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Genetics of α-amylases from rye endosperm*

P. Masojć

Department of Plant Breeding, Academy of Agriculture, Szczecin, Poland

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Summary. Fifteen inbred lines of rye, F_1 and F_2 progenies from crosses between lines were studied using polyacrylamide gel electrophoresis. Conventional genetic analysis of α-amylase zymograms showed that the 19 bands detected in the endosperm of germinating caryopses were controlled by three linked structural loci and one independent modifying locus, which influenced the electrophoretic mobility of isozymes. Two codominant alleles were found at the *α-Amy1, α-Amy2* structural loci and the *M-α-Amy* modifying locus while the *α-Amy3* locus had three alleles. Double-banded expression of the α-amylase alleles was probably due to the simultaneous presence of modified and unmodified forms of isozymes on the zymogram.

Key words: *Secale cereale* L. – Genetics – α-Amylase – Isozymes – Modifiers

Introduction

α-Amylase from cereal caryopses has received much attention because of its adverse influence on bread-making quality (Hagberg and Olered 1975). The reduction of α-amylase activity is the main problem in breeding sprouting-resistant cultivars. However, the efficiency of selection for low activity of the enzyme is limited by a lack of knowledge about the genetic control of its production.

α-Amylase, like many other enzymes, is produced in multiple molecular forms – isozymes (Frydenberg and Nielsen 1965). The inheritance of isozymatic composition of α-amylase has been studied intensively in wheat (Nishikawa and Nobuhara 1971; Nishikawa et al. 1981; Gale et al. 1983), maize (Chao and Scandalios 1971) and barley (Frydenberg et al. 1969; Brown and Jacobsen 1982) while the genetic basis of rye α-amylases still remains unknown. Perez de la Vega et al. (1982) as well as Masojć and Łapiński (1984) detected a considerable polymorphism of α-amylases in rye varieties. Intra-line variation of isozymes suggested that several loci with a number of alleles might be involved in α-amylase production (Łapiński and Masojć 1983).

This paper presents the results of an investigation into the inheritance of α-amylase isozymes in rye endosperm.

Materials and methods

The materials used in the study were 15 inbred lines. It was established experimentally that each line was internally monomorphic with respect to the composition of α-amylases. Since maternal effects influencing banding patterns could be expected, lines were crossed reciprocally. The inheritance of isozymes was analyzed in 18 F_1 and 18 F_2 progenies, using the X^2 test (Sokal and Rohlf 1969). The maximum likelihood equations developed by Allard (1956) were used for the determination of distance between loci.

Electrophoretic analysis of α-amylase was carried out on crude extracts from the starchy endosperm of seeds which had germinated for 5 days at room temperature. A single endosperm was ground in 0.1 ml of 0.1 mM acetate buffer (pH 5.0) containing 2 mM calcium chloride, the whole being thickened with 50 µl 80% sucrose. Approximately 50 µl of extracts were applied to the gel. The enzyme was separated on slabs of 5% polyacrylamide gel containing 0.125% soluble starch. The migration was to the anode. Electrophoretic and staining techniques used have been previously described (Łapiński and Masojć 1983). Additionally, re-electrophoresis of single bands cut out from the gel was carried out. The bands of α-amylase activity were identified by incubating the gels for 30 min at 40 °C against a dextrin-acrylamide film (MacGregor et al. 1974) attached to a glass plate and subsequently staining the film with iodine.

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Results

A total of 19 different bands of α-amylase activity, separated into two zones, were found in materials studied (Figs. 1 and 2). The bands were assigned the numbers 7a to 23 in accordance with the numeration presented in the former papers (Łapiński and Masojć 1983). Additionally, letter symbols were used to differentiate between very closely migrating and previously indistinguishable forms. Zone 1 enclosed 10 bands while 9 bands were grouped in the second zone. There were two types of bands in each of the two zones. Bands 11–15 and 19–23 were usually intensive and sharp (major bands) in comparison with bands 7a, 8, 10a, 10b and 16–18, which were diffuse and sometimes very faint (secondary bands). Bands of a different type were associated in definite pairs, as shown in Fig. 1. The re-electrophoresis of individual bands showed that the secondary bands are generated from appropriate major ones during the separation procedure.

Five crosses between the five homozygous quadruple-banded variants from zone 1 and six crosses between the four homozygous double-banded variants from zone 2 were made (Tables 1 and 2). The F_1 progenies produced patterns in which all parental bands were present. Reciprocal crosses exhibited that bands introduced by the maternal line were more intensive than those of paternal origin. Since the study material was the triploid tissue, differences in bands intensity might be attributed to gene dosage effects. Consequently, two types of heterozygotes could be distinguished in the F_2 generation when the relative intensity of bands was considered. However, in the analysis of segregation the frequencies of these types were combined.

Tests for heterogeneity revealed that in no case was there a significant difference between the F_2 progenies of the same cross type. Thus, only combined data is presented in Table 1. With the exception of one progeny, the segregation ratios observed in the 8 crosses corresponded to the 1:2:1 expectation for a single locus with two codominant alleles. In the remaining 3 crosses (Table 2) apart from parental bands, an additional bands appeared viz. 12a and 12b in cross 9, 20a and 20b in cross 10, 20b and 23 in cross 11. The bands formed nine phenotypes, which segregated in a ratio consistent with 1:2:1:2:4:2:1:2:1 expectation for two independent loci with two codominant alleles each. A definite associations were found between the isozymes of both zones. Bands 14 and 15 were accompanied by bands 20a, 22 or 23 while bands 11, 12a, 13 were always present together with bands 19, 20b or 21.

The genetic interpretation of the segregation data is shown in Fig. 1. Two structural loci, designated as *α-Amy1* and *α-Amy2*, with two codominant alleles each are supposed to encode for α-amylase composition in zone 1. For α-amylase zone 2 one structural locus,

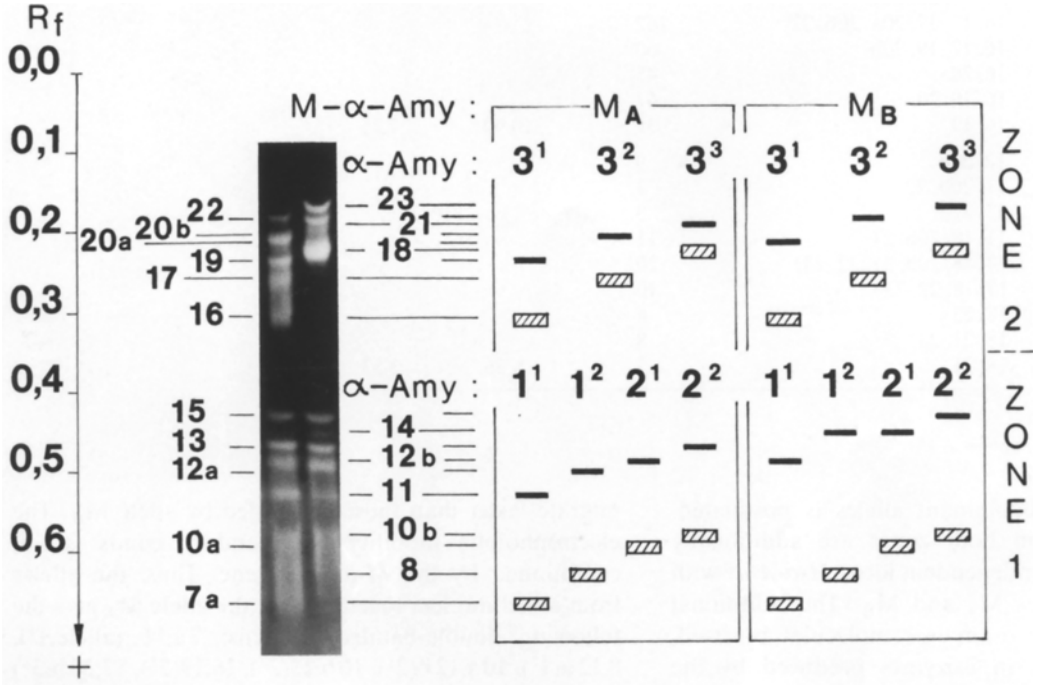

Fig. 1. α-amylase zymograms showing the 19 bands detected in rye endosperm and the banding phenotypes of the α-amylase alleles. Full bands and hatched bands represent major and secondary forms, respectively

Table 1. Single locus segregation of α-amylases in F_2 progenies of crosses between inbred lines

Cross no.	Parental phenotypes				No. of progenies	No. of phenotypes				$X^2_{1:2:1}$
	A		B			A	AB	B	Total	
1	Bands:	7a, 10b, 11, 13	Bands:	8, 10b, 12a, 13	3	174	381	169	724	1.04
2		7a, 10a, 11, 12b		7a, 10b, 11, 13	2	56	130	61	247	0.89
3		8, 10b, 12a, 13		8, 10b, 14, 15	2	161	338	173	672	0.45
4		7a, 10a, 11, 12b		7a, 10a, 12b, 14	1	7	19	6	32	1.19
5		16, 19		17, 20b	2	132	285	121	538	2.35
6		16, 19		18, 21	2	65	112	70	247	2.34
7		17, 20b		18, 21	7	267	589	261	1,117	3.40
8a		17, 20b		17, 22	2	75	160	66	301	1.74
8b		17, 20b		17, 22	1	117	263	160	540	7.18*

* Significant at 0.05 level

Table 2. Two locus segregation of α-amylases in F_2 progenies of crosses between inbred lines

Cross no.	Parentel phenotypes	F_2 phenotypes	No. of phenotypes	X^2 independence	X^2 linkage
9	7a, 10b, 11, 13	7a, 10b, 11, 13	25		
	8, 10b, 14, 15	7a, 8, 10b, 11, 12a, 13	62		
		8, 10b, 12a, 13	30		
		7a, 10b, 11, 12b, 13, 15	63		
		7a, 8, 10b, 11, 12a, 12b, 13, 14, 15[a]	134		
		8, 10b, 12a, 13, 14, 15	66		
		7a, 10b, 12b, 15	42		
		7a, 8, 10b, 12b, 14, 15	84		
		8, 10b, 14, 15	34	9.53	1.58
10	16, 19	17, 22	32		
	17, 22	17, 20b, 22	61		
		17, 20b	33		
		16, 17, 20a, 22	60		
		16, 17, 19, 20a, 20b, 22[a]	162		
		16, 17, 19, 20b	60		
		16, 20a	45		
		16, 19, 20a	67		
		16, 19	35	10.90	7.75
11	18, 21	17, 20b	9		
	17, 22	17, 20b, 22	7		
		17, 22	3		
		17, 18, 20b, 21	11		
		17, 18, 20b, 21, 22, 23[a]	20		
		17, 18, 22, 23	10		
		18, 23	4		
		18, 21, 23	8		
		18, 21	5	6.26	3.53

[a] Phenotype of F_1 progeny

α-Amy3 with three codominant alleles is postulated. Banding patterns from both zones are additionally controlled by a single independent locus *M-α-Amy* with two codominant alleles M_A and M_B. This additional gene does not encode α-amylase molecules by itself, but it causes changes in isozymes produced by the structural genes, which are reflected in alterations of their mobility. Major bands controlled by allele M_A migrate faster than those controlled by allele M_B. The electrophoretic mobility of secondary bands is not conditioned by the *M-α-Amy* gene. Thus, the alleles from structural loci together with the allele M_A give the following double-banded patterns: 7a,11 (allele 1^1), 8,12a(1^2), 10a,12b(2^1), 10b,13(2^2), 16,19(3^1), 17,20b(3^2) and 18,21(3^3). The interaction of the same structural alleles with the allele M_B yields different forms: 7a,12b

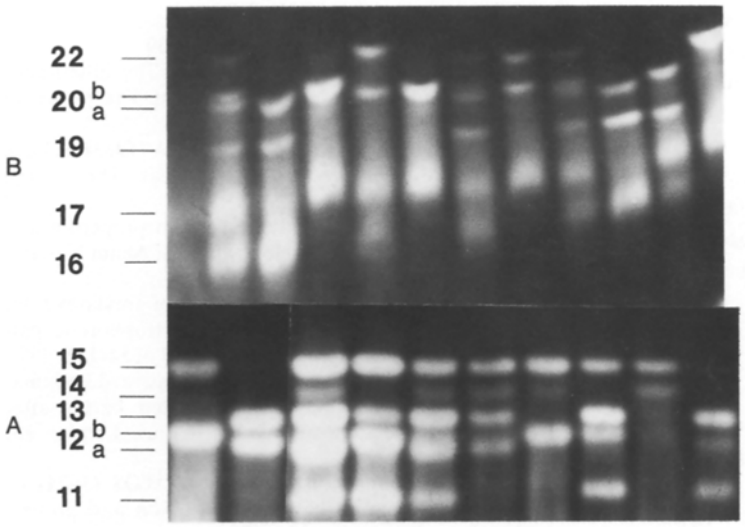

Fig. 2. Zymograms showing the phenotypic variation in F_2 progenies which is due to the independent segregation for *α-Amy1*^{1}/*α-Amy1*^{2} and M_A-*α-Amy*/M_B-*α-Amy* (**A**) and for *α-Amy3*^{1}/*α-Amy3*^{2} and M_A-*α-Amy*/M_B-*α-Amy* (**B**)

(allele 1^1), 8,14(1^2), 10a,14(2^1), 10b,15(2^2), 16,20a(3^1), 17,22(3^2) and 18,23(3^3).

In order to see at what stage, the *M-α-Amy* gene controls α-amylase production, the extracts obtained from two lines having different allelic composition at the *M-α-Amy* and structural loci were mixed together in 1 : 1 ratio. The mixtures were immediately subjected to electrophoresis. Additional, non-parental bands together with bands of each line were found to be present on the zymograms similarly as in appropriate F_1 hybrids. This result indicates that an additional locus controls the α-amylase banding pattern on post-translational level.

Linkage analysis made it possible to obtain only a rough estimate of recombination values for the structural loci. The approximate distances between *α-Amy2* and *α-Amy3* and between *α-Amy1* and *α-Amy3* were 4.5 ± 0.9 M and 7.0 ± 0.7 M, respectively. The recombination value for *α-Amy1* and *α-Amy2* loci was not determined for bands 12a and 12b partially overlapped, which made it difficult to differentiate between the parental and recombinant phenotypes.

Discussion

It is now clear that production of cereal α-amylase is controlled by several structural loci. In wheat, two triplicate gene series on the group 6 and group 7 chromosomes encode for α-amylase (Nishikawa and Nobuhara 1971; Nishikawa et al. 1981; Gale et al. 1983). Isozymes of α-amylase from barley aleurone are governed by two compound loci with a number of alleles located on chromosomes 1 and 6 (Brown and Jacobsen 1982). Two amylase loci have been also detected in maize (Chao and Scandalios 1971).

This study shows that rye α-amylases are controlled by highly organized gene system consisting of three linked structural and one independent modifying locus. Isozymes are supposed to originate as a result of the interaction of two loci, one of which is structural and the other one modifying. In view of this interpretation almost each of the 19 bands may be attributed to a definite allele or a pair of alleles. The two exceptions are the bands 12b and 14. The first can be controlled by *α-Amy2*^{1} and M_A *or α-Amy1*^{1} and M_B alleles while the latter by *α-Amy1*^{2} and M_B or *α-Amy2*^{1} and M_B alleles. Therefore, these bands could represent identical products of different pairs of alleles or, more likely, they may not have been completely resolved by the separation technique employed here. In fact, it is difficult to separate closely migrating α-amylase isozymes because of concentration effects, which usually occur in starch-containing media (Rozhkov 1980).

Products of alleles encoding for monomeric enzymes like α-amylase usually occur on zymograms as single bands. However, in the case of rye α-amylases, definite pairs of bands are inherited as a single units. Segregation data along with the results of re-electrophoresis of individual bands seem to indicate that faster bands of tandems (secondary bands) are unmodified, whereas slower ones (major bands) are modified forms of the enzyme. The generation of unmodified forms from modified ones, which takes place during the separation procedure resembles the conversion of α-amylase III bands to α-amylase II bands observed in barley after heat treatment (MacGregor and Ballance 1980). This phenomenon was explained by Weselake et al. (1983). They showed that the α-amylase III was a complex formed by α-amylase group II and a proteinaceous factor acting as an α-amylase II inhibitor. When the α-amylase II – inhibitor complex disrupts, α-amylase II is liberated and the conversion of α-amylase III to α-amylase II is observed on zymograms. The situation

found in rye may be interpreted similarly. In this case, each major band is supposed to be a complex consisting of the α-amylase produced by the structural gene and the modifying factor encoded by the *M-α-Amy* locus, whereas the secondary band may represent the isozyme free from the modifying factor. It is conceivable that the modifying factor is in fact an endogenous α-amylase inhibitor from rye endosperm.

The existence of two alleles at the *M-α-Amy* locus, acting without dominance, contributes to the maintenance of considerable polymorphism of banding patterns detected within rye varieties (Perez de la Vega et al. 1982; Masojć and Łapiński 1984). The allelic variation at the modifying locus is of significant genetic and evolutionary interest for it may cause functional differentiation of isozymes. This was clearly shown for xanthine dehydrogenase and aldehyde oxidase in *Drosophila melanogaster* (Finnerty and Johnson 1979). At present, the effects of the two forms of the modifying factor on the properties of rye α-amylase are not understood and a detailed studies must be made to clarify this problem.

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