Heredity (1982), **49** (3), 353–357 0018-067X/82/05430353$02.00

MAPPING OF AN ALCOHOL DEHYDROGENASE (Adh-A1) STRUCTURAL GENE ON CHROMOSOME 4A OF DURUM WHEAT

K. N. SUSEELAN, M. V. PRABHAKARA RAO, C. R. BHATIA and I. NARAYANA RAO

Biology and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay-400085, India

Received 1.vi.82.

1. Introduction

The utility of isozymes as chromosome markers in polyploid wheats is well established. Many of the enzyme structural genes are present as triplicate and duplicate series in hexaploid and tetraploid wheats, respectively. Enzyme variants, except null mutations, can be identified in the heterozygous condition because they are normally codominant. Over 75 genes coding for the various isozymes have been located on different chromosomes (arms) of hexaploid wheat, largely on the basis of aneuploid analysis (Hart, 1979 and references therein). Using allelic variants, genes coding for alpha amylase have been mapped on chromosome 6B (*Amy*-6B1 and *Amy*-6B2) and 6D (*Amy*-6D1) (Nishikawa *et al.*, 1981). Hart *et al.* (1976) used alien translocations to map *Got*-D3 (now designated *Got*-Ag3, McIntosh, 1978). One of the reasons for the paucity of linkage data is the lack of reports on allelic isozyme (allozyme) variants. Using a variant of ADH reported earlier (Mahajan, 1975), we have mapped the *Adh*-A1 gene on chromosomal arm 4Ap of *durum* wheat.

NAD-dependent ADH (EC1.1.1.1) observed in wheat seed extracts is a dimeric enzyme, the structural gene(s) for which were located on the "p" arms of the group 4 chromosomes (Hart, 1970, 1979). It was expected that the chromosomal location of the two genes coding for ADH in *durum* wheat would be on 4Ap and 4Bp.

The gene for semi-dwarf plant type (*Gai*1/*Rht*1) is located 15 crossover units from the centromere on the "p" arm of chromosome 4A in bread wheat (McVitte *et al.*, 1978). It was therefore reasonable to infer that a dwarf *durum* cultivar homozygous for the Norin-10 dwarfing gene would carry *Gai*1/*Rht*1 on chromosomal arm 4Ap. The ADH phenotype of this cultivar was ascertained and it was shown to have the common *Adh*-A1a allele.

2. Materials and methods

A tall *durum* wheat (*Triticum turgidum* conv. *durum* (Desf.) Mac Key, cultivar *Bijaga yellow* (*gai*1/*gai*1 *Adh*-A1b/*Adh*-A1b) was crossed reciprocally to a semi-dwarf *durum* cultivar *Malavika* (*Gai*1/*Gai*1, *Adh*-A1a/*Adh*-A1a). The F_1 plants were intermediate in height and their alcohol dehydrogenase zymogram showed a five band pattern. The individual F_2 families were grown separately and scored for gibberellin (GA_3) sensitivity, plant height and seed ADH phenotypes.

For the GA response tests, seeds of the two parents and F_2 were sown in filter paper folds (Myhill and Konzak, 1967) immersed in 10^{-4} M GA_3 solution (Gale and Marshall, 1975). Seedling height was measured on the sixth day after sowing and the seedlings then transplanted into pots. The plant height was measured at maturity to distinguish between homozygous and heterozygous GA-insensitive plants. Extraction and electrophoretic procedures used for the determination of the ADH phenotypes were the same as reported previously (Mitra and Bhatia, 1971).

3. Results and discussion

The ADH zymogram of seed extracts of cv *Malavika*, *Bijaga yellow* and their reciprocal hybrids are shown in fig. 1. Malavika shows the normal ADH pattern consisting of three bands ADH-1-3, 1-4 and 1-5 with relative band intensities of 1:2:1. These bands are formed by the association of the αa and βa monomers. The bands correspond to the $\alpha a \alpha a$, $\alpha a \beta a$, and $\beta a \beta a$ dimers, respectively. The structural genes for αa and βa monomers are located on chromosomal arms 4Ap and 4Bp, respectively. In the variant, the monomer designated αb moves faster than αa and consequently the mobility of the $\alpha b \alpha b$ and $\alpha b \beta a$ is altered resulting in three widely spaced bands.

The F_1 hybrids show five bands ADH-1-1 to 1-5 with the dimeric composition $\alpha b \alpha b$; $\alpha b \alpha a$; $\alpha a \alpha a + \alpha b \beta a$; $\alpha a \beta a$ and $\beta a \beta a$, respectively. The relative intensities of the bands differ in reciprocal hybrids due to the fact that the enzyme phenotype is determined in the endosperm which is a triploid tissue.

(i) *Segregation for ADH in semi-dwarf F_2 plants*

In the first experiment only 27 semi-dwarf F_2 plants were analysed for the ADH phenotypes, of which the numbers showing normal, heterozygous and variant phenotypes were 22, 4 and 1, respectively. If the ADH and *Gai* 1 genes were not linked one would expect a 1:2:1 segregation. The data deviate from this ratio suggesting linkage. Seeds harvested from these semi-dwarf plants were checked for their GA sensitivity and plant height. All were GA insensitive and semi-dwarf. However, as these data were inadequate for the estimation of linkage, another F_2 population was analysed for the segregations of ADH, GA sensitivity and plant height.

(ii) *F_2 segregation for ADH*

When single F_2 seeds were analysed for the ADH phenotype all the four phenotypes shown in fig. 1 were observed. The two five band phenotypes were grouped together. The segregation of the three classes of the normal, heterozygous and variant phenotypes fits the monogenic ratio 1:2:1 (table 1).

(iii) *F_2 segregation for gibberellin sensitivity and plant height*

The F_2 seedlings were tested for their GA response which showed a 3:1 segregation for insensitive and sensitive seedlings. The insensitive

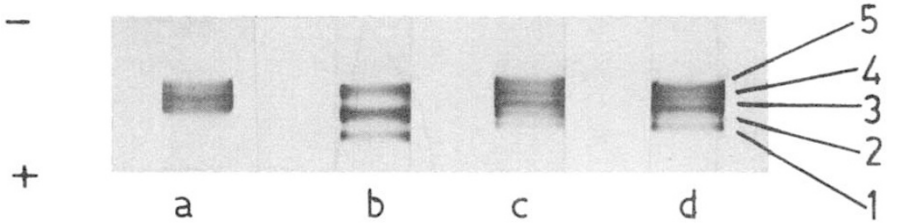

FIG. 1.—Alcohol dehydrogenase zymogram of (a) Normal (Malavika), (b) Variant (Bijaga yellow) and the F_1 hybrids, (c) Malavika × Bijaga yellow (d) Bijaga yellow × Malavika. The dimer composition of the bands in the hybrids are 1 ($\alpha b \alpha b$) 2 ($\alpha b \alpha a$) 3 ($\alpha a \alpha a + \alpha b \beta a$) 4 ($\alpha a \beta a$) and 5 ($\beta a \beta a$).

TABLE 1

Segregation for gibberellin response, plant height and ADH phenotype in the F_2 of a cross between Bijaga yellow (gai1/gai1, Adh-*A1b*/Adh-*A1b*) *and Malavika* (Gai1/Gai1, Adh-*A1a*/Adh-*A1a*)

Segregation for	Number obtained	χ^2 value Calculated	Table (0·05)
GA insensitive vs sensitive	66 : 26	0·522 (3 : 1)	3·841
Tall vs semi-dwarf	52 : 21	0·553 (3 : 1)	3·841
ADH (aa : ab : bb)	25 : 32 : 12	5·261 (1 : 2 : 1)	5·991
Single grains of F_2 ADH (aa : ab : bb)	7 : 24 : 12	1·744 (1 : 2 : 1)	5·991
Joint segregation in the F_2:			
*Gai*1/*Gai*1, *Adh*-A1a/*Adh*-A1a	17		
*Gai*1/*Gai*1, *Adh*-A1a/*Adh*-A1b	6		
*Gai*1/*Gai*1, *Adh*-A1b/*Adh*-A1b	0		
*Gai*1/*gai*1, *Adh*-A1a/*Adh*-A1a	5		
*Gai*1/*gai*1, *Adh-A1a*/*Adh-A1b*	17		
*Gai*1/*gai*1, *Adh-A1b*/*Adh-A1b*	3	53·145 (1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1)	15·50
*gai*1/*gai*1, *Adh*-A1a/*Adh*-A1a	3		
*gai*1/*gai*1, *Adh*-A1a/*Adh*-A1b	9		
*gai*1/*gai*1, Adh-A1b/*Adh*-A1b	9		

seedlings could be homozygous (*Gai*1/*Gai*1) or heterozygous (*Gai*1/*gai*1). These were further distinguished by measuring the plant height at maturity. However, this classification may not be totally unambiguous as can be seen from the non-discontinuity in plant height distribution (fig. 2). GA insensitive tall plants (above 75 cm in height) were classified as heterozygotes (*Gai*1/*gai*1) and dwarfs as homozygous (*Gai*1/*Gai*1). The proportion of the three genotypes classified as above fits the expected 1 : 2 : 1 ratio.

(iv) *Evidence for linkage*

Robinson (1971) has shown that the F_2 with two codominant loci is the most efficient cross for estimating linkage. The F_2 segregation for two

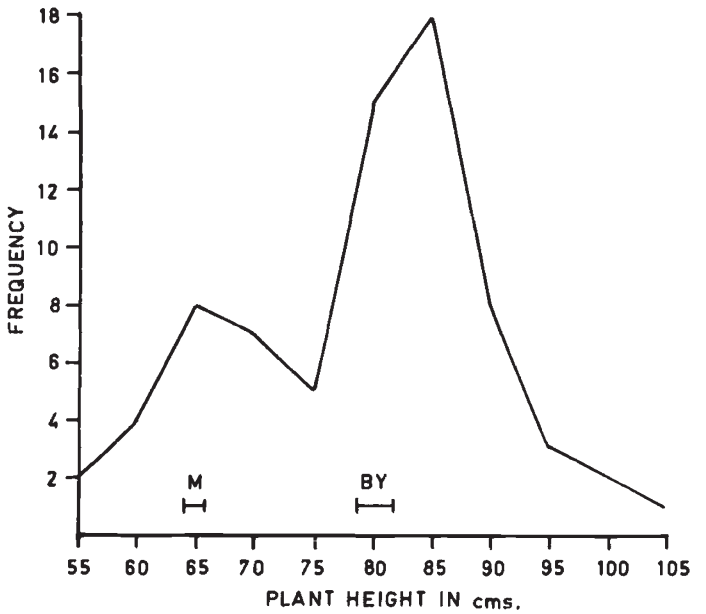

FIG. 2.—The height distribution of the F_2 plants from the cross Malavika × Bijaga yellow. The parental mean ± SEs are given inside. M (Malavika); BY (Bijaga yellow).

co-dominant loci is expected to give a segregation ratio of 1:2:1:2:4:2:1:2:1. The above F_2 was tested for a joint segregation of *Adh*-A1 and *Gai*1. The observed segregation was found to differ significantly from the expected ratio, suggesting linkage between the two genes.

(v) *Estimation of linkage*

The F_2 population was grouped according to the method given by Robinson (1971) for two co-dominant loci in coupling phase and a chi-square test was applied with $p = 0{\cdot}5$. The value differed significantly from the expected, again suggesting linkage. The maximum likelihood estimate for p was calculated by iteration and it was found that the p value of $23{\cdot}1 \pm 4{\cdot}0$ gives the least chi-square value. Hence, it is inferred that the *Adh*-A1 locus and the *Gai*1 locus are $23{\cdot}1 \pm 4{\cdot}0$ crossover units apart. It was earlier shown that the *Gai*1/*Rht*1 locus is 15 crossover units from the centromere. This allows us to infer the order of the two genes with respect to the centromere. This is shown in fig. 3.

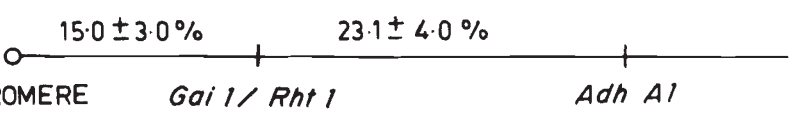

FIG. 3.—Chromosome map of the "p" arm of 4A of tetraploid wheat showing the order of the centromere, *Gai*1/*Rht*1 locus and the *Adh*-A1 locus.

4. References

GALE, M. D., AND MARSHALL, G. A. 1975. The nature and genetic control of gibberellin insensitivity in dwarf wheat grain. *Heredity, 35*, 55–65.

HART, G. E. 1970. Evidence for triplicate genes for alcohol dehydrogenase in hexaploid wheat. *Proc. Natl. Acad. Sci. U.S.A. 66*, 1136–1141.

HART, G. E. 1979. Genetical and chromosomal relationships among the wheats and their relatives. *Stadler Symposium. Vol. 11, University of Missouri, Columbia, 9*, 9–30.

HART, G. E., McMILLIN, D. E., AND SEARS, E. R. 1976. Determination of the chromosomal location of a glutamate-oxaloacetate transaminase structural gene using *Triticum-Agropyron* translocations. *Genetics, 83*, 49–61.

MAHAJAN, B. S. 1975. *Isoenzyme polymorphism in the sub-tribe Triticinae*. Unpublished Ph.D. thesis submitted to the University of Bombay.

McINTOSH, R. A. 1978. A catalogue of gene symbols for wheat. *Proc. 5th Intern. Wheat Genetics Symposium, New Delhi*, 1299–1309.

McVITTIE, J. A., GALE, M. D., MARSHAL, G. A., AND WESTCOTT, B. 1978. The intra-chromosomal mapping of the Norin 10 and Tom Thumb dwarfing genes. *Heredity 40*, 67–70.

MITRA, R., AND BHATIA, C. R. 1971. Isoenzymes and polyploidy, I. Quantitative and qualitative isoenzyme studies in the *Triticinae, Genetical Research, 18*, 57–69.

MYHILL, R. R., AND KONZAK, C. F. 1977. A new technique for culturing and measuring barley seedlings. *Crop Sci. 7*, 275.

NISHIKAWA, K., FURUTA, Y., HINA, Y., AND YAMADA, T. 1981. Genetic studies of amylase isozymes in wheat. IV. Genetic analyses in hexaploid wheat. *Jap. J. Genet. 56*, 385–395.

ROBINSON, R. 1971. *Gene mapping in laboratory mammals*. Part A. Plenum Press. London, New York, 151 pp.