GENETICA POLONICA
Vol. 28 1987 No. 3

INHERITANCE AND GENETIC POLYMORPHISM OF BETA-AMYLASE FROM RYE (*SECALE CEREALE* L.) ENDOSPERM[1]

PIOTR MASOJĆ[2]

Department of Plant Breeding and Experimentation, Academy of Agriculture, Szczecin

Summary. The inheritance of beta-amylase isoenzymes separated by polyacrylamide gel electrophoresis technique was studied using conventional genetic analysis. Results of the studies support the hypothesis that the enzyme is coded by two strongly linked genes with five (*β*-Amy1) or two (*β*-Amy2) codominant and recessive null alleles, which are inherited independently of the genes controlling the composition of alpha-amylases. Ten rye varieties were found to have a significant frequency of null alleles and a high degree of heterozygosity. The genotype distribution generally agreed with the Hardy-Weinberg equilibrium principle.

Beta-amylase from rye endosperm is an enzyme showing significant polymorphism (Buschbeck, Wilp 1982). A set of isoenzymatic forms changes in the course of both maturation and seed germination (Masojć 1984). During 5 - 7 days after the seeds were allowed to germinate it is possible, using polyacrylamide gel electrophoresis, to separate beta-amylase into 7 intensive bands, five of which, faster migrating (1 - 5), form the first zone of the zymogram, and two slower ones (7 and 9) form the second zone of that zymogram (Łapiński, Masojć 1983). As shown by the analysis of inbred lines, in a homozygous state there occurs at most a single intensive band in each zone. Homozygotes with one, rarely two bands or those showing no bands (null phenotype) are encountered among lines. The lack of beta-amylase activity in rye grain was also found by other authors (Daussant et al. 1981). The genetic basis of polymorphism of beta-amylase from rye endosperm is unknown. It has been only established that the enzyme is controlled by the chromosome 5R (Artyomova 1982, Schmidt et al. 1984). Observations on the variation of beta-amylase composition in inbred lines, like in rye cultivars (Masojć Łapiński 1984), suggest that bands of the zymogram first zone are alloenzymes coded by one gene, whereas bands of the second zone are coded by another gene.

The purpose of the present paper was to verify the above hypothesis using conventional genetic analysis.

---

[1] Received for publication: September, 1986. The work was performed under Problem MR II/7 coordinated by the Polish Academy of Sciences.

[2] Dr. Present address: ul. Słowackiego 17, 71 - 434 Szczecin, Poland.

MATERIAL AND METHODS

The studying material consisted of 16 inbred lines, internally homogeneous with respect to beta-amylases, and 10 cultivars and strains of winter rye. As a result of a controlled crossing of lines 12 hybrids of F_1 and F_2 generations were obtained.

Amylases were extracted from the endosperm of grains germinating for 5 days at a room temperature. The amylases were electrophoretically separated in 5% polyacrylamide gel added with 0.125% soluble starch. Details of gel extraction, electrophoresis and staining methods were given earlier (Łapiński, Masojć 1983). The beta-amylase isoenzymes were identified with the use of a β-limit dextrin substrate.

A genetic analysis of inter-line F_2 hybrids was performed using the χ^2-test (Sokal, Rohlf 1969), whereas the frequency of alleles occurring in rye cultivars were determined by the gene counting method (Elandt-Johnson 1971). Heterozygosity was estimated by the method of Nei (Nei, Roychoudhury 1973).

RESULTS AND DISCUSSION

Out of the lines used for the studies 14 displayed single-banded phenotype (band 1, 2, 3, 4, 5 or 7), one — double-banded (2 and 9) phenotype and one — null phenotype (Fig. 1). In the zymograms representing the F_1 generation, bands of the maternal origin were more intensive than bands introduced by the paternal line, which may be explained by the gene dosage effect. Double-banded phenotype of the heterozygote indicate that beta-amylases active in the endosperm of germinating grains have a monomeric structure.

Table 1 shows phenotypic segregations observed in the F_2 progenies and their agreement with the expected values on the assumption of monogenic inheritance. In the hybrids derived from the crosses between the lines showing the presence

Table 1. Segregation ratios of beta-amylase isoenzymes from rye endosperm in F_2 progenies of inter-line hybrids

Hybrid No.	Parental phenotypes		Number of phenotypes				χ^2 1 : 2 : 1 or 3 : 1	P
	A	B	A	AB	B	Total		
1	band No.: 1	band No.: 2	130	292	125	547	2.59	0.20 - 0.30
2	1	3	39	79	37	155	0.11	0.90 - 0.95
3	2	3	119	291	131	541	3.64	0.10 - 0.20
4	5	1	57	104	51	212	0.42	0.80 - 0.90
5	3	5	9	36	15	60	3.60	0.10 - 0.20
6	4	5	64	151	83	298	2.48	0.20 - 0.30
7	2	5	80	179	73	332	2.33	0.30 - 0.50
8	7	9	39	101	51	191	2.14	0.30 - 0.50
9	1	null	240	—	94	334	1.20	0.20 - 0.30
10	2	null	152	—	39	191	1.91	0.10 - 0.20
11	9	null	252	—	80	332	0.10	0.70 - 0.80
12	7	5	47	102	51	200	0.24	0.80 - 0.90
13	5	2.9	80	179	73	332	2.33	0.30 - 0.50
14	7	2.9	39	101	51	191	2.14	0.30 - 0.50

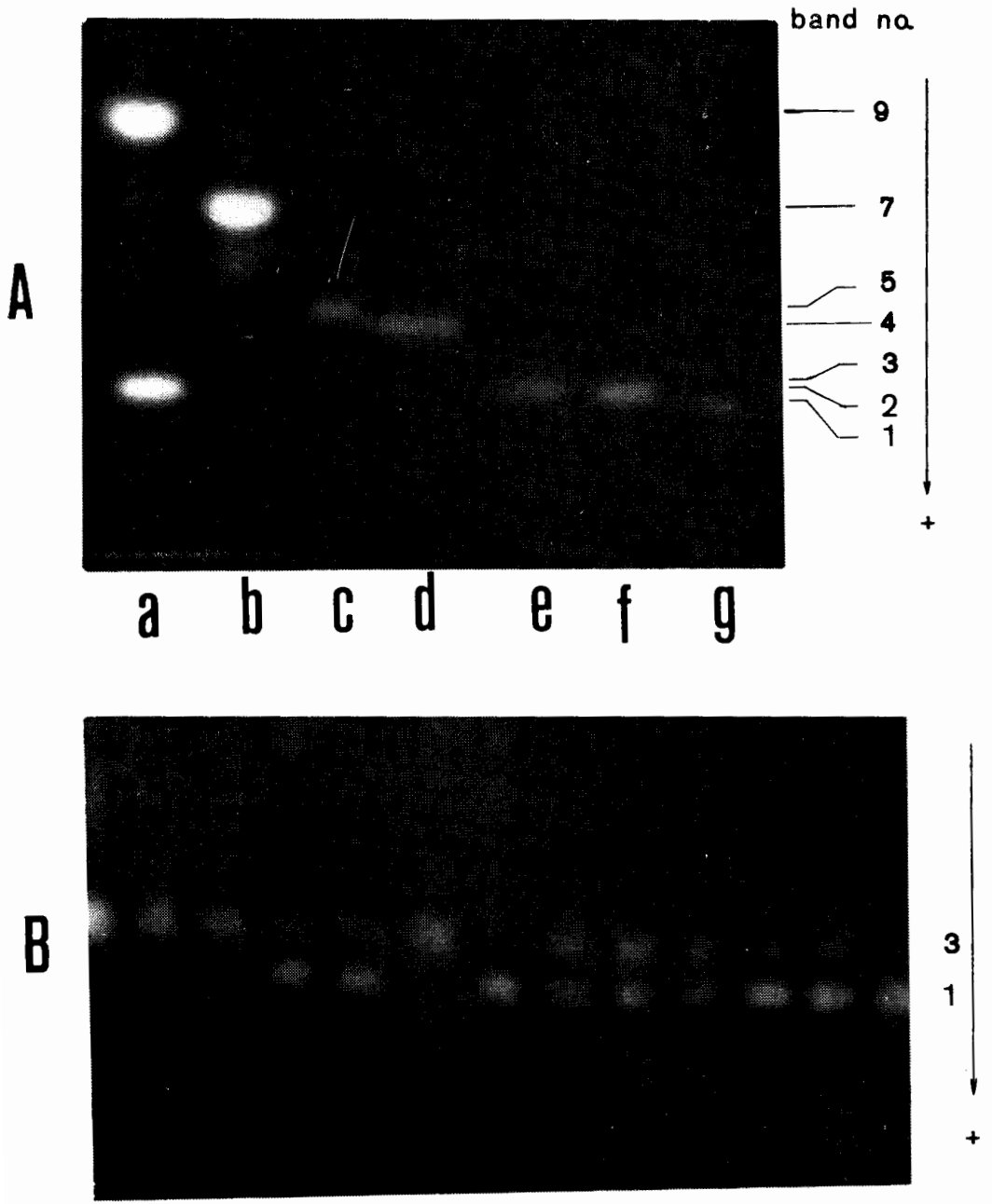

Fig. 1. The β-amylase zygomrams showing one double- (a) and six single-banded (b-g) homozygous phenotypes of the parental lines (A) and the segregation of bands 1 and 3 in the F_2 progeny of an inter line hybrid (B). The β-amylase isozymes are sometimes accompanied by faint secondary bands

Table 2. Independent segregation of alpha- and beta-amylase genes in F_2 and BC_1 progenies of inter-line hybrids

Pair of loci	Type of data	Number of genotypes									χ^2 1 : 4 : 1 : 2 : 2 : 2 : 2 : 1 : 1 or 1 : 1 : 1 : 1	P
		a/aB/B	A/aB/b	A/Ab/b	A/aB/b	a/aB/b	A/AB/b	A/ab/b	a/ab/b	A/AB/B		
α-Amy2 – β-Amy 1	F_2	14	62	16	33	28	29	30	12	12	2.10	0.95 - 0.98
α-Amy1 – β-Amy1	BC_1	–	36	31	–	–	32	33	–	–	0.42	0.90 - 0.95
α-Amy2 – β-Amy1	BC_1	–	21	15	–	–	25	17	–	–	3.02	0.30 - 0.50
α-Amy3 – β-Amy1	F_2	29	114	27	58	44	53	52	23	26	3.31	0.90 - 0.95
α-Amy3 – β-Amy1	BC_1	–	55	54	–	–	49	52	–	–	0.40	0.90 - 0.95
M-α-Amy – β-Amy1	F_2	19	79	20	35	30	46	34	14	18	5.46	0.50 - 0.70

of a single band from the first zone (position 1 - 7) or from the second (position 8) the phenotypes segregated in the ratio consistent with 1 : 2 : 1 expectation for a single locus with two codominant alleles. When one of the parental lines showed no activity of beta-amylase (position 9 - 11), the segregation of a single-banded and null phenotypes observed in the F_2 progeny was not significantly different from the 3 : 1 ratio. These data suggest that isoenzymes of the first zone are products of five alleles of a single gene. The alleles were designated β-Amy1^1 — β-Amy1^5 according to the band numbers. The allele responsible for the lack of band in the first zone was assigned the symbol β-Amy1^N. Isoenzymes of the second zone may be recognized to be allelic forms of the second gene. Its 3 alleles, coding bands 7, 9 and the lack of band in the second zone are proposed to be designated β-Amy2^1 β-Amy2^2 and β-Amy2^N, respectively.

Three last positions in Table 1 present a simultaneous segregation of isoenzymes from the first and second zones. Among 723 grains of the F_2 generation no phenotypes, indicative of β-Amy1 and β-Amy2 gene recombinations, were found. That may mean that these genes are located at two strongly linked loci or form a compound locus, like some genes of esterases (Wehling, Schmidt-Stohn 1984) and peroxidases (Garcia et al. 1982) in rye or like genes of beta-amylases in wheat (Ainsworth et al. 1983).

The electrophoresis method used in the present work make possible the obtaining of the pattern of both beta- and alpha-amylases on a single gel. The genetic analysis of alpha-amylase composition (Masojć — 1987) permitted to distinguish three linked structural genes designated α-Amy1, α-Amy2, α-Amy3 and an independent locus of the modifying M-α-Amy gene. Table 2 presents segregation of alpha- and beta-amylase genes in the F_2 progenies of inter-line hybrids. The observed segregation ratios indicate that the inheritance of these genes is independent, which agrees with the data of Schlegel and Mettin (1985).

Earlier studies (Masojć, Łapiński 1984) showed that population cultivars are polymorphic to a high degree with regard to beta-amylase composition. In the rye grain, depending on the cultivar, the most frequently encountered are the alleles β-Amy1^N (Wiatka, Donar), β-Amy1^1 (Ponsi, Animo, Halo), β-Amy1^2 (Otello,

Table 3. Frequencies of beta-amylase alleles and heterozygosity (h) in rye cultivars

Cultivar	Alleles of β-Amy1						$h_{(\beta-Amy1)}$	Alleles of β-Amy2			$h_{(\beta-Amy2)}$
	N	1	2	3	4	5		N	1	2	
Otello	0.298	0.113	0.528	0.059	0.002	0.000	0.617	0.655	0.232	0.113	0.505
Petkus 1035	0.303	0.195	0.359	0.036	0.005	0.102	0.731	0.534	0.359	0.107	0.576
Donar	0.318	0.183	0.290	0.071	0.015	0.123	0.763	0.589	0.149	0.262	0.564
Ponsi	0.297	0.323	0.086	0.174	0.094	0.026	0.762	0.742	0.165	0.093	0.415
Kungs II	0.276	0.187	0.406	0.033	0.020	0.078	0.718	0.717	0.219	0.064	0.435
Sv 6970	0.208	0.077	0.521	0.118	0.020	0.056	0.664	0.819	0.167	0.014	0.302
Dańkowskie Złote	0.267	0.236	0.081	0.287	0.049	0.080	0.777	0.676	0.173	0.151	0.491
Animo	0.003	0.605	0.004	0.345	0.010	0.033	0.486	0.878	0.077	0.045	0.222
Halo	0.324	0.506	0.189	0.184	0.015	0.056	0.617	0.687	0.205	0.108	0.475
Wiatka 2	0.403	0.306	0.014	0.080	0.162	0.035	0.711	0.569	0.393	0.038	0.521

Table 4. Testing for the Hardy-Weinberg equilibrium principle in rye cultivars at the β-Amyl

Cultivar		Number of phenotypes																Degrees of freedom	χ^2	P
		null	1	2	3	4	5	1,2	1,3	1,4	1,5	2,3	2,4	2,5	3,4	3,5	4,5			
Dańkowskie	1[a]	24	44	13	77	8	14	18	42	9	15	9	4	3	6	12	2	7	11.04	0.10 - 0.20
Złote	2[b]	21.4	54.5	15	70.7	8.6	14.7	11.5	40.6	6.9	11.3	13.9	2.4	3.9	8.4	13.8	2.3			
Halo	1	14	148	6	37	0	13	5	58	0	14	0	0	0	1	4	0	2	5.84	0.05 - 0.10
	2	16.4	147.8	2.8	36	0.2	8.8	5.7	55.9	0.3	17	2	0	0.6	0.3	6.2	0			
Wiatka 2	1	44	103	6	21	56	8	2	21	24	5	0	0	0	3	2	5	3	7.70	0.05 - 0.10
	2	48.7	102	3.4	21.1	47	8.8	2.6	15	29.7	6.4	0.7	1.4	0.3	7.8	1.7	3.4			
Donar	1	20	27	53	14	2	14	23	6	1	10	4	3	20	0	3	0	4	4.56	0.30 - 0.50
	2	20.2	30.0	53.7	10.0	2.3	18.7	21.2	5.2	1.1	9.0	8.0	1.7	14.3	0.4	3.5	0.7			
Ponsi	1	16	57	19	25	11	5	4	31	14	3	4	5	0	4	0	2	4	14.64	<0.01
	2	17.6	59.2	11.7	26.7	12.9	3.1	11.4	22.5	12.1	3.4	6.0	3.2	0.8	6.5	1.8	1.0			

[a] observed number
[b] expected number

Petkus 1035, Kungs II, Sv 6970) or β-Amy1[3] (Dańkowskie Złote). The frequency of β-Amy1[4] and β-Amy1[5] alleles is generally low. All the cultivars exhibit a similar composition of the alleles of the second beta-amylase gene. The β-Amy2[N] allele occurs with the highest, and β-Amy2[2] allele — with the lowest frequency (Table 3). Significant frequency of null alleles revealed in rye cultivars is not exceptional. Such alleles occur equally frequently at the loci of phosphatases or peroxidases (Perez de la Vega et al. 1982, Perez de la Vega, Allard 1984). Using immunochemical techniques it was shown that the cause of the absence of beta-amylase activity in the grain of some rye lines was a low level of the protein (Daussant et al.). It may not, therefore, be excluded that the action of β-Amy1[N] and β-Amy2[N] alleles consists in inhibiting enzyme production.

Table 5. Testing for the Hardy-Weinberg equilibrium principle in rye cultivars at the β-Amy2

Cultivar		Number of phenotypes				Degrees of freedom	χ^2	P
		null	7	7.9	9			
Ponsi	1[a]	109	54	6	29	1	0.00	>0.99
	2[b]	109.0	53.8	6.1	29.1			
Kungs II	1	99	72	4	20	1	0.54	0.25 - 0.50
	2	100.3	70.6	5.4	18.7			
Petkus 1035	1	59	96	20	20	1	2.82	0.05 - 0.10
	2	55.6	99.9	15.0	24.4			
Donar	1	72	35	20	71	1	2.08	0.10 - 0.25
	2	68.7	39.1	15.5	74.4			

[a] observed number
[b] expected number

Heterozygosity of rye cultivars is high and ranges from 0.486 (Animo) to 0.777 (Dańkowskie Złote) in the case of the β-Amy1 gene and from 0.222 (Animo) to 0.576 (Petkus 1035) in the case of the β-Amy2 gene (Table 3). Such a high frequency of heterozygotes occurs also at the loci of phospho-glucoso-isomerase and peroxidase (Perez de la Vega et al. 1982). A significant level of heterozygosity detected at some enzymatic loci of rye results from the maintenance of a high frequency of several alleles and is presumably a symptom of adaptational strategy of allogamous species.

The agreement of the phenotype frequency distribution in a population with the equation of the Hardy-Weinberg equilibrium principle constitutes an additional criterion of allelism for isoenzymatic forms. Tables 4 and 5 present testing results of genetic equilibrium in rye cultivars, separately for isoenzymes of the first and second zones. The revealed phenotype frequencies are generally in agreement with the expected values, which justifies the genetic interpretation accepted in this paper.

REFERENCES

1. Ainsworth C. C., Gale M. D., Baird S. (1983). The genetics of β-amylase isozymes in wheat. 1. Allelic variation among hexaploid varieties and intrachromosomal gene locations. Theor. Appl. Genet., 66: 39 - 49.

2. Artyomova N. V. (1982). Chromosomal control of alcohol dehydrogenase, esterase and beta-amylase isoenzymes in different rye cultivars. Genetika, 18: 661 - 667.
3. Buschbeck R., Wilp R. (1982). Beziehungen zwischen Auswuchsresistenz und Amylase — Isoenzymmustern bei Winterrogen. Tag.-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin, 198: 559 - 569.
4. Daussant J., Zbąszyniak B., Sadowski J., Wiatroszak I. (1981). Cereal β-amylase: immunochemical study on two enzyme — deficient inbred lines of rye. Planta, 151: 176 - 179.
5. Elandt-Johnson R. C. (1971). Probability models and statistical methods in genetics. John Wiley and Sons Inc. New York, London, Sydney, Toronto.
6. Garcia P., Perez de la Vega M., Benito C. (1982). The inheritance of rye seed peroxidases. Theor. Appl. Genet., 61: 341 - 351.
7. Łapiński M., Masojć P. (1983). Polymorphism of amylases from rye endosperm I. Variability of the electrophoretic patterns of amylases from inbred lines. Genet. Polon., 24: 131 - 137.
8. Masojć P. (1984). Amylases in the developing rye grain. Zesz. Nauk. Akad. Roln., Szczecin, 110: 13 - 21.
9. Masojć P. (1987). Genetics of α-amylases from rye endosperm. Theor. Appl. Genet., 73: 440 - 444.
10. Masojć P., Łapiński M. (1984). Polymorphism of amylases from rye endosperm II. Intra- and inter-cultivar variability of the electrophoretic pattern of amylases. Genet. Polon., 25: 17 - 27.
11. Nei M., Roychoudhury A. K. (1973). Sampling variances of heterozygosity and genetic distance. Genetics, 76: 379 - 390.
12. Perez de la Vega M., Allard R. W. (1984). Mating system and genetic polymorphism in populations of *Secale cereale* and *S. vavilovii*. Can. J. Genet. Cytol., 26: 308 - 317.
13. Perez de la Vega M., Vaquero F., Vences J., Garcia P. (1982). Isozymatic polymorphism and mating system in rye (*Secale cereale* L.). Tag.-Ber., Akad. Landwirtsch.-Wiss. DDR, Berlin, 198: 571 - 579.
14. Schlegel R., Mettin D., Melz G. (1985). Chromosome recognition and gene localisation in rye (*Secale cereale* L.) current status. Proceedings of Eucarpia Meeting of the Cereal Section on Rye (Svalöv, Sweden), June 11 - 13: 57 - 72.
15. Schmidt J. Ch., Seliger P., Schlegel R. (1984). Isoenzymes as biochemical markers of rye chromosomes. Biochem. Physiol. Pflanzen., 179: 197 - 210.
16. Sokal R. R., Rohlf F. J. (1969). Biometry. W. H. Freeman and Company, San Francisco.
17. Wehling P., Schmidt-Stohn G. (1984). Linkage relationships of esterase loci in rye. Theor. Appl. Genet., 67: 149 - 153.

DZIEDZICZENIE I POLIMORFIZM GENETYCZNY BETA-AMYLAZY Z BIELMA ŻYTA (*SECALE CEREALE* L.)

Streszczenie

Badano sposób dziedziczenia izoenzymów beta-amylazy, rozdzielonych techniką elektroforezy w żelu poliakrylamidowym. Wyniki badań potwierdzają hipotezę, że enzym jest kodowany przez dwa silnie sprzężone geny z pięcioma (β-Amy1), lub dwoma (β-Amy2) kodominującymi oraz recesywnymi allelami null, które dziedziczą się niezależnie od genów kontrolujących skład alfa-amylaz. U dziesięciu odmian żyta stwierdzono znaczną częstość alleli null oraz wysoki stopień heterozygotyczności. Rozkład genotypów był na ogół zgodny z zasadą równowagi Hardy-Weinberga.

НАСЛЕДОВАНИЕ И ГЕНЕТИЧЕСКИЙ ПОЛИМОРФИЗМ БЕТА-АМИЛАЗЫ ИЗ ЭНДОСПЕРМЫ РЖИ (*SECALE CEREALE* L.)

Резюме

Используя методы обычного генетического анализа, исследовался способ наследования изоферментов бета-амилазы, разделяемых техникой электрофореза в полиакриламидном геле. Результаты исследований подтверждают гипотезу, что фермент кодируется двумя генами, сильно сцеплёнными с пятью (β-Amy1) или двумя (β-Amy2) кодоминирующими и с рецессивными аллелями null, которые наследуются независимо от генов, контролирующих состав альфа-амилаз. У 10 сортов ржи обнаружено высокую частоту аллелей null, а также высокую степень гетерозиготности. Распределение генотипов в общем соответствовало равновесию Харди-Вайнберга.