Response to GA and Variation of the Culm Length in Doubled Haploid Lines of Wheat

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Abstract: An F₁ hybrid with the semi dwarf genes, Rht₁ and Rht₂, was crossed with Hordeum bulbosum and maize (teosinte) (bulbosum method and maize method) to produce doubled haploid (DH) lines, and 102 seedlings of each DH line produced by the two methods were treated with GA. The sensitivity to GA was shown by the GA index (GAI) which is (length of the first leaf sheath (LS) in GA-treated seedlings / length of LS in GA-untreated seedlings) × 100. The scatter diagram of GAI plotted against culm length was divided into three groups, low, medium and high GAI groups. The segregation ratio of these gametophytic phenotypes was close to the expected ratio: 1:2:1 = Rht₁Rht₂: (Rht₁rht₂+rht₁Rht₂): rht₁rht₂, in both DH lines produced by the two methods. The frequency distribution of culm length in DH₃, DH₄, DH₅, and DH₆ populations from DH produced by the bulbosum method showed two peaks, though that in F₀, F₁, F₂, and F₃ populations from the same F₁ hybrid (non-selective population) showed a continual normal distribution. The ratio of individuals with a shorter culm length to those with a longer culm length in DH lines was close to the expected ratio: 1:3 [Rht₁Rht₂: (Rht₁rht₂+rht₁Rht₂+rht₁rht₂)]. The frequency distribution of culm length in F₃ was significantly different from that in F₀-F₂ populations, indicating an unintentional selection during the generation of the selfed hybrid, but the frequency of short culm relative to long culm individuals was lower in F₃, F₄, and F₅ populations than in DH lines. These findings suggest the usefulness of selecting the medium culm-length lines in the DH lines produced by the bulbosum or maize method in addition to examining the GA sensitivity (GAI).

Key words: Culm length, Doubled haploid, Gibberellic acid response, Hordeum bulbosum, Intergeneric cross, Triticum aestivum, Zea mays.

In wheat, three alternative doubled-haploid breeding systems are now available, anther culture (Buyser et al., 1981; Winzeler et al., 1987), crossing with Hordeum bulbosum (Sitch and Snape, 1986; Snape et al., 1988) and crossing with maize (Suenga and Nakajima, 1989; Ushiyama et al., 1991). Ushiyama et al. (2006, 2007) developed two methods for producing doubled haploid (DH) of wheat effectively by using wheat × Hordeum bulbosum (bulbosum method) and wheat × maize crossing (maize method). The maize method may be less genotype dependent than the bulbosum method (Laurie and Reymondie, 1991). Whether or not a genetic variation occurs in the progeny of the DH lines, however, has not been tested. For breeding programs, it is important to select medium culm-length lines having a culm length between 70 and 90 cm for lodging resistance and mechanical harvesting. Yamada (1989, 1990) developed a technique for selecting a semi dwarf gene of wheat based on the response to gibberellic acid (GA) of the first leaf sheath (LS), and identified the semi dwarf line in the Japanese modern wheat varieties by this technique. In Japanese wheat breeding programs aimed to select lines with a high-yielding ability, it is assumed that breeders have unconsciously selected the genotypes carrying semi dwarf gene Rht₁ or Rht₂ (Yamada, 1989).

In this study, we investigated the culm length of DH lines produced by the bulbosum and maize methods and their responses to GA. We examined the possibility of selecting a line with a medium culm length based on the response to GA. In addition, we compared progenies of DH lines produced by the bulbosum method with those obtained by the conventional bulk method.

Materials and Methods

The Rht tester lines shown in Table 1 were kindly supplied by Dr. T. Yamada, National Agriculture Research Center. To determine the GA-insensitivity gene (dwarf gene) in wheat cultivar Tozan 18, we crossed Tozan 18 with each Rht tester line, and selfed the F₁ hybrid to produce F₂ populations. Winter wheat Tozan 18, a landrace bred at the Nagano Prefecture Agricultural Experiment Station, has high yielding, freezing hardiness, snow heaving resistance and lodging resistance but no resistance to pre-harvest sprouting.
Genetic improvement of wheat Tozan18 has been an integral part of Nagano Prefecture Agricultural Experiment Station’s wheat improvement program. About 400 seedlings of each F2 population, 12 seedlings of the F1 hybrid and 12 seedlings of the parental lines were investigated for GA-insensitivity following the method of Yamada (1989). Plants were grown at a temperature of 20/15ºC (day/night) under 16 hour day length irrigating water containing 10 ppm GA for 21 days and the length of the first leaf sheath (LS) was measured.

To evaluate the variation in culm length and GA sensitivity of DH lines, we produced DH lines according to the method of Ushiyama et al. (2006). The detached spikes of F1 hybrid of Tozan 18 × Fukuhokomugi were crossed with H. bulbosum and cultured for 14 days in the sucrose solution to which 2,4-dichlorophenoxyacetic acid (dissolved in sulfuric acid solution) was added at the concentration of 50 mg L⁻¹. The DH plants thus produced were successively selfed to produce DH3 plants. Some of the F1 hybrids were crossed with maize (teosinte) and DH4 plants were produced according to the method of Ushiyama et al. (1991). The DH4 plants produced by each method were treated with GA as reported by Yamada (1990), and grown in the field to determine the final culm length and the sensitivity to GA. The experiment was conducted with two replications. The sensitivity to GA is shown by the response of the LS to GA, and the sensitivity is shown by the GA index (GAI).

\[
\text{GAI} = \left( \frac{\text{length of LS in GA-treated seedlings}}{\text{length of LS in GA-untreated seedlings}} \right) \times 100.
\]

\[\chi^2\text{ test was performed to examine the goodness of fit to the expected ratio.}\]

To examine the variation of culm lengths in the progenies of the hybrid and DH lines, we grew about 500 plants each of F3, F4, F5 and F6 of Tozan × Fukuhokomugi and DH3, DH4, DH5 and DH6 produced by bulbosum method, in the field with a spacing of 3 cm within the row and 60 cm between rows, and measured their culm lengths.

### Results

Table 1 shows the wheat lines used in this study, and their sensitivity to GA (GAI). The GAI value in Tozan 18 was 168 and that in the F1 hybrid of the cross Tozan 18 × Rht3 tester line, D6899, was 141, showing a lower GA sensitivity than other lines. Fig. 1 shows the frequency distribution of the length of LS in the F2 of the cross between Tozan 18 and other tester lines after treatment with 10 ppm GA. An LS longer than 60 mm, which is considered to be induced by GA treatment, was observed in the cross Tozan 18 × Rht1 tester line, Nishikazekomugi and Fukuhokomugi, but not in the cross Tozan18 × Rht2 tester line (Norin 61), and Tozan 18 × Rht1Rht2 tester line (Norin 10).
Fig. 1. Frequency distribution of the length of the first leaf sheath of F2 seedlings of Tozan 18 crossed with Rht tester lines, measured 21 days after the treatment with 10 ppm GA.

Fig. 2 shows the culm length plotted against GAI value in DH lines (DH1) which were produced by the bulbosom and maize methods. This scatter diagram was divided into three groups, low GAI group, in which GAI was over 260 and culm length was longer than 80 cm, and medium GAI group, in which GAI was 130−220 and culm length was 70−100 cm. In DH lines produced by the bulbosum method, the number of plants classified into low, medium and high GAI groups was 27, 49 and 26, respectively, and in DH line produced by maize method, it was 24, 58 and 20, respectively. The ratio was close to 1 : 2 : 1 in both DH lines produced by the bulbosum and maize methods. In addition, the mean culm length of DH lines produced by the bulbosum and maize methods were 78.0 and 78.9 cm, respectively, and no significant difference was observed between them.

The mean GAI in DH lines produced by the bulbosum and maize methods was 178 and 190, respectively, with no significant difference seen between the two methods. The overall mean culm length in DH lines produced by the two methods was 78.5 cm. To compare the distribution pattern of the culm length in the DH lines produced by the two methods, we classified the culm length into thirteen groups; shorter than 51, 51−56, 56−61, 61−66, 66−71, 71−76, 76−81, 81−86, 86−91, 91−96, 96−101, 101−106 and longer than 106 cm, and examined the frequency of each group. Again, no difference was detected in the frequency distribution between the two methods (χ² test of 2×13 contingency table on these classes; df=12, χ²=7.360, p<0.01). The overall mean GAI of all values in DH lines produced by the bulbosum and maize methods was 184. The range of GAI was classified into lower than 104, 104−124, 124−144, 144−164, 164−184, 184−204, 204−224, 224−244, 244−264, 264−284, 284−304, 304−324, and higher than 324, and examined the frequency of each length group. Again, no difference was detected in the frequency distribution between the two methods (df=10, χ²=9.305, p<0.01).

Fig. 3 shows the distribution of culm length in F3 to F6 and DH1 to DH6. The culm length was classified
at 5 cm intervals, and to adjust the yearly deviation of growth, we calculated scores (the number of lines) of each class shown by the difference from the mean value of each class and compared the distribution pattern of the scores. The score of the 14 classes of the $F_3$ corresponded to that of $F_4$ (df = 13, $\chi^2 = 4.609$, $p < 0.10$), and that of $F_4$ also corresponded to that of $F_5$ (df = 13, $\chi^2 = 18.427$, $p < 0.10$). However, $\chi^2$ tests between the score in $F_6$ and that in other generations gave a significant difference ($F_6$ vs. $F_3$; df = 13, $\chi^2 = 36.186$, $p < 0.01$, $F_6$ vs. $F_4$; $\chi^2 = 34.248$, $p < 0.01$, $F_6$ vs. $F_5$; $\chi^2 = 28.623$, $p < 0.01$).

On the other hand, the scores of the 15 classes in the DH line produced by the bulbosum method corresponded to those in all other generations (DH$_3$ vs. DH$_4$; $\chi^2 = 13.165$, $p < 0.10$, DH$_3$ vs. DH$_5$; $\chi^2 = 15.222$, $p < 0.10$, DH$_3$ vs. DH$_6$; $\chi^2 = 10.513$, $p < 0.10$).

The histograms of the scores in the DH line produced by the bulbosum method had two peaks. In DH$_3$, DH$_4$, DH$_5$, and DH$_6$, the percentage of plants with a score of $-10$ or lower was 26, 27, 26 and 26%.

Figure 3. Culm length (score) in successive generations of selfed progenies of hybrid Tozan 18 × Fukuhokomugi and DH line produced by the bulbosum method.

Score: The number of lines.
respectively. On the other hand, in F3, F4, F5 and F6, the scores showed a near normal distribution, and the percentage of the plants with a score of −10 or lower was 20, 19, 19 and 20%, respectively.

**Discussion**

In this experiment, the GA sensitivity of the tester lines was similar to that reported by Yamada (1989) except that Norin 10 showed a very low sensitivity and Tozan 18 showed a lower GA sensitivity than the others (Table 1). If the Rht gene in Tozan 18 was different from that in the tester line, the GA sensitivity of F2 population derived from Tozan 18 × the tester line should be different from that of Tozan 18. As shown in Fig. 1, the distribution pattern of the length of LS in F2 hybrid of Tozan 18 × Norin 61 (Rht2) treated with GA was similar to that in Tozan 18, which shows that the genotype of Tozan 18 was Rht2. In Tozan 18 crossed with Fukuhokomugi and Nishikazekomugi carrying the Rht1 gene, some plants had an LS longer than 60 mm, because the crosses had rht1+rht2 (Fig. 1).

Snape et al. (1988) developed a second generation of DH lines to investigate the generation of gametoclonal variations by the *bulbosum* method, and detected gametoclonal variations in several characters. Laurie and Snape (1990) suggested that the variation in DH lines produced by the maize method was likely to be similar to that in DH lines produced by the *bulbosum* method. Suenaga and Nakajima (1993) reported that the segregation ratio of the DH lines derived from F1 of Fukuhokomugi (Rht1) × Oligo (Rht2) coincided with the expected ratio of 3:1 (insensitive : sensitive). In Fig. 2, GA sensitivity (GAI) and the culm length in the DH lines produced by the *bulbosum* method were similar to those in the DH line produced by the maize method, and there was no difference between the methods. In addition, the low, medium and high GAI groups in Fig. 2 show that they carry the Rht1Rht2 gene, Rht1rht2 or rht1Rht2 gene, and rht1rht2 gene, respectively. The segregation ratio of these gametoclonal phenotypes in the DH lines produced by the two methods was close to the expected ratio of 1:2:1. Therefore, we conclude that the two methods could be useful for breeding of wheat without a genetic drift. Furthermore, we can select medium culm lines carrying one Rht gene by the response to GA before planting, and reduce the number of lines by half. In haploid breeding, we can thus improve the efficiency of developing new wheat varieties. The expected frequency of the plants with homogeneous Rht1Rht2 in F3, F4, F5 was 14.1, 19.1, 22.0 and 23.4%, respectively. The expected frequency of the culm length were nearly the same except for F3. However, the frequency of the score of −10 cm in DH was lower than that in the self-fertilization lines (from F3 to F6), and the frequency of the score between −5 and −35 cm in DH line was higher than that in the self-fertilization lines (Fig. 3). In the later progeny generations of the self-fertilization lines, even in F3, peaks were not observed in the histogram (Fig. 3). This phenomenon indicated a genetic drift of the short culm lines carrying the Rht gene due to inhibition of growth of short culm individuals caused by shading by the long culm individuals when the self-fertilization mass selection was applied in dense planting for several generations. The cross combination between parents with different Rht genes magnified the culm length variation in progeny generations. In this case, medium culm length lines can be selected by examining the response to GA in the DH line produced by the *bulbosum* or maize method before planting in a field.

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