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## CHANGES IN ISOENZYMES OF PEROXIDASE IN GREEN-EAR OF PEARL MILLET

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ALTERED state of oxidative enzymes plays important role in plant metabolism during pathogenesis[1]. *Sclerospora graminicola* causes proliferations in ear-heads of *Pennisetum americanum* (called green-ear of pearl-millet) and affects the activities of oxidative enzymes[2,3]. We have reported higher peroxidase and lower IAA-oxidase activities parallel with increased protein, total phenols, orthodihydroxyphenols, and auxin contents in the diseased millet tissues[2]. We report the isoenzymatic patte.n of peroxidase in healthy and diseased millet tissues.

Peroxidase isoenzymes were separated by zone electrophoresis in polyacrylamide gel using method of Davis[4] and Ornstein[5]. Acetone powder of various sample categories was homogenized in 0.05 Tris-HCl buffer (pH 7.8). Supernatant fraction after centrifugation at 15,000 rpm was taken as representative sample containing 50–60 $\mu$g of protein and mixed with 60% sucrose (1:2) to omit use of sample and spacer gels. It was then layered on the top of running gel (7.5%). Cathode and anode were connected to upper and lower reservoirs respectively. A constant current of 3 m A/tube (150 Volts) was applied, the electrophoresis was accomplished in 70–75 min.

Gels were stained with saturated solution of benzidine (in 25% acetic acid) and 1% $H_2O_2$ for peroxidase activity. The dark blue bands appearing within 1–2 min were noted.

Isoenzyme pattern was studied at various stages of the disease. In the healthy leaves five anodic isoenzymes (gel-1; $A_2$, $A_3$, $A_{10}$, $A_{12}$ and $A_{13}$) were recorded which increased to eight with three new

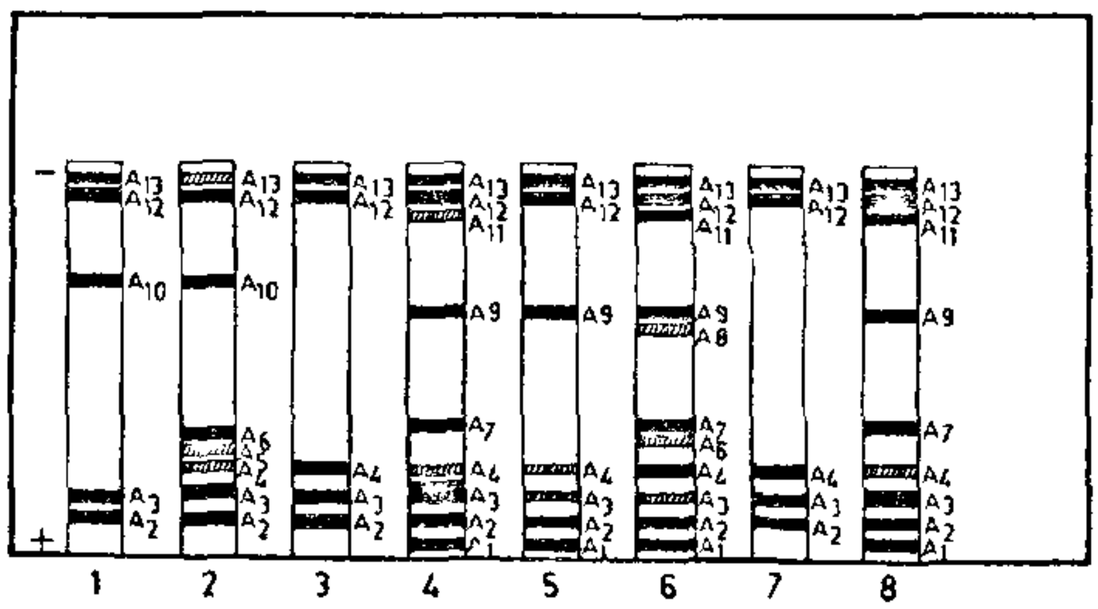


**Figure 1.** Peroxidase isoenzyme pattern in different stages of green-ear in pearl-millet. Gel-1. Healthy leaf, **2.** Diseased leaf, **3.** Healthy ear-head, **4.** Green-ear initial stage, **5.** Completely proliferated ear-head, **6.** Suppressed ear-head, **7.** Healthy half of half-deformed ear-head, **8.** Diseased half-deformed ear-head. ▬ Deep-stained bands, ▨ Light stained bands.

bands (gel-2; $A_2$, $A_3$, $A_4$, $A_5$, $A_6$, $A_{10}$, $A_{12}$ and $A_{13}$) in diseased leaves.

In healthy ear-head and the healthy half of the half-deformed ear-head five isoenzyme bands (gel-3, 7; $A_2$, $A_3$, $A_4$, $A_{12}$ and $A_{13}$) were noted. In the green-ear initial stage and diseased half of half-defomred ear-head nine isoenzymes were recorded (gel-4, 8; $A_1$ to $A_4$, $A_7$, $A_9$ and $A_{11}$–$A_{13}$) out of which three ($A_1$, $A_7$ and $A_9$) were new as compared to healthy ear-head (gel-3) and healthy half of half-deformed ear-head (gel-7). In completely proliferated ear-head, seven (gel-5; $A_1$ to $A_4$, $A_9$, $A_{12}$ and $A_{13}$) isoenzymes band were noted. The isoenzyme $A_7$, common in the other three stages of diseased ear-heads, disappeared in this stage. The suppressed ear-heads showed eleven isoperoxidase (gel-6; $A_1$ to $A_4$, $A_6$, $A_7$, $A_8$, $A_9$ and $A_{11}$ to $A_{13}$).

We have reported highest peroxidase activity in the suppressed ear-heads followed by green-ear initial stage, diseased half of half-deformed ear-heads, completely proliferated ear-heads and diseased leaves over their healthy counter parts[2]. The number of isoenzymes followed the same line. Stahmann and Demorest[6] concluded that appearance of new isoperoxidases was because of *de novo* protein synthesis in the parasitized tissues. Presumably, the appearance of new isoperoxidases and the higher peroxidase activity in the diseased tissues was also a result of higher protein levels recorded in the affected tissues[3].

Shekhawat and Arya[2] reported suppressed IAA-oxidase activity in the diseased tissues (lowest being in

the suppressed ear-heads). Macnicol[7] proposed that IAA-oxidase activity was associated with isoenzyme of peroxidase. Yoneda and Endo[8,9] observed seven isoperoxidases in *Pharbitis nil* but only two of them showed IAA-oxidase activity. In our studies we have also noted that the diseased tissue extracts containing high levels of ortho-dihydroxyphenols induced a lag in the peroxidase catalysed IAA-oxidation *in vitro* and the addition of $MnCl_2$ or $H_2O_2$ in the reaction mixture could reduce the lag period[3] to large extent. Yoneda and Endo[8,9] and Stonier, Stasinos and Murthyreddy[10] concluded that auxin protectors migrated along with some of the isoperoxidases and interferred in IAA-oxidase activity.

It is concluded that infection of *S. graminicola* in pearl-millet, induced formation of new isoperoxidases but they were not able to oxidise auxin in diseased system because of high level of ortho-dihydroxy phenols associated with infection.

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## STERILITY IN *PHRAGMITES COMMUNIS* (RETZ.) TRIN.

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*PHRAGMITES COMMUNIS* is the common reed widely used for thatching houses and in the manufacture of pulps for rayon and paper. During embryological investigations in the family Poaceae it was observed that this grass propagates exclusively by vegetative means by underground rhizomes with no seed set, though it flowers regularly. The present report describes the developmental aspects of the anther and ovule of *P. communis* which was collected from Kolleru Lake (Andhra Pradesh).

In its very early development, the anther comprises of a homogenous mass of cells. The archesporium is hypodermal and the development of the anther wall is of the Monocotyledonous type[1] showing a subepidermal endothecium with fibrous thickenings, single middle layer and uniseriate glandular tapetum of binucleate cells. The microspore mother cells undergo the usual meiotic divisions and give rise to isobilateral microspore tetrads. The microspores do not undergo any further development but degenerate. Thus the anther lobe at maturity encloses only aborted microspores without any protoplasmic contents (figure 1).

The ovary is superior and unilocular with a subbasal, bitegmic ovule. The ovule is campylotropous and tenuinucellar, (figure 4). The archesporium functions directly as the megaspore mother cell (figure 2). Meiosis in the megaspore mother cell results in a linear tetrad of megaspores (figure 3). The embryo sac development conforms to the Polygonum type[2]. The egg which is usually bigger than the synergids is flask-shaped with a large apical vacuole. The three antipodal cells occupy the narrow chalazal part of the embryo sac (figure 4).

There is no fertilization and the embryo sac degenerates. This is followed by enlargement of some of the nucellar cells at the chalazal end which become two or three nucleate due to mitotic divisions (figure 5).

Thus in *P. communis*, ovular degeneration and failure of seed development appear to be due to nonfunctional pollen development which has been compensated by its aggressive vegetative growth.