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Inheritance and geographical distribution of phenol reaction-less varieties of barley

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Summary

The reaction of spikes and grains of barley to phenol was investigated using 8,849 cultivated and 349 wild accessions collected from the world. The pericarp and hull of the grain were stained dark brown by a 1% phenol solution and the reaction of awn was sharpest. Phenol reaction was controlled by a dominant gene, named *Phr* (phenol reaction) which was located on chromosome 2. All the wild strains of various *Hordeum* species showed a positive reaction to phenol indicating it was the prototype of the trait. Only 51 accessions of cultivated barley showed negative reaction to phenol. They were distributed along the so-called 'Silk-road' and the type of variety was limited suggesting that it was a rather new mutation which occurred in the Middle East. Synteny of the chromosome region involving the phenol reaction gene in some gramineous plants was discussed.

Introduction

The inheritance and geographical distribution of the phenol reaction in rice (*Oryza sativa* L.) have been investigated intensively. The hull and pericarp of some rice varieties are stained dark brown with phenol solution. This reaction is controlled by a dominant gene, *Ph* (phenol staining) distributed mainly in *Indica* rice, and it is a good discriminator distinguishing *Indica* from *Japonica* subspecies of rice (Oka, 1953; Kuriyama & Kudo, 1967; Maekawa, 1984).

Enzymatic and molecular genetic studies on phenol oxidase are reported on peach (Wong et al., 1971), mango (Robinson et al., 1993), potato (Hunt et al., 1993) and tomato (Shahar et al., 1992; Newman et al., 1993). On the other hand, in the case of barley (*Hordeum vulgare* L.), varietal variation for the coloring of grains with phenol has been investigated (Takasugi, 1937), but all the varieties tested were stained, more or less, and no inheritance study of the trait has been reported.

Thus, we aimed to find out phenol reaction-less variants of barley and to examine the inheritance and geographical distribution of the variants.

Materials and methods

Varietal variation

A total of 8,849 cultivated barley accessions composed of 7,519 landraces and improved varieties, 557 experimental lines and 773 mutants, and 349 strains of wild relatives composed of *H. agriocrithon* (24 strains), *H. arizonicum* (1), *H. brachyanthrum* (1), *H. bulbosum* (33), *H. dawoense* (9), *H. geniculatum* (11), *H. glaucum* (28), *H. gussoneanum* (2), *H. ischnatherum* (18), *H. laguncliforme* (3), *H. leporinum* (25), *H. marinum* (7), *H. murinum* (22), *H. paradoxon* (9), *H. proskowetzii* (35), *H. spontaneum* (117), *H. stebbinsii* (1) and *H. violaceum* (3) available from the Barley Germplasm Center, Okayama University were tested for the phenol reaction.

After optimization in a preliminary experiment, grains or spikes were treated with a 1% phenol solution, adjusted to pH6, for 2 or 3 days at room temperature to check the reaction. Each spike was soaked in phenol solution in a test tube and grains were put in a petri dish on the filter paper moistened with phenol solution.

Information about agro-morphological and isozyme characters of the accessions were available from the

data base established by the Barley Germplasm Center.

Inheritance

In the first experiment, three accessions with negative phenol reaction, OUC663, OUI677 and OUI750 were crossed with 8 linkage testers with positive phenol reaction, OUL001, 004, 017, 053, 056, 085, 095 and 131, including 16 marker genes belonging to the chromosomes 1 to 7. A total of 18 crosses were thus obtained. About 200 F_2 plants were raised from each cross combination. The marker characters were recorded at the time of full expression, and spikes were harvested to check phenol reaction. Recombination values between the genes were estimated by the productive ratio method (Fisher & Balmukand, 1928) in the F_2 generation.

Because the gene for phenol reaction linked closely with *mt4* (mottled leaf-4), no recessive recombinant was obtained in the first experiment. Thus more than 2,000 F_2 plants each of OUC663/OUL085 and OUI750/OUL085 crosses were tested in the second experiment for estimating the recombination value between the phenol reaction gene and *mt4*.

In the third experiment, about 150 plants each of four F_2 populations (OUT635/OUI633, OUI346/-OUI750, OUI677/OUI750 and OUI750/OUC663) crossed among six accessions with negative phenol reaction were tested for the reaction to check the genic composition of the accessions.

Results and discussion

Varietal variation

The pericarp and hull of the grain were stained by phenol solution and the reaction of awn was the sharpest. Neither awn nor hull of sterile grain was stained by phenol solution indicating the signal of phenol reaction was derived from the fertile endosperm or embryo.

Positive reaction to phenol may be the prototype of the trait, because all the wild strains examined showed a positive reaction.

In most of the cultivated barley, awns or grains were clearly stained by the phenol solution, but in some of the accessions the reaction was not clear. Those accessions were examined repeatedly and finally 51 accessions showing negative reaction to phenol were detected (Table 1).

Table 1. Number of barley accessions tested and number of accessions with negative reaction to phenol

Region or group	No. acc. tested		No. negative acc.
Japan	781	(98)*	0
Korea	493	(0)	1
China	650	(71)	12
Nepal	879	(2)	1
S.W. Asia[1)]	557	(78)	32
Turkey	836	(518)	3
Europe	538	(338)	2
N. Africa[2)]	280	(3)	0
Ethiopia	975	(232)	0
US & others[3)]	149	(46)	0
Modern vars.[4)]	1381	(529)	0
Exp. lines[5)]	557	(135)	0
Mutants	773	(349)	0
Total	8849	(2399)	51

Number of accessions in the parentheses indicate two-rowed type.
[1)]: India to Cyprus. [2)]: Except Ethiopia. [3)]: New continent. [4)]: Mostly from Japan, Europe & North America. [5)]: Linkage testers, isogenic lines,*etc.*

Among the cultivated barley accessions about a quarter was two-rowed. But two-rowed varieties were very few in the east Asia, because originally the two-rowed type was not distributed east of Nepal (Takahashi, 1955). Very few accessions were polymorphic or mixture for this trait, and they were omitted from Table 1, because they might be risen by outcrossing. The accessions with negative reaction were mainly distributed along the so-called 'Silk-road', an old trade route constructed in the central Asia before Christ, which had been an important route to link Europe and Asia until the sea route between the regions was established. One accession with negative reaction was found in 493 Korean landraces and two accessions were found in 24 accessions from Spain, but none was in Europe, except Spain, New continent and Africa (Figure 1).

The accessions with negative reaction to phenol were most frequent in Iran (12/51), followed by Afghanistan (12/100) and Pakistan (7/134). In China five accessions with negative reaction were found in Xinjiang, the northwest part, four accessions in the east part Zhejiang, one each from south-central Yongnan and north-central Neimenggu, and one of 54 local varieties from Tibet highland showed a negative reaction to phenol. Because Ethiopia is a secondary center of diversity of barley (Vavilov, 1926), as many as 975

Figure 1. Geographical distribution of the phenol reaction-less varieties of barley.

landraces collected from the region were tested, but all of the accessions showed a positive reaction.

Both of two accessions from Spain were common for six-rowed, covered, lax spike, blue aleurone, short haired rachilla, spring type and *Ca*, *Fr*, *At* for esterase isozyme *Est-1*, *-2* and *-4* loci indicating these two may be closely related. The three accessions from Turkey were six-rowed, covered, lax spike, long haired rachilla, white aleurone, winter type, bt_2 or Occidental (W) type for brittle rachis (Takahashi, 1955) and two of them had the same isozyme genotype for three loci mentioned above, again indicating these accessions may be closely related.

These 51 accessions were all six-rowed, and with long awn except one Chinese accession with hooded lemma. However, the gene for hooded lemma (*K*) is epistatic to long awn. Many of them were with covered grain (39/51), lax spike (47/51) and long haired rachilla (44/51). Accessions with blue aleurone (14/51 or 27%) were rather frequent in comparison with the proportion of blue-aleurone accessions on the whole (22%). Frequency of *bt* gene for the Oriental type (E) of brittle rachis (Takahashi, 1955) was 75%. The proportion of spring and winter types was similar. Esterase isozyme pattern for *Est-1*, *-2* and *-4* loci was determined in 41 accessions and 28 of them showed only three types of isozyme pattern; *Al*, *Fr*, *At* (13 accessions), *Ca*, *Fr*, *At* (8) and *Pr*, *Fr*, *At* (7).

This limited distribution and type of the accessions with negative phenol reaction indicates that it is a rather new mutant which occurred in the Middle East in a plant with six-rowed, covered, lax spike, long haired rachilla, long awn, Oriental type for the brittle rachis and *Al*, *Fr*, *At* for esterase isozyme. The phenol reaction-less varieties with other traits such as naked, dense spike, short haired rachilla may be risen by outcrossing. The variant might be expanded in distribution by migration and outcrossing.

Table 2. Segregation of phenol reaction in the F_2 generation (total of 18 pop.)

	Positive	Negative	Total	$\chi^2(3:1)$	p
Obs.	2779	925	3704	0.001	>0.9
Exp.	2778	926			

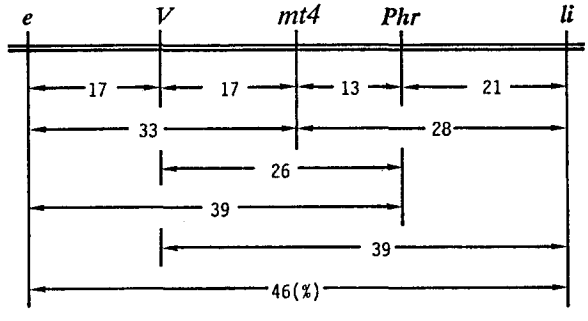

Figure 2. Linkage relation of *Phr* and other marker genes on chromosome 2.

Table 3. Joint segregation of *Phr* and marker genes on chromosome 2 in C663/L085 and I750/L085 F_2 populations

A : B	A-B-	A-bb	aaB-	aabb	χ_L^2	RCV(%)
[C663/L085 n=2361]						
Phr : *mt4*	1229	520	601	11	194.89**	14.3 ± 2.0
Phr : *V*	1465	284	262	350	417.08**	25.9 ± 1.1
Phr : *li*	1210	539	582	30	166.86**	22.5 ± 1.9
Phr : *e*	1297	452	522	90	31.26**	40.1 ± 1.7
V : *li*	1250	477	542	92	45.11**	38.6 ± 1.7
V : *e*	1202	525	617	17	201.85**	17.1 ± 2.0
mt4 : *V*	1212	618	515	16	196.43**	16.8 ± 2.0
mt4 : *li*	1534	296	258	273	255.27**	28.3 ± 1.1
mt4 : *e*	1508	322	311	220	118.83**	33.9 ± 1.2
li : *e*	1405	387	414	155	7.42**	45.6 ± 1.5
[I750/L085 n=2147]						
Phr : *mt4*	1131	467	542	7	173.88**	12.3 ± 2.1
Phr : *V*	1350	248	250	299	335.73**	26.5 ± 1.1
Phr : *li*	1107	491	529	20	162.68**	19.7 ± 2.1
Phr : *e*	1156	442	472	77	41.27**	38.1 ± 1.8
V : *li*	1171	429	465	82	30.92**	39.7 ± 1.8
V : *e*	1097	503	531	16	179.23**	17.4 ± 2.1
mt4 : *V*	1141	532	459	15	149.04**	18.0 ± 2.1
mt4 : *li*	1416	257	220	254	266.91**	26.7 ± 1.1
mt4 : *e*	1379	294	249	225	163.05**	31.2 ± 1.2
li : *e*	1258	378	370	141	4.14*	46.6 ± 1.6

χ_L^2: Chi-square value for linkage.
* and **: Significant at the 5% and 1% levels, respectively.

Inheritance

A total of 3,704 F_2 plants from 18 crosses segregated into the 3 to 1 ratio (Table 2). Thus, the phenol reaction in barley is controlled by a single dominant gene. In the case of rice, the gene for phenol reaction is named *Ph*, however the authors propose a gene symbol *Phr* for preventing possible confusion with *Ph* (pairing homoeologus) gene in wheat, where a similar locus may be found in the genus *Hordeum*. As mentioned above, the signal of phenol reaction derived from the fertile endosperm of embryo, however metaxenia phenomenon was not observed in the crossed seeds nor grains on the heterozygous plants for the *Phr* gene.

A total of 581 plants from four F_2 populations crossed between six phenol reaction-less accessions from China (OUC663), Pakistan (OUI346 and OUI677), Afghanistan (OUI750), Iran (OUI633) and Turkey (OUT635) showed negative reaction to phenol without exception, and all of F_2 plants derived from about 30 crosses between positive parents showed positive reaction to phenol (data were not shown). These indicate that phenol reaction is controlled by a single gene.

Analyzing the joint segregation of *Phr* and marker genes, *Phr* was independent of marker genes belonging to chromosome 1 and 3 to 7: namely, *n* (naked caryopsis) and *br* (brachytic plant) on chromosome 1, *als* (absent lower laterals), *uz* (uzu or semi-brachytic growth) and *al* (albino lemma) on chromosome 3, *K* (hooded lemma), *gl-3* (glossy seedling-3) and *Bl* (blue aleurone) on chromosome 4, *trd* (third outer glume) and *B* (black lemma and pericarp) on chromosome 5, *o* (orange lemma) on chromosome 6 and *s* (short haired rachilla) on chromosome 7.

On the other hand, the *Phr* was linked with four marker genes located on the chromosome 2: namely, *e* (wide outer glume), *V* (two-rowed), *mt4* (mottled leaf-4) and *li* (ligule-less). However, because *Phr* and *mt4* closely linked in the repulsion phase, no double recessive recombinant was obtained. Therefore in the second experiment, 2,361 F_2 plants of OUC663/OUL085 and 2,147 F_2 plants of OUI750/OUL085 were examined.

Recombination values among *Phr* and four marker genes estimated from the joint segregation in the F_2 populations are shown in Table 3. Recombination values estimated in OUC663/OUL085 population coincided with those in OUI750/OUL085.

Weighted mean of the recombination values are shown in Figure 2. The *Phr* is inserted between *mt4* and *li*.

In addition, it will be interesting to point that the genes for phenol reaction of grain are located on chromosome 2A and 2D of wheat and 2R in rye (Wrigley & McIntosh, 1975) which are homoeologous with barley chromosome 2 (Devos et al., 1993) and *Ph* (phenol staining) gene in the rice plant is linked with *lg* (ligule-less) in 21 cM distance (Kinoshita, 1984) on rice chromosome 4 which shows a homology with group-2 chromosome of wheat (Kurata et al., 1994).

Thus *Phr*/*phr* gene is an useful marker on chromosome 2 of the tribe Triticeae. And physiological studies on the phenol reaction in relation with biotic stress such as insects and diseases or with grain quality became possible using newly found phenol reaction-less variants.

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