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RELATIONSHIP BETWEEN PHOSPHATASE ACTIVITY AND SEX EXPRESSION IN *COCCINIA INDICA* W. & A.

MADAN LAL and V. S. JAISWAL

Department of Botany, Banaras Hindu University,
Varanasi 221 005, India

THE formation of stamen and ovary are the result of two different embryological events leading to the differentiation of male and female reproductive organs¹. Plant growth regulators have been shown to regulate sex organ differentiations²⁻⁴. The activities of several enzymes seem to be affected by exogenous growth regulators^{5,6}. Studies have been carried out to establish a correlation between enzyme activities and sex expression⁶⁻⁸. This report describes the patterns of acid and alkaline phosphatase distribution in vegetative and reproductive parts of male and female plants of *Coccinia indica*.

For enzyme analysis, vegetatively growing shoot tips, flower buds (5 mm in size) and mature flowers (at anthesis) were harvested from male and female plants of *C. indica* growing in the campus of the university. Known amounts of different tissues were homogenized in 0.1 M Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol⁹ using a chilled pestle and mortar. The homogenates were centrifuged at 12,000 rpm for 20 min at 0-4°C. The clear supernatants were used as enzyme source.

Acid and alkaline phosphatase activities were determined at pH 4.8 and 9.0 respectively disodium

p-nitrophenylphosphate as substrate. The total and specific activity of each enzyme were expressed as units per gram fresh weight and units per milligram protein respectively. The units represent μg of *p*-nitrophenol produced per minute at 30°C. Protein was estimated colorimetrically by the procedure of Lowry *et al.*¹⁰ using bovine serum albumin as standard. The experiments were repeated twice.

Acid phosphatase isoenzymes were separated electrophoretically on 7.5% polyacrylamide slab gels (1 mm thick). After electrophoresis, the gels were rinsed for about 30 min in sodium acetate buffer (pH 4.8). The buffer was changed every 10 min to lower the pH of the gel from 9.0 to 4.8. The gel was then transferred to a reaction mixture containing α -naphthyl phosphate and Fast Garnet GBC salt, and incubated at 30°C until brown bands appeared¹¹.

Changes in acid phosphatase and alkaline phosphatase activity in male and female plants/flowers were recorded (table 1). Marked differences in both total and specific acid phosphatase activity were observed between reproductive tissues of male and female plants. Male flower buds and mature flowers had significantly higher levels of acid phosphatase activity compared to female flowers. Total and specific activity of the enzyme increased in developing male reproductive organs. Specific acid phosphatase activity increased in developing female flowers also, but the total activity in female tissues was less than in vegetative tissues. Acid phosphatase activity in vegetative shoot tips of male and female plants were similar.

The highest alkaline phosphatase activity was recorded in male mature flowers and the lowest in female mature flowers (table 1). There was no marked difference in alkaline phosphatase activity between male and female vegetative tissues.

Three isoenzymes of acid phosphatase, designated A, B and C, were observed in vegetative shoot tips of

Table 1 Acid and alkaline phosphatase activity in vegetative and reproductive tissues of male and female plants of *Coccinia indica*

Sex	Developmental stage	Acid phosphatase		Alkaline phosphatase	
		(Units/g fr. wt)	(Units/mg protein)	(Units/g fr. wt)	(Units/mg protein)
Male	Vegetative shoot tips	41.0 ± 1.8	0.49 ± 0.02	10.0 ± 0.9	0.12 ± 0.01
	Flower buds	43.5 ± 1.8	0.91 ± 0.04	11.2 ± 0.2	0.23 ± 0.01
	Mature flowers	45.5 ± 4.2	1.20 ± 0.11	11.5 ± 0.7	0.29 ± 0.05
Female	Vegetative shoot tips	43.2 ± 1.3	0.48 ± 0.02	10.5 ± 1.1	0.12 ± 0.01
	Flower buds	33.0 ± 2.3	0.56 ± 0.04	3.3 ± 0.7	0.06 ± 0.01
	Mature flowers	33.3 ± 1.8	0.61 ± 0.03	2.8 ± 0.6	0.05 ± 0.01

Data represent mean of 3 replicates ± S.D.

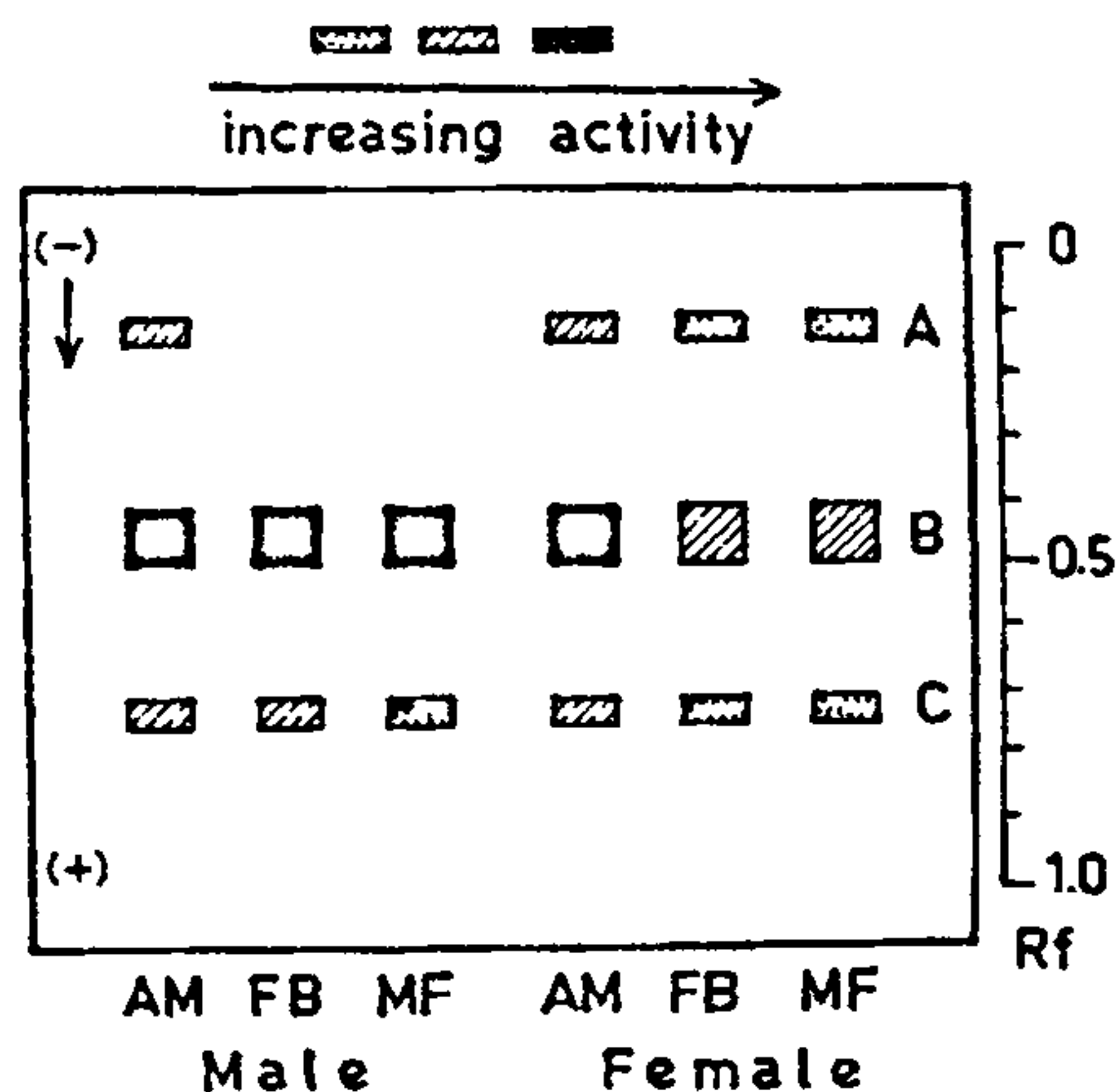


Figure 1. Zymograms showing the patterns of acid phosphatase (pH 4.8) isoenzyme distribution in vegetative and reproductive tissues of male and female plants of *Coccinia indica*. [AM, vegetative shoot tips; FB, flower buds; MF, mature flowers.]

both male and female plants, each with similar relative electrophoretic mobility and intensity in both sexes (figure 1). However, band A was absent in developing and mature male flowers, and the intensity of band C decreased during flower development. All three bands were present in female reproductive tissues but bands A and C were less intense in flowers than in vegetative tissues.

Gibberellins are known to favour male sex expression in many plants, including cucurbitaceous species. A correlation between higher gibberellin content and male sex expression has been established^{4,12}. It has been demonstrated that gibberellin treatment enhances acid phosphatase activity and its secretion in plant tissues^{5,13}. It appears that acid and alkaline phosphatase activities are enhanced in male reproductive tissues as a result of higher endogenous gibberellin content of these tissues, as suggested earlier⁸.

Changes in isoenzyme patterns during organ differentiation and growth have been reported in several plants¹⁴. The present results suggest that acid phosphatase isoenzyme A, present in vegetative tissues, disappears in male reproductive organs during their formation but not in female reproductive organs. It is likely that repression of certain gene(s), specific to acid phosphatase isoenzyme A, is

responsible for male sex expression, whereas female sex expression is favoured by the continued expression of such gene(s) in *C. indica*.

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INDUCTION OF MUTATION THROUGH INTERSPECIFIC GRAFTING

S. B. DANDIN, V. R. MALA, S. R. RAMESH and R. BALAKRISHNA

Central Sericultural Research and Training Institute, Mysore 570 008, India

PHYSICAL and chemical mutagens are the major agents used to induce mutations. It has also been