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AN α-AMYLASE GENE FROM *AEGILOPS VENTRICOSA* TRANSFERRED TO BREAD WHEAT TOGETHER WITH A FACTOR FOR EYESPOT RESISTANCE

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SUMMARY

VPM 1, a bread wheat with eyespot resistance derived from *Aegilops ventricosa*, was shown to have a characteristic α-amylase allele on chromosome 7D, *α-Amy-D2b*, also derived from *Ae. ventricosa*. Chromosome substitution lines confirmed that the eyespot resistance of VPM 1 was also determined by chromosome 7D. Among F_3 families from a cross between VPM 1 and a line lacking this eyespot resistance and the α-amylase marker allele, the two characters segregated independently. This finding reduces the value of the allele as a marker for eyespot resistance, but suggests that VPM 1 possesses a large segment of chromosome from the D genome of *Ae. ventricosa*.

1. INTRODUCTION

Resistance to eyespot (*Pseudocercosporella herpotrichoides*) in European cultivars of bread wheat, *Triticum aestivuum* (AABBDD, $2n = 6x = 42$), is mainly derived from the French cultivar Cappelle-Desprez. It has proved durable despite widespread exploitation for about 30 years. More potent resistance, derived from *Aegilops ventricosa* (DDM^vM^v, $2n = 4x = 28$), has been introduced into a hexaploid wheat line, VPM 1 (Maia, 1967; Doussinault and Dosba, 1977). Although its durability is untested it is a potentially valuable source of resistance, particularly if combined with resistance from Cappelle-Desprez, and it might render unnecessary the use of fungicides to control eyespot.

Law *et al.*, (1975) showed that the major nuclear component of resistance in Cappelle-Desprez was carried on chromosome 7A. They suggested that the resistance of VPM 1 might also be determined by the group 7 chromosomes. Jahier *et al.*, (1979) showed by F_2 monosomic analysis that the resistance of Roazon, a cultivar derived from VPM 1, was associated with chromosome 7D.

Selection for resistance to eyespot among breeding lines is laborious, and an easily scored marker on chromosome 7D, with no deleterious agronomic effects and sufficiently closely linked to the factor for eyespot resistance, would be extremely valuable. The group 7 chromosomes of wheat carry the triplicate α-amylase structural gene series *α-Amy-2* (Nishikawa and Nobuhara, 1971) which encodes 16 distinguishable *α-AMY-2* isozymes (Gale *et al.*, 1983). This paper describes the analysis of these isozymes in VPM 1. It also reports experiments which examine the association between eyespot resistance and the isozyme phenotype in some substitution lines and segregating lines derived from VPM 1.

2. Materials and Methods

(i) *Genotypes*

Aegilops ventricosa (line No. 11 supplied by G. Doussinault and Accession 'A', PBI collection); *Triticum durum* cv. Cando; and the following lines of *T. aestivum*: VPM 1 (Doussinault and Dosba, 1977); Fundin (Vilmorin/Vg 8058//Cappelle-Desprez///TJB 16-8), susceptible to eyespot; Hobbit'S' (Marne/Vg 9144//Professeur Marchal///TJB 16-8), with moderate eyespot resistance derived from Cappelle–Desprez; CWW 3319/5, a breeders line derived from VPM 1 with a similar degree of eyespot resistance (Bingham, 1983); Roazon (VPM 1/Moisson; Jahier *et al.*, 1979), with similar eyespot resistance; Chinese Spring (CS), susceptible to eyespot and the CS nullisomic 7D tetrasomic 7B stock.

Segregating lines. Twenty-seven F_3 families derived from random F_2 plants from the cross VPM 1 × Fundin.

Intervarietal chromosome substitutions. A substitution of chromosome 7D from VPM 1 into Hobbit'S' produced by four or five backcrosses onto Hobbit'S' monosomic 7D before extraction of the 42 chromosome disome. These lines are designated Hobbit'S' (VPM-7D) (Law and Worland, 1982).

(ii) *α-Amylase analysis*

The enzyme was generated by gibberellic acid induction of half-grains and the isozymes assayed on flat bed polyacrylamide isofocussing gels as described in Gale *et al.*, (1983).

(iii) *Assessment of eyespot resistance*

Resistance was assessed on seedlings grown in the glasshouse, inoculated by placing a cylinder of straw infested with *P. herpotrichoides* around the coleoptile. The degree of penetration of the pathogen through leaf sheaths was assessed as described by Scott (1971). There were eight to ten replicate plots of each line, arranged in randomised blocks; each plot consisted of four inoculated seedlings.

3. Results

(i) *α-Amylase analysis*

Sixteen isozyme bands are encoded by the *α-Amy*-2 loci on chromosomes 7A, 7B and 7D. Analysis of the CS nulli-tetrasomic series has shown that three of these isozymes are the products of *α-Amy-D2* on 7D (Gale *et al.*, 1983).

Confirmation of this result is shown by comparisons between the CS and CS nullisomic 7D tetrasomic 7B tracks and the Fundin and *T. durum* tracks in fig. 1(a). In both pairs the genotypes lacking chromosome 7D (the tetraploid lacks the entire D genome) also lack isozymes 9, 13 and 16. The CS genotypes also differ from Fundin and the tetraploid in that they show isozyme 11 rather than B1. This is due to the presence on chromosome 7B of the *α-Amy-B2b* allele instead of the '*a*' allele in CS, and is irrelevant to the present investigation.

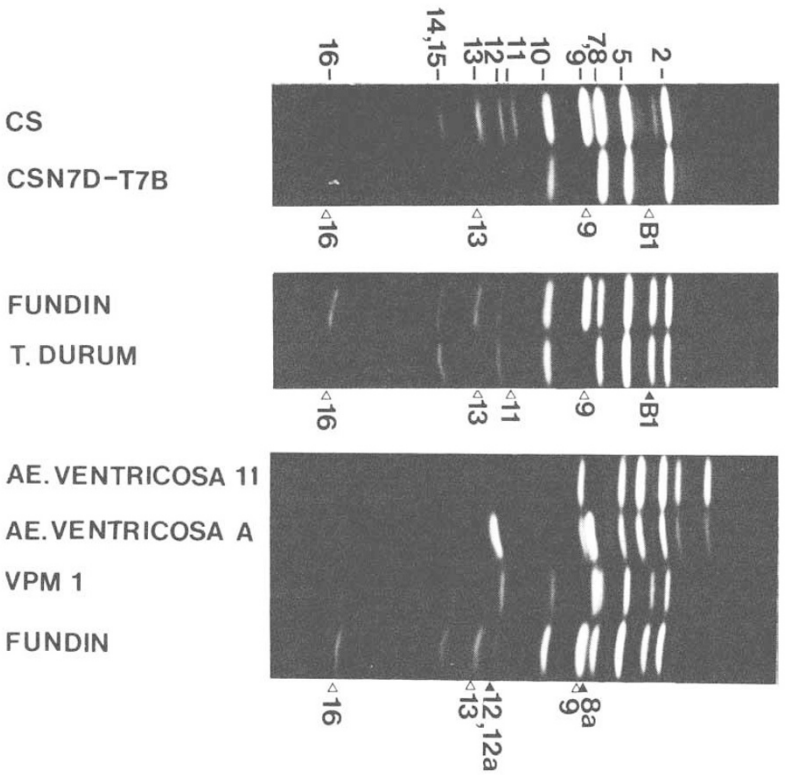

PLATE. 1 *α-AMY-2* phenotypes. (a) Genotypic comparisons showing the isozymes controlled by *α-Amy-D2* in bread wheat and the exchange in VPM 1 of the *α-Amy-D2a* allele for *α-Amy-D2b* from *Ae. ventricosa*. (b) A sample of VPM 1 × Fundin F_3s segregating at the *α-Amy-D2* locus. (For explanations see text).

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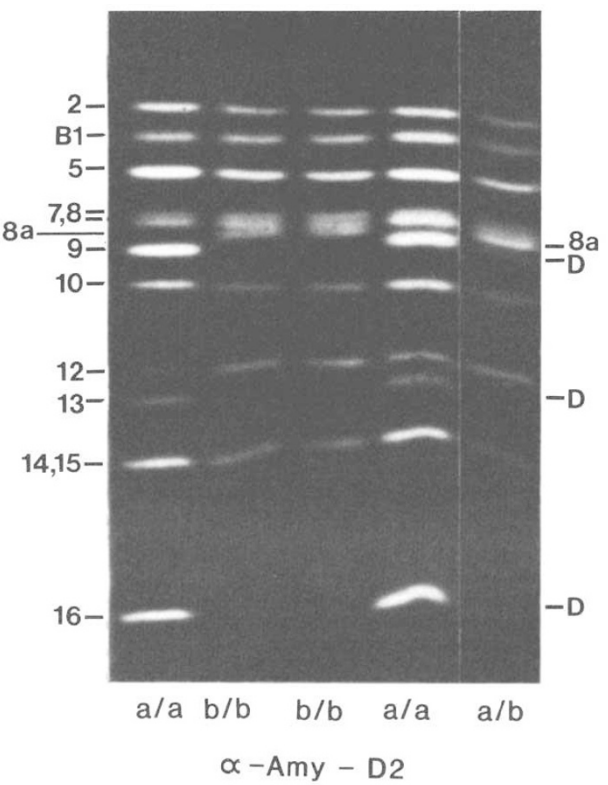

α-Amy - D2

The zymogram of VPM 1 shows that all three *α-Amy-D2* isozymes (9, 13 and 16) are absent but are replaced by two new isozymes (8a and 12a). Isozymes 12 and 12a have similar pIs and are often seen separated although they are superimposed in plate 1(a). Isozyme 12a is always stronger than 12. Examination of the two accessions of *Ae. ventricosa* shows that isozymes with pIs very similar to 8a and 12a are present in Accession 'A' but not in 'No. 11', from which eyespot resistance was originally transferred. The correspondence is, in fact, so precise that it seems likely that at some time in the last twenty years, since the original hybrid was made, the stocks have altered. Heterozygosity at the *α-Amy-D2* locus in the original 'No. 11' stock could be responsible.[1]

The *α-AMY-2* patterns obtained from three grains from each of 27 segregating F_3 families derived from VPM 1×Fundin (sample shown in plate 1(b)) show clearly that the isozymes 8a and 12a represent the product of an alternative allele, *α-Amy-D2b*, to that carried by Fundin, *α-Amy-D2a*.[2] The segregation of 8 *α-Amy-D2a/a*:13*a/b*:6*b/b*, with no non-parental combinations indicates that, for mapping or marker purposes, the two *α-Amy-D2* phenotypes are produced by a single allelic difference. Although the numbers are low there is no indication of any reduction in the rate of transmission of *α-Amy-D2b*.

The *α-Amy-D2b* phenotype of VPM 1 was also displayed by the Hobbit'S' (VPM-7D) substitution, but not by CWW 3319/5 or Roazon, both of which are eyespot-resistant breeders' lines derived from VPM 1.

(ii) *Eyespot resistance*

The resistance of the Hobbit'S' (VPM-7D) substitution line was assessed in three separate tests, together with the parental lines (table 1(a)). In each test the substitution line was significantly more resistant ($P<0{\cdot}001$) than the recipient parent, Hobbit'S', and was comparable in resistance with VPM 1.

Resistance was assessed in seven VPM 1×Fundin F_3 families that were homozygous for *α-Amy-D2a* and in five families homozygous for *α-Amy-D2b*, together with the parental lines. Resistance of the F_3s showed a continuous distribution between the parental values (table 1(b)). There was no association between α-amylase phenotype and eyespot resistance.

4. Discussion

The variant *α-AMY-2-D* phenotype of VPM 1 is clearly entirely due to an allelic difference at the *α-Amy-D2* locus on chromosome 7D. This is shown by the absence in VPM 1 of the isozymes produced by the compound *α-Amy-D2a* allele, and by the cosegregation of the novel isozymes found in VPM 1 with the three *a* allele isozymes. The novel isozymes correspond with two displayed by an accession of *Ae. ventricosa*. This difference confirms the genotype of the Hobbit'S' (VPM-7D) substitution line. The eyespot

[1] Note added in proof. Dr. Doussinault (pers. comm.) has resolved this problem by confirming that, while Accession No. 11 has been used in later interspecific crosses, another accession, 'Np. 10', was actually employed in the breeding of VPM 1.

[2] The system of allele designation follows that used by Ainsworth *et al.*, (1984). By convention the *a* allele is that carried by Chinese Spring.

TABLE 1

Eyespot score and α-Amy-D2 genotype for the (a) Hobbit'S' and Hobbit'S' (VPM 7D) substitution line and (b) the parents and 12 F_3 families derived from VPM 1 × Fundin

(a)	Line	1981	1982	1983	*α-Amy-D2* alleles
	Hobbit'S'	6·16	7·51	7·53	*a/a*
	VPM 1	4·16	5·26	5·06	*b/b*
	Hobbit'S' (VPM 7D)	4·39	5·18	4·30	*b/b*
	LSD (5%)	1·09	0·82	0·82	
		Fundin		6·04	*a/a*
(b)	Parents	VPM 1		3·79	*b/b*
	F_3 families	25/6/1		5·42	*a/a*
		26/5/5		4·15	*a/a*
		25/4/5		3·88	*a/a*
		25/5/3		5·15	*a/a*
		25/6/5		4·44	*a/a*
		25/7/1		4·88	*a/a*
		26/7/5		4·48	*a/a*
		25/6/6		4·72	*b/b*
		26/2/2		5·94	*b/b*
		26/3/5		4·88	*b/b*
		26/5/4		4·17	*b/b*
		26/8/2		6·00	*b/b*
	LSD (5%)			0·84	

resistance of this line confirms chromosome 7D as the carrier of the resistance factor transferred from *Ae. ventricosa*.

In the VPM 1 × Fundin F_3 families, independent segregation of eyespot resistance and the *α-Amy-D2* alleles showed that the loci concerned are widely separated on chromosome 7D. This suggests that a large segment of the *Ae. ventricosa* chromosome is present in VPM 1. By analogy with *α-Amy-B2* on chromosome 7B, the α-amylase locus is expected to lie on the long arm of 7D close to the centromere (6 per cent recombination) (Gale *et al.*, 1983). The linkage area would therefore extend well out on both arms, indicating that the eyespot factor is located in a distal region and would probably not be mapped by telocentric location methods.

The finding that the alien segment is greater than 50 recombination units suggests that there is considerable scope for increasing the wheat 7D content of eyespot-resistant breeding lines by selection, to eliminate any deleterious effects on yield or quality often associated with large alien transfers (Law, 1981).

The high apparent recombination within the alien segment also indicates that the transfer was made from the D genome rather than the M^v genome of *Ae. ventricosa*.

The *α-Amy-D2* phenotype provides a simple test for chromosome 7D from VPM 1, in the development of chromosome substitution lines to improve the eyespot resistance of wheat breeding lines (Scott and Hollins, 1982). Unfortunately it cannot be used as a marker for the VPM 1 eyespot resistance in conventional breeding programmes, as was confirmed by the absence of the *α-Amy-D2b* in the eyespot-resistant breeding line CWW 3319/5 and the cultivar Roazon. Other enzyme loci are known on chromo-

some 7D and these are being investigated to find a variant in the transferred segment closer to the eyespot resistance factor.

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