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Importance of the Secondary Genepool in Barley Genetics and Breeding II. Disease Resistance, Agronomic Performance and Quality

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Abstract: In this second paper on the use of secondary genepools in barley improvement, we describe the characterisation of leaf rust resistant recombinant lines (RLs) derived from *Hordeum vulgare* × *H. bulbosum* crosses. Twelve RLs were inoculated with leaf rust and the early stages of disease development were observed. Several RLs showed complete resistance to the pathogen, but others had a high level of partial resistance, which may be durable. Some of these RLs and others were tested in yield trials to determine the effects of introgressed chromatin from *H. bulbosum* on yield and quality. We conclude that there are no major adverse effects that cannot be overcome through normal breeding techniques.

Keywords: barley; *Hordeum bulbosum*; leaf rust; *Puccinia hordei*; introgressions; yield; malting quality

Disease resistance

Resistance to several diseases has been transferred from *Hordeum vulgare* L. into *H. bulbosum* L. (Pickering & Johnston 2004). Since *H. bulbosum* is probably not a host to barley leaf rust (*Puccinia hordei* Otth; Xu & Snape 1989; Anikster 1989) it would be desirable to exploit the genes for nonhost resistance of that species in barley breeding. Such genes may confer a more durable type of resistance than the genes available in the cultivated host species. Little is known about the genetics of nonhost resistance but resistance to heterologous rust species may be the joint effect of many quantitative genes for non-hypersensitive resistance (Hoogkamp *et al.* 1998). The resistance of wheat to the powdery mildew forma specialis of *Agropyron* has been reported to be based on a gene-for-gene response (Tosa 1989).

In New Zealand 33 lines carrying introgressed DNA from *H. bulbosum* (called here recombinant lines, RLs) showed complete or incomplete hypersensitivity resistance to *P. hordei* in the field and glasshouse, or had a reduced rate of infection compared to the recipient cultivars Emir and Golden Promise. Twelve of these RLs were screened to determine more precisely the level and mechanism of their resistance to leaf rust. The research was carried out in the Netherlands and Spain (Dr Diego Rubiales) using similar methods.

Agronomic performance and quality

In this part of the programme we investigated whether introgressions of DNA from *H. bulbosum* into cultivated barley may affect yield and quality in the presence or absence of disease. Some of the RLs tested are resistant to one or more diseases whereas others are as susceptible as their barley parents. Yield trials were carried out in two years in New Zealand. Half the plots were treated with fungicide to control powdery mildew and leaf rust (caused by *Blumeria graminis* f.sp. *hordei* L. and *Puccinia hordei* Otth, respectively), the prevalent diseases on spring-sown barley in Canterbury (New Zealand). Malting quality was assessed on

samples from harvest year 1 only. Five of the lines had also been tested in the leaf rust experiments described above.

MATERIALS AND METHODS

Disease resistance

The 12 RLs used in the experiments are shown in Table 1. The parental *H. vulgare* cultivars are designated as E (Emir) and G (Golden Promise) followed by the chromosomal location of the *H. bulbosum* introgression(s). Where two or more RLs have the same chromosomal introgression location, another letter is added (a, b, or c). The full identity including original code numbers of each RL can be supplied on request.

Eleven days after sowing, first leaves were fixed horizontally and inoculated with leaf rust isolates 1.2.1, Córdoba and Tunisia. The inoculum was mixed with 9 times the volume of Lycopodium powder, and applied in a settling tower at about 200 spores per cm^2. After inoculation the plants were incubated overnight (10 h) in a dark mist chamber, and then transferred to a greenhouse at 20 to 25°C. After 6 days uredia were counted daily in a marked area containing about 20 to 50 pale flecks before mature uredia appeared. The latency period (LP) was estimated as the time from the start of incubation to the time when 50% of the uredia had appeared. Infection type (IT) was assessed on a 0–9 scale (McNeal *et al*. 1971).

Three RLs (E-1HL, E-2HL-a and G-2HL-b) with contrasting infection types to *P. hordei* were used to determine the infection frequency (IF) and resistance mechanism with isolate 1.2.1. On the fourth day after inoculation we collected central segments from three leaves per line and stained them for UV microscopy (Hoogkamp *et al*. 1998). At least 60 infection units were evaluated per leaf segment and classified according to their stage of development (Hoogkamp *et al.* 1998). Infection units that were arrested after primary infection hyphae had formed but before the formation of six mycelial branches per infection unit were classed as "early aborted". Infection units with six or more mycelial branches were classified as "established". Yellow autofluorescence of plant cells directly associated with infection units was recorded as "necrosis" and occurred after haustorium formation indicating post-haustorial resistance. The diameter of 20 established colonies per leaf segment was measured and the IF on any unsampled leaves or leaf stubs was determined by counting the number of pustules in 1 cm^2. On leaves with a low IF a larger segment of the leaf was cut, measured and the number of pustules counted to calculate the IF in uredia per cm^2.

In a third experiment the three RLs were inoculated with four different *P. hordei* isolates (1.2.1., 17, 26 and Uppsala) to check the race-specificity of the resistance by measuring LP and IF.

Agronomic performance and quality

Trials were laid out in a randomised block design with five replicates of 30 plots per replicate; plot area was 4 m^2. Each replicate consisted of 15 entries × 2 plots, one of which was treated with fungicide. The 15 entries comprised 13 recombinant lines (RLs) derived from *H. vulgare* × *H. bulbosum* crosses and their respective barley parents, Emir (feed barley) and Golden Promise (GP; malting barley) (Table 3). Ten of the 13 RLs were trialled again in year 2 with extra replicates for GP and E-4HL-a. Code numbering follows the same format as previously. The RLs were all different selections apart from G-4HL-b, which was derived from G-4HL-a after reducing the size of the 4HL introgression through backcrossing to GP. In year 1, the fungicides applied to control powdery mildew and leaf rust were Opus® (epoxiconazole) or Twist® (trifloxystrobin) + Folicur® (terbuconazole), each applied twice with a final application of Merit® (propiconazole + fenpropimorph). In year 2 three applications of Opus® + Fortress® (quinoxyfen) were made during the growing season. Disease in year 1 was scored on four dates for powdery mildew and once for leaf rust, which only appeared later in the season. Area under the disease progress curve (AUDPC) was calculated for the mildew data using standard methods. Other agronomic characters such as earliness, height and lodging were recorded.

Eight months after harvest year 1, thousand grain weights (tgw) and germinations were carried out at Lincoln (New Zealand) and 120 g of grain taken from each plot were kindly micromalted by Coors Brewing Company (Golden, Colorado, USA). Samples from two complete replicates were then analysed for malting quality at University of Adelaide (Australia) using standard methods (Logue *et al.* 2002). Owing to financial constraints only grain protein, diastatic power, malt protein, hot water extract (IOB), viscosity, soluble protein and the Kolbach Index were determined.

Data were analysed with analysis of variance; a few plots were excluded because of sowing errors. There were some spatial trends, particularly for the harvest data, but adjusting for these trends produced similar results to those from the simple analysis of variance presented here. Comparisons among the RLs and their respective parents were made as contrasts within the analysis.

RESULTS AND DISCUSSION

Disease resistance

Table 1 shows the LPs and ITs of the 12 RLs with isolate 1.2.1. Results from the Córdoba and Tunisia isolates were generally similar (data not presented). On four RLs no pustules had developed (IT = 0 or 1), or a few pustules appeared but were associated with strong necrosis or chlorosis. In these RLs the *H. bulbosum* introgressed DNA must contain a gene(s) for hypersensitive resistance to *P. hordei.* L94 was the most susceptible accession since the leaf rust fungus had the shortest LP. The LP on Vada was 34% longer than on L94 due to its high level of partial resistance. The LPs on Emir and Golden Promise were generally intermediate to L94 and Vada, indicating a moderate level of partial resistance. On four lines (E-1HL, E-5HL, G-2HL-b and G-5HL-6HS) the LPs were longer than on Emir or Golden Promise, and equal to or longer than on Vada. The level of partial resistance of G-2HL-b was outstanding since the LP was even longer than on Vada and 46% longer than on L94.

Table 1. Latency period (LP, hours in parenthesis for L94; and as a % of L94), and infection type (IT) of isolate 1.2.1 of *Puccinia hordei* on 12 barley recombinant lines with introgressed chromatin from *Hordeum bulbosum*

Barley line[§]	*H. bulbosum* parent	Relative LP	IT[#]
L94		100 (136 h)	9
Vada		134	9
Emir		111	9
E-4HL-a	Cb2920/4	113	9
E-4HL-b	Cb2920/4	114	9
E-5HL	Cb2920/4	124	9
E-1HL	A17	129	9
E-4HL-c	A17/1	–*	0
E-2HL-a	HB2032	–	0
E-2HL-b	HB2032	–	0
E-2HS-a	Cb2920/4	–	4
E-6HS-7HS-7HL	Cb2920/4 × Cb2929/1	–	5
Golden Promise		113	9
G-5HL-6HS	Cb2920/4	129	9
G-2HL-b	A17/1	146	9
G-2HL-a	Cb2920/4	–	1

[§]In bold the lines used in further leaf rust experiments

[#]Infection type (IT): 0 = no symptoms; 1 = minute necrotic flecks; 4, 5 = necrotic flecks and pustules surrounded by necrotic or chlorotic tissue; 9 = fully compatible pustules that may be surrounded by pale green halos

*LP could not be determined because of low numbers of uredia, due to the low IT

The second experiment (Table 3) confirmed the resistance rankings. No uredia were formed on E-2HL-a and its resistance was complete and the response very rapid. Almost all infection units were arrested within 24 hours before they formed at least six mycelial branches. Most (90%) of such aborted colonies were associated with autofluorescent plant cells indicating a hypersensitive reaction. The high degree of partial resistance in G-2HL-b was apparent too, since the number of pustules was much lower than on Vada and not associated with chlorosis or necrosis (infection type 9), which would have indicated hypersensitivity. High partial resistance is also associated with high frequencies of early aborted infection units. Although the percentage of early abortion was unexpectedly low for Vada, RLs E-1HL and G-2HL-b had even higher percentages of early abortion and smaller established colonies than Vada and Emir, indicating a higher level of partial resistance. Since most of the early aborted colonies were unassociated with autofluorescent plant cells, the resistance mechanism is probably not based on hypersensitivity but on blocking haustorium formation at most of the plant cell wall penetration sites.

The resistances of E-1HL, E-2HL-a and G-2HL-b were confirmed in the third experiment (data not presented). Compared with Emir, the introgression in E-1HL caused a longer LP and lower IF to all four isolates. The complete resistance of E-2HL-a was also effective against the four isolates and the level of partial resistance in G-2HL-b was higher than in Vada since the LP was longer and the IF lower. Both these RLs contain introgressions on 2HL although the sizes differ and they have different *H. vulgare* and *H. bulbosum* parents. We will

Table 2. Means of fungicide treated and untreated plots for ear emergence (days ± Emir), straw height and lodging (maximum of two scores) on 13 recombinant lines and their respective barley parents. Disease incidence is for untreated plots only. Leaf rust incidence: mean % leaf area infected for one scoring date. AUDPC: area under the disease progress curve for powdery mildew incidence recorded as % leaf area infected on four dates

Code	*H. bulbosum* parent	Ear emergence	Straw height (cm)	Lodging (%)	Leaf rust incidence	Powdery mildew AUDPC
E-2HS-a	Cb2920/4	+2.3	84	12	3	1942
E-2HS-b	A17/1	+6.4	77	0	62	1624
E-2HL-a	HB2032	-0.3	93	17	0	1505
E-4HL-a	Cb2920/4	+6.9	78	21	10	342
E-6HS	Cb2920/4 × Cb2929/1	+4.0	84	18	33	1744
E-7HS	Cb2920/4	+10.4	76	0	44	2308
E-7HL	A17/1	+1.0	90	6	27	120
Emir		**0**	**92**	**23**	**42**	**1634**
G-2HL-a	Cb2920/4	+3.2	71	3	1	1712
G-2HL-b	A17/1	+3.5	75	22	3	528
G-4HL-a	Cb2920/4	+9.5	70	30	7	455
G-4HL-b	Cb2920/4	+3.4	71	14	70	1633
G-5HL	Cb2920/4	+6.3	73	0	38	3212
G-6HS	Cb2920/4	+3.5	74	10	54	2054
Golden Promise		**+1.2**	**75**	**17**	**74**	**1698**
Lsd 5%*		**0.6**	**3**	**10**	**10**	**291**

*Lsd 5%: Least significant difference between the means at the 5% level. Associated degrees of freedom are 111, 111, 111, 53, 49 for the measurements, respectively

establish whether the gene conferring the hypersensitive resistance in E-2HL-a is allelic to the gene for non-hypersensitive resistance in G-2HL-b.

In conclusion, the resistances in these RLs must be due to the introgressed segments of DNA from *H. bulbosum*. Although the complete resistance in some of the RLs may not be durable, the partial resistances in E-1HL and G-2HL-b, which are manifested by long LPs, low IFs and early abortion of infection units, may be long-lived.

Agronomic performance and quality

Yield trial

Agronomic data (year 1) are presented in Table 2. Two RLs, E-2HS-b and G-5HL, were highly susceptible to leaf rust and powdery mildew, respectively. Fungicide applications did not have a significant effect ($P > 0.2$) on ear emergence or straw height, but lodging was increased slightly (by about 3%; $P = 0.09$) with fungicide, perhaps because of greater spike weight.

Ear emergence for all RLs was significantly ($P < 0.05$) later than their parents for all RLs except E-2HL-a. GP was significantly later than Emir but by just over one day. All RLs were shorter strawed than their respective parents except for E-2HL-a.

Yield data (year 1) are presented in Figure 1. GP and its RLs were generally higher yielding than Emir and the Emir RLs. Of the 13 RLs tested, seven equalled or exceeded the yield (4–22% increases) of the barley parent when leaf rust and powdery mildew were present (i.e. without fungicide application). Conversely, when disease was controlled most of the RLs were similar to or lower ($P < 0.05$) in yield than their parents and a yield penalty of up to 36% was associated with the *H. bulbosum* introgression. Yields of all the RLs and the recurrent parents increased when disease was controlled by fungicides

Table 3. Infection frequency (IF in uredia/cm^2), percentage of early aborted (EA) infection units (cessation of growth before at least 6 mycelial branches), and average longitudinal diameter of established colonies of *Puccinia hordei* isolate 1.2.1. in seedling leaves of three recombinant lines and two barley cultivars

Barley line	IF	% EA	% EA without autofluorescent plant cells	Length of established colonies (μm)
Emir	115	10	93	322
Vada	76	12	94	314
E-1HL	65	26	72	228
G-2HL-b	27	51	86	212
E-2HL-a	0	96	10	156

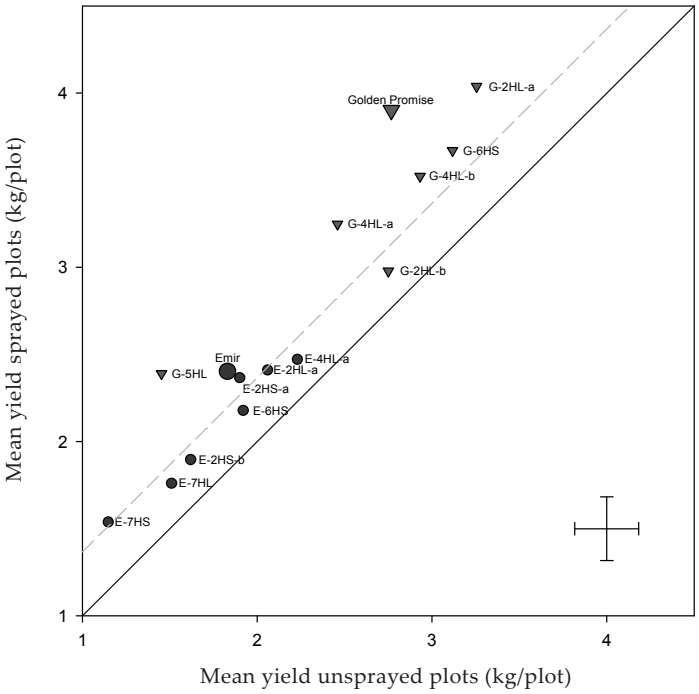

Figure 1. Grain yields (after screening over a 2.4 mm sieve) of recombinant lines derived from Golden Promise (▼) and Emir (●) and their *H. vulgare* parents. Error bars (bottom right corner) are Lsd 5% (df = 111) to compare between means. The solid line indicates where sprayed mean yield equals that for untreated plots and the dotted line is the solid line plus the Lsd 5%. Values outside and to the left of this line were significantly higher yielding in the fungicide treated plots

($P < 0.001$ for the overall effect of the fungicide), and this effect varied among genotypes ($P = 0.041$ for the genotype × fungicide interaction).

There were some inconsistencies in the fungicide effect. Two RLs with low disease incidence (G-2HL-a and G-4HL-a) responded positively to fungicide applications whereas these had only slight effect on the disease susceptible RLs E-2HS-b and E-7HS. Thus, other factors, such as the use of strobilurin fungicides, must explain these anomalies. This was confirmed statistically, since including disease data in the analysis (leaf rust incidence, mildew incidence or AUDPC) did not fully account for the fungicide effect or the significant interaction.

In year 2, E-2HL-a, E-7HS and E-7HL were omitted. There was little lodging or disease and negligible fungicide effect on yield (no strobilurin fungicides were applied). The RL rankings for ear emergence and straw height were similar to year 1. Mean yields were higher than year 1 by 57 and 29% for fungicide treated and untreated plots, respectively. G-5HL again yielded poorly, whereas all the other GP RLs gave similar or lower yields (G-2HL-b and G-4HL-a) than GP. E-2HS-b was the lowest yielding Emir RL, but E-4HL-a significantly outyielded Emir before taking into account screening losses. In both years screening losses (2.4mm sieve size) were most pronounced for RLs with low tgw's (E-4HL-a, G-2HL-b, G-4HL-a and G-5HL).

Table 4. Year 1 means for fungicide-treated plots (2 replicates only) for grain and malting parameters

Line	Tgw	GP	MP	SP	DP	HWE	V	KI
E-2HS-a	38.4	16.3	15.9	3.91	605	67.4	1.58	25.6
E-2HS-b	40.5	18.0	18.0	4.85	670	65.9	1.50	27.7
E-2HL-a	41.3	16.7	16.0	3.95	550	66.9	1.60	25.8
E-4HL-a	34.8	16.9	15.6	3.92	630	67.7	1.59	26.2
E-6HS	38.4	17.6	17.3	4.05	669	65.0	1.45	24.1
E-7HS	40.7	15.0	17.4	4.05	671	65.6	1.50	24.0
E-7HL	39.7	16.7	16.0	4.07	592	66.1	1.53	26.4
Emir	**40.2**	**17.9**	**16.2**	**3.51**	**644**	**64.7**	**1.86**	**22.5**
G-2HL-a	39.2	16.2	14.5	3.82	614	68.4	1.78	27.9
G-2HL-b	35.6	14.4	14.3	3.74	587	68.0	1.69	27.8
G-4HL-a	31.7	15.6	14.5	4.06	658	67.7	1.71	29.7
G-4HL-b	38.1	16.0	14.5	4.20	707	66.3	1.86	30.6
G-5HL	32.3	16.7	16.4	4.28	741	63.8	1.56	27.0
G-6HS	38.3	16.0	14.3	4.70	696	66.2	1.61	35.1
Golden Promise	**39.4**	**14.0**	**12.8**	**3.89**	**637**	**69.5**	**1.93**	**32.9**
Lsd 5%* (df = 27)	2.0	2.8	1.2	0.43	93	2.0	0.15	3.8
Mean change with no fungicide	1.2	0.5	0.3	–0.06	-4	-0.9	0.04	–1.1
Lsd change (df = 27)	0.5	0.7	0.3	0.11	24.0	0.5	0.04	1.0

*Lsd 5%: Least significant difference between the means at the 5% level
Tgw – 1000 grain weight (g); GP – grain protein (%); MP – malt protein (%); SP – soluble protein (%); DP – diastatic power (μmoles maltose equivalent/min/g dry weight); HWE – hot water extract (% dry weight – IOB); V – viscosity (centipoise); KI – Kolbach Index

Malting analysis (Table 4)

Fungicide did not have a significant effect on % germination, diastatic power, grain protein or soluble protein levels ($P > 0.1$). For other traits, the effect of fungicide was similar for all genotypes ($P > 0.2$ for the genotype × fungicide interaction). Germinations were all above 89% but there were some differences in tgw, which ranged from 31.7 g (G-4HL-a) to 41.3 g (E-2HL-a). Tgw from fungicide-treated plots were on average 1.2 g higher than those for untreated plots.

Grain protein contents were generally lower for the RLs derived from the malting cultivar GP than those derived from the feeding variety Emir. Grain and malt protein contents of all genotypes were high, and consequently diastatic power (DP) was also high and extracts low with rather poor modification. The RLs had higher (Emir) or lower (GP) HWEs than their respective parents with some significant differences among the RLs. Unfortunately, it is hard to draw conclusions about the effects of particular introgressions on quality. This is not surprising since the traits contributing to malting quality have complex inheritance (Hayes & Jones 2000). However, tgw's were significantly lower for two of the three 4HL RLs, whereas G-4HL-b, which was derived from G-4HL-a, has a smaller introgression following further recombination and its tgw was similar to GP. The differences in tgw and protein between E-2HS-a and E-2HS-b, and for tgw between G-2HL-a and G-2HL-b, might be due to differences in introgression size as well as the genotype of the *H. bulbosum* parent.

In conclusion, the introduction of new genetic material can be accommodated in the barley genome without too many detrimental effects on

agronomic performance and quality. Hence, useful disease resistant lines can be developed. No firm conclusions can be drawn about the effects of particular introgressions on yield and quality or the introgression size in all RLs.

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References

Anikster Y. (1989): Host specificity versus plurivority in barley leaf rusts and their microcyclic relatives. Mycol. Res., **93**: 175–181.

Hayes P.M., Jones B.L. (2000): Malting quality from a QTL perspective. In: 8th Int. Barley Genetics Symp., Adelaide, Australia, Vol. 1: 99–105.

Hoogkamp T.J.H., Chen W.-Q., Niks R.E. (1998): Specificity of prehaustorial resistance to *Puccinia hordei* and to two inappropriate rust fungi in barley. Phytopathology, **88**: 856-861.

Logue S., Tansing P., Roumeliotis S. (2002): 2000 Season Barley Quality Report. University of Adelaide, Waite Campus, Glen Osmond, South Australia: 20–22.

McNeal F.H., Konzak C.F., Smith E.P., Tate W.S., Russell T.S. (1971). In: A uniform system for recording and processing cereal research data. USDA Agricultural Research Service, Washington: 34–121.

Pickering R., Johnston P. (2004): Recent progress in barley improvement using wild species of *Hordeum*. Cytogenetic and Genome Research (in press).

Tosa Y. (1989): Evidence on wheat for gene-for-gene relationship between formae speciales of *Erysiphe graminis* and genera of gramineous plants. Genome, **32**: 918–924.

Xu J., Snape J.W. (1989): The resistance of *Hordeum bulbosum* and its hybrids with *H. vulgare* to common fungal pathogens. Euphytica, **41**: 273–276.

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Abstrakt

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V druhém příspěvku o užití sekundárních a terciárních genofondů ve šlechtění ječmene podáváme charakteristiku rekombinantních linií (RL) odolných ke rzi ječné, odvozených z křížení *Hordeum vulgare* × *H. bulbosum*. Rzí ječnou jsme inokulovali dvanáct RL a pozorovali jsme rané fáze vývoje choroby. Některé RL jevily úplnou rezistenci k patogenu, ale jiné měly vysokou hladinu částečné rezistence, která může být trvanlivá. Některé z těchto RL jsme testovali ve výnosových pokusech, abychom zjistili vlivy introgrese chromatinu z *H. bulbosum* na výnos a kvalitu. Docházíme k závěru, že se neprojevily větší nepříznivé vlivy, které by nemohly být překonány běžnými šlechtitelskými technikami.

Klíčová slova: ječmen; *Hordeum bulbosum*; rez ječná; *Puccinia hordei*; introgrese; výnos sladařská jakost

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