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STRUCTURE OF THE ISOZYMES OF THE AAT-2 AND AAT-3 SYSTEMS OF ASPARATE AMINOTRANSFERASE IN WHEAT, RYE AND TRITICALE

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SUMMARY

The electrophoretic isozyme phenotypes for the AAT-2 and AAT-3 regions of leaves of *Triticum turgidum*, *T. aestivum*, *Secale cereale*, × *Triticosecale* (hexaploid) and *T. aestivum*/ *S. cereale* 6R addition line are described. The phenotypes varied in distribution and relative intensity of the isozyme bands, which were densitometrically measured. The results are consistent with a hypothesis of the dimeric structure for the AAT-2 and AAT-3 systems.

INTRODUCTION

Isozymes of aspartate aminotransferase (AAT, L-aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1. GOT, glutamate oxaloacetate transaminase) have been studied in several higher plant species and evidence on their dimeric structure has been obtained in *Zea mays* (MACDONALD & BREWBAKER, 1972; SCANDALIOS et al., 1975), in *Stephanomeria exigua* (GOTTLIEB, 1973) and in *Triticum aestivum* and the related species *Secale cereale, Agropyron elongatum, Hordeum vulgare* (HART, 1975; TANG & HART, 1975; HART et al., 1976; HART & LANGSTON, 1977; HART et al., 1980; HART & TULEEN, 1983; SALINAS & BENITO, 1985).

Genetic and biochemical analyses of active AAT isozymes in hexaploid wheat (2n = 6x = 42 and genome composition AABBDD) provide evidence on the expression of three genetically independent AAT systems: AAT-1, AAT-2 and AAT-3 in the zymogram phenotype of cv. Chinese Spring, which migrate from fast to slow rates during electrophoresis at slightly alkaline pH.

The chromosomal location of AAT structural genes of hexaploid wheat in cv. Chinese Spring was investigated by HART (1975). This analysis was carried out with appropriate aneuploid derivatives and provided evidence that the AAT-2 isozymes are encoded by a triplicate set of structural genes, designated as *Aat-A2, Aat-B2* and *Aat-D2* and located in the long chromosome arm of homoeologous group 6. The AAT-3 structural genes *Aat-A3, Aat-B3* and *Aat-D3* are located respectively on the chromosome arms 3AL, 3BL and 3DL.

In studies on *Secale cereale* (2n = 14 and genome formula RR) chromosome additions to *T. aestivum* (TANG & HART, 1975; SALINAS & BENITO, 1985) provide evidence on the existence of two structural genes, *Aat-R2* and *Aat-R3* which were placed on chromosomes 6R and 3R respectively.

This paper deals with an analysis of the AAT isozymes over horizontal polyacrylamide gels in different combinations of the genomes A, B and D of wheat and R of rye using densitometry to measure the relative intensity of the isozyme bands.

MATERIAL AND METHODS

The following material was chosen on account of the genome compositions:

AABBDD	*T. aestivum* cv. Chinese Spring
AABBDD+6R''	*T. aestivum* cv. Chinese Spring/*S. cereale* cv. Imperial 6R addition line
RR	*S. cereale*, line 'C-4'
AABBRR	6x-triticale cv. Torote
AABB	*T. turgidum* cv. Arcipreste

The adition line was supplied by Dr. E. R. Sears (University of Missouri, Columbia, USA) and the remaining material by Dr C. Soler (INIA, El Encín, Madrid, Spain).

The aspartate aminotransferase isozymes were separated using horizontal 8% polyacrylamide gel slab electrophoresis. Individual samples of plant leaf tissue were crushed and immersed in 25 µl 0.1 M. sodium acetate solution (pH = 7.2). Small pieces of filter paper (Whatman 3 MM of 2 × 10 mm) were soaked in the extract and then inserted into 180 × 280 × 2 mm polyacrylamide gels. The gel buffer was 0.015 M Tris-citric (pH 7.75) and the electrode buffer was 0.3 M NaOH-boric acid (pH 8.6). The samples were subjected to electrophoresis at 4 °C for 3 h. The mobility was directed from the cathodic to the anodic side. The gels were stained according to the method of HART (1975) and were scanned using a Joyce and Loebl Chromostan densitometre.

RESULTS

All material showed the three AAT regions in the zymogram phenotypes: AAT-1, AAT-2 and AAT-3 (Fig. 1) which were also well defined in the densitometric plots.

The densitometric patterns for AAT-3 are given in Fig. 2. A total of three AAT-3 bands which migrate at relatively fast, intermediate and slow rates AAT-3a, AAT-3b and AAT-3c, were observed with different relative intensities in the phenotype of common and durum wheats, 6x-triticale and 'Chinese Spring' 6R addition line. The relative intensity of the AAT-3b band of *T. turgidum* was twice as strong as the relative intensity of both AAT-3a and AAT-3c. The AAT-3a and AAT-3b bands of *T. aestivum* cv. Chinese Spring and the wheat-rye 6R addition line were more intensely stained than AAT-3c. For 6x-triticale the AAT-3b and AAT-3c bands were more intensely stained than AAT-3a. *S. cereale* cv. C-4 showed only one band in its zymogram phenotype at AAT-3c.

The AAT-2 densitometric plots are presented in Fig. 3. Among the material examined we observed a total of five AAT-2 bands which migrate at relatively different rates from fast to slow: AAT-2a, AAT-2b, AAT-2c, AAT-2d and AAT-2e. The line

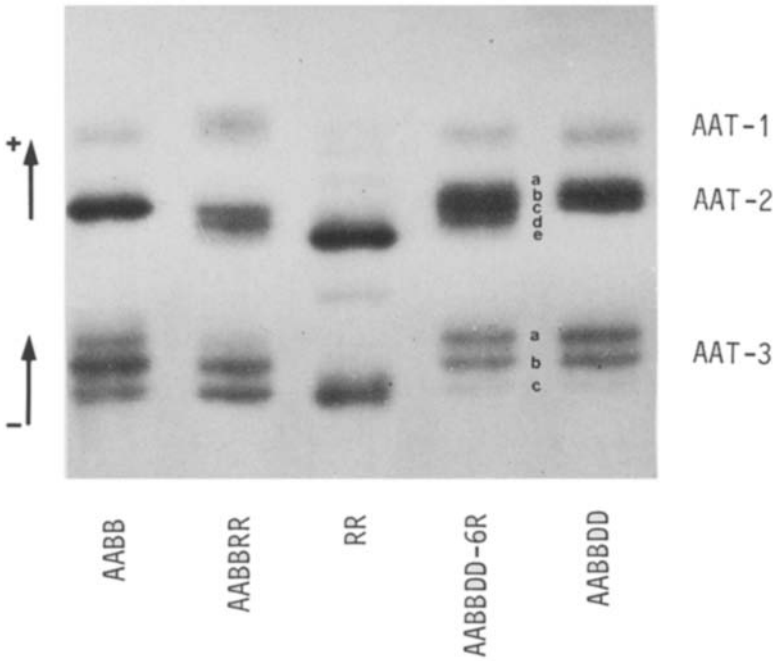

Fig. 1. Zymogram of AAT in polyacrilamide gel. AABB: 'Arcipreste' durum wheat cultivar; AABBRR: 'Torote' 6x-triticale cultivar RR; 'C-4' rye cultivar; AABBDD-6R: Chinese Spring/Imperial 6R disomic wheat-rye addition line; AABBDD: 'Chinese Spring' common wheat cultivar.

'C-4' of *S. cereale* only showed the AAT-2e isozyme band which has an electrophoretic mobility that coincides with the slow migration rate of 6x-triticale and the wheat-rye 6R addition line. The cv. Arcipreste of *T. turgidum,* expressed only the AAT-2c isozyme band with an intermediate rate of migration. The relative intensity of the AAT-2b and AAT-2c bands of *T. aestivum* cv. Chinese Spring was the same, but higher than that of AAT-2a. The AAT-2c and AAT-2d bands of 6x-triticale had a similar staining intensity, but higher than the AAT-2e band. The five isozyme bands of the wheat-rye 6R addition line exhibited the following relative staining intensities: AAT-2c > AAT-2b = AAT-2d > AAT-2a = AAT-2e.

DISCUSSION

The results obtained for the AAT-3 system coincide well with the hypothesis of HART (1975, 1979) who assumed that AAT-3 isozymes would be constituted as dimers having approximately equal quantities of four subunits designated as α^3, β^3, δ^3 and ρ^3 (respectively assigned to genomes A,B,D and R) (Table 1). These subunits associate randomly into approximately equal active dimers that give three isozyme bands in the zymogram. HART & LANGSTON (1977) carried out studies of reassociation of the subunits into active enzymes which served to demonstrate that these molecules exist functionally as dimers. Thus, AAT-3c band of cv. Chinese Spring would be composed of $\alpha^3\alpha^3$ homodimers, AAT-3a of $\beta^3\beta^3$ and $\delta^3\delta^3$ homodimers and $\beta^3\delta^3$ heterodimers, and AAT-3b

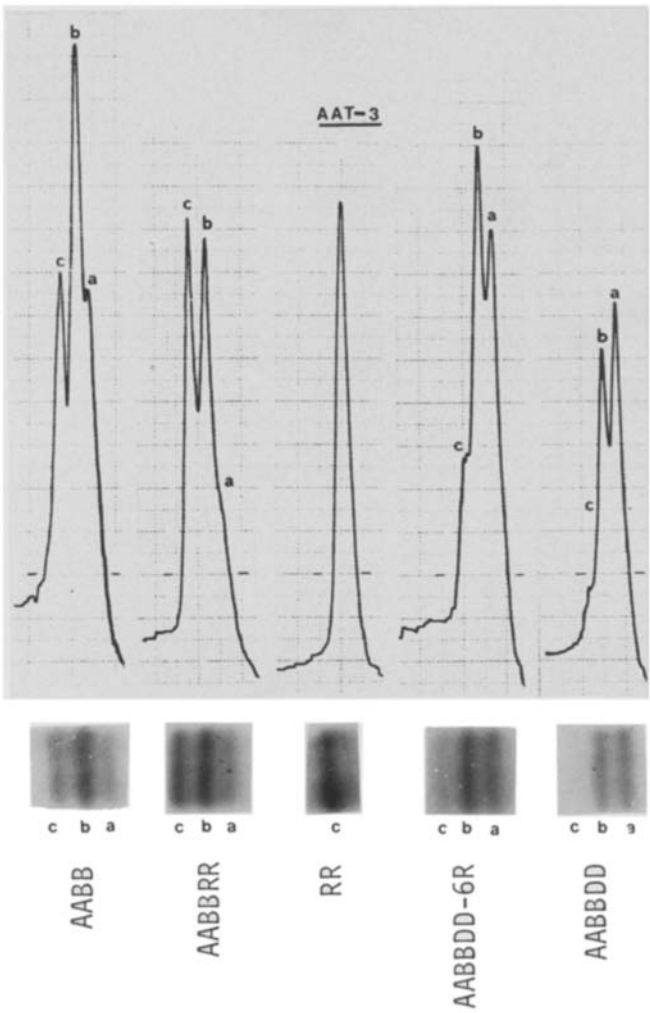

Fig. 2. AAT zone 3 densitometry of same material presented in Fig. 1.

of $\alpha^3\beta^3$ and $\alpha^3\delta^3$ heterodimers. The products of triplicate *Aat-3* structural genes of wheat and of the homoeologous in rye could combine to produce different dimeric molecules.

The results on the intensity and mobility of bands of the AAT-2 system also are consistent with the production of dimeric forms of active isozymes. These are formed by random association of the subunits produced by the structural genes *Aat-A2, Aat-B2* and *Aat-D2* of wheat and *Aat-R2* of rye that are respectively located on the chromosome arms 6AL, 6BL and 6DL (HART, 1975, 1979, 1984; TANG & HART, 1975) and 6RL (SALINAS & BENITO, 1985). These subunits could be designated α^2,β^2,δ^2 and ρ^2 respectively (Table 1). The following subunit combination can be assumed for the formation of the active isozyme molecules in each material:

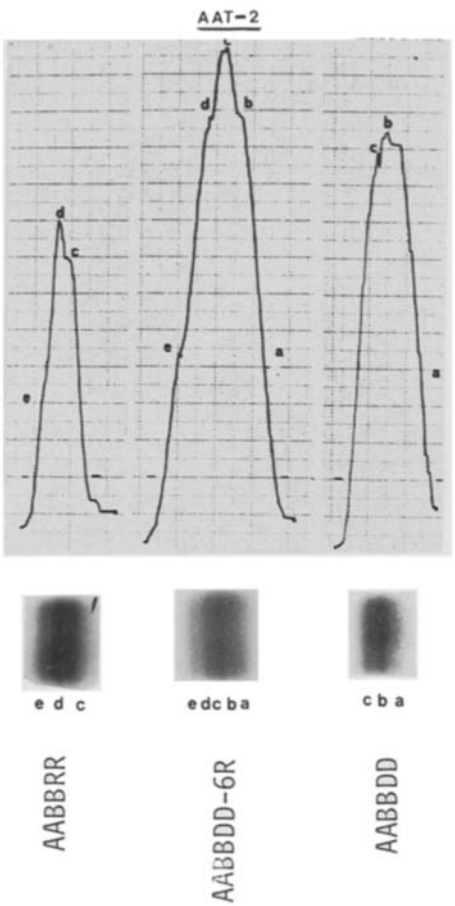

Fig. 3. AAT zone 2 densitometry of same material presented in Fig. 1.

Table 1. Schematic model for the subunit composition of the AAT-2 and AAT-3 systems in *T. turgidum*, hexaploid triticale, *S. cereale*, *T. aestivum*/*S.cereale* 6R addition line and *T. aestivum*.

Isozymes	Genomes				
	AABB	AABBRR	RR	AABBDD+6R″	AABBDD
AAT-2a				$\delta^2\delta^2$	$\delta^2\delta^2$
AAT-2b				$\alpha^2\delta^2,\beta^2\delta^2$	$\alpha^2\delta^2,\beta^2\delta^2$
AAT-2c	$\alpha^2\alpha^2,\beta^2\beta^2,\alpha^2\beta^2$	$\alpha^2\alpha^2,\beta^2\beta^2,\alpha^2\beta^2$		$\alpha^2\alpha^2,\beta^2\beta^2,\alpha^2\beta^2$	$\alpha^2\alpha^2,\beta^2\beta^2,\alpha^2\beta^2$
AAT-2d		$\alpha^2\rho^2,\beta^2\rho^2,$		$\alpha^2\rho^2,\beta^2\rho^2$	
AAT-2e		$\rho^2\rho^2$	$\rho^2\rho^2$	$\rho^2\rho^2$	
AAT-3a	$\beta^3\beta^3$	$\beta^3\beta^3$		$\beta^3\beta^3,\delta^3\delta^3,\beta^3\delta^3$	$\beta^3\beta^3,\delta^3\delta^3,\beta^3\delta^3$
AAT-3b	$\alpha^3\beta^3$	$\alpha^3\beta^3,\beta^3\rho^3$		$\alpha^3\beta^3,\alpha^3\delta^3$	$\alpha^3\beta^3,\alpha^3\delta^3$
AAT-3c	$\alpha^3\alpha^3$	$\alpha^3\alpha^3,\rho^3\rho^3,\alpha^3\rho^3$	$\rho^3\rho^3$	$\alpha^3\alpha^3$	$\alpha^3\alpha^3$

Table 2. χ^2 tests comparing the observed densitometric values and the expected ones on the assumption of random association in equal active dimeric molecules of different subunits (densitometric values are refered to mms. from the basis to the top for each plot).

Isozymes	Genomes											
	AABB			AABBRR			AABBDD+6R''			AABBDD		
	obs.	exp.	χ^2	obs.	exp.	χ^2	obs.	exp.	χ^2	obs.	exp.	χ^2
AAT-2a							25	23		24	22.2	
AAT-2b							98	92		94	92.4	0.30⁻
AAT-2c				60	64.4		117	138	5.89⁻	89	92.4	
AAT-2d				70	64.4	0.87⁻	98	92				
AAT-2e				15	16.2		30	23				
AAT-3a	66	64.7		20	20		81	92		53	58.2	
AAT-3b	123.0	129.6	0.7⁻	78	80	0.1⁻	100	92	2.40⁻	63	58.2	0.87⁻
AAT-3c	10	64.7		82	80		26	23		15	14.6	

⁻P value not significant to the 5% level.

i) The AAT-2e isozyme band of rye, which showed the slowest rate of migration, could be due to the formation of the $\rho^2\rho^2$ homodimers. This band also is observed in all material having the R genome or the 6R chromosome pair.
ii) The AAT-2c is the only isozyme band observed in *T. turgidum* which has the genome composition AABB. Consequently, it is formed of the $\alpha^2\alpha^2$, $\beta^2\beta^2$ and $\alpha^2\beta^2$ dimers.
iii) The 6x-triticale shows a 4:4:1 relative staining intensity distribution for the AAT-2c, AAT-2d and AAT-2e isozyme bands, respectively. This observation is enterely consistent with the expectations if the following dimers are formed: AAT-2c = $\alpha^2\alpha^2$, $\beta^2\beta^2$ and $\alpha^2\beta^2$, AAT-2d = $\alpha^2\rho^2$ and $\beta^2\rho^2$, and AAT-2e = $\rho^2\rho^2$.
iv) *T. aestivum* cv. Chinese Spring exhibits a 1:4:4 AAT-2a, AAT-2b, AAT-2c quantitative distribution of staining intensity, and the random association of subunits corresponds well to the hypothesis that AAT-2a is formed by the $\delta^2\delta^2$ homodimer, being AAT-2b = $\alpha^2\delta^2$ and $\beta^2\delta^2$ and AAT-2c = $\alpha^2\alpha^2$, $\beta^2\beta^2$ and $\alpha^2\beta^2$.
v) Five AAT-2 isozyme bands in *T. aestivum* cv. Chinese Spring/*S. cereale* cv. Imperial 6R addition lines are present. These are the expression of all possible combinations of the subunits α^2, β^2, δ^2 and ρ^2. This is in agreement with the expected 1:4:6:4:1 random distribution.

Table 2 shows the expected mobility and quantitative distribution of the possible dimeric molecules for the material studied for both the AAT-3 and AAT-2 systems. χ^2 tests comparing the densitometric values with the expected ones were not significant for all material in both AAT-3 and AAT-2 systems. Consequently, the densitometric values for the relative staining intensity of the three zones of AAT-3 activity are in agreement with the expected ones on the assumption that dimeric molecules are formed. Moreover, an excellent adjustment between the model for the subunit composition of the AAT-2 isozymes and the zymogram observed was found in all the genome combinations here studied.

The hypotheses of dimeric structure and genetic control of AAT-3 and AAT-2 system in wheat, rye, and 6x-triticale that were originally derived from genetic and bio-

chemical studies (HART, 1975; TANG & HART 1975; HART & LANGSTON, 1976, 1977; HART et al., 1976; HART et al., 1980) are strongly supported by the results here presented.

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