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BRIEF COMMUNICATION

Distribution of Cu^{2+}-diamine oxidase during ontogeny of seedlings of *Vigna radiata* cultivars

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Abstract

Activity of Cu^{2+}-diamine oxidase (DAO; E.C.1.4.3.6.) was measured in *Vigna radiata* (L.) Wilczek cultivars K851, MH8320 and Pusa Baisakhi in light and in dark during ontogeny of seedlings. DAO activity was always the highest in cv. K851. In both light and dark grown seedlings maximum DAO activity was detected on day 2 after germination. Thereafter, in light grown seedlings it declined consistently upto non-detectable levels. In dark, DAO activity was higher than in light and it had the second maximum on day 7 following a similar declining pattern as observed for the light grown seedlings. The DAO activity was higher in a shoot apex alongwith leaves than in roots and shoot axis.

Additional key words: mungbean, polyamines, senescence.

The diamine putrescine, triamine spermidine; and tetramine spermine are ubiquitous in plant cells while other polyamines (PA) are of more or less limited occurrence. The importance of PAs in plant growth and development remains yet a matter of debate (Tiburcio *et al.* 1994). Most of the physiological functions of PAs are similar to those of cytokinins. These include role of PAs in cell division, organogenesis, senescence and stress responses (Tiburcio *et al.* 1993, Bagni and Torrigiani 1992, Flores *et al.* 1984). The amine oxidases catalyse the oxidation of amines, in particular di- and polyamines to the corresponding aldehydes. Diamine oxidases (E.C.1.4.3.6.) have been isolated and purified from many plant species including legumes and the molecular and functional

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properties have been reviewed recently (Medda *et al.* 1995). Diamine oxidase activity during seed germination has been reported in *Cicer arietinum, Glycine max, Lens esculenta, Lathyrus sativus, Phaseolus vulgaris, Pisum sativum, Vigna mungo,* and *Zea mays* (Federicio and Angelini 1988, Maccarrone *et al.* 1991, Suresh *et al.* 1976, Torrigiani and Scoccianti 1995, Scoccianti *et al.* 1990, De Tomaso *et al.* 1992, Srivastava *et al.* 1981, Lahiri *et al.* 1992). Accumulation of polyamines has been reported during early growth phase in the mungbean hypocotyl (Goldberg and Perdrizet 1984). The present study is planned to report the distribution of DAO in three cultivars of mungbean, commonly grown as legume crop in tropics, during early growth phases.

Vigna radiata (L.) Wilczek (cultivars K851, MH8320 and Pusa Baisakhi) seeds procured from I.A.R.I. Pusa, New Delhi were surface sterilised with 90 % ethyl alcohol for 4 min and washed thoroughly with distilled water. The seeds were soaked for 1 h in distilled water before planting and germinated at 25 ± 2 °C in Petri dishes on moist filter papers at 16-h photoperiod (irradiance of 80 μmol m^{-2} s^{-1}; fluorescent-tubes and incandescent bulbs) or total darkness for 15 d.

At specified period 500 mg of the fresh mass from whole seedlings was homogenised in prechilled mortars at 0 - 4 °C in 0.01 M phosphate buffer [pH 7.0; ratio 1:4 (m/v)] and the extract was filtered through double layered muslin cloth as described by Naik *et al.* (1981). The crude homogenate was centrifuged at 14 300 *g* and the supernatant was used for the enzyme assay using the method of Naik *et al.* (1981) with a slight modification. The assay mixture contained 50 μM phosphate buffer (pH 7.5), 10 μM putrescine, 0.2 cm^3 enzyme extract in a total volume of 3.5 cm^3. After incubation at 37 °C for 30 min the reaction was terminated by adding 0.5 cm^3 of 10 % trichloroacetic acid. The centrifuged supernatant (1 cm^3) from the assay tubes was taken and (1 cm^3) warm ninhydrin reagent was added followed by 1.5 cm^3 of acetic acid. The tubes after mixing were kept in a boiling water bath for 30 min to develop the colour of Δ^1-pyrroline and ninhydrin reagent. The tubes were then cooled and 2.5 cm^3 acetic acid was added to make up the volume to 6.0 cm^3. The orange to red color of the samples was measured by spectrophotometer at 510 nm (*Model 106, Systronics,* Ahmedabad, India). The enzyme activity was determined by calculating the amount of Δ^1-pyrroline produced using a standard curve drawn for the pure commercial DAO obtained from *Sigma Chemicals Co.* (USA).

Mungbean seedlings grown in dark showed maximum DAO activity on day 2 followed by a decrease thereafter (Fig.1*A*). The enzyme activity, however, again increased slightly on day 7 and decreased further in all the three cultivars. A higher DAO activity was noticed in cv. K851, than in the cultivars MH8320 and Pusa Baisakhi (Fig. 1*A*). Light grown seedlings showed similar pattern as in dark except for the another peak on the day 7 (Fig. 1*B*).

Since the maximum DAO activity has been detected in all three cultivars on day 2, it was thought necessary to find out the trend of the enzyme activity during germinating phase (Fig. 2). DAO activity was measurable from 6 h after seed sowing, increased to a peak (24 h) and then gradually declined (Fig. 2).

This pattern was observed in both dark and light grown seedlings. DAO activity was higher in dark than in light grown seedlings (from 6 h to 15 d after germination). In 7-d-old etiolated seedlings of cv. K851 the young primary

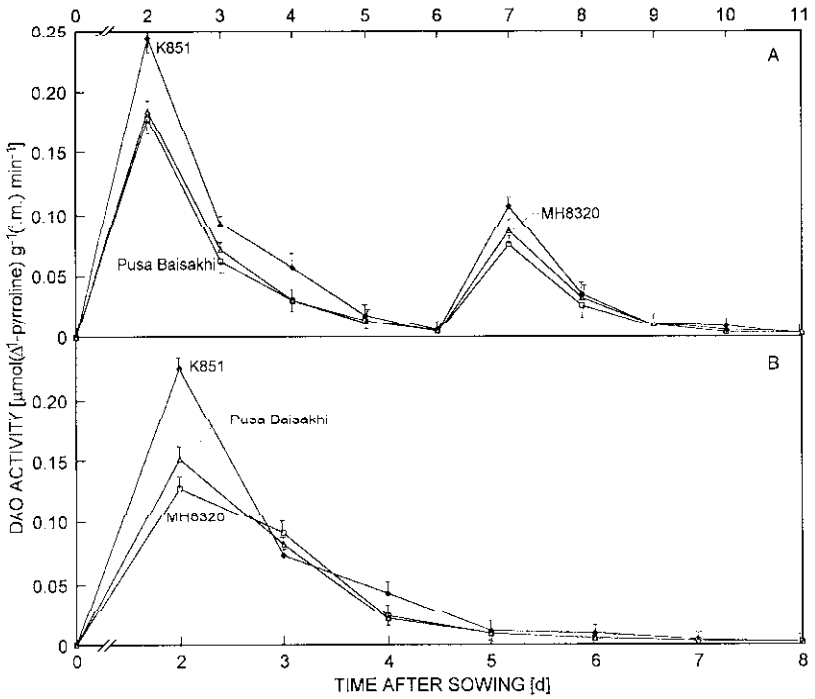

Fig. 1. Diamine oxidase activity during ontogeny of seedlings of mungbean cultivars K851 (*rhombs*), MH8320 (*triangles*), and Pusa Baisakhi (*squares*) grown in the dark (*A*) or in the light (*B*).

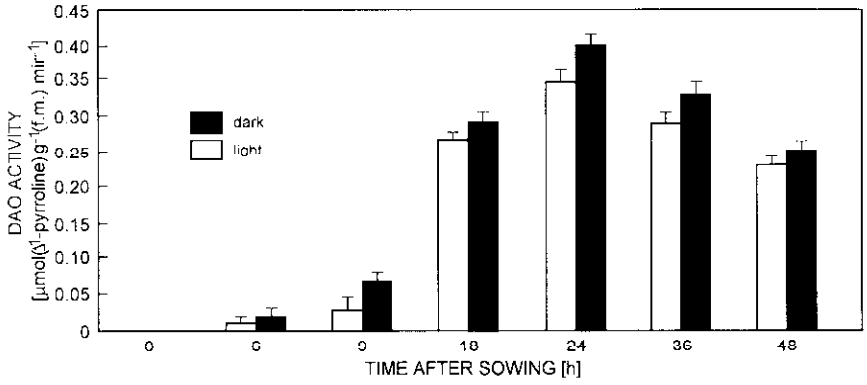

Fig. 2. Diamine oxidase activity during early ontogeny of seedlings of mungbean cultivar K851 grown in the dark (*full columns*) or in the light (*open columns*).

leaves alongwith the shoot apex had maximum DAO activity [0.119 ± 0.008 μmol(Δ^1-pyrroline) g^{-1}(f.m.) min^{-1}] which was 2 -3 fold higher than that in the roots and shoot axes [0.050 ± 0.005 and 0.034 ± 0.007 μmol(Δ^1-pyrroline) g^{-1}(f.m.) min^{-1}, respectively].

The results indicate that DAO activity in seedlings of three mungbean cultivars might have a role in the regulation of PAs level. The purification and the characterisation of the possible isoforms are under progress.

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