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Soluble Forms of Invertases in Mungbean (*Vigna radiata*) During Germination and Developmental Stages of Various Tissues

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Abstract: Three varieties of mungbean NM 19-19, NM 20-21 and NM 121-125 were germinated to investigate the changes in activity of soluble forms of invertases with respect to their pH optima during germination and various developmental stages of leaves, flowers and pods. High alkaline invertase activity was present after 24 h of germination in all varieties and during subsequent stages of germination and it was replaced by acid invertase. Acid invertase seemed to be present in tissues undergoing rapid growth and development, where hexoses were quickly utilized and sucrose was rapidly hydrolyzed. Immature stages of leaves, flowers and pods primarily contained acid invertase but as the growth proceeded, alkaline invertase or neutral invertase were appeared alone or along with acid invertase activity. Alkaline invertase was supposed to hydrolyze the sucrose in cells of storage organ.

Key words: Invertase, mungbean, leguminosae, germination, pH optima

Introduction

Expansion growth of plant cells depends upon synthesis of RNA and proteins and in some cases also of DNA synthesis (Nitsan and Lang, 1965). It seems possible that among the proteins which are synthesized during cell growth are enzymes which in turn play a regulatory function in growth i.e. by determining the availability of substrates. One such enzyme could be invertase. Invertase can be envisaged to control cell growth by regulating the hydrolysis of sucrose and also its transport to cells as well between different compartments of the cell (Seitz and Lang, 1968).

Invertase i.e. β -fructofuranoside fructohydrolase (EC 3.2.1.26) have been isolated from several plant tissues especially those engaged in active growth and development (Ap-Rees, 1974). Three forms of invertases are present in higher plants i.e. 'acid' invertase, 'alkaline' invertase and 'neutral' invertase (Sacher *et al.*, 1963; Darren and Frederik, 1998). Acid invertase converts sucrose into glucose and fructose (Beruter and Studer, 1997; Isla *et al.*, 1999). It is present in the cell wall in insoluble form and in the cytoplasm and vacuole in soluble form (Quick and Schaffer, 1996; Nguyen-Quoc and Foyer, 2001). Measurements of alkaline or neutral invertase activity during development have been reported in carrot and sugar cane (Hatch and Glasziou, 1962). In both plants the function of the alkaline invertase is to catalyze sucrose hydrolysis in cells of storage organ that lack appreciable acid invertase activity. Neutral invertase is present in considerable amount in mature tissues and catalyze the hydrolysis of terminal non-reducing β -fructofuranoside residues in β -D-fructofuranoside like sucrose. It also may play a key role in the control of hexose concentrations in the cytosol of sugar cane stem cells, thus affecting control over the expression of sugar responsive genes (Vorster and Botha, 1998).

Current study is an attempt to investigate changes in the activity of soluble forms of invertases during germination and developmental stages of various tissues. Earlier wall bound invertase activity in different tissues of mungbean was also studied (Mansoor and Naqvi, 2000).

Materials and Methods

Mungbean (*Vigna radiata*) seeds viz; cv. NM'19-19', cv. NM'20-21' and cv. NM'121-125', were supplied by Pakistan Agricultural Research Council Islamabad and used as experimental material. Flowering started within 30-40 days whereas wilting take place approximately 60-70 days after germination.

Culture practice: Seeds were sterilized with 1% sodium hypochlorite solution. The experiment was conducted in randomized complete block design with four replications. Seeds were sown in a 14 x 10 inches plastic trays, filled with sandy loam soil. The seeds were sown approximately half inch apart. Moisture

was maintained by watering trays on alternate days. Four samples (24, 48, 72 and 96 h after germination) of 2 g/rep/variety were harvested. They were first washed thoroughly with tap water and then with distilled water, packed in moisture proof plastic bags and stored at -4°C till enzyme extraction.

Field experiment: Seeds of three varieties were sown in the field in randomized complete block design with four replications. Plots (3x5 feet) were prepared by mixing sandy loam soil with manure in 1:1 ratio. Four hundred seeds of each variety were sown half inch apart in each plot i.e. hundred seeds in each row. Soil moisture was maintained by irrigating the crop three times a week. Samples of leaves were collected from young plant (30 days after sowing), blooming plant (50 days after sowing) and mature plant (66 days after sowing). NM 19-19 was used for flower and pod analysis. Three stages of flower, bud, closed flower (pre-anthesis) and open flower (post anthesis) were collected. Four samples of pods were collected i.e. immature pods, young pods, mature pods and dry pods. All the samples were washed with tap water then with distilled water. Packed in moisture proof plastic bags and stored at -4°C till further processing.

Enzyme extraction: Samples were homogenized in ice chilled extraction buffer (0.02 M Tris HCl pH 7.2 and 0.01 M NaCl, pH 7.0, at a ratio of 2:1 (v/w) and filtered through four layers of muslin cloth. Insoluble material was removed by centrifugation at 10,000 g for 10 min at 4°C. Supernatant was collected and used for enzyme assay.

Enzyme assay: Two estimating buffer systems were used for the estimation of invertase (0.1 M NaOAc buffer, pH 3.0-6.0 and 0.1 M potassium phosphate buffer, pH 6.5-8.0). Crude enzyme extract (0.5 ml) was incubated in a mixture containing 0.5 ml of estimating buffer (pH 3.0- 8.0) at 37°C for 30 min. The reaction was stopped by adding 1 ml of alkaline copper reagent (Somogi, 1937). Reducing sugars were determined by the method of Nelson (1944) with minor modifications. Total proteins were estimated by the method of Lowry (1951).

Results and Discussion

Estimation of invertase from different stages of germination: A continuous shift in the synthesis pattern of the enzyme was seen from germination till maturation of mungbean. The invertase activity (μ g sugar/mg protein/30 min.) pH profile of variety NM 19-19 at different germination times was determined (Fig. 1a). At 24 h after sowing, invertase activity was found to be maximum at pH 7.0, acid invertase was appeared as growth proceeded. This pattern was also same for NM 20-21 (Fig. 1b). The pattern of invertase activity in NM 121-125, showed that at 24 h of

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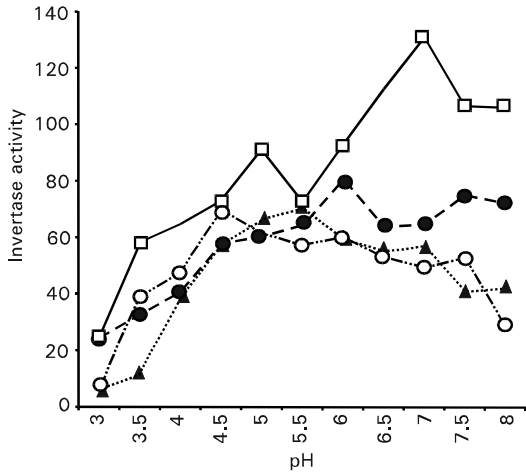


Fig. 1a: Invertase activity (µg sugar/mg protein/30 min.) pH profile at different times of germination in '19-19' of mungbean.

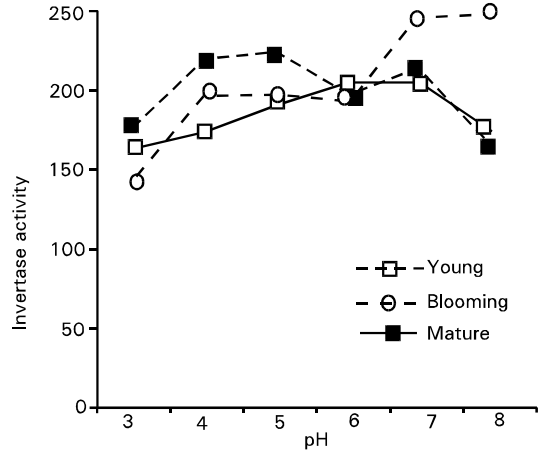


Fig. 2a: Invertase activity (µg sugar/mg protein/30 min.) pH profile of leaves in '19-19' of mungbean.

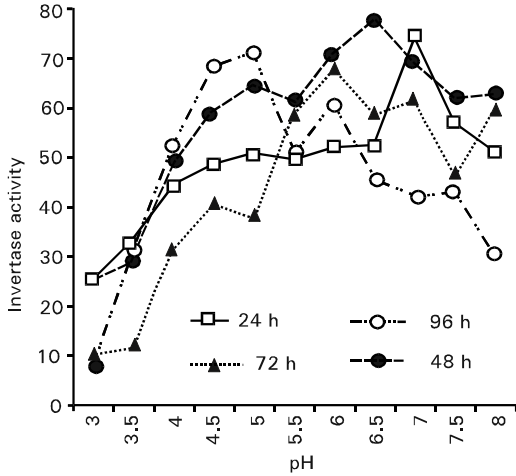


Fig. 1b: Invertase activity (µg sugar/mg protein/30 min.) pH profile at different times of germination in '20-21' of mungbean.

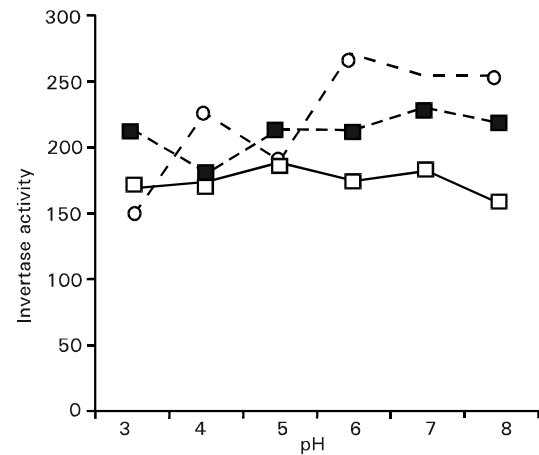


Fig. 2b: Invertase activity (µg sugar/mg protein/30 min.) pH profile of leaves in '20-21' of mungbean.

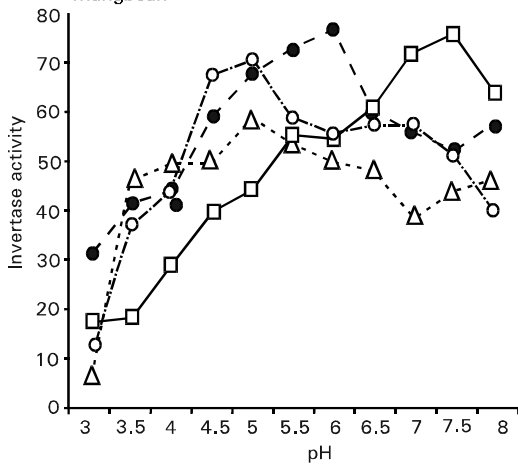


Fig. 1c: Invertase activity (µg sugar/mg protein/30 min.) pH profile at different times of germination in '121-125' of mungbean.

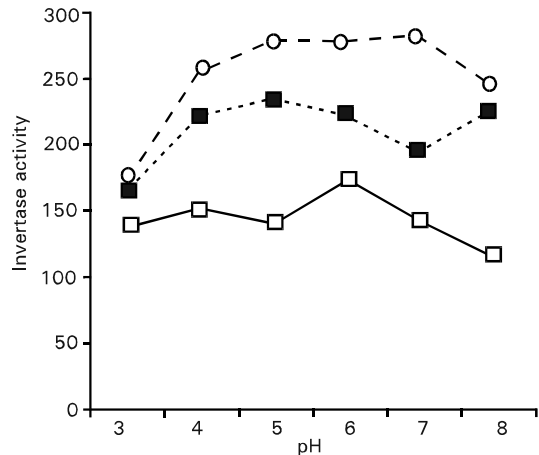


Fig. 2c: Invertase activity (µg sugar/mg protein/30 min.) pH profile of leaves in '20-21' of mungbean.

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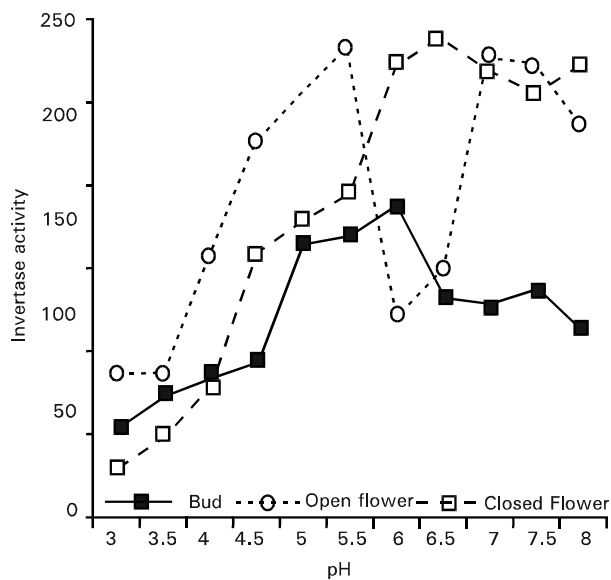


Fig. 3: Invertase activity (μg sugar/mg protein/30 min.) pH profile at different stage of flower in '19-19' of mungbean

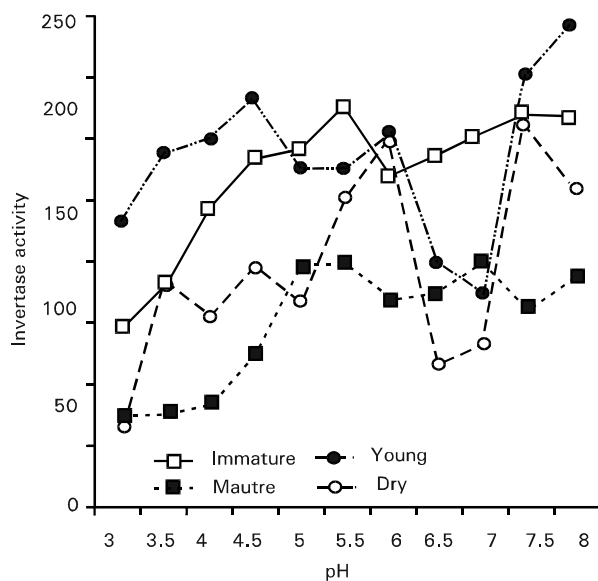


Fig. 4: Invertase activity (μg sugar/mg protein/30 min.) pH profile during pod maturation in '19-19' of mungbean

germination, maximum activity was seen at pH 7.5, whereas high peak was observed at acidic pH during 48, 72 and 96 h of germination (Fig. 1c).

These results are completely agreed with the findings of Dey (1986), who reported that high levels of alkaline invertase activity occur in dormant mungbean seeds and during germination its activity decreased rapidly and replaced by high acid invertase activity and the synthesis of acid invertase may be a key step in the developmental process related to seed viability and seedling vigour. He also suggested that the normal rapid rise of acid invertase activity on germination was a result of denovo synthesis of the enzyme. Furthermore, the progressive rise in the acid invertase activity equates with the theory that this form of the

enzyme is required by the developing tissues to convert sucrose to free hexoses.

In NM 19-19 and 20-21, neutral invertase was present with no acid invertase activity at 24 h of germination. Presence of neutral invertase activity was also reported in mature carrot and sugar cane (Hatch and Glasziou, 1962; Vorster and Botha, 1998). Isla *et al.* (1999) reported that in the absence of alkaline invertase, neutral invertase take over the role of acid invertase.

Estimation of invertase from different stages of leaves: There was a transition between acid and alkaline invertase during leaf development of all the three varieties. Invertase activity in leaves of NM 19-19 was shown (Fig. 2a), where the maximum activity was seen at acid pH in young leaves. When leaves were taken from blooming plant, peak activity was observed at alkaline (8.0) and acid pH in mature leaves. In NM 20-21, acid invertase was present in young and blooming leaves, having activity at pH 5.0 and 6.0 respectively. However in mature leaves peak invertase activity was seen at neutral pH (Fig. 2b). Young and mature leaves of NM 121-125 possessed acid invertase, while the leaves from blooming plant showed the presence of neutral invertase activity (Fig. 2c). Hatch and Glasziou (1962) reported that acid invertase is present in immature sugar-cane tissues which is replaced by an alkaline invertase activity in mature storage tissue, the presence of acid invertase indicated the availability of fructose for cell growth and formation of structural organelles availability. These findings correlated with the current investigations where in leaves of young plant acid invertase was present while, the leaves of blooming plant showed predominantly the alkaline invertase activity. Acid invertase could also be present in cell wall (insoluble form), cytoplasm and vacuole (soluble form) (Quick and Schaffer, 1996; Nyuyen-Quoc and Foyer, 2001), but the activity of cell wall bound acid invertase would be significantly higher than soluble acid invertase at any stage of apple fruit development (Zhang *et al.*, 2001) and has been shown to be present in tissues undergoing rapid growth and development, namely young roots and shoots, where hexoses are rapidly utilized and sucrose are rapidly hydrolyzed (Schaffer, 1986; Beruter and Studer, 1997). However in mature tissues the presence of highly efficient acid invertase is undesirable, therefore alkaline invertase was present, but present studies showed the presence of acid invertase in mature leaves of NM 19-19 and NM 121-125. This could be explained by the earlier findings of Pollock and Lloyd (1977) that in leaf of *Lolium temulentum*, acid invertase activity fell during leaf extension but rose again after ligule formation.

Estimation of invertase from different stages of flower and pod: During current study, when different stages of flower in NM 19-19 were analyzed for invertase activity (Fig. 3), it was detected that acid invertase activity was high at bud, closed flower and open flower stages with pH 6.0, 6.5 and 5.5 respectively. It was interesting to find that in all the developmental stages of flower high invertase activity was detected only at acidic pH. It might be due to the fact that there was rapid morphological and physiological changes for the conversion into fruits, due to which it might need more hexoses that were obtained by the hydrolyzing sucrose through acid invertase.

Four stages of pod were used for the estimation of invertase activity and noticed that the highest activity was found to be present at acidic pH in immature and young pods, this high activity of acid invertase indicate that in these stages of pod development, the sucrose content was either very low or declining very rapidly (Fig. 4). These results are supported by the findings of Venkataramana and Naidu (1993), reported high acid invertase activity in young sugar cane stem with low sucrose concentration indicate hydrolysis of sucrose under conditions where there is a heavy demand for hexoses. In mature pods the activity was high at acidic and neutral pH, these findings are almost similar with the findings of Moriguchi *et al.* (1991) who reported that both acid and alkaline invertases were active in mature mandarin fruits with

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a low sugar content, that is, the former was involved in the uptake of unloaded sugar into cells, while the latter was involved in the consumption of deposited sugar as energy. In dry pods only alkaline invertase was present. As matured tissues possess either no or low acid invertase activity and the absence of acid invertase may indicate the sucrose storage.

Briefly it can be concluded that the immature tissues possessed acid invertase activity which was replaced by alkaline or neutral invertase as growth proceeded. This was the reason that the seed (fully matured tissue) when germinated, alkaline or neutral with no acid invertase activity was seen at 24 h of germination. Later stages of germination were the young or immature stages of plant where we found acid invertase activity.

Invertases which hydrolyze sucrose to glucose and fructose are widely distributed in higher plants and often occur as multiple forms differing in pH optima, isoelectric points and subcellular locations in many tissues. Among the functions proposed for invertase are regulation of hexose levels in tissues and utilization of sucrose stored in vacuole (Ricardo, 1994).

Three forms of invertases are present in higher plants; 'acid' invertase, 'alkaline' invertase and 'neutral' invertase (Sacher *et al.*, 1963; Vorster and Botha, 1998). The function of 'acid' invertase is to catalyze the irreversible cleavage of sucrose to glucose and fructose (Isla *et al.*, 1999). The 'alkaline' invertase was confined to mature tissues and hydrolysis of sucrose in cells of storage organs (Dey, 1986). 'Neutral' invertase catalyzes the hydrolysis of terminal non-reducing β -fructofuranoside residues in β -D-fructofuranoside like sucrose, it also may play a key role in the control of hexose concentrations in the cytosol of sugar cane stem cells, thus affecting the control over expression of sugar responsive genes (Vorster and Botha, 1998).

Dey (1986) observed that high level of alkaline invertase was present in resting seeds of mungbean and rapidly replaced by acid invertase activity on germination. The activity of 'acid' invertase remained high at the mature stage of tomato fruit which do not store sucrose (Manning and Maw, 1975). In case of sugar cane 'acid' invertase activity was high in young stem which declined as the cane matured with the concomitant increase in sucrose (Venkataramana and Naidu, 1993). Both 'acid' and 'alkaline' invertases were active in immature mandarin fruits with a low sugar content, the former was involved in the uptake of unloaded sugar into cells, while the latter was involved in the consumption of deposited sugar as energy (Moriguchi *et al.*, 1991). Present study is an attempt to investigate the changes in soluble forms of invertases during germination and various phases of development.

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