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Inheritence, Linkage and Possible Use of Fractured Starch Mutant in Barley (*Hordeum Vulgare* L.)

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Abstract

In order to breed barley lines with reduced viscosity of wort, a fractured starch mutant of naked barley cultivar, Nubet, was obtained from the M_2 seeds mutated by the diethyl sulfate treatment. Seeds of this fractured starch mutant were opaque and the endosperm consists of angular, irregular and fractured starch. The mutant was caused by single recessive mutation and assigned by the symbol *fra*. The gene was located on chromosome 4, distal in long arm by linkage recombinations using translocation homozygote lethal test set. The linkage value between the fractured starch mutant and T2-4a, T2-4d were 26.0 ±4.9, 34.2 ±3.1 percent respectively. In addition to the reduced seed size, fewer kernels per spike and higher tillering ability, lower β-glucan viscosity and higher lysine content of the grain were associated with this mutant. β-glucan viscosity of the Nubet grains increased from 3 weeks after anthesis to matury and most of the viscose substances appeared to be stored in the middle of the endosperm tissue. Since the mutant grains showed better milling property as compared to Nubet, it can be used as breeding resources to develope new barley cultivars for malting and milling purpose.

Introduction

Starch in barley grains has been widely consumed not only as animal feed and human food but also as malt for industrial purpose. However, due to high viscosity of gums and malt extracted from barley with warm water, stiring and filtering sweet wort are difficult to perform (Djurtoff, 1958). The main cause of high viscosity of worts is β-glucan of barley endosperm (Scott, 1972; Izydorczyle et al., 2000). The barley β-glucan are essentially linear polymer of glucose with β-1,4 and β-1,3 glycosidic linkages which are randomly arranged (Preece and Mackenzie, 1952; Igarashi and Sakurai, 1965). For breeding purpose, a rapid method of viscosity evaluation for estimating β-glucan was developed by Greenberg and Whitemore (1974). They reported that extracted viscosity was closely related to actual β-glucan content in a logarithmic fashion, with a correlation coefficient of 0.89.

Barley starch granules are in spherular structure. In barley endosperm, two different types of starch granules, large oval type bigger than 25 μm and small granule which is about 5 μm in size, are embedded in a matrix of protein as observed by scanning electron microscopy (Pomeranz, 1974). A wide range of varietal differences in the ratio of small to large granules were reported by Goering et al. (1973). On the other hand, the starch granules of rice and wrinkled-seed pea are fairly angular as illustrated by microscopic photographs (Banks and Greenwood, 1975).

Induced mutation are a suitable breeding method for the extending genetic variability in barley. A number of chemical mutagens have been used for the mutation and diethyl sufate is one of the important mutagens inducing high proportion of point mutation with negligible frequence of chromosome abbrevation and very little physiological

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injury (Nilan, 1964). Barley translocation have been used by a number of workers to assign new mutant gene onto a specific chromosome and to test linkages (Hanson, 1952; Burnham, 1962; Ramage, 1966; Kunzel et al., 2001). Homozygous translocations have little or no effect on the phenotype of barley and heterozygous translocations are phenotypically recognized by semisetrility as a result of female abortions. The aborted spores and ovaries are caused by deficiencies and duplications of chromosome segment resulting from adjacent disjunction. An important consequence of this alternate disjunction in barley is that few recombinants are recovered from genes occurring between the centromeres and breakpoints of translocation (Ramage, 1963). When an individual heterozygote for a translocation and a gene pair is selfed, four phenotypic classes can be recognized in the F_2 generation. Since a translocation involves an exchange of chromosome pieces between two non-homologous chromosomes, linkage obtained is an evidence for the location of assigned gene on either one or both chromosomes. To determine which of these possibilities is acceptable, a test must be made with an additional translocation involving a breakpoint of different chromosomes (Eslick, 1979). A translocation homozygote lethal test set including seven barley chromosomes have been developed by Biggerstaff (1981). The possible advantages of these test set are maintaining recessive male sterile genes without rogueing homozygote dominant plants and increasing precision of linkage values (Eslick, 1979).

In this study, we report inheritance and linkage of fractured starch mutant induced from barley cultivar, Nubet using translocation homozygous lethal test set. For the developing possible usage of fractured starch mutant, grain qualities related to brewing and milling were compared with parent cultivar, Nubet.

Materials and Methods

A fractured starch mutant, Franubet, was selected from the M_2 seeds of naked barley cultivar, Nubet, treated with 0.01 M of diethyl sulfate. Opaque seeds were screened visually among the vitreous wild-type seeds. The selected opaque seeds were then stained with iodine and only dark blue seeds were selected finally because waxy endosperm also showed opaque. The fractured starch phenotype of mutant seeds was characterized by the microscopic observation at different developmental stages from the begining of starch synthesis to maturation. For the genetic analysis, the mutant was crossed with Nubet, Nupana and translocation homozygote lethals of all possible combinations of the seven barley chromosomes. Crossed seeds were selfed and F_2 seeds were harvested.

To evaluate the agronomic characters, the yield trails were performed in the field of Crop Experiment Stations in Montana State University located near Bozeman Montana, USA, with four replications of randomized complete block design. Harvested seeds from each experiment station were bulked for the evaluation of grain quality. The quality determinations of Franubet and Nubet were performed by the following procedures.

1) Proximate analyses including protein by Kjeldahl method, ash, ether extract and crude fiber content were determined by AOAC standard methods.
2) Starch content of grains was evaluated with enzymatic procedure described by Banks and Greenwood (1971).
3) Amino acid analysis was performed using a Beckman 120℃ automatic analyzer and conducted by A.A.A laboratories : 6206, 89th Avenue, Southeast, Mercerland, Washington, USA.
4) β-glucan viscosity was determined by Brabender viscometer based on a method described by Fox (1981).

Results and Discussion

Inheritance and linkage of fractured starch mutant

Endosperms of Nubet and Franubet grains (fractured

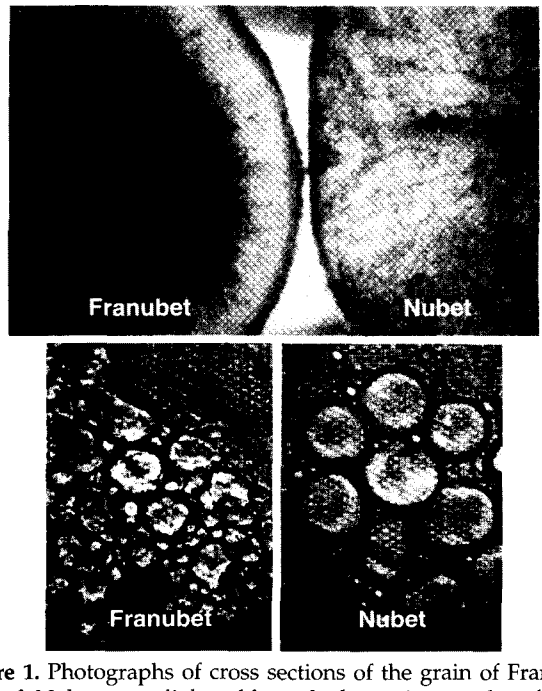

Figure 1. Photographs of cross sections of the grain of Franubet and Nubet on a light table and photomicrographs of their starch granules.

starch mutant) were observed to verify fractured starch granules under light microscope. The starch granules of Franubet were fractured and irregular in shape and size compared with Nubet starches (Figure 1). These shape formed at early stage of grain filling. A few round granules were also found in the mutant, however, most of them were fractured or cracked. Hybrid seeds between Nubet and Franubet from reciprocal cross showed the round starch granules like as Nubet. This results revealed that characteristic of normal round starch granules were dominant over that of fractured starch granules. The result of F_2 segregation ratio of mutant cross fit a 3 dominant : 1 recessive ratio with over 95% probability regardless of genotype background(Table 2). Backcross F_1 seeds were also segregated to normal and fractured starch granules and phenotypic ratio were fit to 1 dominant : 1 recessive with 75~50% probability. These results indicated that fractured starch character is a recessive single gene inheritance with xenia effect. Based on these observations, the genotypes of normal and the mutant were assigned as 'Fra' and 'fra', respectively.

F_2 segregation of cross combination between test set of translocation homozygote lethal and Franubet are presented in Table 3. The segregation of T2-4a and T2-4d were significantly different from the expected segregation ratio and linkage recombination were 26.0 ± 4.9% and 34.2 ± 3.1%, respectively. Independent assortments were shown for the crosses between Franubet and T1-6j, T1-7c, T3-4d, T3-4b, T4-5e, T5-7g and T6-7c. This results revealed that the gene of fractured starch granules are located on chromosome 4. The translocation of T3-4d and T4-5e also involved chromosome 4, however, translocation breakpoint of these lines were reported to the short arm of chromosome 4 and it is possible to segregate independently when exchange segments are short arm of chromosome 4. Therefore, fractured starch gene is located at the distal in long arm of chromosome 4. The position of translocation breakpoints and fra gene are diagrammed in Figure 2.

Agronomic character and grain quality of fractured starch mutant

Agronomic characters and grain quality of fractured

Table 1. Description of translocation homozygote lethals test stocks used.

Translocation* designation	Background cultivar	Breakpoint		Authority
T1-6a	Mars	S	Sat	Ramage et al (1961)
T1-6j	Bonus	L	Sat	Ramage (1971)
T1-7c	Mars	S	Sat	Nilan (1964)
T2-3a	Gull	S?	S	Kasha (1965)
T2-4a	Mars	-	-	Eslick (1978)
T2-4d	Bonus	S	L	Ramage et al (1961)
T3-4b	Bonus	-	-	
T4-5e	Bonus	S	L	Persson (1965)
T5-7g	Bonus	L	L	Tuleen (1974)
T6-7c	Bonus	S	S	Ramage et al (1971)

S=short arm; L = long arm; Sat = Satellite.
?=probably in that arm; - = breakpoint not determined.
*; Interchange chromosome with lower number listed first, higher number listed second.

Table 2. Segregation ratios of fractured starch granules in the hybrid between Franubet (fractured starch mutant) and Nubet.

Corss	Generation	No. of seeds			Expected ratio	Chi-square	P
		Fra-	frafra	Total			
Nubet / Franubet	F_1	25	0			-	
Franubet / Nubet	F_1	35	0			-	
Nubet / Franubet // Franubet	BC_1	71	64	135	1 : 1	0.363	0.75-0.50
Nubet / Franubet	F_2	436	148	584	3 : 1	0.039	〈0.95
Franubet / Nubet	F_2	104	35	139	3 : 1	0.002	〈0.95

fra fra : Recessive class of fractured starch mutant.
Fra - : Dominant class of fractured starch mutant (Round).

Table 3. F_2 segregation of fractured starch mutant crossed with translocation homozygote lethal lines and their linkage recombination values.

Translocation	Semisterile		Fertile		Total	X^2(6:2:3:1)	Recombination
	Fra-	fra fra	Fra-	fra fra			
T1-6j	21	13	15	4	53	3.26	Independent
T1-7c	78	32	46	9	165	3.17	Independent
T2-4a	74	21	21	11	108	23.41**	26.0±4.9
T2-4d	42	13	10	8	73	8.87*	34.2±3.1
T3-4b	65	13	43	10	131	6.86	Independent
T4-5e	60	11	25	5	101	5.18	Independent
T5-7g	49	20	32	8	109	1.57	Independent
T6-7c	67	24	28	7	126	2.34	Independent

fra fra : Recessive class of fractured starch mutant.
Fra- : Dominant class of fractured starch mutant (Round).

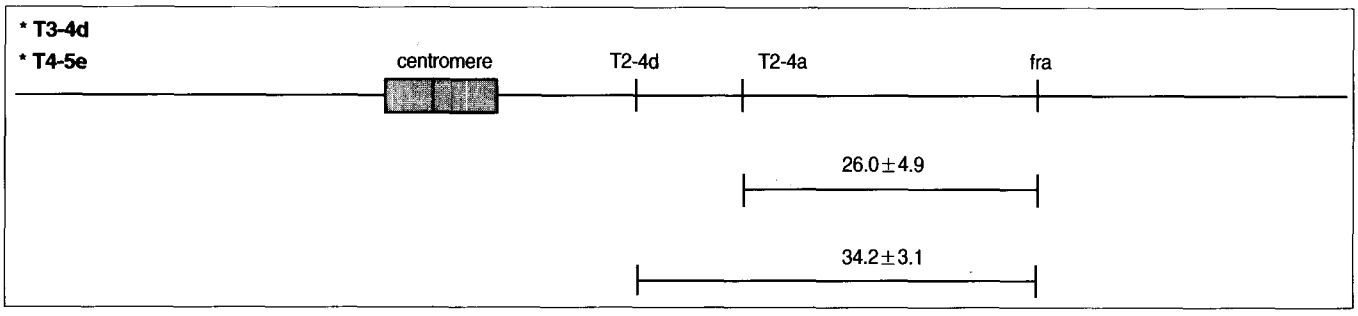

Figure 2. Linkage recombination between fractured starch mutant (fra) and transloctions on the barley chromosome 4. *; Breakpoint location considered to somewhere in short arm.

starch mutant are shown in Table 4. The number of kernels per spike and kernel weight of Franubet were significantly lower than those of Nubet. However, when the number of spikes per unit area planted (m^2), the mutant yielded much higher spike number than Nubet. Presumably, due to such difference in spike number, Nubet and Franubet showed similar yield performances. Other significant phenotypic differences between Franubet and Nubet are shorter plant height and delayed maturing of the mutant. However, no significant differences were observed between Nubet and Franubet grains in chemical compositions determined by proximate analysis.

Table 4. Agricultural characteristics and grain quality of fractured starch mutant grown in the 3 locations.

Characteristics	Nubet	Franubet
Number of kernel per spike	23.7	20.1 NS
Kernel weight (mg)	40.4	33.3*
Number of spike per m^2	344.0	451.0*
Yield (kg/10 a)	317.4	297.5 NS
% protein in grain	14.34	15.14 NS
% starch	57.7	59.8 NS
% ether extract	2.61	2.90 NS
% crude fiber	0.38	0.39 NS
% ash	1.86	1.97 NS

* : Significant differences from Nubet at p=0.05 level.
NS : No significant differences from Nubet.

Amino acid content of grains of Nubet and Franubet were listed in Table 5. Glutamic acid and proline were the major amino acids in both genotypes. Significant differences between Franubet and Nubet were determined in isoleucine, lysine, glutamic acid and proline. Fraunubet contain higher amount of lysine and lower amount of glutamic acid, isoleucine and proline as compared with Nubet. High lysine barley has been developed by many breeders (Munck et al., 1970; Ingverson et al., 1975; Jarvi and Eslick, 1975; Ullrich and Eslick, 1978; Davy et al., 2000) and mostly high lysine mutants were associated with shrunken endosperms with lower grain yield. Since Fraunubet showed plump kernels with high lysine, this mutant is worth to be considered as a new source of high lysine barley.

In Figure 3, β-glucan viscosity of the fra mutant are illustrated. The samples of Franubet and Nubet were harvested periodically after anthesis. The β-glucan viscosity

Table 5. Average amino acid contents of grain fractured starch mutant (Franubet) compared to Nubet, from samples grown in the 3 locations.

Amino acid	As % of grain (DMB)			As % of protein (DMB)		
	Nubet (x)	Franubet (y)	Difference (y-x)	Nubet (x)	Franubet (y)	Difference (y-x)
Alanine	0.599	0.602	+0.003	3.33	3.56	+0.23*
Arginine	0.607	0.682	+0.075	3.36	4.06	+0.69
Aspartic acid	0.982	0.983	+0.001	5.45	5.81	+0.36**
Glutamic acid	5.755	4.995	-0.760**	31.91	29.44	-2.47*
Glycine	0.491	0.504	+0.013	2.73	2.98	+0.26**
Histidine	0.235	0.257	+0.002	1.30	1.53	+0.22
Isoleucine	0.615	0.583	-0.032**	3.42	3.44	+0.03
Leucine	1.080	1.070	-0.010	6.01	6.35	+0.34*
Lysine	0.400	0.473	+0.073*	2.22	2.81	+0.59*
Methionine	0.308	0.302	-0.006	1.71	1.78	+0.07
Phenylalanine	0.953	0.879	-0.074	5.28	5.21	-0.07
Proline	2.400	2.140	-0.260*	13.28	12.62	-0.66
Serine	0.828	0.804	-0.024	4.60	4.75	+0.14
Threonine	0.597	0.593	-0.004	3.32	3.51	+0.19**
Tyrosine	0.474	0.492	+0.018	2.63	2.91	+0.28*
Valine	0.807	0.781	-0.026	4.48	4.61	+0.13
Taurine	0.923	0.782	-0.141	5.14	4.61	-0.53
Total	18.000	16.900	-1.100**			

*,** : Significant differences from Nubet at p=0.05 and 0.01 levels, respectively.

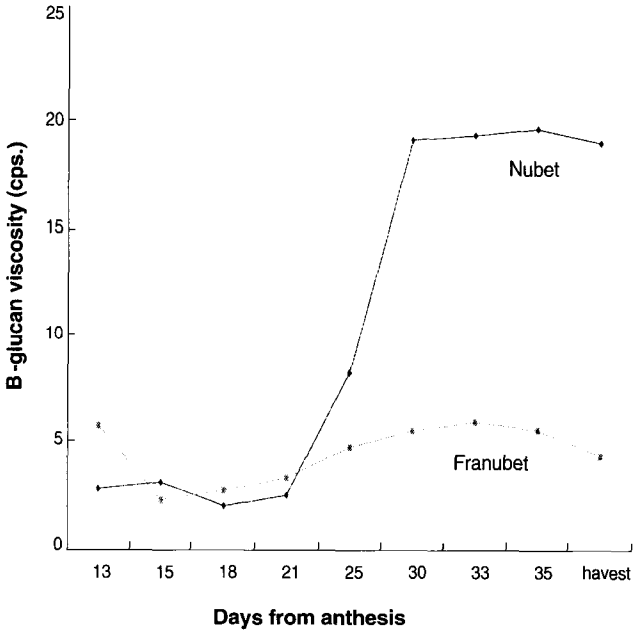

Figure 3. Change of β-glucan viscosity as the grain of Nubet and Franubet developed.

curve of developing endosperms in Nubet showed the viscosity started to increase from 21 days after anthesis and reached to maximum at physiological maturity. The viscosity of Franubet also changed in similar pattern of Nubet. However, much lower viscosity level of fra mutant was observed than those of Nubet. This result appeared to agree with Coles (1979), suggesting that starch accumulation was nearly complete when the moisture content of endosperm decline 40%, whereas the accumulations of β-glucan and hemicellulose continue to near physiological maturity. Fulcher et al. (1977) reported that main deposition of β-glucan occurs at the sub-aleuron cell walls, but results shown by Gohl et al. (1977) indicated that the highest deposition of β-glucan were near the center of endosperm.

The synthesis of enzymes such as α-amylase and β-glucanase are of primary importance in malting and brewing process to promote enzymatic hydrolysis of carbohydrate. Because of the chemical nature of β-glucans, the degradation of the starch endosperm is prevented by the presence of the cell wall and β-glucan. As Goering et al. (1973) pointed out, high β-glucan viscosity causes the stiring problem during brewing and delays the degradation of carbohydrate and protein. Since Franubet showed

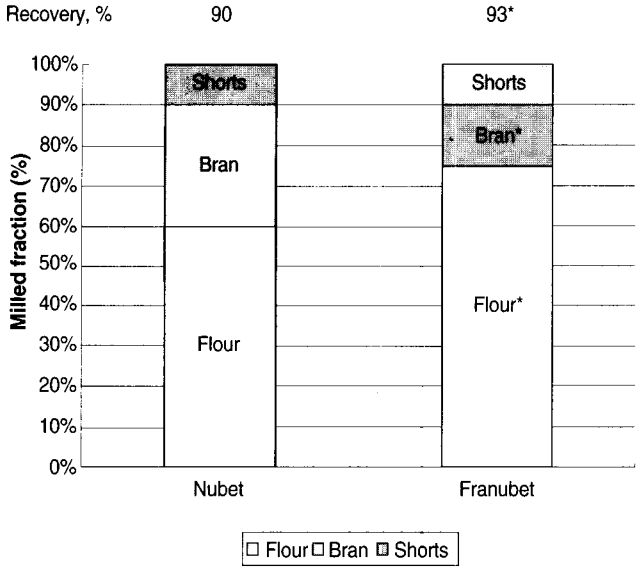

Figure 4. Comparison of average percent recovery and Buhler milled fractions for barley starch mutants grown in several environments. * ; Significant difference from Nubet at p=0.05 level.

significantly lower β-glucan viscosity, the fractured starch mutant can be used as a source of germ line to develop high quality of malting barley cultivar with lower viscosity of wort.

Nubet and Franubet grains were evaluated in terms of milling properties by using Buhler test mill developed for wheat milling (Figure 4). Significant differences were observed for percent recovery, flour yield and bran between Nubet and Franubet. The flour yield of Franubet ranged from 70 to 81% and average 75% which was 13% higher than those of Nubet. Barley flour in general is light and fluffy and inferior to wheat flour. And barley bran is usually crumbled and some endosperm starch remains on the bran. A considerable amount of samples were lost during milling because of plugging of the test mill. In spite of milling problems, grains of Franubet showed better milling properties than those of Nubet.

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◐ 알려 드립니다

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