ENZYME ACTIVITY AND ISOENZYME PATTERNS OF *COCCINIA GRANDIS* IN RELATION TO SEX EXPRESSION

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ABSTRACT

Qualitative and quantitative changes in peroxidase (EC 1.11.1.7) and acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatases of the shoot tips, nodal segments with buds, internodal segments, leaves and mature flowers in male and female plants of *Coccinia grandis* were analysed. Higher peroxidase and phosphatase activity was noted in the explants from male plants over those from the female plants. Variation in isoenzyme patterns were exhibited by different explants obtained from both male and female plants.

INTRODUCTION

SEX expression in flowering plants has been a subject of much interest to plant scientists. As cucurbits are both monomorphic and polymorphic in their sex expression, they have been studied extensively for understanding the genetic and physiological basis of the underlying phenomenon[1-4]. Retig and Rudich[5], and Jaiswal and Kumar[6] have correlated sex expression in cucurbits with enzyme activity. *C. grandis*, a dioecious cucurbit in which a chromosomal basis of sex determination has been established was chosen for the present study. The objective was to recognize whether sex specific isoenzyme markers exist in this system. Some work has already been carried out on this plant relating to qualitative differences in free amino acids of male and female plants[7], and also on enzyme activity and isoenzyme pattern in relation to sex differentiation[8,9]. This paper reports the qualitative and quantitative changes in peroxidase, acid and alkaline phosphatases in male and female plants of *C. grandis* and their possible relation to sex expression.

MATERIALS AND METHODS

As the sexes cannot be distinguished before flowering, shoot tips, isolated nodes, each bearing an immature flower bud, mature flowers, internodal segments and leaves were collected from flowering male and female plants growing in the Botanical Garden of this University.

One gram tissue from each type of explant was homogenized with 0.02 ml β-mercaptoethanol in 10 ml of 50 mM phosphate buffer at pH 7.5; 0.02 ml β-mercaptoethanol in 10 ml of 0.1 M Tris-HCl buffer at pH 8 and 0.1 M Tris-HCl buffer at pH 8 for extraction of peroxidase, acid phosphatase and alkaline phosphatase respectively. The macerates were centrifuged at 12,000 rpm for 30 min at 0°C. The clear supernatants were used for estimation of activities of the above mentioned enzymes and to trace their isoenzyme patterns. Lowry's[10] method was used to determine the total protein content in the extract, using bovine serum albumin as the standard.

Aliquots of the supernatants were assayed for total peroxidase activity using guaiacol in the presence of hydrogen peroxide. One ml of 1% guaiacol in 7.8 ml phosphate buffer (pH 6.1), and 1 ml of 2% H_2O_2 were added to 0.2 ml of the extract. Enzyme activity was determined spectrophotometrically (Bausch and Lomb UV spectrophotometer) by measuring the increase of absorbancy per minute at 420 nm.

The samples were incubated in the medium containing 15 mM of *p*-nitrophenyl phosphate and 0.5 M sodium acetate buffer at pH 5.5 for acid phosphatase activity and in 10 mM Tris (hydroxymethyl) amino methane (Sigma) at pH 9 for alkaline phosphatase activity. The reaction was terminated by adding 1 ml of IN NaOH. The amount of *p*-nitrophenol released was spectrophotometrically measured at 410 nm.

Separation of isoenzymes was carried out by disc gel electrophoresis using 7.5% polyacrylamide gel. The protein (100 mg) was loaded in each tube. Bromophenol blue was used as a tracking dye. Each tube was subjected to 1 mA electric current till the dye reached the bottom of the gel. Electrophoresis was carried out in a deep freeze (−14°C) tempera-

ture; the temperature of the running buffer was 2–4°C. Tris-HCl buffer (pH 8.3) was used for separation. To localize the peroxidase the gels were incubated in a mixture of 0.6 ml guaiacol and 0.5 ml 3% H_2O_2 (added just before staining) in 100 ml of 0.2 M acetate buffer (pH 4) at 26°C for 15–20 min. Acid phosphatases were visualized by incubating the gels in 1 mg/ml of Fast Garnet GBC salt (Sigma), α-napthyl phosphate (Sigma) and 10 drops of 1% $MgCl_2$ in 0.5 M sodium acetate buffer at pH 5.5. Incubation was carried out at 37°C. The bands appeared after 30–40 min. Gels for alkaline phosphatase could not be stained.

RESULTS AND DISCUSSION

Table 1 shows that the activity of the enzyme varies in the explants from both male and female plants. In the extracts of the shoot tip, peroxidase, acid and alkaline phosphatase activities are higher in the male plants. Alkaline phosphatase and peroxidase activities in the female flower buds (with nodal segments) are higher than those in the male flower buds. However, alkaline phosphatase activity is very low in the mature female flower.

In the male plants the shoot tip showed high activity of peroxidase, acid and alkaline phosphatases. The flower buds at the nodes, in both the sexes, have low enzyme activity except that of the alkaline phosphatase in female plants. But as the flower matures the peroxidase, acid phosphatase and alkaline phosphatase activity (in male plant) increases while alkaline phosphatase activity in female plant decreases. Leaves of male plants have highest alkaline phosphatase and mature flowers have highest acid phosphatase activity. The activity of these enzymes is higher in leaves as compared to internodal segment in both the sexes.

Relatively high peroxidase, acid and alkaline phosphatase activities are observed in the shoot tips. However, as the flowers mature the activity of these enzymes decreases except in respect of alkaline phosphatase in the female. The relation between peroxidase activity and growth, differentiation and ageing in various plant systems is reported earlier[4, 11–13]. These enzymes seem to affect the maturation and ageing of the flower as their activity was found to be enhanced in the mature flowers. The marked difference in the amount of enzyme activity in male and female plant parts suggests their role in the sex expression in *C. grandis*. In nodal segments with flower bud, peroxidase activity is higher in female than in the male plants. In internodal segment and mature flower, the peroxidase activity is higher in the male than in the female plants. In shoot tip and leaves the differences are only marginal. High phosphatase activity is reported to be correlated with high amount of GA_3. It is established that a relatively high GA_3 content favours male sex expression[14–16]. In the present study higher phosphatase activity is observed during initiation and maturation of male flowers.

The numbering of isoenzyme bands on the gels was done on the basis of R_f values. The shoot tip of the male plant has two peroxidase isoenzymes numbered 3 and 5, whereas that of the female has only band 4 (figure 1A–D). The node with male flower bud has bands numbered 1, 2, 3 and 5. In the node with female bud band 1 is absent, there are additional bands 3a, 3b and 4. In the mature flower, the number of isoperoxidases is reduced to two in male (band 3 and 4) and three in the female (2, 3 and 5). The leaves and the internodes also show variation in the number of isoenzymes of peroxidase. The leaves of the male plant have bands 1, 2, 3 and 5 (3 and 5 being prominent in leaves) while the female has bands 1, 2, 3a, 3b and 5. In the internodal segment the female plants lack band 4. Instead they have additional bands 3a and 3b. The isoperoxidase numbered 5 is more intense in male plants parts than in the female (figure 1A, B) except in the internodal segment.

The isoenzymes of acid phosphatase also show variation in the tissues of the two sexes (figure 2A–D). The male shoot tip has bands B-F while that of the female has bands B, D, E and F. The flower buds at the nodes have six bands each in male and female. They are A–F in the male and O, A, B, D, E, F in the female. O is absent in the male and C is absent in the female. The mature flowers have bands A, C, D, F in the male and A, B, D, F in the female plant.

Table 1 *Total enzyme activity*

Plant part	Peroxidase (μg/min)		Acid phosphatase (μg/min)		Alkaline phosphatase (μg/min)	
	Male	Female	Male	Female	Male	Female
Shoot tip	63.7	56.2	215.6	138.6	27.566	5.159
Node with flower bud	16.0	32.0	99.33	92.4	0.077	2.387
Flower	50.6	40.5	338.8	115.5	0.154	0.077
Leaf	51.2	54.0	200.2	123.2	62.986	44.275
Internode	34 8	18.8	115.5	50.05	0.097	0 077

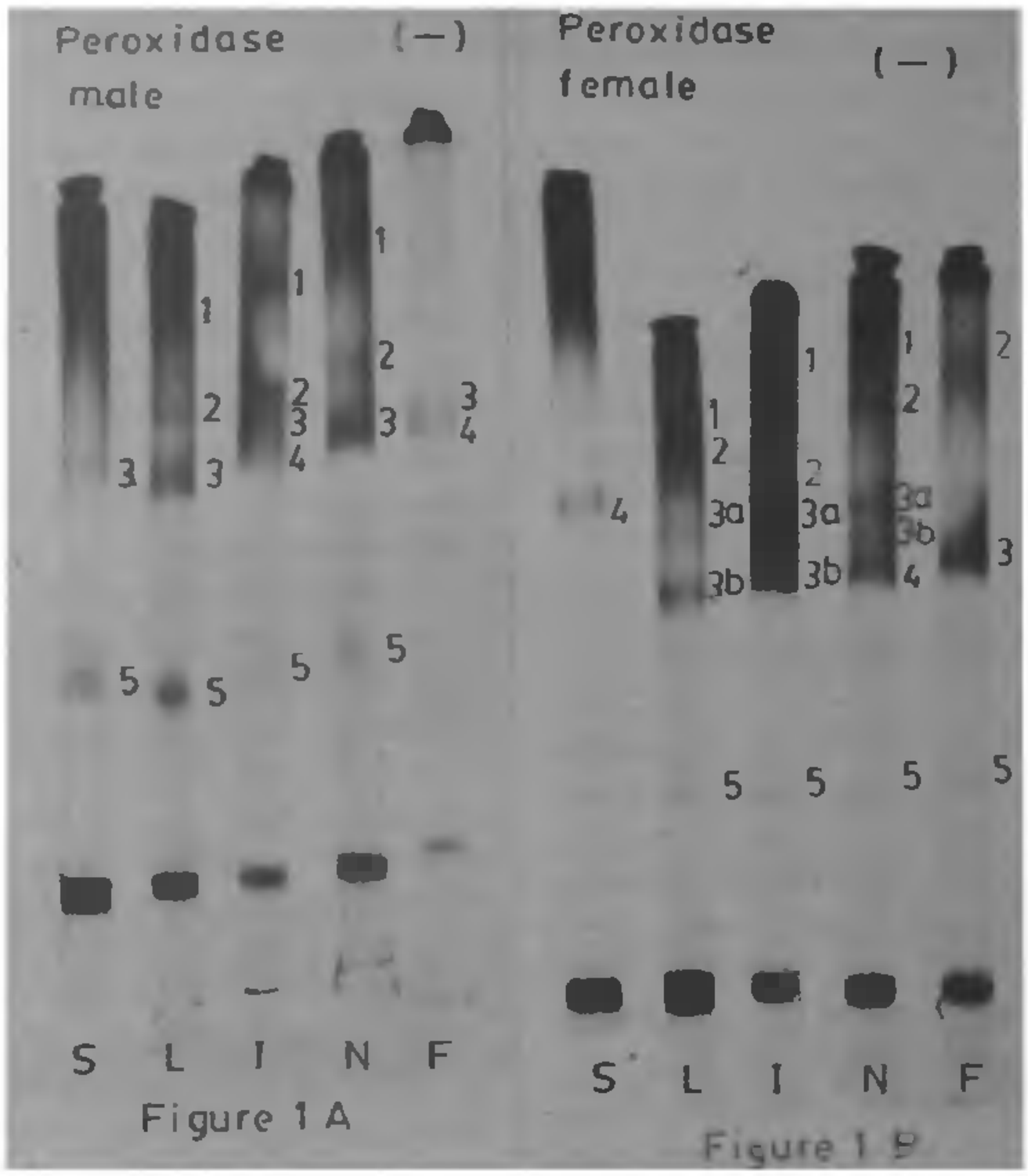

Figure 1A, B. Isoenzymes of peroxidase of male and female plants of *Coccinia grandis*; [S, shoot tip; L, leaves; I, internodal segments; N, nodal segments with buds; F, mature flowers].

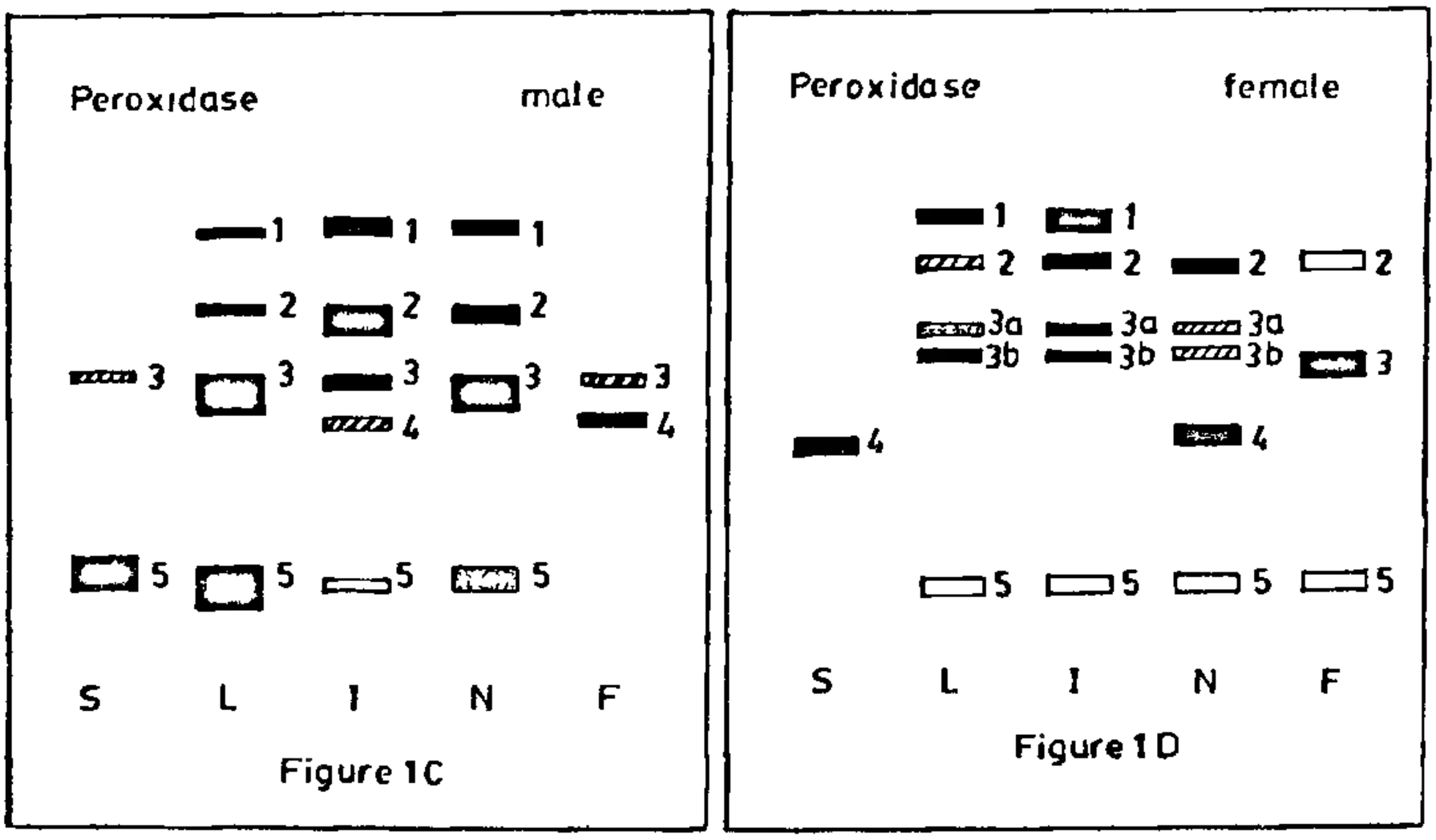

Figure 1C, D. Zymogram showing variation in electrophoretic pattern of peroxidase in male and female plant. [S, shoot tip; L, leaves; I, internodal segments; N, nodal segments with buds; F, mature flowers].

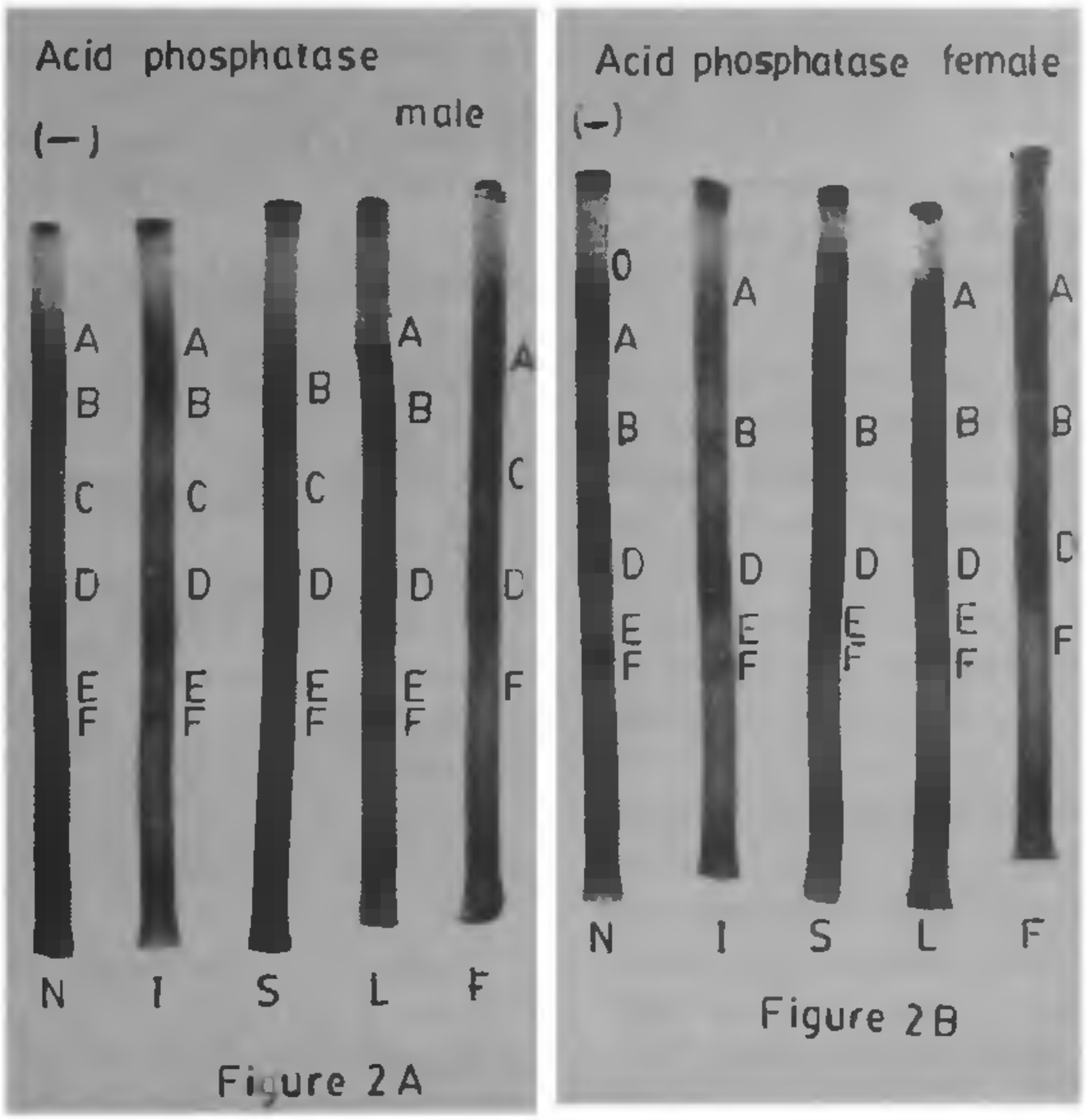

Figure 2A, B. Isoenzymes of acid phosphatase of male and female plant of *C. grandis*; [N, nodal segment with buds; I, internodal segments; S, shoot tips; L, leaves; F, mature flowers].

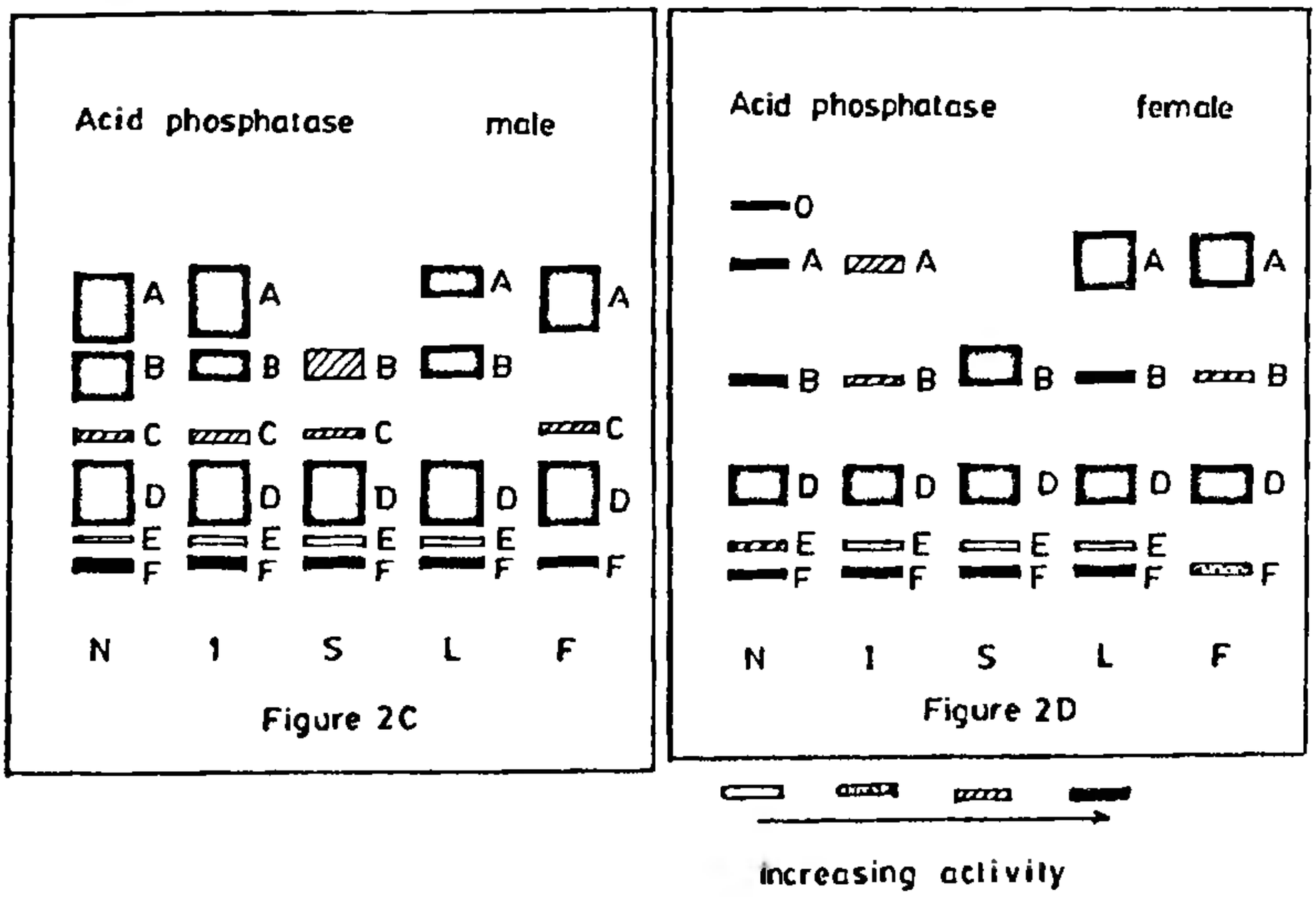

Figure 2C, D. Zymogram showing the patterns of acid phosphatase isoenzyme of male and female plants. [N, nodal segment with buds; I, internodal segments; S, shoot tips; L, leaves; F, mature flowers].

However, in the male flowers, band F has a higher intensity than that in the female flowers (figure 2A–D).

In both male and female plants the leaves have the same isoenzyme bands A,.B, D, E and F. The internodal segments of the male have A–F and female have A, B, D, E, F.

The present study has shown that the isoenzyme patterns of peroxidase and acid phosphatase are different in shoots of the male and female plants. It is also noted that the isoenzyme pattern changes as the flowers mature. This variation indicates that the isoenzymes may be involved in sex expression in *Coccinia*. In *Cannabis sativa* the male flowers are characterized by the presence of three specific isoperoxidases[17].

The isoenzymes of alkaline phosphatase could not be stained even after using different staining methods. Brewbaker *et al*[18] have also reported their inability to stain the bands of alkaline phosphatase with fast blue RR and α-napthyl phosphate using plant extract.

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