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Analysis of Soluble and Wall Bound Acid Phosphatases During Various Phases of Development in Mung Bean (*Vigna radiata*)

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Abstract: Acid phosphatase is a hydrolytic enzyme which catalysis the hydrolysis of variety of phosphate esters that may involve in many biochemical steps. During present investigations, qualitative analysis of acid phosphatases from soluble and wall bound fraction was performed from different tissues of four varieties of mung bean. It was detected that number of bands varies from 1-6. Acid phosphatase (AP-II and AP-III) were present in leaves, flowers and pods whereas AP-V was specific to leaves and pods. More number of soluble acid phosphatase was observed in younger stages of leaves and pods, it decreased at the older stages. In case of wall bound fraction, young leaves and flowers showed lesser number of bands whereas an increase in the total number of bands was prominently seen as the growth proceeds.

Key words: *Vigna radiata*, leguminosae, soluble, wall bound, acid phosphatase, isozymes, polyacrylamide gel electrophoresis

troduction

wo types of phosphatases acid and alkaline are reported in living ells but plant cells mainly exhibit activity at acidic pH (Krishan, 964). Acid phosphatases are widely distributed in animals, plants nd bacteria (Lynn and Clevette-Radford, 1987). No alkaline hosphatase activity is seen in roots, coleoptile and leaf of barley Upadhya and Yee, 1968) and cell wall of *Convolvulus arvensis* (Klis *t al.*, 1974). Acid phosphatases play an important role in the obilization of phosphate reserves during germination (Yamagata *t al.*, 1980) and catalysis the hydrolysis of variety of phosphate sters (Park and Van Etten, 1986). Acid phosphatases are lycoproteins and have pH optima around 5.0-6.0 (Lynn and levette-Radford, 1987).

lany seeds contain acid phosphatase activity which increases uring germination and slowly disappears (Rossi *et al.*, 1981). Acid hosphatase isozymes are present in diverse plant genera tissues. 'any differences are reported in isozymic patterns between the enera, and between the tissues of plant. Some isozymes are of ommon occurrence (Hall *et al.*, 1969).

cid phosphatases can be extracted from soluble as well as wall-ound fractions. The cell wall acid phosphatase is an intrinsic mponent of plant cell walls and is useful in the study of protein afficking mechanisms to the cell surface (Kaneko *et al.*, 1998). Its ultiple molecular forms may show fast or slow migration. In maize, rmant and at first 48 hours of seed germination, a fast migration nd is observed at 72 hours of germination along with a slow grating band. This slow migrating band persists even after 96 urs of germination, suggesting that the presence of slow grating enzyme is important for full development (Teno *et al.*, 87). In flax genotrophs there is relative mobility shifts in acid osphatases which is controlled by dominant homozygous for ter, and recessive homozygous for slower alleles (Tyson *et al.*, 86)

terials and Methods

eds of four varieties of mung bean (*Vigna radiata*) viz. cv. NCM89 , cv. 121-123 (B), cv. 19-19 (C), and cv. 20-21 (D) were sown andomized complete block design with three replications. Third of all the plants was tagged as soon as they appeared and were ected from young (L_1), blooming (L_2), matured (L_3) and escent (L_4) plants; two stages of flower i.e. bud (F_1) and pre-nesis (closed) flower (F_2), and two stages of pods i.e. young , and mature pods (P_2) were collected. These samples were used for the qualitative separation of acid phosphatase from soluble and wall-bound fractions (Vattuone *et al.*, 1981). Polyacrylamid gel electrophoresis was performed (Maurer, 1971) with minor modifications. Gels were incubated in the stain till the brown bands of acid phosphatase appeared (Fields and Tyson, 1983).

Results and Discussion

The electrophoretic pattern of acid phosphatases from soluble and wall bound fractions from different tissues of all the four varieties are exhibited in Fig. 1 and 2, respectively.

Soluble acid phosphatase (AP): Soluble acid phosphatases were present in all the tissues at all the developmental stages. Fig. 1 is a compiled zymogram by which temporal and spatial comparison among different varieties can be made. Immature leaves possessed AP-I, AP-IV, AP-V, and AP-VI isozymes of almost same intensities in all the four varieties. Young leaves contained AP-I, AP-III and AP-VI in cv. NCM89, cv. 121-123 and cv. 20-21, whereas AP-V was present instead of AP-III in cv. 19-19. In matured leaves AP-I, AP-IV, AP-V and AP-VI were present in all the four varieties with same intensities. At senescent common bands of all cultivar were AP-II, AP-III and AP-V with slight differences in their intensities and width. It may be concluded that AP-I and AP-VI were consistently present in immature, young and mature leaves of all varieties. In senescent leaves AP-I and AP-VI were missing whereas AP-II could be considered as the specific band of senescence. In *Xanthium* leaves 2-7 acid phosphatases had been reported (Chen *et al.*, 1970). Oat and barley leaves possessed 4-5 isozymes (Wyen *et al.*, 1971).

In bud and closed flower a dark band, AP-II was present except in cv. 19-19 where AP-III could be seen instead of AP-II. AP-VI appeared in closed flower of all the varieties. In pods AP-I, AP-III and AP-V isozymes were present in young stage in all varieties except cv. 19-19 where AP-I, AP-V and AP-VI were found. The matured stage of pod AP-I was missing in all varieties, AP-II was present in cv. NCM89 and cv. 20-21, whereas AP-III appeared in c.v. 19-19 and cv. 121-123. Varietal differences could be seen during flowering and fruiting stages. According to Biswas and Cundiff (1991) four different isoforms of acid phosphatases were present in germinating seeds of *Vigna sinensis*. Two of these (AP-I and AP-II) were constitutively expressed and another two (AP-III and AP-IV) were developmentally regulated (Biswas *et al.*, 1996). Current results revealed that AP-I and AP-V appeared in leaf, disappeared from flower and reappeared in pods.

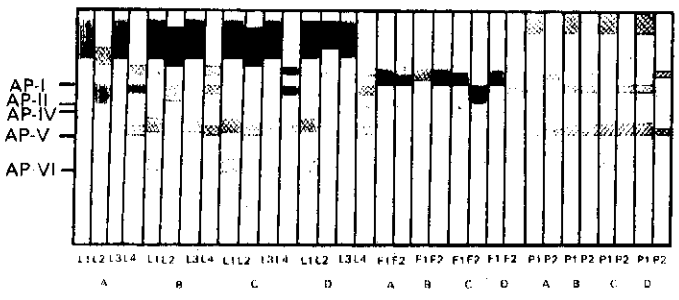

Fig. 1: Electrophoretic pattern of acid phosphatase isolated from soluble fraction. L1, young leaves; L2, blooming leaves; L3, mature leave; L4, senescent leaves; F1, bud; F2, closed flower; P1, young pod; P2, matured pod. A, Cv-NCM 89; B, Cv-121-123, C, Cv-19-19; D, Cv-20-21.

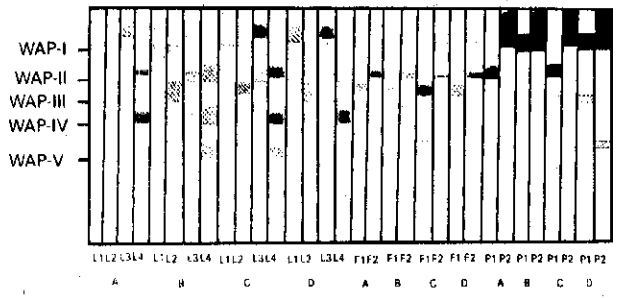

Fig. 2: Electrophoretic pattern of acid phosphatase isolated from wall bound fraction. L1, young leaves; L2, blooming leaves; L3, matured leaves; L4, senescent leaves; F1, bud; F2, closed flower; P1, young pod; P2, mature pod. A, Cv-NCM 89; B, Cv-121-123; C, Cv-19-19; D, Cv-2021.

Wall bound acid phosphatase (WAP): Fig. 2 represents isoforms of acid phosphatase isolated from wall bound fraction of different developmental stages of mung bean. Immature leaves possessed only one broad band of WAP-I, whereas young leaves WAP-II and WAP-III appeared along with WAP-I, in all varieties however WAP-II was absent from cv. 20-21. In matured leaves WAP-I and WAP-II were present in all the samples studied, with different intensities. Senescent leaves contained isozymes WAP-II and WAP-IV. In wheat five bands of acid phosphatase are observed in dormant seed and coleoptile whereas four are detected in germinated seeds and first leaf (Macko *et al.*, 1967). It had been noted that buds showed the appearance of WAP-III only whereas closed flower possessed WAP-I, and WAP-II in all cultivars. Tobaco protoplasts secreted two isoforms of acid phosphatase during regeneration of the cell wall (Kaneko *et al.*, 1996). WAP-III was specific to young pods except in cv. 20-21 and WAP-V was specific to mature pods. In case of leaves and flowers, number of wall bound acid phosphatases were less at the early stages of development which were increased at later stages.

During present investigations it was detected that slow and fast migrating acid phosphatases were present in leaves and pods isolated from both the fractions. However slow migrating acid phosphatase persisted for longer period. In maize, slow migrating acid phosphatase along with fast migrating acid phosphatases are present from 48 hours of germination. The slow migrating band persists even after 96 hours of germination indicating that this slow band is required for full development (Teno *et al.*, 1987).

From the current research, temporal and spatial expression of soluble and wall bound acid phosphatase was studied. These acid phosphatases showed differences in the isozymic pattern, which may be responsible for developmental and functional specialization.

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