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NOTE
GROWTH OF *PUCCINIA HORDEI* ON LEAF SURFACE OF SUSCEPTIBLE AND RESISTANT BARLEY CULTIVARS[1]

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ABSTRACT

Growth of the races F, BRS 7612 and BR/EST of *Puccinia hordei*, the cause of barley brown rust, on leaf surface of three barley cultivars Cebada Capa, CI 1243 and Ribari was studied. In most cases there were no significant differences in urediniospore germination, length and width of germ tube, number of branches per germ tube and percentage of germ tubes forming appressoria among races or cultivars.

However, the number of branches per germ tube of race F was significantly less than that of race BR/EST on cv. Ribari (Ribari is resistant to race F and susceptible to race BR/EST). Race BR/EST also produced wider germ tubes than did race F on leaf of cv. Ribari at 21/26°C.

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رشد قارچ عامل بیماری زنگ قهوه‌ای جو *Puccinia hordei* روی سطح برگ ارقام حساس و مقاوم جو

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چکیده

رشد نژادهای F، BRS7612 و BR/EST از قارچ عامل بیماری زنگ قهوه‌ای جو (*Puccinia hodei*) روی سطح برگهای سه رقم جو Cebada Capa، CI 1243 و Ribari مورد بررسی قرار گرفت. در بیشتر حالات هیچ اختلاف معنی داری بین نژادها و ارقام مختلف جو از نظر جوانه‌زدن اسپور، طول و عرض لوله تندش، تعداد انشعابهای لوله تندش و درصد لوله تندش مولد اپرسوریوم وجود نداشت. با وجود این، هنگامیکه گیاهان در دمای ۲۱/۲۶ درجه سانتی گراد نگهداری شدند میانگین تعداد انشعابها در هر لوله تندش در نژاد F بطور معنی‌داری کمتر از نژاد BR/EST روی سطح برگ رقم Ribari (Ribari نسبت به نژاد BR/EST حساس و در برابر نژاد F مقاوم می‌باشد) بود و همچنین نژاد BR/EST لوله‌های تندش قطورتری نسبت به نژاد F روی برگ رقم Ribari تولید کرد.

INTRODUCTION

Barley genotype resistant to *Puccinia hordei* Otth., the cause of the brown rust, seems to be relatively rare. The single dominant resistance gene designated pa7 carried by the spring cv. Cebada Capa appears to

be effective in most parts of the world but is temperature sensitive and becomes ineffective to races F, BRS 7612 and BR/EST at 5°C or lower. The gene pa9 carried by genotype CI 1243, on the other hand, becomes ineffective to these races at temperatures above 25°C (2,3). Obviously temperature sensitivity of this sort imposes limits on the utilization of the resistance. For a better understanding of the mechanism of temperature sensitivity of barley cvs., it seemed appropriate to carry out investigation of the behavior of *P. hordei* on leaf surface of barley plants. The objective of this study was to investigate growth of races F, BRS 7612 and BR/EST of *P. hordei* on water agar at 5°C and 22°C and development of the races on leaf surface of three barley cultivars at 5°C and 26°C.

MATERIALS AND METHODS

Plastic petri-dishes containing 0.25% sterile water agar were inoculated using a settling tower (8) with urediniospores of races of *P. hordei* and incubated at 5°C and 22°C. Approximately 200 spores were applied per cm^2 in all replications. The inoculated petri-dishes were maintained in an illuminated incubator with 16 h light at an intensity of 17 microeinsteins m^{-2} sec^{-1}. Three petri-dishes were used for each treatment. Observations were made after 6 and 11 days at 5°C and 4 after days of incubation at 22°C. Approximately 100 spores were randomly chosen for assessment of germination percentage in each petri-dish and about 15 germ tubes were observed for measurement of length, width and number of branches per germ tube in each treatment.

Seeds of barley cultivars Cebada Capa, CI 1243 and Ribari (Ribari is resistant to race F and susceptible to race BR/EST) were sown in 7.5 cm diameter plastic pots containing John Innes compost No. 2 in an air-conditioned spore-proof glasshouse at temperatures ranging from 15 to 22°C with a 16 h photoperiod. Supplementary light was supplied by 400 W mercury vapor lamps providing a light intensity of 200 microeinsteins m^{-2} sec^{-1} at the soil surface. The original stock of urediniospore of *P. hordei* and seed of barley cultivars were provided by Dr. Brian Clifford of the Welsh Plant Breeding Station. Races of F, BR/EST and ERS 7612 were used in all experiments. One pot with four plants at two unfolded leaf stage was inoculated with each race in each cultivar and in each time of observation. One mg fresh urediniospores was mixed with 25 mg talc, divided into 4 parts and each part used for inoculation of one plant. Leaves were inoculated with a small paint brush, sprayed with water and covered by a polythene bag for 24 h. Before inoculation the leaves were rubbed lightly between moistened fingers to remove surface wax (4). To study growth of *P. hordei* on the leaf surface of barley cultivars at 21/26°C, inoculated plants were maintained in an illuminated incubator with 16 h light at an intensity of 13 microeinsteins m^{-2} sec^{-1} and 8 h dark at 26 and 21°C, respectively. For study of growth of *P. hordei* after 24 h and 48 h leaves inoculated with race BRS 7612 were each cut into tip, mid and base segments (9) and stained using the method of Skipp and Samborski (12). The leaves inoculated with races BR/EST and F were similarly cut and stained 48 h after inoculation. To study growth of *P. hordei* at 5°C, inoculated plants were incubated at 5°C and leaves were harvested 48 h after

inoculation. The remaining methods and materials were the same as described for experiment at 21/26°C. In all treatments ca. 100 spores and germ tubes were examined randomly for percentage of germination and percentage of germ tubes producing appressoria on the leaves of each plant. Length and width of germ tube and number of branches per germ tube were determined using the methods of other investigators (1,10).

The percentage of germination and percentage of germ tubes forming appressoria when greater than 40 were arc sin transformed before analysis ($X = \sin^{-1}x$ where X is the transformed and x the percentage value). Duncan's multiple-range test was used for comparing the mean levels after analysis of variance as described by Little and Hills (6).

RESULTS

Urediniospores germinated usually by production of a single germ tube per spore that grew close to the surface of the leaf and conformed to its wavy configuration. Approximately 80% of germ tubes exhibited the wavy configuration. The other 20% did not conform to any decipherable pattern. Growth on the leaf surface seemed otherwise undirected. Germ tubes produced branches before forming appressoria. The number of branches varied. For example, 48 h after inoculation at 21/26°C on cv. Ribari, the average number of branches was 1.70 for race F and 4.11 for race BR/EST. The width of germ tube also varied.

For example, in cv. Ribari the width of germ tube of race F was 4.20 µm and that of race BR/EST was 4.25 µm 48 h after inoculation (Table 1).

The growth of germ table was erratic. Sometimes very long germ tubes passed over stomata without forming appressoria. Other times they were short and produced an appressorium and penetrated through stoma. No germ tube penetrated a stoma without forming an appressorium. In most instances, thin infection pegs developed from appressoria and penetrated the stoma.

The results in Table 2 indicate that there were no significant differences between races in spore germination, width of germ tube and number of branches/germ tube after 4 days of incubation at 22°C on water agar among the races. The germ tube of race BRS 7612 was significantly longer than that of race F. Similar results were obtained after 6 and 11 days of incubation at 5°C. The results in Table 2 indicate that there were no significant differences among cultivars regarding percentage of spore germination, length of germ tubes and percentage of germ tubes forming appressoria on leaf surface at higher temperature (21/26°C) of incubation. The average number of branches per germ tube of race F was significantly less than in races BR/EST and also race BR/EST produced wider germ tubes than did race F on cv. Ribari, 48 h after inoculation. Percentage of spore germination, number of branches per germ tube and percentage of germ tube forming appressoria were similar in all treatments, when plants were incubated at 5°C (Table 3).

Table 1. Growth of three races of *P. hordei* on leaf surface of barley cultivars at 21/26°C†.

Factors	Cultivars	Race F after 48 h		Race BRS 7612 after 24 h		Race BR/EST after 48 h	
		n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$
% Spore germination§	Cebada Capa		39.30		41.00		41.65
	CI 1243		33.30		32.30		41.56
	Ribari		43.50		45.09		43.93
Length of germ tube (µm)	Cebada Capa	21	193.03	38	251.70	43	252.68
	CI 1243	19	322.35	25	313.70	24	218.76
	Ribari	21	245.89	35	219.16	26	217.77
Width of germ tube (µm)	Cebada Capa	19	B 5.85 a	26	A 4.13 a	23	A 4.151 a
	CI 1243	18	A 4.64ab	19	A 4.20 a	20	A 5.05 a
	Ribari	22	B 4.20 b	28	B 4.83 a	20	A 6.54 b
No. of branches per germ tube	Cebada Capa	22	A 2.68	34	A 3.29	12	A 2.81
	CI 1243	14	A 2.14	16	A 3.50	19	A 3.05
	Ribari	17	A 1.70	34	B 3.79	26	B 4.11
% Germ tube forming appressoria¶	Cebada Capa		45.56		46.30		50.40
	CI 1243		43.66		56.63		62.46
	Ribari		46.20		45.30		41.17

† Plants maintained in an illuminated incubator with 16 h light and 8 h dark at 26 and 21°C, repectively.

§ Percentages are expressed as the mean of arc sin transformation. Significant differences are denoted by different small letters within each column and by capital letters within each row for each factor. In the cases where the letters are not presented there were no significant differences between the treatments.

¶ Number of germ tubes examined. One hundred checked for percentage of spore germination and germ tube forming appressoria in each plant.

Table 2. Characteristics of growth of *P. hordei* on water agar.

Temperature	Factors	Days after inoculation	Race F		Race BRS 7612		Race BR/EST	
			n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$
22°C	% Spore germination	3		17.66		10.66		12.33
		4		22.66		13.66		19
	Length of germ tube (µm)	3	16	A 128.39 b†	13	B 346.20 b	15	AB 69.5 c
		4	15	B 207.3 b	15	A 413.14 b	14	A 428.8 b
	Width of germ tube (µm)	3	13	6.110	14	5.675	15	5.675
		4	15	5.675	14	5.675	14	5.675
	No. of branches per germ tube	3	15	1.4615	15	1.6	15	1.33
		4	15	1.60	13	1.3	14	1.28
5°C	% Spore germination	6		5.6		7.66		8.33
		11		12.33		13.33		11
	Length of germ tube (µm)	6	14	A 436.1 a	14	A 365.6 b	16	A 436.9 b
		11	13	B 488.9 a	15	AB609.11 b	14	A 652.6 a
	Width of germ tube (µm)	6	15	5.675	14	5.67	16	5.67
		11	14	5.675	15	5.48	14	5.47
	No. of branches per germ tube	6	14	1.57	15	1.46	15	1.33
		11	14	1.21	15	1.33	14	1.42

† Significant differences are denoted by different small letters within each column and with capital letters within each row for each factor. In the cases where the letters are not presented, there were no significant differences between treatments. n = number of measurement. Three hundred spores were checked for assessment of germination percent.

Table 3. Growth of three races of *P. hordei* on leaf surface of barley cultivars after 48 h incubation at 5°C.

Measurement	Cultivars	Race F		Race BRS 7612		Race BR/EST	
		n	$\bar{X}$	n	$\bar{X}$	n	$\bar{X}$
% Spore germination	Cebada Capa		10.3		11.3		13.30
	CI 1243		11.22		13.47		14.65
	Ribari		10.67		12.35		12
Length of germ tube (μm)	Cebada Capa	27	A 187.46	27	A 142.08	32	194.21
	CI 1243	28	A 251.68	21	A 199.4	28	A 194.3
	Ribari	22	AB 196.01	27	B 145.72	20	A 252.53
Width of germ tube (μm)	Cebada Capa	23	A 5.17	24	B 5.64	24	AB 5.25
	CI 1243	24	A 5.22	18	B 5.93	23	A 5.514
	Ribari	23	A 5.51	20	A 5.62	17	A 5.19
No. of branches per germ tube	Cebada Capa	24	1.83	26	1.92	28	2.50
	CI 1243	30	1.96	19	2.10	25	2.32
	Ribari	25	1.72	27	1.62	22	49.50
% Germ tube forming appressoria†	Cebada Capa		47.74		40.41		44.50
	CI 1243		48.97		48.7		43.20
	Ribari		48.52		48.52		49.50

† Percentages are expressed as the mean of arc sin transformation. Significant differences are denoted by different small letters within each column and with capital letters within each row for each factor. In the cases where the letters are not presented, there were no significant differences between the treatments. n = number of germ tubes examined. One hundred spores were checked for percentage of spore germination and germ tube forming appressorium.

DISCUSSION

On water agar in most cases the length of germ tubes of race F was less than that of races BRS 7612 and BR/EST. Apparently race F grows slower than the other races tested at higher or lower temperatures of incubation. The percentage of germination at 22°C was greater than that at 5°C. The results are in agreement with the report of Simkin and Wheeler (11) who found that percentages of germination of *P. hordei* were lower at 5°C than at 20°C on cover-slips and leaves of barley cv. Zephyr. Politowski and Browning (7) also found that the percentage of germination of *P. coronata avenae* on leaves of oat was lower at 10°C than at 15.5°C and 26.5°C.

The results of experiments conducted on leaf surface of barley cultivars indicated that there were no significant differences between cultivars in spore germination and percentage of germ tube forming appressoria. This is in agreement with Clifford (1) who reported that the percentage of germination of *P. hodei* culture BR/E/71d and germ tube association with stomata were similar on resistant and susceptible barley varieties.

From water agar measurements it was concluded that the width and number of branches per germ tube of the three races were similar but the results of experiments on leaf surfaces when the plants were incubated at 21/26°C showed that the width and number of branches per germ tube of race F were less than those of race BR/EST on cv. Ribari. It may be suggested that there are some biochemical or physical factors on leaves of cv. Ribari to inhibit the normal growth of germ tubes of race F. Alternatively some biochemical compounds may be produced

because of the interaction between leaves of Ribari and race F. Kono (5) reported that the rate of germination of *P. arachidis* Speg. on abaxial leaf surfaces of the Kintoki peanut variety was greater than on the adaxial surface. He reported that sugars and amino acids exuded from leaf surfaces were greater in quantity on the abaxial than on the adaxial surface. Grambow and Riedel (3) stated that the dependence of *P. graminis* on morphogenically active biochemical factors from the host plant, even at a very early stage of development, leads us to the assumption that the occurrence of these factors may play a role in some forms of resistance.

The results in Tables 2 and 3 generally suggested that there were no significant differences between temperature sensitive cultivars Cebada Capa and CI 1243 on leaf surface development of *P. hordei* when plants were incubated at 5°C and 21/26°C. Chemical or physical factors on the leaf surface do not have any role in temperature sensitivity of these two cultivars. Santoso *et al.* (10) also suggested that there were no pre-formed compounds at the leaf surface of resistant wheat varieties to inhibit spore germination and early development of *P. graminis tritici.*

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