Short Communication

RFLPs Analysis of High pI α -amylase Multigene Families in Barley (*Hordeum vulgare* L.)

Chikako KIRIBUCHI, Genkichi TAKEDA and Tetsuo TAKANO¹⁾

*Laboratory of Plant Breeding, Faculty of Agriculture, The University of
Tokyo, Bunkyo-ku, Tokyo 113*

Analysis of restriction fragment length polymorphisms (RFLPs) revealed that there was at least 3 types of high pI α -amylase multigene families in barley. It was estimated that the isozyme banding patterns detected by the isoelectric-focusing (IEF) method were not directly determined by these 3 types of structural genes, but affected by a regulatory gene (or part). It was assumed that there was a linkage between the structural genes and the regulatory gene, but new combinations of the structural genes and the regulatory gene could be produced by genetic recombination. The bred lines with the new isozyme banding patterns may have resulted from the new combinations of the genes.

KEY WORDS: *Hordeum vulgare* L, malting barley, α -amylase, RFLPs, isoelectric-focusing, multigene family.

Introduction

One of the important objectives in the breeding of malting barley is to improve the activity of α -amylase synthesized during germination. Thus it is important to determine how the α -amylase activity of barley is regulated genetically.

Previously we classified barley varieties into three isozyme patterns (type 1~type 3) by the isoelectric-focusing (IEF) based on the major bands in the high pI group (TAKANO and TAKEDA 1985). Molecular biological studies have showed that high pI α -amylase in barley is controlled by multigene family consisting of about seven genes or pseudogenes (MUTHUKRISHNAN *et al.* 1983, CHANDLER *et al.* 1984, ROGERS 1985). However it remains to be determined whether there is a polymorphism for the α -amylase structural genes corresponding to the isozyme patterns. To investigate this problem, RFLPs (restriction fragment length polymorphisms) analysis was considered to be suitable. For example SHARP *et al.* (1988) observed the strong associations between RFLPs at the β -amylase loci and isozyme polymorphisms for β -amylase in wheat.

In addition, we observed that the new IEF patterns in the cross-combination of type 1 $\times$ type 2 occurred at a low frequency, and estimated that the gene coding for the band detected only in type 1 was transferred from type 1 to type 2 with genetic recombination and the recombination value ranged from 5.3 to 9.7% (TAKANO *et al.* 1988, KIRIBUCHI and TAKEDA 1988). RFLPs analysis may enable to verify this assumption.

Materials and Methods

Nine varieties of two-rowed barley belonging to the three IEF types (type 1~type 3) and

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¹⁾ Present address: *Experimental Farm, Faculty of Agriculture, The University of Tokyo, Tanashi, Tokyo*
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11 bred lines exhibiting homozygous new banding patterns (type 1 minus band 10, type 2 plus band 10) were used (Fig. 1). The bred lines with homozygous new banding patterns were obtained in the F_4 generation of the cross combination of type 1 $\times$ type 2 ("Haruna Nijo $\times$ Klages", "Clipper $\times$ Haruna Nijo").

DNA was isolated from barley shoots by the method described by DALLAPORTA *et al.* (1983), and purified by centrifugation to equilibrium in cesium-chloride ethidium bromide density gradients.

Barley DNA (10 μ g per sample) was digested with 50 units of *Hind* III. After electrophoresis on 0.8% agarose gels, the DNA was blotted onto a Biodine A membrane (Pall Corporation) as described by MANIATIS *et al.* (1982). The entire pM/C cDNA insert for the high pI α -amylase coding sequence (ROGERS 1985) was used as a probe. Nonradioactive DNA labeling and detection system using dioxigenin and anti-dioxigenin alkaline phosphatase conjugate (Boehringer Mannheim) was employed for the detection of the α -amylase genes.

Results and Discussion

The banding patterns of the α -amylase isozymes detected by IEF are shown in Fig. 1.

RFLPs analysis revealed that there was a polymorphism for the high pI α -amylase structural genes among the varieties. We identified 3 RFLP patterns and designated them as type A, type B and type C (Fig. 2). All the type 1 varieties (Haruna Nijo, Kou A, Kirin Choku 1) showed type A and the type 3 varieties (Amagi Nijo, Domen, Zephyr) type C (Table 1). As for the type 2 varieties, Clipper and Kenia showed type B and Klages showed type C. The results obtained in the lines of new isozyme banding pattern were more confusing.

Fig. 1. Banding patterns of α -amylase detected by isoelectric-focusing. Only bands 10-16 are shown.

Previously we assumed that the lines of type 2 plus band 10 had structural genes of type 2 and one gene coding for band 10 and that the lines of type 1 minus band 10 had the genes of type 1 except for the gene coding for band 10 (TAKANO *et al.* 1988). So we expected that the lines of type 2 plus band 10 derived from the progeny of "Clipper $\times$ Haruna Nijo" would show a RFLP pattern similar to type B, and that the other lines of type 2 plus band 10 derived from the progeny of "Haruna Nijo $\times$ Klages" would show a pattern similar to type C. But in practice all the lines of type 2 plus band 10 showed type A. Moreover, though expected to show the RFLP pattern similar to type A, all the lines of type 1 minus band 10 showed type B (Table 1).

This discrepancy can be explained by assuming that a part of the members of the high pI α -amylase multigene family was detected in the RFLPs analysis. However this assumption seems improbable because we used a highly conserved coding sequence as a probe.

Therefore the existence of a regulatory gene (or part) linked to the α -amylase structural genes was postulated and the gene was tentatively designated "R". The following is our assumption. If a variety or line has the regulatory gene "R1" which is present in the type

Fig. 2. RFLP patterns detected by a high pI α -amylase probe.

Plate 1; Variety 'Haruna Nijo' (Type 1)
 2; Variety 'Klages' (Type 2)
 3; Variety 'Kenia' (Type 2)
 4; Variety 'Amagi Nijo' (Type 3)

1 varieties, IEF bands 11-14 appear. If the variety or line has "R2" which is present in the type 2 varieties, bands 12 and 14-16 appear. If the variety or line has "R3" which is present in the type 3 varieties, bands 11-15 appear. As for band 10, regardless of the kinds of the regulatory gene, whenever the structural genes of type A are present, band 10 necessarily appears. On the basis of this assumption, the appearance of new isozyme banding patterns considered to result from the recombination between the structural genes and the regulatory gene (or part). That is, the lines of type 1 minus band 10 must have the structural genes of type B and the regulatory gene "R1", and the lines of type 2 plus band 10 must have the structural genes of type A and the regulatory gene "R2". Klages which showed the RFLP pattern of type C as well as the type 3 varieties must have the "R2" gene instead of "R3".

It remains to be determined whether the regulatory gene mentioned above is related to the control of the gene expression. However, as band 10 is observed only in Japanese varieties (TAKANO and TAKEDA

Table 1. Relationship between IEF and RFLP patterns

Variety or line	IEF pattern	RFLP pattern
Haruna Nijo	Type 1	Type A
Kou A	Type 1	Type A
Kirin Choku 1	Type 1	Type A
Clipper	Type 2	Type B
Kenia	Type 2	Type B
Klages	Type 2	Type C
Amagi Nijo	Type 3	Type C
Domen	Type 3	Type C
Zephyr	Type 3	Type C
CH 27-1 ¹⁾	Type 1 minus Band 10	Type B
CH 86-4 ¹⁾	Type 1 minus Band 10	Type B
CH 154-5 ¹⁾	Type 1 minus Band 10	Type B
CH 164-1 ¹⁾	Type 1 minus Band 10	Type B
CH 167-4 ¹⁾	Type 1 minus Band 10	Type B
CH 77-2 ¹⁾	Type 2 plus Band 10	Type A
CH 84-8 ¹⁾	Type 2 plus Band 10	Type A
CH 138-9 ¹⁾	Type 2 plus Band 10	Type A
CH 169-4 ¹⁾	Type 2 plus Band 10	Type A
HK 6-2-17 ²⁾	Type 2 plus Band 10	Type A
HK 6-6-101 ²⁾	Type 2 plus Band 10	Type A

¹⁾ Progeny of "Clipper × Haruna Nijo"

²⁾ Progeny of "Haruna Nijo × Klages"

1985), it is likely that the gene coding for band 10 is under a different control of the expression. Meanwhile it is necessary to consider the occurrence of post-translational modifications. There is still much left to be studied. RFLPs analysis using the promoter/upstream region of a high pI α -amylase genomic clone as a probe and isoelectric-focusing analysis of the *in vitro* translation products of mRNA may provide more information.

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Literature Cited

- CHANDLER, P. M., J. A. ZWAR, J. V. JACOBSEN, T. J. V. HIGGINS and A. S. INGLIS 1984. The effects of gibberellic acid and abscisic acid on α -amylase mRNA levels in barley aleurone layer studies using an α -amylase cDNA clone. *Plant Mol. Biol.* **3**: 407~418.
- DALLAPORTA, S. L., J. WOOD and J. B. HICKS 1983. A plant DNA miniprep: version II. *Plant*

- Mol. Biol. Reporter. **1**:19~21.
- KIRIBUCHI, C. and G. TAKEDA 1988. Genetic analysis of α -amylase isozymes and fixation of new banding patterns in barley. Japan. J. Breed. **38** (suppl. 2):228~229.
- MANIATIS, T., E. F. FRITSCH and J. SAMBROOK 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- MUTHUKRISHNAN, S., G. R. CHANDRA and E. S. MAXWELL 1983. Hormonal control of α -amylase gene expression in barley. J. Biol. Chem. **258**:2370~2375.
- ROGERS, J. C. 1985. Two barley α -amylase gene families are regulated differently in aleurone cells. J. Biol. Chem. **260**:3731~3738.
- SHARP, P. J., S. DESAI and M. D. GALE 1988. Isozyme variation and RFLPs at β -amylase loci in wheat. Theor. Appl. Genet. **76**:691~699.
- TAKANO, T. and G. TAKEDA 1985. Polymorphism for α -amylase in germinating seed and malt of barley varieties detected by isoelectric-focusing gel electrophoresis. Japan. J. Breed. **35**:9~16.
- , C. KIRIBUCHI and G. TAKEDA 1988. Genetic analysis on banding patterns and activity of α -amylase isozymes in barley (*Hordeum vulgare* L.) Japan. J. Breed. **38**:65~71.

オオムギ高等電点 α -アミラーゼ多重遺伝子族の 制限酵素断片長多型による解析

桐渕千雅子・武田元吉・高野哲夫¹⁾

(東京大学農学部, 文京区弥生, 〒113)

(¹⁾東京大学農学部田無農場, 田無市, 〒188)

9品種について, オオムギ高等電点 α -アミラーゼ遺伝子のコード領域をプローブとしてオオムギゲノムの制限酵素断片長多型(RFLPs)を調べた結果, 多重遺伝子からなるオオムギ高等電点 α -アミラーゼ構造遺伝子群には3つの型があることが明らかになった。(Fig. 2, Table 1). 1品種を除いて, RFLPの型と等電点電気泳動法で示される α -アミラーゼアイソザイムのバンドパターンの型との間には対応関係が見られた。一方, 交雑後代において, 組換え型と考えられた新しいアイソザイムパターンを示す系統では, 予想されるRFLPの型と実際にあらわれたRFLPの型とが違っていた(Fig. 1, Table 1)。この結果から α -アミラーゼアイソザイムのバンドパターンは, 構造遺伝子によって一義的に決定されるものではなく, 構造遺伝子群とこれと連鎖関係にある調節遺伝子との働き合いによって決定されるものと推察された。