P L A N T B R E E D I N G A N D S E E D S C I E N C E
Volume 56 2007

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LEAF RUST RESISTANCE IN SELECTIONS FROM BARLEY LANDRACES COLLECTED IN SARDINIA

ABSTRACT

Leaf rust caused by fungus *Puccinia hordei* has economic importance in many barley growing regions. Breeders are constantly looking for new effective sources of resistance to this pathogen. Landraces were proven to be rich source of resistance genes for resistance to major pathogens of barley. A total of 240 lines selected from 12 populations of barley landraces collected in Sardinia were tested for leaf rust resistance. Eight differential isolates of *P. hordei* were used. Plants of only 8 lines (4974, 4997, 5041, 5055, 5086, 5102, 5121 and 5132) originating from 5 populations of barley landraces (Sard 2, Sard 5, Sard 7, Sard 8, Sard 9) showed resistance to leaf rust. From total 8 lines showing resistance to leaf rust in 7 lines (4974, 4997, 5055, 5086, 5102, 5121 and 5132) were observed both plants resistant and susceptible after inoculation with isolate of leaf rust. Next after selection of resistant plants single plant lines were created (4974-4, 4997-1, 5041-3, 5055-1, 5086-3, 5102-1, 5121-1 and 5132-1) and resistance tests were performed with differential isolates of leaf rust. Only one line (4974-4) was resistant to infection with all isolates used. Identified leaf rust resistant lines, especially line 4974-4 should be used as source of leaf rust resistance in barley breeding programmes. Different strategies for control of barley leaf rust were discussed.

Key words: barley landraces, *Hordeum vulgare*, leaf rust, *Puccinia hordei*, resistance genes

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide. There are regions of the word in which it is the most importat crop and often it is still grown as landraces (Bothmer *et al.*, 1995; Fischbeck 2003). Barley landraces are grown in place where other cereals are not suitable for farming due to harsh environmental conditions such as high altitude, low rainfall (<300 mm annually), or soil salinity (Bothmer *et al.*, 1995; Yahyaoui *et al.* 1996; Ceccarelli *et al.* 1999; ICARDA 2005). Growing of barley landraces is especially crucial in dry areas such as the highlands and mountains of Central Asia, the Horn of Africa, the Andes, the Atlas Mountains in North Africa and Southern Europe including Mediterranean islands Cyprus, Crete, Sicylia, Corsica

Communicated by Edward Arseniuk

and Sardinia. In the mountainous and dry regions of North Africa and West Asia (WANA) barley grain often is used for human consumption. This consumption is considerably higher during dry years (Damania 1988; Bothmer *et al.* 1995; Ceccareli *et al.* 1999; Grando 2002; Fischbeck 2003).

Barley was and still is very important crop in southern Europe, including Sardinia. Barley landraces are still grown in Sardinia and are called by farmers *S' orgiu sardu* (Attene *et al.* 1996). Wild barley (*H. spontaneum*) is absent in Sardinia. Based on this fact the cultivation of barley in Sardinia most probably originated from introduced cultivated forms from Aegean and Near East regions (Attene *et al.* 1996; Papa 1993). Over centuries barley in form of landraces was the most important crop used for internal consumption (especially in mountainous regions), while wheat was generally exported. After the Second World war the cultivation of barley in Sardinia declined. Currently barley is second most widely- cultivated cereal in Sardinia, after wheat. It is used mainly for animal feed as green fodder, grain and straw. Sardinian barley landraces have some unique characteristics, especially the presence of strong awns which very often are solidly connected to the lemma even after threshing. In addition they are showing resistance to abiotic and biotic stresses (Attene *et al.* 1996; Papa 1993, 1994; Papa *et al.* 1991, 1994, 1998).

Leaf rust caused by fungus *Puccinia hordei* has great economic importance in many barley growing regions in Europe, North America, Australia and West Asia and North Africa (WANA) (Parlevliet *et al.* 1981; Reinhold and Sharp 1982; Lim and Gaunt 1986; Yahyaoui and Sharp 1987; Park *et al.* 1992; Park 2003; Woldeab *et al.* 2006). The yield losses due to infection by *P. hordei* may reach 30% in susceptible cultivars in experimental conditions. However, the average yield losses of barley due to this disease usually reach less than 10% (Griffey *et al.* 1994; Dreiseitl and Jurecka 1996; Whelan *et al.* 1997; Niks *et al.* 2000). It has to be stressed that often more important than lowering of barley yield is loss of its quality. This is especially concerns plantations of barley for malting purpose. Recently the breeders interest in resistance to barley leaf rust has increased in Europe (Mazaraki and Grabowska, 1998; Niks *et al.* 2000; Czembor *et al.* 2006; Czembor and Czembor 2007a, 2007b). This interest is caused by observations of increases in fitness of leaf rust populations to many currently grown barley cultivars and to cultivar Vada. Cultivar Vada is well known for possessing high level of partial resistance and it was used wildly as parent in major European barley breeding programs (Czembor and Czembor 2007a, 2007b).

Based on many studies on the genetics of barley-leaf rust host-pathogen system 19 loci with major genes for resistance were identified: *Rph1*, *Rph2bj*, *Rph2k*, *Rph2l*, *Rph2m*, *Rph2n*, *Rph2q*, *Rph2r*, *Rph2s*, *Rph2t*, *Rph2u*, *Rph2y*, *Rph3c*, *Rph3w*, *Rph3aa*, *Rph4*, *Rph5*, *Rph6*, *Rph7g*, *Rph7ac*, *Rph8*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph13*, *Rph14*, *Rph15*, *Rph16*, *Rph17*, *Rph18*, *Rph19*) (Franckowiak *et al.* 1997; Park and Karakousis 2002; Steffenson 2002; Park *et al.* 2003; Weerasena *et al.* 2004). In last 70 years the use of disease-resistant

barley cultivars has been an efficient means for controlling major diseases and preventing yield losses (Brooks *et al.* 2000; Finckh *et al.* 2000; Fischbeck 2003; Weibull *et al.* 2003; Czembor 2005). However, barley breeders, geneticists and plant pathologists are constantly looking for new efficient sources of resistance to leaf rust to combine them with already used in modern cultivars in order to increase the resistance durability (Levine and Cherevick 1952; Nover and Mansfeld 1959; Nover and Lehmann 1968, 1974; Tan 1977; Walther and Lehmann 1980; Sharp and Reinhold 1982; Yahyaoui *et al.* 1988; Alemayehu 1995; Brian *et al.* 1995; Alemayehu and Parlevliet 1996; Chicaiza *et al.* 1996; Backes *et al.* 2003; Bonman *et al.* 2005).

Archaeological data are proving that the original area of cultivation of *H. vulgare* L. was the area of the Fertile Crescent. In this area barley was derived from its wild ancestor *Hordeum spontaneum* C. Koch. when Neolithic men selected spikes with tough rachis. *H. spontaneum* is common in West Asia often growing on the same fields as cultivated barley and it occurs also in Egypt and Libya (Bothmer *et al.* 1995; Hawkes 1995; Badr *et al.* 2000). In addition, the discovery of stands of wild barley in southern Morocco has been reported (Molina-Cano and Conde 1980; Molina-Cano *et al.* 1982). This report suggests that the area of North Africa may be considered as possible additional center of origin for cultivated barley (Molina-Cano *et al.* 1987, 1992, 2002, 2005). Considering this fact, barley landraces collected in Mediterranean region including Sardinia may be rich source of new genes for resistance to leaf rust due to their high degree of diversification resulting from the long co-evolution with populations of pathogen (Wolfe, 1988).

In many studies it was proven that barley landraces can be valuable source of many breeding characteristics including resistance to major pathogens (Caddel 1976; Sharp and Reinhold 1982; Yahyaoui *et al.* 1988; Leur *et al.* 1989; Czembor 1996, 2001, 2005; 2007a, 2007b, Czembor and Czembor 2001, 2007c; Martinez *et al.* 2001; Shtaya *et al.* 2006a). The objective of this study was to study leaf rust resistance in lines selected from barley landraces collected in Sardinia.

MATERIALS AND METHODS

Plant material

A total of 240 lines selected from barley landraces collected in Sardinia were tested. These lines were created in Istituto di Agronomia Generale of the University of Sassari, Sardinia. They were selected from 12 populations of barley landraces collected in Sardinia in 1990 (Table 1) (Attene *et al.* 1996). Each population was represented by 20 randomly chosen lines (two cycles of single head progeny) (Papa *et al.* 1998).

Table 1

Collection sites of twelve populations of Sardinian barley landraces.

Name of landrace population	Collection site	Latitude	Elevation [m.a.s.l.]
Sard 1	Nurra 3 (near Sassari)	40°47'40"	170
Sard 2	Nurra 2 (near Sassari)	40°43'50"	85
Sard 3	Orosei	40°19'50"	240
Sard 4	Cuglieri 1	40°04'00"	80
Sard 5	Sinis North 2 (near Riola)	40°01'00"	4
Sard 6	Sini North 3 (near Riola)	39°58'00"	20
Sard 7	Cordedu 2	39°51'00"	10
Sard 8	Senorobi	39°32'50"	200
Sard 9	S. A. Frius	39°28'50"	300
Sard 10	S. N. Gerrei	39°29'00"	600
Sard 11	Sestu	39°22'00"	105
Sard 12	Quartu 2 (near Cagliari)	39°15'00"	35

Pathogen

Eight differential isolates of *P. hordei* were used (Table 2). These isolates originated from IHAR Radzikow collection and were chosen according to differences in virulence spectra observed on 12 differential cultivars. None of the isolates used was able to differentiate genes *Rph4* from *Rph8* and *Rph1* from *Rph10* and *Rph11*.

Table 2

Differential isolates and their infection types on differential set.

Accession name	Accession number	Gene	Isolates							
			Ph- 9	Ph-5	Ph-4	Ph-6	Ph-31	Ph-21	Ph-17	Ph-25
Sudan	CIho 6489	Rph1	4	4	4	4	4	4	4	4
Peruwian	CI 935	Rph2	4	4	4	4	4	4	2	4
Estate	CI 3410	Rph3	0	4	0	4	4	0	4	4
Gold	CI 1145	Rph4	4	4	0	4	4	4	4	4
Magnif	CI 13860	Rph2+Rph5	4	1	4	0	0	0	1	4
Bolivia	CI 1257	Rph2+Rph6	4	4	4	4	0	4	4	4
Cebada Capa	CI 6193	Rph7	0;	0;	0;	0;	0;	0;	0;	0;
Egypt 4	CI 6481	Rph8	4	4	0	4	4	4	4	4
HOR 2596	CI 1243	Rph9	4	4	4	4	4	1	4	4
Cliper C8	None	Rph10	4	4	4	4	4	4	4	4
Cliper C67	None	Rph11	4	4	4	4	4	4	4	4
Triumph	PI 290195	Rph12	4	4	4	4	4	0;	4	4

Resistance tests

In preliminary test about 30 plants per line were evaluated in a greenhouse with the Ph-25 isolate of *P. hordei*. Isolate Ph-25 represented the most virulent isolate available allowing the expression of highly effective (in breeders point of view) resistance to leaf rust. Resistance reactions after inoculation with this isolate showed only 8 lines from 5 populations: lines 4974 and 4997 selected from population Sard 2; lines 5041, 5055 selected from population Sard 5; line 5086 selected from population Sard 7; line 5102 selected from population Sard 8; lines 5121, 5132 selected from population Sard 9. From total 8 lines showing resistance to leaf rust in 7 lines (4974, 4997, 5055, 5086, 5102, 5121 and 5132) were observed plants resistant and susceptible after inoculation with isolate Ph 25. From one to four resistant plants per each landrace were grown in the greenhouse to obtain their seed. In this manner single plant lines were created and 8 lines 4974-4, 4997-1, 5041-3, 5055-1, 5086-3, 5102-1, 5121-1 and 5132-1 were further tested with 2 isolates of leaf rust – Ph-25 and Ph-5 (Table 3).

Table 3

Reaction of 8 selections from barley landraces to infection by 2 isolates of *Puccinia hordei*.

Lines	Isolates	
	Ph-25	*Ph-5*
4974-4	2	0;
4997-1	0	4
5041-3	0	3
5055-1	3	2
5086-3	3	3
5102-1	3	3
5121-1	3	3
5132-1	3	4

Only one line 4974-4 showed resistance to both isolates. This line was tested in greenhouse with 8 differential isolates of *P. hordei* (Table 4).

Table 4

Reaction of selection 4974-4 from barley landraces to infection by 8 isolates of *Puccinia hordei*.

Lines	Isolates							
	Ph- 9	*Ph-5*	*Ph-4*	*Ph-6*	*Ph-31*	*Ph-21*	*Ph-17*	*Ph-25*
4974-4	0:	1	0	0	0	0;	0;	0;

Testing procedure

This study was carried out in the IHAR Radzikow greenhouse. Cultivar L94, which does not carry any known genes for resistance to *P. hordei,* was used as a susceptible control. The plants were grown with 16 h light and temperature

range of 18-22°C. Urediniospores of *P. hordei* were suspended in deionized water with couple drops of "Tween 20" and inoculated onto one-week old seedling plants (primary leaf fully expanded) using a rate 3 mg urediniospores and 10 ml of water for 100 plants. Inoculated plants were incubated for 24 hours in a chamber in which the humidity was maintained near saturation by mist from ultrasonic humidifiers n complete darkness and with a temperature range of 12-15°C. Then plants were transferred to a greenhouse bench.

Disease assessment

Reactions of each accession were evaluated after an incubation period of 12-14 days in a greenhouse at 20-24°C. Disease symptoms were assessed on the primary leaf of the seedlings according to 0-4 scale adapted from Levine & Cherewick (1952) (Table 5). Infection types 0, 0;, 1 and 2 were considered indicative of incompatibility whereas infection types 3 and 4 of compatibility.

Table 5

Description of infection types and codes used (adapted from Levine and Cherewick 1952).

Infection Type	Host Response	Symptoms
0	Immune	No vivible uredia
0;	Very resistant	Hypersensitive flecks
1	Resistant	Small uredia with necrosis
2	Moderately resistant	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis
3	Moderately susceptible	Medium sized uredia with or without chlorosis
4	Susceptible	Large uredia without chlorosis

Postulation of leaf-rust resistance genes

Hypotheses about the specific resistance genes present were made by comparing the reaction spectra of the tested lines with those of differential lines.

RESULTS

Only 8 lines (3.3%) (4974-4, 4997-1, 5041-3, 5055-1, 5086-3, 5102-1, 5121-1 and 5132-1) from total 240 lines tested originating from 5 populations of barley landraces (Sard 2, Sard 5, Sard 7, Sard 8, Sard 9) showed resistance to leaf rust. From these lines only one line (4974-4) was resistant to infection with 2 most avirulent isolates available (Table 3).

Based on obtained results of testing with 8 differential isolates it was impossible to postulate which specific genes for resistance are present in line 4974-4 (Table 4). This line was resistant to all isolates used.

DISCUSSION

Based on screening tests it may be concluded that barley landraces collected in Sardinia posses leaf rust genes for resistance. Obtained results are proving the practical advantage of preserving the genetic diversity of barley in the form of landraces. However, only 8 lines (3.3%) from total 240 lines tested originating from 5 populations showed resistance reaction to leaf rust. After preliminary testings only one line (4974-4) showed resistance to all isolates used. This line can possess unique resistance and should be used in breeding programes of barley.

Barley landraces collected in Mediterranean region are very diverse and often heterogenous concerning many breeding characteristics including disease resistance (Sharp and Reinhold 1982; Yahyaoui *et al.* 1988; Leur *et al.* 1989; Czembor 1996, 2001, 2005; Czembor and Czembor 2001; Martinez *et al.* 2001; Shtaya *et al.* 2006a). This diversity results from big contrasts in this region, both in geographical conditions (e.g. climate, altitude) and in agricultural practices, between the highlands and lowlands (Attene *et al.* 1996; Papa 1993). This fact was confirmed in presented study because from total 8 lines showing resistance to leaf rust in 7 were observed plants resistant and susceptible after inoculation with isolate Ph 25.. Collecting missions in Mediterranean region are recommended by many investigators because barley landraces in on this area are subject to rapid genetic erosion (Attene *et al.* 1996; Papa 1993; Perrino *et al.* 1986; Damania 1988; Podyma 1989; Hammer *et al.* 1996).

Many gene banks worldwide possess collections of barley and the total number of these barley accessions is estimated to be about 350 000. Approximately 40% of these collections refers to landraces collected in the field or selections from landraces (Valkoun and Konopka 2004). However, almost in all collections only field observations are available concerning disease resistance and only small percentage of these accessions was studied for resistance to major pathogens under controlled conditions and with set of differential isolates. Presented in this paper studies provide not only valuable results for barley geneticists and plant pathologists but first of all they provide needed characteristic of gene banks barley collections for practical use by breeders (Yahyaoui *et al.* 1988; Leur *et al.* 1989; Czembor 1996, 2001, 2005; Martinez *et al.* 2001; Fischbeck, 2003, Weibull *et al.* 2003, Shtaya *et al.* 2006a)

Identified in this study new source of resistance to leaf rust originated from landraces should be a relatively easier incorporated into a barley breeding programs in comparison to those originating from mutants or wild barley. Moreover undesirable agronomic traits that are usually derived from wild relatives do not have to be bred out when using landraces as a source of leaf rust resistance. In addition using barley landraces in breeding programs may have also other advantage which is incorporation of desirable agronomic traits e.g. good adaptation to dry land conditions (Ceccarelli *et al.* 1987, 1995; Weltzien 1988; Weltzien and Fischbeck 1990; Yahyaoui *et al.* 1996; Fischbeck 2003).

In many regions of the word many barley varieties had to be discarded because they were the far too disease susceptible to be of any further value. This susceptibility was due to not only by the lost of resistance determined by specific major genes but also by host erosion of partial resistance during breeding of barley for leaf rust race-specific resistance (Parlevliet 1983; Brown and Hovmřller 2002; McDonald and Linde 2002; Ochoa and Parlevliet 2007). Because of all of these facts there is strong need to identify and use new sources of resistance to this pathogen in barley breeding programmes (McDonald and Linde 2002; Backes *et al.* 2003; Czembor and Czembor 2007a, 2007b).

The durability of the resistance genes to leaf rust present in barley cultivars may be increased by using many different strategies for deploying resistance genes. (Parlevliet 1983; Wolfe 1988; Finckh *et al.* 2000; McDonald and Linde 2002). These strategies are: combining partial (minor genes) and race-specific (major) resistance genes, multiline cultivars, partial resistance, combining different race-specific resistance genes into one cultivar and deploying many cultivars with different resistance genes in space (e.g. cultivar mixtures) or time (winter versus spring barley) (Parlevliet 1983; Finckh *et al.* 2000; Brown and Hovmřller 2002; McDonald and Linde 2002). Very important for durability of resistance to leaf rust in agricultural practice is proper use of new sources of resistance to this pathogen (Steffenson *et al.* 1993; Walther 1996; Brown and Hovmřller 2002; McDonald and Linde 2002; Park 2003; Shtaya 2006c).

In presented study seedling resistance tests were used in order to describe infection types expressed by barley plants after inoculation with differential isolates of *P. hordei.* This kind of testing as sufficient for screening for disease resistance and it is used commonly in many breeding programs to postulate the presence of specific genes for resistance (Parlevliet 1976; Jin *et al.* 1995; Czembor 1996, 2005; Brooks *et al.* 2000; Shtaya 2006b; Czembor and Bladenopoulos 2007; Czembor and Czembor 2007a, 2007b). However, using this kind of tests it is not possible or at least it is difficult to identify and describe partial resistance. For description of this kind of resistance we need conduct additional to infection type measurements characteristic for this kind of resistance. In addition, partial resistance is generally better expressed at the adult plant stage (Parlevliet and van Ommeren 1975; Smit and Parlevliet 1990; Martinez *et al.* 2001; Shtaya *et al.* 2006a; Ochoa and Parlevliet 2007). It will be very interesting if further studies of described lines will also include parameters describing partial resistance and extension of resistance studies to plants at adult stage. Final determination of the number of resistance genes and the type of their action in tested selection may be established by crosses and backcrosses among appropriate genotypes (Jin and Steffenson 1994; Alemayehu 1995; Czembor 1996, 2005; Czembor and Czembor 2001; Czembor *et al.* 2006).

Studies resulting in description of new sources of resistance are also important because future strategies for the control of barley pathogens will have to focus increasingly on more ecologically acceptable methods. Any usage of chemicals (pesticides, fungicides, herbicides, and mineral fertilizers) in agri-

culture is increasingly criticized in societies of many countries. Breeding for resistance represent such ecologically safe method. In addition to ecological also economical arguments (use of fuel, labour, special machines) are in favour of breeding for resistance versus chemical control (Nierobca *et al.* 2003; Gullino and Kuijpers 1994).

Results of presented study confirmed findings in other investigations that barley landraces can possess leaf rust resistance genes different from genes present in cultivated varieties (Levine and Cherevick 1952; Nover and Mansfeld 1959; Nover and Lehmann 1968, 1974; Tan 1977; Sharp and Reinhold 1982; Yahyaoui *et al.* 1988; Alemayehu 1995; Brian *et al.* 1995; Alemayehu and Parlevliet 1996; Bonman *et al.* 2005; Czembor 2007a, 2007b, Czembor and Czembor 2007c). The use of new sources of leaf rust resistance described in this study, especially highly resistant line 4974-4 should result in increasing of leaf rust resistance genes diversity in barley cultivars.

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