BIOSYSTEMATIC STUDIES ON THE *HORDEUM MURINUM* AGGREGATE

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INTRODUCTION

The genus *Hordeum* is recognized to comprise more than 30 species. Åberg (1940) classified the genus into four sections, namely, *Stenostachys* Nevski, *Campestria* Ands., *Bulbohordeum* Nevski, and *Cerealia* Ands. Among these sections, the plants of *Stenostachys* and *Bulbohordeum* are perennial, while those of *Campestria* and *Cerealia* annual. Barley is only one cultivated species, *Hordeum vulgare* L. *s. l.*, in the genus, and belongs to the section of *Cerealia*.

In the section of *Campestria*, there are a group of species called *Hordeum murinum* aggregate. According to Bowden (1962), they are easily distinguishable from other species in the same section by having leaf-blades with prominent auricles at the bases, flat and ciliate glumes, and large inflated lateral spikelets. The aggregate consists of diploid, tetraploid and hexaploid plants with the basic number of chromosomes (n=x=7). However, since they are very similar in their features, the classification of species is still complicated and undefinite. For instance, Bowden (1962) classified the aggregate into three species, *H. glaucum* Steudel (=*H. stebbinsii* Covas) (2x), H. murinum L. (4x) and *H. leporinum* Link (4x and 6x). Among them, *H. murinum* is characterized by subsessile, rarely sessile central spikelets, whereas two other species, *H. glaucum* and *H. leporinum*, have pedicel ones. Meanwhile, Booth and Richards (1976) recognized that the aggregate consisted of *H. leporinum* ssp. *glaucum* (2x), *H. murinum* (4x) and *H. leporinum* ssp. *leporinum* (4x and 6x). Baum and Bailey (1984a) classified the aggregate into *H. murinum* (*sensu lato*) and *H. glaucum*, but later they (1984b) concluded that the aggregate comprised *H. glaucum, H. murinum* (*sensu stricto*), and *H. leporinum*.

From the viewpoint of biosystematics, the present author aimed to reconsider the taxonomic and evolutionary problems of the *H. murinum* aggregate, after examining morphological and biochemical characteristics, using many strains of the aggregate preserved in the Barley Germplasm Center of Okayama University.

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MATERIALS AND MOTHODS

Sixty-two strains of the *H. murinum* aggregate collected from southwestern Asia, the Mediterranian region, and also from some other regions of the world, were used as the materials. The plant were grown in a glasshouse, and their chromosome numbers were counted in mitotic cells of root-tips by the usual smear method. To find a useful key character for species identification at the seedling stage, pigmentation and hairiness of the lower leaf sheath, and also hairiness of leaf blade were examined. After maturity, lengths of pedicel, palea and empty glume (including awn) taken each from the central and lateral spikelets in the middle portion of 10 spikes per strain were measured. Awn length was variable under the growing conditions, and the boundary between lemma and awn was very difficult to determine. Therefore, lengths of these characters were excluded for measuring.

For electrophoresis of isozymes, the plants were grown under a 12 hr illumination in a growth cabinet kept at 18°C. Electrophoresis of esterase isozymes was conducted by the horizontal starch gel method described by Hvid and Nielsen (1977), using the crude extract from the first leaf blade of a 10 day-old seedling per strain. For the electrophoreses of peroxidase and aspartate aminotransferase isozymes, 0.2g of the first leaves of the seedlings from each strain were crushed and homogenized with an extraction buffer (0.2ml of 0.0625M Tris-HCl, pH6.8, containing 18% glycerin and 0.02% polyvinylpyrrolidone K30 (PVP), and 20μl of 10% melcaptoethanol). The homogenate was centrifuged at 13,000 rpm for 30 min at 5 °C, and the supernatant was applied to electrophoresis, using a vertical polyacrylamide slab gel, 15×12×0.2cm. The polyacrylamide solution was made, after Davis (1964) with some modifications. The starch and polyacrylamide slab gels were stained by procedures listed by Nielsen and Johansen (1986).

EXPERIMENTAL RESULTS

Classification of the strains based on chromosome number

According to the somatic chromosome numbers, these strains could

TABLE 1 Origin of diploid and tetraploid strains of the *H. murinum* aggregate examined

	Number of strains examined						
	Japan	S. W. Asia	Caucasus	Europe	Egypt	USA	Total
Diploid		32	1		1	1	35
Tetraploid	3	6	1	14		3	27
Total	3	38	2	14	1	4	62

TABLE 2 Characteristics of spikelets in the diploid (2x) and tetraploid (4x) strains of the *H. murinum* aggregate

Character	Mean		Range		C. V. (%)	
	2x	4x	2x	4x	2x	4x
Central spikelets (mm)						
Pedicel length	1.08	1.39	0.6- 1.4	0.7- 2.0	21.9	25.4
Palea length	7.86	9.97	7.0- 9.1	8.0-12.0	8.2	9.1
Glume length	23.47	24.58	19.7-30.9	20.6-29.9	11.1	12.2
Lateral spikelets (mm)						
Pedicel length	1.64	1.84	1.2- 2.0	1.6- 2.2	12.4	10.8
Palea length	10.85	12.86	8.6-12.9	9.5-14.4	8.3	10.0
Glume length	27.20	26.17	19.1-33.1	19.5-34.3	11.6	19.4

be classified into two groups, diploid (2n=14) and tetraploid (2n=28) strains. No hexaploid (2n=42) strain was included in the present materials.

As seen in Table 1, all the strains from Japan and Europe proved to be tetraploid, with no exception. On the other hand, most of those from southwestern Asia, including Afghanistan, Iran, Iraq, Lebanon and Jordan, were found to be diploid species.

Comparison of spikelet characteristics between the diploid and tetraploid strains

Several spikelet characteristics have been used in general as key characters for species identification within the genus *Hordeum* (for instance, Nevski 1934, Bowden 1962, Booth and Richards 1976, Bothmer and Jacobsen 1985).

To distinguish between the diploid and tetraploid strains, lengths of pedicel, palea and glume in the central and lateral spikelets were measured. As shown in Table 2, these organs were somewhat smaller in the diploid strains than in the tetraploids, except for glume length in the lateral spikelets. However, the range of variation in the diploid and tetraploid strains overlapped for all of the characters, which makes it impossible to classify distinctly the strains into the two groups of diploid and tetraploid, as to the length of spikelet characters. It should be noted that all the pedicels of the central spikelets were elongated to some extent (0.6-2.0mm) in both diploid and tetraploid strains, to say nothing of those of the laterals.

Principal component analysis was made with these data for spikelet characters. Table 3 shows the eigen vectors and eigen values for each character. The eigen vectors of the first principal component were all positive, and those for palea lengths in the central and lateral spikelets

TABLE 3 Eigen vectors and eigen values of principal component analysis for six characters of spikelets in the *H. murinum* aggregate

Character	Eigen vectors of principal component		
	I	II	III
Central spikelets			
Pedicel length	0.310	−0.516	0.242
Palea length	0.496	−0.044	−0.542
Glume length	0.406	0.482	0.144
Lateral spikelets			
Pedicel length	0.361	−0.383	0.592
Palea length	0.530	−0.082	−0.357
Glume length	0.285	0.589	0.387
Eigen value	2.881	1.751	0.569
Contribution (%)	48.0	29.2	9.5

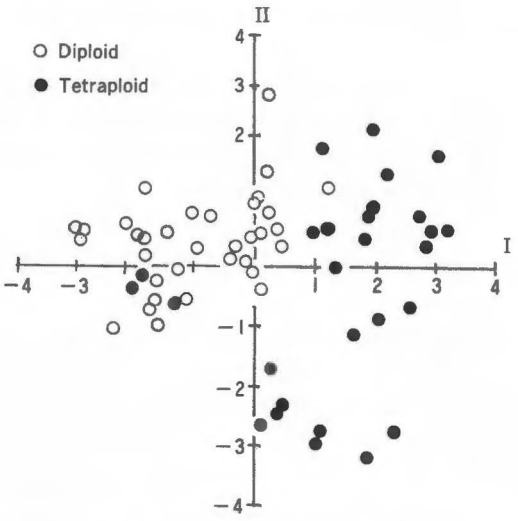

FIG. 1 Scattered diagram of the first and second scores of the diploid and tetraploid strains of *H. murinum* aggregate.

were larger. The eigen vectors of the second component were large for pedicel and glume lengths in the central and lateral spikelets, but the formers were negative and the latters were positive. The contribution of the eigen values for the first and second principal components was 48.0 and 29.2%, respectively, which indicates that more than 70% of all the variation of these characters could be explained by the two principal components, I and II. Furthermore, the scores of these two principal components were calculated for each strain, and Fig. 1 shows the scattered diagram of scores obtained. Most of the diploid strains clustered on the left side of the figure, while all but three tetraploid strains were on

the right side with a considerable range for the second component (vertically). Among the tetraploid strains, the three exceptional strains were of Iranian and two of Hungarian origin. Italian strains were scattered on the lower right side. The scattered diagram indicates that the former three exceptional strains had smaller spikelets, and the Italian strains were characterized by longer pedicels and shorter glumes. From these results, it may be possible to distinguish roughly between the diploid and tetraploid strains by the principal component analysis of these spike characteristics, with a few exceptions.

Seedling characteristics of the diploid and tetraploid strains

At the seedling stage, it is desirable to distinguish between the diploid and tetraploid strains from their appearances. As the characteristics of seedlings, anthocyanin pigmentation and hairiness of leaf sheath, and hairiness of leaf blade were examined. Table 4 shows that all of the diploid strains were characterized by anthocyanin pigmentation and hairlessness of leaf sheath, and 83% of them had hairs on the leaf blade, Meanwhile, among the tetraploid strains, about one half of them had colorless leaf sheath, and all the strains had hairs on leaf sheath and leaf blade.

From these results, it is apparent that the classification between the diploid and tetraploid strains at the seedling stage is possible by examining the presence or absence of hairs on leaf sheath; the diploid strains are hairy, while the tetraploids are glabrous.

Isozyme banding patterns of the diploid and tetraploid strains

Comparison of isozyme banding patterns is sometimes effective to identify the barley varieties (Nielsen and Johansen 1986, Linde-Laursen *et al.* 1987, and others), and also to classify the species of *Hordeum* (Jørgensen 1986). The present author examined isozyme banding patterns of the strains for finding the possiblity of classification into the diploid and tetraploid strains by means of electrophoresis.

Among the strains, differences in banding patterns of esterase and

TABLE 4 Frequencies of strains characterized by pigmentation and hairiness of leaf sheath, and hairiness of leaf blade at the seedling stage in the diploid and tetraploid strains of the *H. murinum* aggregate

	Pigmentation of leaf sheath	Hairiness of leaf sheath	Hairiness of leaf blade
Diploid (N = 35)	100%	0%	83%
Tetraploid (N = 27)	52	100	100

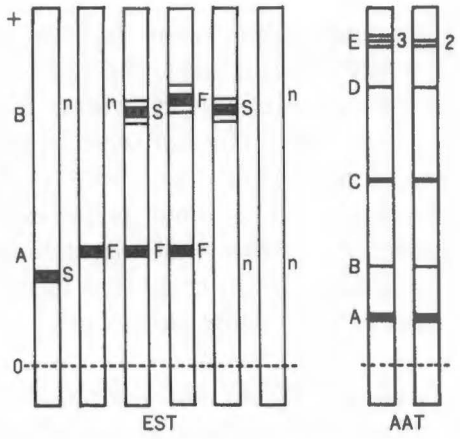

FIG. 2 Banding patterns of esterase (EST) and aspartate aminotransferase (AAT) in the diploid and tetraploid strains of *H. murinum* aggregate.

TABLE. 5 Frequencies of strains showing different banding patterns of esterase and peroxidase isozymes in the diploid and tetraploid strains of *H. murinum* aggregate

	Esterase						Peroxidase	
	A zone			B zone			E zone	
	Fast	Slow	Null	Fast	Slow	Null	2 bands	3 bands
Diploid (N = 35)	77%	20%	3%	0%	0%	100%	100%	0%
Tetraploid (N = 27)	59	7	33	7	85	7	0	100

peroxidase isozymes were observed, but there was no variant for aspartate aminotransferase isozymes. Clearly stained bands of esterase isozymes were detected at two zones of the anodal side, which were tentatively named zones A and B. Six types of variants were easily detected by different migrating distances of the bands, which were composed of the combinations of fast, slow and null bands at the two zones, as illustrated on the left side of Fig. 2. Meanwhile, peroxidase isozyme bands were observed at five zones of the anodal side. Among them, variants of banding patterns were found at zone E which was the fast migrating one. At the zone, the strains could be divided into two groups of which they had two and three bands, respectively, as shown on the right side of Fig. 2.

Table 5 shows the frequencies of the diploid and tetraploid strains having these different isozyme patterns. Every diploid strain had a null band at zone B of esterase isozymes and two bands at zone E of peroxidase, while the esterase isozyme banding patterns at zone A varied among the strains. On the other hand, all of the tetraploid strains were characterized by three bands at zone E of peroxidase isozymes. At

both zones A and B of esterase isozymes, however, fast, slow and null bands were observed among the tetraploid strains, respectively. These results indicate that the distinction between the diploid and tetraploid strains could be made by examining the number of bands at zone E of the peroxidase isozymes.

DISCUSSION

Booth and Richards (1976) classified the *H. murinum* aggregate into two major taxa by the multivariate analysis for several spikelet characters. They were recognized as *H. murinum* (4x) and *H. leporinum* which comprised ssp. *leporinum* (4x and 6x) and ssp. *glaucum* (2x), indicating the trend to have extreme *leporinum* values in characters such as pedicel length of central floret, ratio of glume widths of central and lateral florets, rachilla hair length. However, the present observation at the seedling stage revealed that diploid and tetraploid strains were easily distinguishable each other by the presence or absence of hairs on the lower leaf sheath; the diploid strains were hairy, while the tetraploids are glabrous.

Electrophoresis of isozymes and proteins is a useful tool for taxonomic and evolutionary studies. Booth and Richards (1978) showed that diploids, tetraploids and hexaploids of the *H. murinum* aggregate could be distiguished by analysis of seed protein electrophoresis. Giles (1984) made an electrophoretic survey of 58 populations of *H. murinum* collected from Britain, Europe and the Mediterranean region, by the horizontal starch gel system. The results indicated that little genetic differentiation in enzyme characters had occurred between populations and that most populations tended to be monomorphic. Of the six enzyme systems, the esterase pattern was the most variable. Sixteen banding patterns of esterase isozymes were encountered, but 14 of them occurred rather rarely and unusually. One of the two major patterns indicated both slow bands at zones A and B, and the other had no band at zone A and slow bands at zone B. Furthermore, the former pattern was observed frequently, especially in North European populations collected in Britain and Belgium, and the latter pattern was abundant in the Mediterranean region. The former pattern could not be observed among the present tetraploids. While, the latter pattern was frequently found in many strains, especially in strains collected from the Mediterranean region and Hungary, as mentioned by Giles (1984).

The peroxidase isozyme patterns of an anodal migrating system were uniform in the *murinum* populations of Giles (1984), as is the same as in the present materials. However, the present tetraploid strains had in common 3 bands at zone E, although Giles indicated that only 2 bands

were detected at the same zone. The difference in the number of bands at zone E might suggest that the polyacrylamide gel system was more recommendable for separation of bands than the starch gel system, since the number of bands at zone E was a useful key character for the classification of the diploid and tetraploid strains.

As to the origin of polyploidy in the *H. murinum* aggregate, the tetraploid and hexaploid strains were thought to be allopolyploidy. From the karyotype study and the pairing behaviour of chromosomes in triploid and pentaploid hybrids of the *H. murinum* aggregate, Rajhathy and Morrison (1962) suggested that *H. murinum* (4x, GGMM genome) was produced by the hybridization between *H. glaucum* (2x GG gemone) and an unidentified diploid (MM gemone), and that the hexaploid *murinum* (GGMMHH gemone) was derived from the hybridization between *H. murinum* (4x) and the other unidentified diploid with HH gemone. Similar relationships of the *H. murinum* aggregate were found in the C-banded karyotype of the chromosomes (Vosa 1976) and banding patterns of seed proteins (Booth and Richards 1978). The present results also indicate that the tetraploid strains were not similar to the diploids in several characteristics. This suggests that the tetraploids were not the autopolyploidy of the diploids, but the allopolyploidy derived from the hybridization between the diploid strain and other unknown diploid. From the comparison of the characteristics between the present diploid and tetraploid strains shown in Tables 4 and 5, the unknown diploid parent might have the following characteristics; hairiness of the lower leaf sheath, fast or slow bands at zone B of esterase isozymes, and at least the third band at zone E of peroxidase isozymes. Because, these characteristics could not be found in the diploid strains.

Finally, the present materials of the *H. murinum* aggregate were classified into two groups, the diploid and tetraploid strains. The diploid strains are identified as *H. glaucum* Steudel, while the tetraploid strains might be recognized as *H. leporinum* Link because these tetraploid strains have long pedicels in the central spikelets of 0.7-2.0mm. According to the taxonomy of Bowden (1962), *H. leporinum* has long pedicels of central spikelets, ranging (0.8)−1.0−2.0mm, whereas the central spikelets of *H. murinum* are usually subsessile of rarely sessiled, 0.2−0.5−0.8mm long pedicels.

SUMMARY

Morphological and biochemical variations were examined, using 62 strains of the *H. murinum* aggregate. They were classified into two groups, 35 diploid strains (2n=14) and 27 tetraploid strains (2n=28), after examining their chromosome numbers. However, it was difficult to

distinguish the two groups by comparing morphological characteristics of spikelets, lengths of pedicel, palea and glume in the central and lateral spikelets because their range of variation for each character overlapped, although the diploid strains were generally smaller than the tetraploids for these characters. Among seedling characteristics examined, the hairiness at the lower leaf sheath was thought to be a useful key character for classification of the two groups; the diploid strains had hairs and the tetraploids no hair. An electrophoretic survey also indicated that it was possible to distinguish between the two groups by different banding patterns of peroxidase isozymes. Furthermore, some of the esterase and peroxidase isozyme bands in the tetraploid strains were the same as those of the diploids, and the others were specific to the tetraploids. These results suggested that the tetraploid species was an allopolyploid derived from a hybridization between the diploid and other unknown diploid species. Finally, from the fact that all the diploid and tetraploid strains had long pedicels in the central spikelets, the diploid and tetraploid strains were identified as *H. glaucum* Steudel and *H. leporinum* Link, respectivery.

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