Acta Phytopathologica et Entomologica Hungarica 55 (1), pp. 3–10 (2020)
DOI: 10.1556/038.55.2020.001

Identification of Barley Lines with Resistance to Powdery Mildew Based on Seedling and Adult Plant Responses

M. I. E. ARABI*, M. JAWHAR and E. AL-SHEHADAH

Department of Molecular Biology and Biotechnology, AECS, P. O. Box 6091 Damascus, Syria

(Received: 25 August 2019; accepted: 7 November 2019)

Powdery mildew (*Blumeria graminis*) is a major fungal disease of barley causing economical yield losses worldwide. Breeding for resistance to this disease is crucial due to the rapid change in pathotype patterns of *B. graminis* in fields. In the present work, powdery mildew-resistant barley germplasm was developed by crossing four cultivars currently used in Europe and West Asia. Out of 265 doubled haploid lines derived from these crosses, 40 lines were evaluated at seedling and adult stages. Data showed significant differences among barley lines with a continuum of resistance levels ranging from highly susceptible to tolerant which were consistent during the two growth stages. Two promising lines were more tolerant to powdery disease than the others. Across lines, there was a high correlation between field and greenhouse reaction ($r = 0.80$, $P < 0.01$), indicating the utility of greenhouse evaluations for screening barley for powdery mildew. This study suggests that, the newly identified resistance lines can serve as potential donors for ongoing powdery mildew resistance breeding program, and both types of seedling and adult plant resistance identified here can offer promising genetic stocks for accumulating both resistances to acquire durable resistance and long lasting control against *B. graminis* in Mediterranean and similar environments.

Keywords: Barley (*Hordeum vulgare* L.), powdery mildew, seedling resistance, adult plant resistance.

Barley powdery mildew caused by the fungal pathogen, Blumeria graminis f. sp. hordei, is among the most devastating fungal diseases causing significant yield losses across the world (Rsaliyev et al., 2017). In recent years, this disease has also become more significant because of the rapid change in pathotype patterns and agricultural practices (Dreiseitl, 2016). In Syria, with the increasing demand for barley and more intensive management practices, powdery mildew have assumed greater significance (Leur van et al., 1989; ICARDA, 1998,).

The economic damage caused by powdery mildew can be avoided by using large amounts of fungicides, but their extensive use may lead to the development of resistant pathogen strains (Hysing et al., 2012), therefore, utilization of breeding for resistance is economical, and carries no health and environmental hazards especially in developing countries where most farmers are small to marginal and unable to afford costly fungicides and other technologies (Dreiseitl and Zavřelová, 2018).

*Corresponding author; e-mail: ascientific1@aec.org.sy

0238–1249/$ 20.00 © 2020 Akadémiai Kiadó, Budapest

Efforts to breed cereals against powdery mildew have identified resistances that are expressed at seedling growth stages and that usually remain effective throughout the seedling adult plant growth stages (Kundsen et al., 1986; Das and Griffy, 1994). Genetic studies have indicated that the heritability to this disease resistance ranged from low to high in the progeny of crosses between resistance and susceptible lines (Jørgensen, 1994; Bargougul, 2016). Therefore, a considerable proportion of the variance may be attributed to the environmental factors. Causal field observation prone to variability due to the time and intensity and level of inoculum, cultivar and environmental interactions make it impossible to obtain error-free estimates (Ceccarelli et al., 1995).

Major difficulties in previous breeding efforts have been concentrated on screening plants for this disease resistance and transferring of resistance genes. Therefore, finding new sources of resistance and improved knowledge about the reaction of barley genotypes at seedling and adult stages would be of great value to breeders in improving resistance to powdery mildew.

This study was carried out to evaluate barley seedlings and adult plants to Syrian powdery mildew populations under experimental conditions that are typical of a large part of the barley-growing areas of western Asia. Barley cultivars presently grown in Europe and West Asia were used in this study to identify new sources of resistance in adapted genetic backgrounds and in plant types agronomically acceptable to farmers.

Materials and Methods

Plant material

A total of 40 out of 265 barley double haploid lines produced according to Kasha and Kao (1970) were screened on the basis of agronomic characteristics and evaluated in this work (Table 1). These lines were produced through four barley crosses made between six parents possessing different mildew reactions. Arabi Abiad (susceptible) is a Syrian local cultivar, Arrivate (moderately susceptible) was received from USA, Igri (resistant; possessing the resistance allele *Mlra*) from Germany, CI5791 (susceptible) from Ethiopia, PK30-136 (moderately resistant) is a Pakistan cultivar and IC-9 (moderately resistant) is a new genotype developed at ICARDA (International Center for Agricultural Research in the Dry Areas). Haploids were produced by crossing these six parents using the standard procedure. Briefly, spikes were manually emasculated and pollinated with fresh pollen. Pollinated spikes were treated with 2,4-dichlorophenoxyacetic acid (2,4-D). Tillers from donor plants were collected when the majority of microspores were at mid- to late-uninucleate stage of development. The tillers were placed in Hoagland's salt solution and stored at 4 °C in the dark for 21 days. Then, under sterile conditions, cold-treated (spikes were sterilized with 96% ethanol, and the anthers were excised and transferred to FW culture medium (Foroughi-Wehr et al., 1976; Arabi et al., 2005). Haploid plants were vernalized for 8 weeks. Subsequently, haploids were treated with colchicine solution for chromosome doubling (Subrahmanyam and Kasha, 1975).

Seedling test

Seeds of each genotype were planted in plastic flats (60 × 40 × 8 cm) filled with sterilized peatmoss with three replicates. Each experimental unit consisted of 10 seedlings. They were placed under greenhouse conditions at temperatures 20–22 °C (day) and 16–18 °C (night) with a daylength of 16 h and a relative humidity of 85–95%. At growth stage (GS) 11–12 (Zadoks et al., 1974), and in order to detect the seedling reaction to the same field population, seedlings at emerge leaves stage were placed for four nights in the field and the symptoms development was checked 10 days later and scored at GS 32. The experiment was repeated twice.

Adult plant test

Field experiments were performed under natural disease infection in Syria, at a site of 970 m altitude (550-mm rainfall average). The location of the experiment was chosen to be favorable for the development of powdery mildew disease, since *B. graminis* infects barley in this location annually. Seeds were sown in a randomized complete block design, with three replicates. Individual Plots were 1 × 1 m with a 1-m border. Each plot consisted of five rows, 25 cm apart and with 50 seeds sown per row. Soil fertilizers; 50 kg/ha of nitrogen in the form of Urea (46%) were drilled in equal portions before sowing and after tillerring, and 27 kg/ha superphosphate (33% P_2O_5) were drilled before sowing. The experiments were surrounded by spreader rows of the powdery mildew-susceptible cv. Golf, which had already been infected by the virulent isolate Pt1m (Arabi and Jawhar, 2012) before the experiments were sown. An additional spreader row was also placed approximately in the middle of each experiment. In addition, powdery mildew – infected stubble was distributed in the field when seedlings were at the second leaf stage and plants were wetted twice a day by applying water using a high-pressure sprayer to enhance disease infection.

Disease assessment

At seedling (GS 32) and adult plant (GS 80) stages, mildew infections were visually assessed according to the scale described by Moseman and Baenziger (1981) whereas; plants having infection types 0–30% were considered as tolerant, those having infection types 30–60% as moderate, while those having infection types over 60% of leaves covered by mildew were rated as severe. For each method, the mean value was used for analysis.

Statistical analysis

Data was subjected to analysis of variance using the STAT-ITCF statistical programme (2nd Version). Differences between means were evaluated for significance by using Newman-Keuls test at 5% probability level (Anonymous, 1988).

Results and Discussion

In the current study, six barley parents with different resistance levels to mildew infections were used. As shown in (Figs 1, 2) mildew caused more severe infection on the susceptible parents 'Arrivate', 'CI5971' and 'Arabi Abiad' as compared with the resistant ones. Furthermore, the disease symptoms were typically observed in infected seedlings and adult plants with the severity values being consistently higher in the susceptible parents. These results are in agreement with our previous observations under natural conditions (Arabi and Jawhar, 2012).

According to a scale described by Moseman and Baenziger (1981) the reactions of the 40 progeny lines to mildew under greenhouse and field tests were classified into 13 lines as tolerant, 14 moderate and 13 severe group (Table 1). However, significant differences ($P < 0.05$) in mean severity values were detected among different lines, and a continuum of genotypic reactions to the disease from tolerant to severe was observed (Table 2).

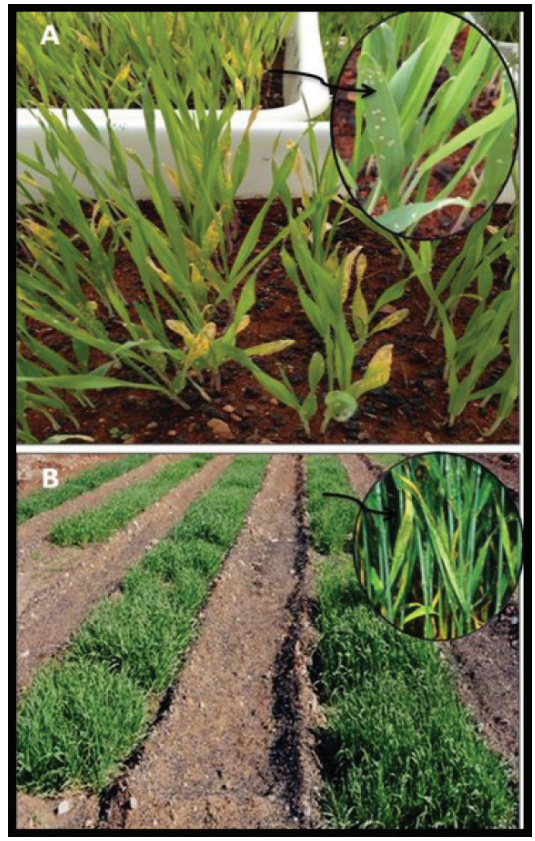

Fig. 1. Powdery mildew symptoms on the barley susceptible Arrivate genotype under greenhouse (A) and field (B) conditions

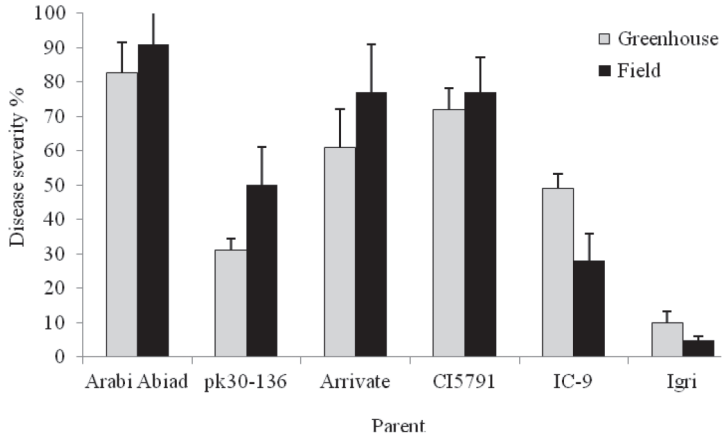

Fig. 2. Frequency of powdery mildew reactions incited on the barley parents used in the study, under greenhouse and field conditions. Error bars are representative of the standard error (Mean ± SD, $n = 3$)

Table 1

Parental cross and progeny susceptibility to powdery mildew*

Cross	Progeny		
	Tolerant	Moderate	Severe
Arabi Abiad X IC-9	5	4	6
Arrivate X PK30-136	7	8	3
CI5791 X Igri	1	2	1
Arabi Abiad X Arrivate	–	–	3
Total	13	14	13

*Based on a scale described by Moseman and Baenziger (1981)

The data showed that four lines (B08-AS-19, 29, 35 and 36) were classified as highly tolerant under both greenhouse and field conditions, whereas lines B08-AS 2, 11, 12, 31 and 32 were the most susceptible lines (Table 2). Significant correlation coefficient ($r = 0.80$, $P < 0.01$) was found between the two tests for mildew reaction, indicating that lines reacted similarly to *B. graminis* populations under both conditions.

The identification of new sources of resistance to powdery mildew and their introduction into cultivated crops is a very important element of breeding programs (Dreiseitl and Zavřelová, 2018). Therefore, inclusion of this diverse germplasm in the barley breeding program might increase the dominance effect and epistatic variations controlling quantitative traits such as mildew resistance (Halward and Wynne, 1991; Bargougul, 2016). These would also lead to extend segregation for various traits and in obtaining useful recombinants/transgressive segregants in the further generations.

One of the major objectives of the Syrian barley breeding program is to develop high-yielding cultivars with resistance to mildew disease through the transfer of genes from resistant sources. In this work, all the 13 tolerant lines maintained their reaction during seedling and adult stages, and these stability levels may have been evidence of their resistant levels. Moreover, the population of crosses Arrivate /PK30-136 and CI5791/Igri

Table 2

% Powdery mildew severity on barley lines tested at seedling and adult plant stages

Cross	No.	Lines	Seedling	Adult plant
Arabi Abiad X IC-9	1	B08-AS-1	43.3g*	59.0e
	2	B08-AS-2	90.0b	80.3b
	3	B08-AS-3	13.3j	25.0h
	4	B08-AS-4	6.6k	30.0h
	5	B08-AS-5	32.3h	29.6h
	6	B08-AS-6	63.3d	72.2c
	7	B08-AS-7	21.6i	26.3h
	8	B08-AS-8	7.05c	82.3b
	9	B08-AS-9	9.30j	18.0i
	10	B08-AS-10	38.3h	65.6d
	11	B08-AS-11	90.0b	91.5a
	12	B08-AS-12	73.3c	91.6a
	13	B08-AS-13	45.0g	55.2e
	14	B08-AS-14	13.3j	37.3g
	15	B08-AS-15	10.0j	29.3h
Arrivate X PK30-136	16	B08-AS-16	53.3e	60.8d
	17	B08-AS-17	41.6g	44.5f
	18	B08-AS-18	28.3i	13.0j
	19	B08-AS-19	8.30j	9.10j
	20	B08-AS-20	26.6i	27.0h
	21	B08-AS-21	66.6d	49.6f
	22	B08-AS-22	56.6e	33.3g
	23	B08-AS-23	56.6e	30.0h
	24	B08-AS-24	37.6h	16.60i
	25	B08-AS-25	26.3i	44.3f
	26	B08-AS-26	13.3j	6.30k
	27	B08-AS-27	6.60k	26.6h
	28	B08-AS-28	34.0h	7.60k
	29	B08-AS-29	6.60k	6.60k
	30	B08-AS-30	13.3j	3.30k
	31	B08-AS-31	98.3a	72.9c
	32	B08-AS-32	96.6a	85.0b
	33	B08-AS-33	33.3h	38.3g
CI5791 X Igri	34	B08-AS-34	26.6i	33.8g
	35	B08-AS-35	1.6k	18.6i
	36	B08-AS-36	3.3k	37.8g
	37	B08-AS-37	58.3e	80.2b
Arabi Abiad X Arrivate	38	B08-AS-38	33.3h	63.3d
	39	B08-AS-39	50.8f	77.0c
	40	B08-AS-40	55.3e	75.5c
LSD			6	5.1

*Values within a column followed by different letters are significantly different at $P < 0.05$ according to Newman-Keuls test.

will be used for mapping genes associated with mildew resistance. Some lines from these crosses (B08-AS-19, 29, 35 and 36) were recovered with a high degree of resistance to mildew. These will be tested in multilocation trials to test their stability and adaptability.

It is well known that barley resistance to powdery mildew is encoded by the *Mlo* (Jørgensen, 1992) and *Mla* (Boyd et al., 1995) loci. The *Mlo* genes mediate resistance by directing the formation of subcellular cell wall appositions at the sites of infection, which prevents the penetration of the fungus into the epidermal cells (Jørgensen, 1992; Wolter et al., 1993). On the other hand, hypersensitive cell death (HR) in the leaves of plants with the *Mla* locus is an incompatibility response that is thought to mediate resistance by preventing both conidial development at the haustorial stage and hyphal elongation (Koga et al., 1990). Therefore, it is likely that lines B08-AS-19, 29, 35 and 36 resist powdery mildew by preventing the development of secondary mycelia.

This work showed that the barley breeding lines had diversity for infection response to the powdery mildew pathogen. Thirteen promising sources of resistance were identified that could be considered as possible donors in further barley breeding programs. In addition, a positive correlation between field and greenhouse reactions was found, this can speed up the breeding process by allowing assessment at seedling stage when field screening is ineffective due to unsuitable environmental conditions

Acknowledgements

The authors wish to thank the Director General of AECS and the Head of the Molecular Biology and Biotechnology Department for their continuous support throughout this work. We also thank Dr. A. Al-Daoude for critical reading of the manuscript.

Literature

Anonymous, (1988): STAT-ITCF, Programme, MICROSTA, realized by ECOSOFT, 2nd Version. Institut Technique des cereals et des Fourrages, Paris, 55 p.

Arabi, M. I. E. and Jawhar, M. (2012): Expression of resistance to *Blumeria graminis* in barley genotypes (*Hordeum vulgare* L.) under field and controlled conditions. J. Plant Biol. Res. 2, 107–112.

Arabi, M. I. E., Al-Safadi, B., Jawhar, M. and Mir-Ali, N. (2005): Enhancement of embryogenesis and plant regeneration from barley anther culture by low doses of gamma irradiation. *In Vitro* Cell. Dev. Biol. 41, 762–764

Bargougul, M. A. (2016): Diallel analysis of the resistance to powdery mildew (*Erysiphe graminis* f.sp. *hordei*) in barley (*Hordeum vulgare L.*). J. New Sci. 27, 1466–1473.

Boyd, A., Smith, P. H., Foster , E. M. and Brown, J. K. M. (1995): The effect of allelic variation at the *Mla* resistance locus in barley on the early development of *Erysiphe graminis* f.sp. *hordei* and host responses. The Plant J. 7, 959–968.

Ceccarelli, S., Grando, S., and Van Leur, J. A. G. (1995): Barley landraces of the Fertile Crescent offer new breeding options for stress environments. Diversity 11, 112–113.

Das, M. K. and Griffy, C. A. (1994): Diallel analysis of adult–plant resistance to powdery mildew in wheat. Crop Sci. 34, 948–952.

Dreiseitl, A. (2016): Emerging *Blumeria graminis* f. sp. hordei pathotypes reveal 'Psaknon' resistance in European barley varieties. The J. Agri. Sci. 154, 1082–1089.

Dreiseitl, A. and Zavřelová, M. (2018): Identification of barley powdery mildew resistances in gene bank accessions and the use of gene diversity for verifying seed purity and authenticity. PLoS ONE 13(12).

Foroughi-Wehr, B., Mix, C., Gaul, H. and Wilson, H. M. (1976): Plant production from cultured anthers of *Hordeum vulgare* L. Z. Pflanzenzuchtg. 77, 198–204.

Halward, T. M. and Wynne, J. C. (1991): Generation means analysis for productivity in two diverse peanut crosses. Theor. Appl. Genet. 82, 784–792.

Hysing, S. C., Rosenqvist, H. and Wiik, L. (2012): Agronomic and economic effects of host resistance vs. fungicide control of barley powdery mildew in southern Sweden. Crop Prot. 41, 122–127.

ICARDA (1998): ICARDA Annual Report (1997): International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.

Jørgensen, J. H. (1992): Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. Euphytica 63, 141–152.

Jørgensen, J. H. (1994): Genetics of powdery mildew resistance in barley. Crit. Rev. Plant Sci. 13, 97–119.

Kasha, K. J. and Kao, K. N. (1970): High frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225, 874–876.

Koga, H, Bushnell, W. R. and Zeyen, R. J. (1990): Specificity of cell type and timing of events associated with papilla formation and the hypersensitive reaction in leaves of *Hordeum vulgare* attacked by *Erysiphe graminis* f.sp. *hordei*. Can. J. Bot. 68, 2344–2352.

Kundsen, J. C. N., Dalsgaard, H. H. and Jørgensen, J. H. (1986): Field assessment of partial resistance to powdery mildew in spring barley. Euphytica, 35, 233–243.

Leur van, J. A. G., Ceccarrelli, S. and Grando, S. (1989): Diversity for disease resistance in barley landraces from Syria and Jordan. Plant Breed. 103, 324–335.

Moseman, J. G. and Baenziger, P. S. (1981): Genes conditioning resistance of *Hordeum spontaneum* to *Erysiphe graminins* f. sp. *Hordi.* Crop Sci. 21, 229–232.

Rsaliyev, A., Pahratdinova, Z. and Rsaliyev, S. (2017): Characterizing the pathotype structure of barley powdery mildew and effectiveness of resistance genes to this pathogen in Kazakhstan. BMC Plant Biol. 17, 178.

Subrahmanyam, N. C. and Kasha, K. J. (1975): Chomosome doubling of barley haploids by nitrous oxide and colchicines treatments. Can. J. Genet. Cytol. 17, 573–583.

Wolter, M., Hollricher, K., Salamini F. and Schulze-Lefert, P. (1993): The mlo resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defence mimic phenotype. Mol. Gen. Genet. 239, 122–128.

Zadoks, J. C., Chang, T. T. and Konzak, C. F. (1974): A decimal code for the growth stages of cereals. Weed Res. 14, 415–421.