http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (10): 1550-1552, 2000

Changes in Wall Bound Invertase Activity in Young and Mature Tissues of Mung Bean (*Vigna radiata*)

Simeen Mansoor and Farzana N. Naqvi
Department of Genetics, University of Karachi, Karachi-75270, Pakistan

Abstract: A study of wall bound invertases in different tissues of mung bean cultivar has been undertaken. In young and mature leaves, acid as well as alkaline invertase activity was prominent. Senescent leaves showed the presence of alkaline invertase only. During flowering and fruiting, flowers and pods both posses acid invertase activity at initial stages of development whereas closed flower and mature pods showed the presence of alkaline invertase. Present results revealed that the young tissues of mung bean required acid invertase activity for their growth and development where hexoses were rapidly utilized with rapid sucrose hydrolysis. In mature and senescent tissues the appearance of alkaline invertase indicate the involvement of enzymes in the storage of sucrose. During present investigations, isozymes of invertases were studied by eluting them in sucrose solution of their respective pH optima after performing 7.5% polyacrylamide gel electrophoresis. Each tissue appears to possess wall bound invertases with predominance of a different activity in each case.

Key words: *Vigna radiate,* leguminosae, wall bound, invertase, pH optima, isozymes, polyacrylarnide gel electrophoresis

Introduction

β-Fructofuranosidse fructohydrolase (EC 3.2.1.26) or invertase enzyme is responsible for the hydrolysis of sucrose into glucose and fructose (Ouelhazi *et al.,* 1992). It often occurs in multiple forms deferring in pH optima, isoelectric points and subcollular locations in many tissues (Pressey, 1994). Wall bound or soluble fractions may contain acid, alkaline or neutral invertases. Acid enzyme catalysis the irreversible cleavage of sucrose to glucose and fructose (Isla *et al.,* 1999), while alkaline enzyme hydrolysed sucrose in cell of storage organs (Dey, 1985). Neutral invertase catalysis the hydrolysis of terminal non-reducing β-fructofuranoside residues in β-D-fructofuranosides like sucrose (Vorster and Botha, 1998). This paper is an attempt to perform comparative study of the cell wail invertases from different tissues of mung bean at various developmental stages.

Materials and Methods

Seeds of cv. 19-19 of mung bean (*Vigna radiate*) were obtained from Pakistan Agricultural Research Council, Islamabad. Seeds were sown in the field in randomized complete block design with three replications. Third leaf was collected from young, blooming, mature and senescent. Bud and closed flower (preanthesis) were taken as two different developmental stages of flower, whereas immature, young and mature pods were the three different stages of pod.

From these tissues, wall bound fraction was obtained by the method of Vattuone *et al*. (1981). pH optima from these tissues was detected by the method of Prado *et al.* (1980). Then 7.5% polyacrylamide gal electrophoresis (PAGE) was performed according to the method of Maurer (1971) with minor modifications. After PAGE, each gel was cut into tan pieces of 0.5 cm. Each piece was then placed in 1 ml of 0.1 M acetate buffer and 0.2 M sucrose solution to elute enzyme in the solution. These tubes were shaken vigorously and incubated overnight at 4°C. Invertase activity (Erg sugar/mg proteins/30 minutes) was estimated by measuring the amount of reducing sugars after enzymatic hydrolysis of sucrose by the method of Nelson (1944). Graph was plotted, each peak will represent an isozyme.

Results and Discussion

A major concern of investigators interested in cellular differentiation is to discern the biochemical steps that accompany the development of cells. The isozymic pattern of plant organs and tissues may be a direct reflection of particular genes that are operating at a given stage of growth and differentiation (Trinh *et al.,* 1981).

In the present investigations, various tissues of mung bean were taken from different developmental stages and wall bound invertase activity (μg sugar/mg proteins/30 minutes) was observed in buffer of different pH (3.0-8.5). invertase isozymes at respective pH optima were detected.

pH Optima of Various Tissues: During present work, young leaf possessed maximum invertase activity at acidic pH and alkaline pH (Fig. 1). Leaf at blooming stage possessed acidic and neutral invertase. Leaves at mature stage had acidic and alkaline invertase, whereas senescent leaf contained neutral and alkaline invertase activity with no acid invertases, according to Isla *et al.* (1988) in the absence of alkaline invertase, neutral invertase take over the role of acid soluble invertase. It was noted that acid invertase persist within young, blooming and mature stages of leaf whereas there was appearance and disappearance of alkaline and neutral invertases. Presence of soluble invertase with pH optima of 7.2 in mature sugarcane stem tissue was also reported by Vorster and Botha (1998).

Bud and closed flower showed highest peak at acidic and at alkaline pH (Fig. 2). Immature pod has maximum invertase activity at acidic pH . Young and mature pod possessed only alkaline invertase (Fig. 3). During present studies it was observed that acid invertase was present at early stages of development in leaves, flowers and pods, whose presence indicated the hydrolysis of sucrose into glucose and fructose for carbon and energy source (Krishnan *et al*., 1985). In sugar cane, a high acid invertase in young stem with low sucrose concentration was also reported by Venkataramana and Naidu (1993). As matured tissues posses either no or very low acid invertase activity and the absence of acid invertase activity may indicate sucrose storage, also reported by (Isla *et al*., 1988).

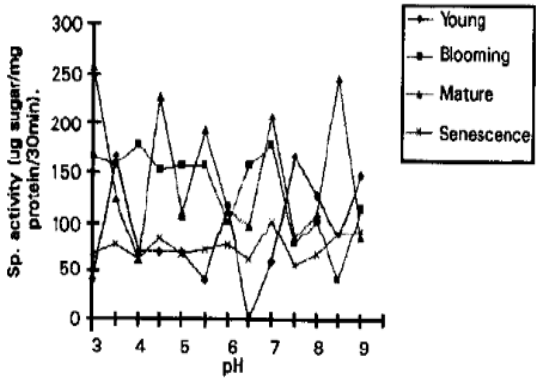

Fig. 1: Invertase activity, pH profile at different stages of leaf in cv, '19-19'

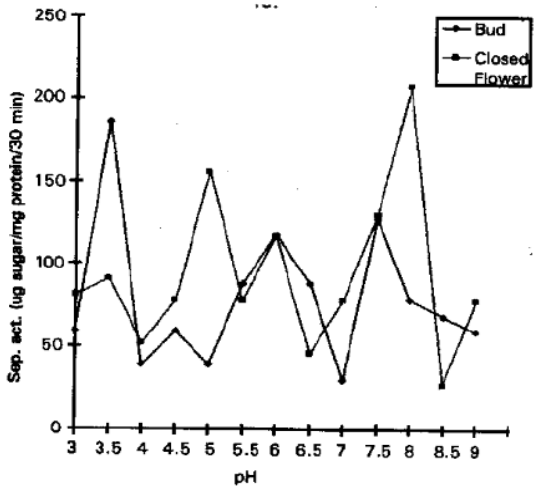

Fig. 2: Invertase activity, pH profile at different stages of flower in cv, '19-19'

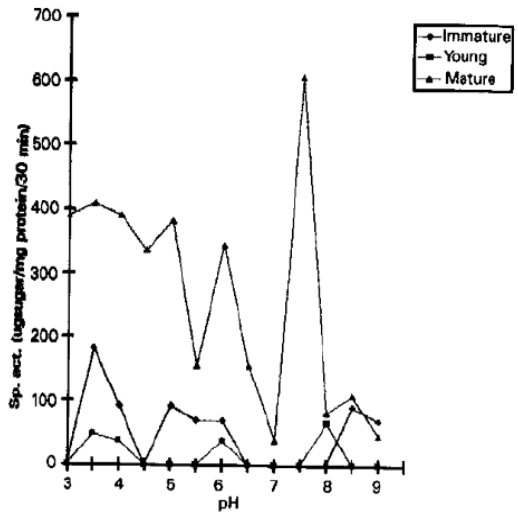

Fig. 3: Invertase activity, pH profile at different stages of pod in cv, 19-19

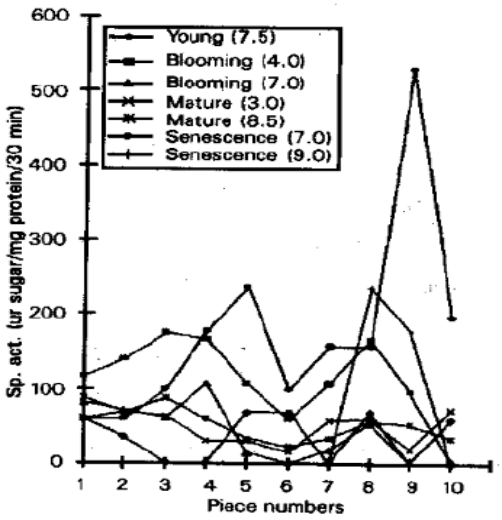

Fig. 4: Invertase isozymes in leaves at their specific pH optima

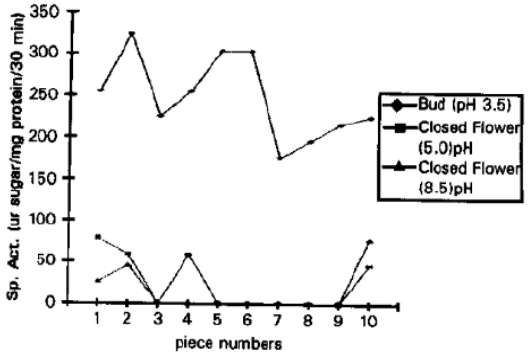

Fig. 5: Invertase isozymes in flower at respective pH optima

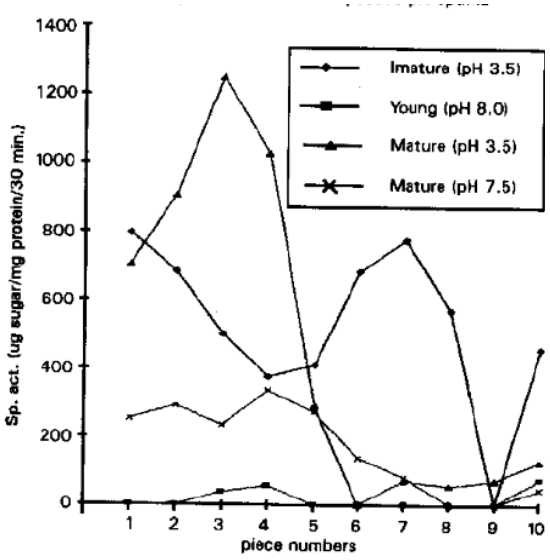

Fig. 6: Invertase isozymes in pod at their respective pH optima

Invertase Isozymes at their Specific pH Optima: Another way of detecting invertase isozymes is to elute the enzyme in the sucrose solution from the gel pieces (after PAGE) and then the amount of sucrose hydrolysed by the enzyme could be detected, each peak will indicate an isozyme (Humphreys and Echeverria, 1980). Figure 4 showed that young leaf possessed acid and alkaline invertases however data of acid invertase was not reported. Young leaf contained Iv_1, Iv_2, Iv_5 Iv_6, Iv_8 and Iv_{10} alkaline isozymes. Leaf at blooming stage have Iv_1 to Iv_9 acid isozymes and Iv_1, Iv_2, Iv_3, Iv_4, Iv_5, Iv_7, Iv_8 and Iv_9 neutral isozymes. Mature leaf had acid and alkaline enzyme activity in all ten pieces with different intensities. In senescent leaf all the ten neutral invertase isozyrnes of different quantities were present however only two alkaline isozymes i.e. Iv_8 and Iv_9 were present. In case of leaves, Iv_1, Iv_2, Iv_5 and Iv_8 were present in all the stages.

Flower at bud stage had Iv_1 to Iv_{10} acid isozymes, with no neutral or alkaline invertases. Closed flower showed both acidic and alkaline invertase activity, in which Iv_1, Iv_2, Iv_4 and Iv_{10} acid isozymes and Iv_1, Iv_2 and Iv_{10} alkaline isozymes were present (Fig. 5). In flowers Iv_1, Iv_2 and Iv_{10} were present in both the developmental stages, Whereas Iv_3, Iv_5, Iv_6, Iv_7, Iv_8 and Iv_9 were specific to bud stage only.

Pods at immature stage possessed invertase activity only at acid pH where Iv_1 to Iv_8 and Iv_{10} were present. In young pod iv_3, Iv_4 and Iv_{10} alkaline isozymes were present with no neutral or acidic activity. Mature pod had both acid and neutral invertases, Iv_1, Iv_2, Iv_3, Iv_4, Iv_5, Iv_7, Iv_8, Iv_9 and Iv_{10} acid isozymes were present whereas Iv_1 to Iv_7 and Iv_{10} alkaline invertases were present (Fig. 6). In pod, Iv_3 and Iv_{10} were present in all stages.

In the present studies, expression of invertase isozymes was detected from various tissues at different devlopmental stages. It was noted that acid invertase was present in the young stages and persists for longer period. It was also observed that Iv_1, Iv_2 and Iv_{10} is present almost in all the tissues at all the pH optima so we may considered it as house keeping isozyme.

References

Dey, P.M., 1985. Changes in the forms of invertase during germination of mung bean seeds. Phytochemistry, 25: 51-53.

Humphreys, T. and E. Echeverria, 1980. Invertase and maltase in the free space of the maize scutellum. Phytochemistry, 19: 189-193.

Isla, M.I., M.A. Vattuone, M.I. Gutierrez and A.R. Sampietro, 1988. Acid invertase from *Tropaeolum* leaves. Phytochemistry, 27: 1993-1998.

Isla, M.I., M.A. Vattuone, R.M. Ordonez and A.R. Sampietro, 1999. Invertase activity associated with the walls of *Solanum tuberosum* tubers. Phytochemistry, 50: 525-534.

Krishnan, H.B., J.T. Blanchette and T.W. Okita, 1985. Wheat invertases: Characterization of cell wall bound and soluble forms. Plant Physiol., 78: 241-245.

Maurer, H.R., 1971. Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis. Walter de Gruyter, Berlin, New York.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153: 375-380.

Ouelhazi, N.K., R. Ghrir, K.D. Le and F. Lederer, 1992. Invertase from *Opuntia ficus-indica* fruits. Phytochemistry, 31: 59-61.

Prado, F.E., O.L. Fleischmacher, M.A. Vattuone and A.R. Sampietro, 1980. Cell wall invertases of sugar cane. Phytochemistry, 21: 2825-2828.

Pressey, R., 1994. Invertase inhibitor in tomato fruit. Phytochemistry, 36: 543-546.

Trinh, T.H., T. Gaspar, K.T. Thanh Van and J.L. Marcotte, 1981. Genotype, ploidy and physiological state in relation to isoperoxidases in *Nicotiana*. Physiol. Plant., 53: 153-157.

Vattuone, M.A., F.E. Prado and A.R. Sampietro, 1981. Cell wall invertases from sugar cane. Phytochemistry, 20: 189-191.

Venkataramana, S. and K.M. Naidu, 1993. Invertase-sucrose relationship in young and mature stem of sugarcane. Phytochemistry, 32: 821-822.

Vorster, D.J. and F.C. Botha, 1998. Partial purification and characterization of sugarcane neutral invertase. Phytochemistry, 49: 651-655.