reported that over 90 % of the germ tubes of *P. g. tritici* differentiate appressoria then penetrating pegs for penetration on encountering stomata opening and/ or wounds (Staples and Macko, 1984; Read, *et al.* 1997). This confirms the role of wounding in enhancing the tissue susceptibility during inoculation thus increasing the sensitivity of the inoculation technique used.

The time interval of 3 weeks used in the present study for evaluation of uredial pustule development of *P. g. tritici* on the entire plants and leaf-pieces is adequate and reasonable because the first macroscopic symptom of *P. g. tritici* infection usually appears as a small chlorotic fleck few days after inoculation. About 2 weeks later, a pustule is formed by rupturing of the host epidermis from pressure of a mass of brick-red urediospores produced in the infection site. These uredial pustules are generally circular to slightly elongate shape and may enlarge up to 4 or 5 mm diameter in the susceptible cultivars (Leonard and Szabo, 2005).

The above-mentioned method of susceptibility evaluation and rating has generally revealed that Anbar and Kamatat were the least susceptible or resistant cultivars to *Puccinia graminis tritici* (strain PG1) infection, whereas Hetiya safra and Senf 870 showed moderate susceptibility or good tolerance to the infection. These cultivars could be involved in breeding program for developing resistant varieties of wheat against the frequent biotype (PG1) of stem rust in Palestine. One important issue that should be undertaken for this involvement is to specify the identity of resistant genes in the selected cultivars through molecular biology techniques and the type of resistance (partial or induced) in these cultivars. Similar evaluation methods of susceptibility of wheat and other cereals were followed by other investigators as the first step in breeding program for developing resistant varieties against infection with rust diseases (Brown-Guedira, *et al.* 1996; Heath, 1997; Adhikari and McIntosh, 1998; Bariana, *et al.* 1998), and many successful examples were cited in the literature in various countries stating the engagement of the selected resistant cultivars of wheat in successful breeding programs for developing resistant varieties of wheat to rust (McIntosh, 1988; Van Asch, *et al.* 1992; Niks *et al.* 2000; Pfender, 2001; Hayden, *et al.* 2004; Knott. *et al.* 2005).

Finally, it is important to ensure that, in breeding program for resistant varieties of wheat to *P. g. tritici*, the resistance should be durable (McIntosh, 1988). Moreover, two types of resistance may be also found in the resistant varieties of wheat to stem rust; the first is partial resistance which is controlled by R-genes such as *Sr2* resistance gene (Spieimeyer, *et al.* 2003; Hayden, *et al.* 2004), and the second is induced resistance which is restricted, for example, to oat seedlings of line possessing the *Pg-a* source of oat stem resistance (Adhikari and McIntosh, 1998), and the resistance induced in wheat seedlings against *P. g. tritici* by infection with avirulent race of *P. recondita* f. sp. *tritici* (Van Asch, *et al.* 1992).

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