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Inheritance of some marker genes in *Setaria italica* (L.) P. Beauv.

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Summary. A study on a series of genetic markers was run on five hybrids of foxtail millet, *Setaria italica,* and on one interspecific hybrid *S. viridis* × *S. italica* (*S. viridis* is the wild relative of *S. italica*). Seven enzymatic systems were investigated using starch gel electrophoresis (esterase, alcohol dehydrogenase, glutamate oxaloacetate transaminase, acid phosphatase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, cathodic peroxidase). This genetic analysis of the 6 F2 has allowed us to define 12 polymorphic loci: *Est-1, -2* and *-3, Adh-1, Got-1* and *-2, Acph-1, Mdh-1* and *-2, Pgd-1* and *-2,* and *Pox-1.* All of them behaved like dimers, except *Est-1* and *Est-2* which showed monomeric structures. Two other markers were examined: waxy endosperm, which appeared to be controlled by one locus, and anthocyanic pigmentation of the collar, for which at least two loci are responsible. Studies of linkage carried out on three F2 showed two linkage groups: *Mdh-1, Pox-1, Wx, Est-3,* and a locus for collar colour, and *Est-2,* and one or two other loci of colouring.

Key words: *Setaria italica* – *Setaria viridis* – Isozymes – Linkage – Waxy endosperm

Introduction

Setaria italica is a highly self-pollinated diploid cereal of the Old World which shows a remarkable morphological diversity (Nguyen Van and Pernes 1984). *S. viridis* is the closest wild relative of *S. italica,* as is shown by the possibilities of hybridization between these two species and the existence of intermediate forms (Williams and Schreiber 1976; De Wet et al. 1979).

The well established method of isozyme analysis through electrophoresis can be used advantageously in the genetic and taxonomic studies of the complex of *S. viridis* and *S. italica.* This article deals with the genetics of some markers – enzymatic and others – of these two species.

Material and methods

Plant material

Six hybrids and their F2 progenies were used for genetic analysis, one being an interspecific *S. viridis* × *S. italica.* The origins of the parental strains used are given in Table 1. They were chosen from a sample of 223 strains of *S. italica* and from 45 strains of *S. viridis* and studied for all markers presented here.

Electrophoresis and staining techniques

Two types of organs were used: well-developped leaves, and 3-day-old germinations (grown at 24 °C).

Two classical gel systems were used to separate the isoenzymes. The first system (Li Bo) was a tris-citrate and lithium

Table 1. Codes and origins of parental strains of the six hybrids studied

Hybrid no.	Parent ♀		Parent ♂	
	Code	Origin	Code	Origin
H1	37-80	China	511-80	West Germany
H2	64-79	China	65-79	China
H3	176-82	France	67-79	China
H4	43-80	Denmark	8-79	Nepal
H5	50-79	China	678-80	France
H6	65-79	China	14-80	France

hydroxide-boric acid buffer system, pH 8.3 (Scandalios 1969). The second gel system (His 5.7) was a L-histidine citrate gel, pH 5.7 (Stuber et al. 1977).

Staining methods were those of Brewer (1970); Stubber et al. (1977); Goodman et al. (1980b), with a few modifications (staining pH, reaction concentrations).

Test of the waxy endosperm in seeds

Seeds were crushed in a few drops of Lugol (4.4 g KI, 2.2 g I_2, 100 ml distilled water, which was diluted 50 times just before using). Waxy starch showed up pink, while non-waxy starch showed up blue.

Results and discussion

For each enzyme, one to three levels of polymorphism was found. The study of geographical variability and of phenotypes and alleles frequencies will be presented separately (Jusuf et al., in preparation).

Genetical analysis

Enzymes. Results are presented in Table 2. Only two enzymes, Est-1 and Est-2, showed a monomeric behaviour: Est-1 showed two closely-linked bands in the strains tested. Hybrids between "fast couple of bands" and "slow couple of bands" showed the four parental bands and no intermediate band. Variations observed at the Est-2 level concerned both migration mobility and the colouring of the only band (brownish or red). As we used two substrates in our staining solution (α- and β-naphtyl acetate), and according to Zouros and Van Delden (1982), variations in colouring can be interpreted by the substrate specificity of the different alleles. In hybrids 1 and 3, the red phenotype (β-specific) appeared to be dominant over the brownish one. For hybrid 4, which was a cross between plants with slow and fast brownish bands phenotypes, two parental bands and no intermediate band were seen.

Other enzymes showed dimer behaviour. These results were surprising only for peroxidase since these enzymes generally have active states as monomers in higher plants (Garcia et al. 1982).

Interpretation of zymograms was more complicated for Got-2, Mdh-1 and Pgd-2, since the interaction of two loci had to be considered. Thus, the presence of three band phenotypes in strains suggested the existence of interloci heterodimers. Genetic analysis confirmed this interpretation (Table 2). In particular, hybrids 1 and 3 had five bands for *Mdh-1*, including all parental bands (one of which was common to both parents). For each of these enzymes, we had to consider the existence of 1) a monomorphic locus (not coded here), as being at the origin of one band common to all the zymograms, and 2) a polymorphic locus (here coded *Got-2, Mdh-1* or *Pgd-2*) with active or null alleles. Such interloci heterodimers have been frequently described for other species and in particular in maize (Goodman et al. 1980a).

Waxy endosperm. The study of F2 seeds of hybrid 2 allowed us to define four classes of colouring with lugol, ranging from blue (Wx) to pink (wx). The F2 segregation ratio was 1 : 1 : 1 : 1 (400 seeds, $\chi^2_{(3)}=0.18$, $P=0.98$), suggesting that this character, expressed in triploid tissue (albumen), is controlled by only one locus. In order to define a posteriori the genotype for waxy endosperm of F2 plants studied in electrophoresis, we tested F3 seeds (20 for each plant F2), and confirmed these results (segregation ratio 1 : 2 : 1, $\chi^2_{(3)}=3.38$, $P=0.18$).

Anthocyanic colouring of the collar (stem base). The segregations studied here were of the ratio 3 red: 1 green (H2, 3, 4 and 5, χ^2 from 0.03–0.62) or 9 : 7 (H1 $\chi^2=0.04$), and suggested the interaction of at least two independent loci in colar colour. These loci will be coded here *Col* with a sub-index indicating progeny: $Col1_{H1}$, $Col2_{H1}$ Col_{H2}, etc.

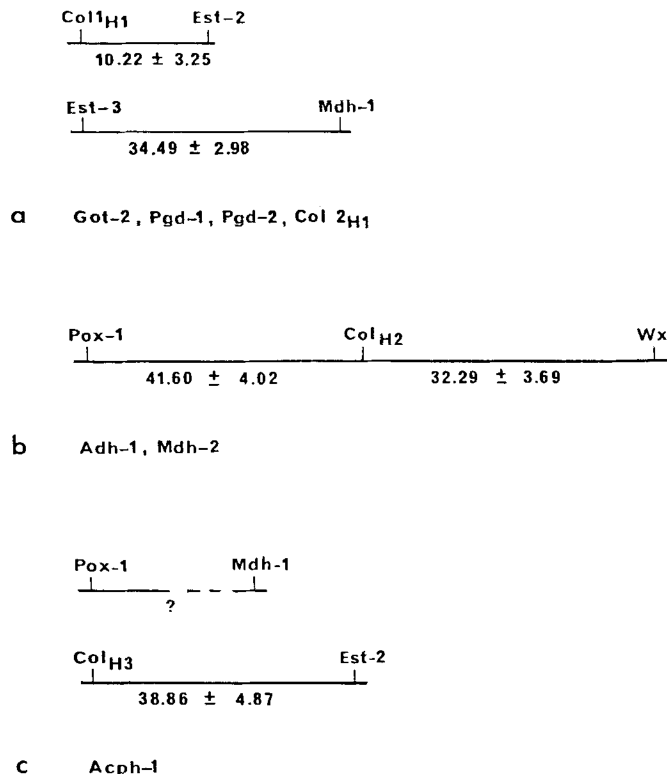

Fig. 1 a–c. Linkage maps for H1, 2 and 3 progenies. Unlinked loci are mentioned under linkage groups. **a** H1 progeny; **b** H2 progeny; **c** H3 progeny

Table 2. List of polymorphic loci studied by electrophoresis and summary of the principal results obtained

<table>
<tr><th>Enzyme</th><th>Gel system</th><th>Locus</th><th>Activity[a]</th><th>Alleles[b]</th><th>Hybrid studied</th><th>Segregation ratio</th><th colspan="3">Observed frequencies</th><th>$P(\varkappa^2)$</th><th>Structure of active enzyme[c]</th></tr>
<tr><td>Esterase</td><td>Li Bo</td><td>Est-1</td><td>L</td><td>$a^i b c^v d^{v0}$</td><td>H5</td><td>1 : 2 : 1</td><td>37|a|</td><td>80|a/b|</td><td>39|b|</td><td>0.28</td><td>Monomer</td></tr>
<tr><td></td><td></td><td>Est-2</td><td>L</td><td>$a b c d^v$</td><td>H1</td><td>3 : 1</td><td>173|b|</td><td>43|a|</td><td></td><td>0.10</td><td>Monomer</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td>H3</td><td>3 : 1</td><td>218|b|</td><td>77|a|</td><td></td><td>0.66</td><td>Monomer</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td>H4</td><td>1 : 2 : 1</td><td>38|a|</td><td>75|a/c|</td><td>36|c|</td><td>0.97</td><td>Monomer</td></tr>
<tr><td></td><td></td><td>Est-3</td><td>G, L</td><td>a b</td><td>H1</td><td>1 : 2 : 1</td><td>68|a|</td><td>99|3 bands|</td><td>49|b|</td><td>0.11</td><td>Dimer</td></tr>
<tr><td>Alcohol dehydrogenase</td><td>Li Bo</td><td>Adh-1</td><td>G</td><td>a b</td><td>H2</td><td>1 : 2 : 1</td><td>61|a|</td><td>103|3 bands|</td><td>57|b|</td><td>0.56</td><td>Dimer</td></tr>
<tr><td>Glutamate oxaloacetate transaminase</td><td>Li Bo</td><td>Got-1</td><td>G, L</td><td>$a b^i$</td><td>H1</td><td>1 : 2 : 1</td><td>63|a|</td><td>97|3 bands|</td><td>56|c|</td><td>0.26</td><td>Dimer</td></tr>
<tr><td></td><td></td><td>Got-2</td><td>G, L</td><td>$a^3 b$</td><td>H6</td><td>3 : 1</td><td>116|3|bands|</td><td>40|b|</td><td></td><td>0.86</td><td>Dimer*</td></tr>
<tr><td>Acid phosphatase</td><td>Li Bo</td><td>Acph-1</td><td>G, L</td><td>a b</td><td>H3</td><td>1 : 2 : 1</td><td>70|a|</td><td>155|3 bands|</td><td>71|b|</td><td>0.72</td><td>Dimer</td></tr>
<tr><td>Malate dehydrogenase</td><td>His 5.7</td><td>Mdh-1</td><td>G, L</td><td>$a^3 b^3 c^{v3}$</td><td>H1</td><td>1 : 2 : 1</td><td>56|a|</td><td>106|5 bands|</td><td>54|b|</td><td>0.95</td><td>Dimer*</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td>H3</td><td>1 : 2 : 1</td><td>91|a|</td><td>136|5 bands|</td><td>69|b|</td><td>0.07</td><td>Dimer</td></tr>
<tr><td></td><td></td><td>Mdh-2</td><td>G, L</td><td>$a b^i$</td><td>H2</td><td>1 : 2 : 1</td><td>58|a|</td><td>119|3 bands|</td><td>44|b|</td><td>0.29</td><td>Dimer</td></tr>
<tr><td>6-phospho gluconate dehydrogenase</td><td>His 5.7</td><td>Pgd-1</td><td>G, L</td><td>a b</td><td>H1</td><td>1 : 2 : 1</td><td>51|a|</td><td>107|3 bands|</td><td>48|b|</td><td>0.51</td><td>Dimer</td></tr>
<tr><td></td><td></td><td>Pgd-2</td><td>G, L</td><td>$a b^3 c^3$</td><td>H1</td><td>3 : 1</td><td>136|b|</td><td>56|a|</td><td></td><td>0.18</td><td>Dimer*</td></tr>
<tr><td>Cathodic peroxidase</td><td>His 5.7</td><td>Pox-1</td><td>(G), L</td><td>a b</td><td>H2</td><td>1 : 2 : 1</td><td>44|a|</td><td>125|3 bands|</td><td>52|b|</td><td>0.11</td><td>Dimer</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td>H3</td><td>1 : 2 : 1</td><td>91|a|</td><td>128|3|</td><td>77|c|</td><td>0.03</td><td>Dimer</td></tr>
</table>

[a] L = leaves; G = germinations

[b] i = specific to *S. italica;* v = specific to *S. virdis*; 0 = null allele; 3 = allele associated with a three band phenotype. For Esterase, β-alleles are written in thick characters, other alleles are null or α-alleles

[c] * = existence of an interaction with another monomorphic locus

Linkage

Analysis of genetic linkages, according to Allard (1956), was only done on three hybrid progenies (H1, 2 and 3).

One or two linkage groups showed up for each cross (Fig. 1). Loci denoted Col_{H2} and Col_{H3}, both linked to *Est-2*, showed very different recombination rates with this locus in the two progenies. It is probable that Col_{H2} and Col_{H3} are different loci, but further studies are required for confirmation. In this case, at least three loci are probably involved in collar colour determination. In foxtail-millet as for other species, colour characters must be studied carefully.

References

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Brewer GJ (1970) An introduction to isozymes techniques. Academic Press, New York London

de Wet JMJ, Oestry-Stidd LL, Cubero JI (1979) Origins and evolution of foxtail millets. J Agric Trad Bot Appl 26:53–63

Garcia P, Perez de la Vega M, Benito C (1982) The inheritance of rye seed peroxidases. Theor Appl Genet 61: 341–351

Goodman MM, Stuber CW, Lee CN, Johnson FM (1980a) Genetic control of malate dehydrogenase isozymes in Maize. Genetics 94:153–168

Goodman MM, Stuber CW, Newton K, Weissinger HH (1980b) Linkage relationships of 19 enzyme loci in Maize. Genetics 96:697–710

Nguyen Van E, Pernes J (1984) Genetic diversity of foxtail millet (*Setaria italica*). In: Plant population biology symposium. Springer, Berlin, Heidelberg, New York, pp 98–104

Scandalios JG (1969) Genetic control of multiple molecular forms of enzymes in plants. A review. Biochem Genet 3:37–79

Stubber CW, Goodman MM, Johnson FM (1977) Genetic control and racial variation of β-glucosidase isozymes in maize (*Zea mays* L.). Biochem Genet 15:383–394

Williams RD, Schreiber MM (1976) Numerical and chemotaxonomy of the Green Foxtail complex. Weed Sci 24:331–335

Zouros E, Van Delden W (1982) Substrate-preference polymorphism at an esterase locus of *Drosophila mojavensis*. Genetics 100:307–314