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Peroxidase isozyme patterns in primary trisomics of pearl millet *

J. S. Sidhu, Ravi and J. L. Minocha
Department of Genetics, Punjab Agricultural University, Ludhiana-141 004, India

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Summary. The peroxidases zymogram phenotypes of seven primary trisomics of pearl millet (*Pennisetum typhoides*) and their disomic sibs were determined. It was found that each of the trisomics can be differentiated from its disomic sib and from other trisomics on the basis of the isozyme band intensities.

Key words: Peroxidases – Primary trisomics – *Pennisetum typhoides*

Introduction

Trisomics ($2n+1$) are important cytogenetic tools. A complete series of primary trisomics of pearl millet, *Pennisetum typhoides* (Burm.) S. & H. ($2n=14$), was developed by Gill et al. (1970). Identification of the extra chromosomes in the trisomic series is important for its use in linkage tests and chromosome maps. The addition of an extra chromosome may cause a unique profile of isozymes to be expressed and thus electrophoretic techniques have been used to characterize trisomics in such crop plants as barley (McDaniel and Ramage 1970), *Datura* (Carlson 1972; Smith and Conklin 1975); Sorghum (Suh et al. 1977) and tomato (Tanksley 1980; Fobes 1980).

Material and methods

The primary trisomic series developed in the inbred line BIL-4 of pearl millet by Gill et al. (1970) was used. The selfed progenies of the seven trisomics (named 'tiny', 'dark green', 'lax', 'slender', 'spindle', 'broad' and 'pseudonormal', representing trisomy for chromosome-1, -2, -3, -4, -5, -6 and -7 respectively) were field-grown. The leaves were collected from the plants at the stage just before the ear emergence. Two leaves, flag and subsequent, were taken from the first tiller of the plant. Early in the mornings leaf samples were collected in chilled acetone from the identified trisomics and their disomic sibs. A crude extract of each sample was prepared in distilled water (3 ml/gm of leaf sample). The extracts were centrifuged at 10,000 g for 15 min and the supernatant was stored at 4 °C.

Starch was hydrolysed according to the method of Smithies (1955). Starch gels were prepared in (pH ˙.6) Tris-citrate buffer. Sodium borate buffer of pH 8.6 was used as a bridge buffer. Electrophoresis was performed at 4 °C. The gels were run at a voltage of 15 V/cm and a current of 2.5 mA/sample. Gels were stained in a mixture of 100 ml of 0.1% benzidine in 0.2 M sodium acetate buffer (pH 5.0) and 5 ml of 2% hydrogen peroxide, and incubated at 10 °C for about half an hour. The homology of different isozyme bands was identified through working out their relative mobility (Rm) in each gel.

The intensity of the bands was approximated with visual ratings by using the following quantitative scoring; 5 = very strong, 4 = strong, 3 = moderate, 2 = light, 1 = faint and 0 = absent. Mean values were worked out from a number of readings (5–10) for each sample. The isozyme band intensities of trisomics were compared using the *t*-test.

Results

Both anodal and cathodal bands of peroxidase isozymes were observed. A total of eight anodal (A-1 to A-8) and seven cathodal (C-9 to C-15) bands were observed (Fig. 1 a, b). Of these, the patterns of three anodal (A-1, A-2, A-4) and four cathodal (C-10, C-12, C-14, C-15) bands were consistent. The results for the mean peroxidase isozyme band intensity for the seven bands in primary trisomics and their disomic sibs are given in Table 1. Peroxidase C-12 had the highest intensity in standard diploid inbred BIL-4 when compared to all of the trisomics and their disomic sibs. Two additional bands, A-3 and C-9, were present in BIL-4.

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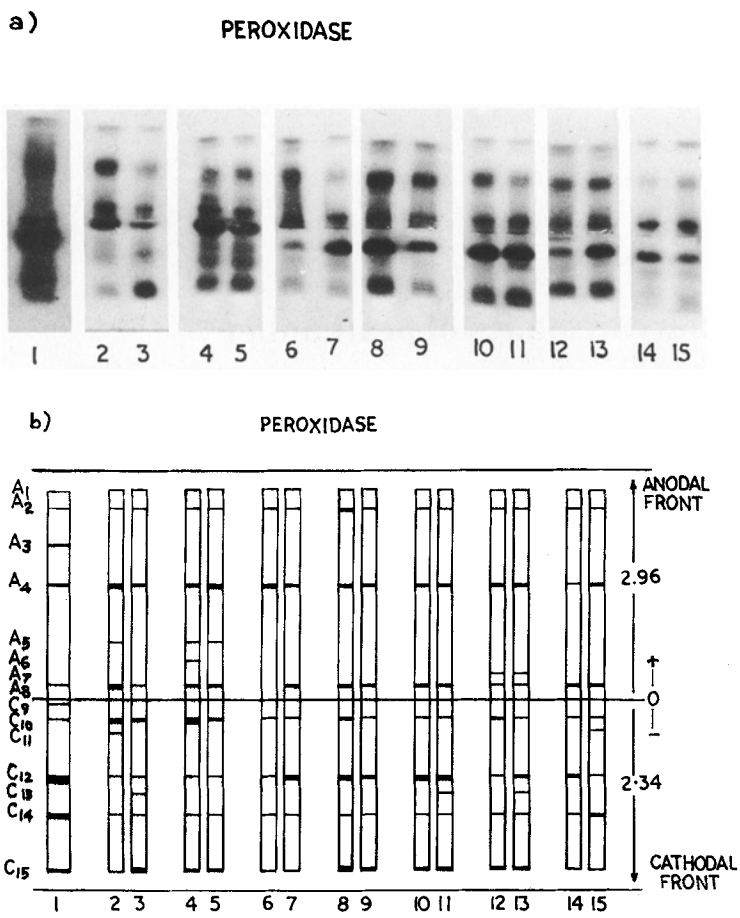

Fig. 1. **a** Photographs of peroxidase zymotypes. *Channels 1* BIL-4 *2* and *3* Trisomic-1 and its disomic sib; *4* and *5* disomic and its trisomic-2 sib; *6* and *7* disomic and its trisomic-3 sib; *8* and *9* disomic and its trisomic-4 sib; *10* and *11* disomic and its trisomic-5 sib; *12* and *13* disomic and its trisomic-6 sib; *14* and *15* disomic and its trisomic-7 sib. **b** Interpretive diagram of part **a**

Table 1. Mean peroxidase isozyme band intensity in primary trisomics and their disomic sibs

Material	Band A-1 (Rm 0.92)	Band A-2 (Rm 0.82)	Band A-4 (Rm 0.56)	Band C-10 (Rm 0.18)	Band C-12 (Rm 0.42)	Band C-14 (Rm 0.62)	Band C-15 (Rm 0.91)
Trisomic-1	2.5±0.19	1.7±0.19	3.5±0.08*	4.1±0.16*	2.9±0.20	1.7±0.18	2.0±0.11*
Disomic sib	2.5±0.16	2.0±0.21	2.5±0.21	2.5±0.19	3.0±0.18	2.0±0.15	3.1±0.19
Trisomic-2	2.7±0.17	2.2±0.19	3.4±0.17*	3.0±0.15*	3.2±0.16	2.2±0.17	2.7±0.12
Disomic sib	2.6±0.17	2.0±0.10	2.0±0.19	3.9±0.10	3.0±0.15	2.4±0.13	2.5±0.20
Trisomic-3	2.0±0.15	2.2±0.22	2.0±0.01*	2.6±0.21	3.7±0.13*	2.9±0.15	2.7±0.15
Disomic sib	2.4±0.20	2.6±0.17	2.9±0.12	2.3±0.19	1.8±0.18	2.6±0.19	3.0±0.17
Trisomic-4	2.8±0.21	0.6±0.16*	2.0±0.12*	0.6±0.13*	2.6±0.14*	2.6±0.17	3.0±0.01
Disomic sib	2.7±0.19	2.3±0.19	2.9±0.10	1.9±0.17	4.1±0.18	2.6±0.17	3.0±0.12
Trisomic-5	2.2±0.12	2.3±0.11	2.0±0.09	2.4±0.19	3.5±0.18	2.5±0.13	2.2±0.17
Disomic sib	2.0±0.16	2.2±0.14	2.4±0.14	2.9±0.21	3.3±0.20	2.3±0.19	2.5±0.19
Trisomic-6	2.8±0.15	2.0±0.18	1.7±0.13	1.2±0.18*	4.1±0.21*	2.2±0.13	3.2±0.20
Disomic sib	2.7±0.14	2.1±0.16	2.0±0.16	2.1±0.14	3.0±0.11	2.5±0.14	3.4±0.21
Trisomic-7	2.5±0.13	2.0±0.09	2.5±0.12	2.6±0.16	1.0±0.09*	2.5±0.16*	2.5±0.05
Disomic sib	2.5±0.14	2.0±0.11	2.3±0.14	3.0±0.11	2.9±0.08	1.6±0.19	2.5±0.13
BIL-4	2.5±0.18	2.0±0.11	2.5±0.01	3.3±0.11	4.9±0.13	3.5±0.11	3.0±0.17

* Significant at 0.01 level of significance

Table 2. Peroxidase bands differentiating between different primary trisomics of pearl millet

	Trisomic-1	Trisomic-2	Trisomic-3	Trisomic-4	Trisomic-5	Trisomic-6	Trisomic-7	BIL-4
Trisomic-1		C_{10}	$A_4C_{10}C_{12}C_{14}$	$A_2A_4C_{10}C_{15}$	$A_4C_{10}C_{12}$	$A_4C_{10}C_{12}C_{15}$	$A_4C_{10}C_{12}$	A_4C_{10} $C_{12}C_{14}C_{15}$
Trisomic-2		–	A_4C_{12}	$A_2A_4C_{10}C_{12}$	A_4	$A_4C_{10}C_{12}$	A_4C_{12}	$A_4C_{12}C_{14}$
Trisomic-3		–	–	$C_{10}C_{12}$	–	$C_{10}C_{15}$	C_{12}	C_{12}
Trisomic-4		–	–	–	$C_{10}C_{12}C_{15}$	$C_{10}C_{12}$	$C_{10}C_{12}$	$C_{10}C_{12}C_{14}$
Trisomic-5		–	–	–	–	C_{10}	C_{12}	$C_{12}C_{14}C_{15}$
Trisomic-6		–	–	–	–	–	$A_4C_{10}C_{12}$	$C_{12}C_{14}$
Trisomic-7		–	–	–	–	–	–	$C_{12}C_{14}$

A comparison of different trisomics with respect to differences in isozyme intensities among different trisomics is given in Table 2. Trisomic-1 showed a significantly higher band intensity for peroxidase A-4 and C-10 and lower intensity for C-15 as compared to its disomic-sib. The former had the highest activity for peroxidase C-10 and lowest for C-14 when compared to other trisomics. Two additional bands, A-5 and C-11, were found in Trisomic-1. Trisomic-2 and trisomic-3 had significantly higher band intensities for peroxidases A-4 and C-12, respectively, than their disomic sibs. Trisomic-2 expressed a lower activity for peroxidase C-10 as compared to trisomic-1, and a higher one than the other trisomics. Peroxidase A-6 was absent in trisomic-2, while present in its disomic sib. Trisomic-3 had a lower activity for peroxidase A-4 than trisomic-1 and -2; and a higher activity for A-2, C-10 and C-12 than trisomic-1, trisomic-2 and trisomic-7, respectively. Trisomic-4 could be distinguished from its disomic sib on the basis of significantly lower band intensities of peroxidase A-2, A-4, C-10 and C-12. This trisomic exhibited the lowest activity for peroxidases A-2 and C-10 when compared to other trisomics. In trisomic-5, all the peroxidases were similar to those found in its disomic sib except for an additional band, C-13. In trisomic-6, the activity of peroxidase C-10 was significantly lower than that found in its disomic sib whereas C-12 activity was significantly higher. Thus, it could be distinguished from other trisomics on the basis of peroxidase C-12 being highest in intensity. Trisomic-7 had a significantly higher activity of peroxidase C-14 than its disomic sib. It expressed the lowest activity for C-12 when compared to other trisomics. C-11 was found to be an additional band which was missing in its disomic sib.

Discussion

Electrophoretic analysis is important in determining genetic variation at the biochemical level. The study of isozymes is advantageous over conventional morphological markers in genetic analysis because genotypic differences are not always expressed at the gross phenotypic level. The primary trisomics offer an important tool for studying the dosage effect of a chromosome. Since the trisomic series of pearl millet was developed from the open-pollinated progenies of the autotetraploid, there exist genetic differences amongst different plant progenies. Thus, different trisomics, along with their respective disomic sibs, can be used for comparing the isozyme bands. The inferences are based on the assumption that there exists a positive correlation between the visual scoring of band intensity and the enzyme activity. Such correlations have been demonstrated in *Datura stramonium* by comparing the band intensities with the densitometer tracings of the typical zymograms on photographic films (Smith and Conklin 1975). Carlson (1972) analysed the primary trisomics of *Datura stramonium* for peroxidases from the cultured tissue derived from hypocotyl, and it was found that the trisomics had either increased or decreased intensity of bands than the disomics. Similar results were obtained by Smith and Conklin (1975) for peroxidase isozyme patterns of the primary trisomics of *Datura*. When composing enzyme intensity of peroxidase bands in trisomics and disomics, it was found that in trisomics it increased in eleven cases and decreased in only two cases.

Significant differences (increases or decrease) in band intensity between trisomics and disomics have been recorded in pearl millet. It is speculated that the increased intensity of a specific band in a trisomic is due to dosage effects of a 'structural gene'. In six different cases, the trisomics had increased band intensity when compared to their disomic sibs. The presence of the extra chromosome results in the increased production of the specific isozyme. Thus, the alleles responsible for such isozymes appear to specify the product in an additive manner. The trisomics showing a significant decrease in the intensity of the specific band(s) from their respective disomics may be due to extra doses of 'regulatory gene(s)' responsible for the production of higher amounts of repressor molecule. The enhanced activity due to an increase in dosage of the structural gene(s) is balanced by an increased production of repressor(s) gene activity due to an increased dosage of 'regulatory genes'. This is evident from the additional

dose of chromosomes 1, 2, 3 and 7: the increased intensity of one or more bands is balanced by the reduced intensity of other bands. Thus, it can be concluded that such controls are 'balanced' in disomics whereas in genetically 'unbalanced' types the extra amounts of 'structural' or 'regulatory' gene products are produced which alter the isozyme patterns. There could also be non-allelic interactions resulting in decreased band intensity in trisomics. Two additional bands (C-11 in trisomic-1 and trisomic-7, C-13 in trisomic-5) were observed in trisomics. The presence of the novel bands in trisomics, lacking in disomics, usually in close proximity to another band, has also been reported in barley (McDaniel and Ramage 1970). Apparently, the enzymes, being polymers of subunits, may show such deviations where genetic differences between alleles involving only a single base pair may act through trisomy to specify an isozyme with electrophoretically detectable differences.

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