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A Comparative Study of Soluble Invertases During Germination and Growth of Four Cultivars of Mung Bean (*Vigna radiata*)

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Abstract: Changing pattern of invertases during germination (1-8 days) of mung bean seeds was studied. Two types of invertases acid and alkaline were found to be present. The alkaline form was predominant in early germinative tissue (day old seedlings) but was quickly replaced by the acidic form which persisted upto eight days of age. Administration of chloramphenicol (20 µg/ml) resulted in an inhibition in the activity of acid invertase indicating its *denovo* synthesis during germination. Comparative studies were also made at various ages for six different tissues viz. embryo, cotyledons, shoots, roots, petals and anthers among four mung cultivars. Results indicate significant differences in the level of invertase among the four cultivars of mung bean which commence from pregerminative stage and persist through the early postgerminative stages to the young shoots, roots and floral tissues. We therefore suggest an early selection amongst varieties for reducing sugar content with respect to this biochemical parameter.

Keywords: *Vigna radiata*, mung bean, Leguminosae, invertase, reducing sugars, pH profile, cultivars

Introduction

Invertases (βfructofuranosidase fructohydrolase) which hydrolyse 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 vacuoles (Ricardo, 1994). Two types of invertase, acid and neutral are found widely distributed in higher plants (Myrback, 1960). In germinating seeds of mung bean, acid invertase rapidly replaces the alkaline form prevalent in dormant condition and the former then persists (Dey, 1986). High levels of acid invertase have been reported in immature fruits of acid lime (Echeverria, 1990) and in young and rapidly growing tissues of sugarcane (Venkataramana and Naidu, 1993). Both acid and alkaline invertases were detected in roots and nodules of mung bean (Chopra *et al*., 1998), while alkaline form only has been reported in mature stem of sugarcane (Vorster and Botha, 1998) and in tubers of *Solanum tuberosum* (Isla *et al*., 1999). Present study was undertaken to observe the changing pattern of expression of acid and alkaline invertases during germination and early growth of mung bean. Moreover, a comparative account of invertase activity was also made in different tissues from four mung cultivars, in order to determine any quantitative differences that may exist between them and thus aid in the identification followed by a suggested early selection of the best performing variety with respect to the conversion and efficient utilization of sucrose and related sugars as regulated by this biochemical parameter.

Materials and Methods

Seeds of four varieties of mung bean (*Vigna radiata*), viz. cv. 19-19, 20-21, 121-123 and NCM 89 were supplied by the Pakistan Agricultural Research Council, Islamabad.

Culture practice: For the determination of pH optima, seeds of cv. 19-19 were sown in petriplates lined with filter paper moistened with a measured volume of distilled water. For enzyme inhibition studies, filter papers were soaked with a solution of the antibiotic chloramphenicol (CAP) at a concentration of 20 µg/ml. Pregerminative tissue (8 hours old embryo), for varietal comparison was collected by imbibing seeds in distilled water followed by embryo excision at 8 hours. Similarly, cotyledons (axis removed) were collected from seeds, two days post-germination. Samples obtained were frozen at 4°C in plastic bags. Individual experiments were designed in randomized complete block with three replications.

Field experiment: 100 seeds/variety/replication were sown in field prepared in the premises of Genetics department, in RCBD with three replications. Whereas young plants of age 16 days served as the source of shoot and root tissue, floral samples of anthers and petals were obtained from mature plants of age 40 and 45 days respectively. Samples harvested were washed with distilled water, blot dried and frozen at -4°C until further analysis.

Extraction of invertase (soluble): Frozen tissues were cut to small pieces and homogenized on ice in a prechilled pestle and mortar with 0.1 M NaCl (2 ml/gm f. wt.). The homogenate was passed through four layers of muslin cloth and centrifugation of the filtrate at 10,000 rpm, released soluble fraction as the supernatant. Two estimating buffers, 0.1 M sodium acetate (pH 3.0-6.0) and 0.1 M potassium phosphate (pH 6.5-8.0) were used for determination of pH optima.

Enzyme assay: Invertase was estimated by measuring the reducing sugars released after enzymatic hydrolysis of sucrose. Reaction mixture contained 40 µl buffer (of desired pH), 10 µl sucrose (0.25 M) and 50 µl crude extract which was incubated at 37°C for 30 minutes. Reaction was terminated by addition of 1 ml alkaline copper reagent of Somogi (1937). Free hexoses were measured by Nelson's modification of Somogyi's method (Nelson, 1944), using glucose as standard. Proteins were estimated by Lowry's folin phenol method (Lowry *et al*., 1951).

Results

Acid and alkaline invertases during germination: The pH profile of invertase obtained from extracts of mung bean seeds at different days of germination is shown in Fig. 1. As can be seen, in day old seedlings, peak activity is recorded in the alkaline range (pH 7.5) which is soon replaced by the acidic form (pH 6.0) at two days of germination. A more acidic peak (pH 5.5) is detected in seedlings of age three days which

Table 1: ANOVA of invertase activity (µg sugar/mg protein/30 min) in different tissues from 4 cultivars of mung bean

Source of variance	df	Embryo (MS value)	Cotyledons (MS value)	Roots (MS value)	Shoots (MS value)	Petals (MS value)	Anthers (MS value)
Between cultivars	3	1087.66*	786.75*	2.08	45.00*	143.60**	27.33**
Between replications	2	6.25	27.25	10.75	1.08	2.33	1.58
Error	6	3.14	92.58	12.75	5.14	6.22	0.58

*Significance at $p<0.05$
**Significance at $p<0.01$

Table 2: Duncun's multiple range test on mean invertase activity (µg sugar/mg protein/30 min.) in different tissues of four cultivars of mung bean

Tissue/Variety	19-19	20-21	121-123	NCM-89
Embryo	46.15[a]	122.00[b]	200.00[c]	58.33[d]
Cotyledon	137.84[a]	189.97[b]	265.83[c]	195.68[b]
Shoots	184.40[a]	180.58[a]	190.93[b]	160.82[c]
Petals	177.76[a]	189.62[b]	190.90[b]	163.41[c]
Anthers	70.00[a]	125.00[b]	87.50[c]	150.00[d]

Means with different letters in a row are significant at $p<0.01$
Means with similar letters in a row are non-significant

further shifts to pH 4.5 at fourth day of germination. When extracts from eight days old seeds are analysed, a prominent acidic peak (pH 4.0) is found again, indicating the presence of acid invertase.

Thus, as germination proceeds, there is a shift from alkaline to the acidic isoform which continues in the acidic range throughout the early post-germinative period of development checked. Enzyme inhibition studies with CAP (Fig. 2), indicate reduction in enzymatic levels at all days with the exception of day old seedlings, indicating *denovo* synthesis of acid invertase during germination while the alkaline form is likely to be zymogen activated.

Invertase activity in different tissues of mung cultivars: Analysis of variance (ANOVA) of the data of invertase activity in different tissues from four cultivars of mung bean is shown in Table 1. Results indicate significant differences among cultivars for embryo, cotyledon and shoot tissue while highly significant differences exist for the petals and anthers. Root tissue on the contrary does not reflect these intervarietal differences, the MS value being non-significant in this case. To pin point the mean values that differ from each other, Duncuns multiple range test was applied (Table 2). As can be seen, in two of the five tissues i.e. embryo and anthers, all means are different from each other while for the cotyledons, shoots and petals, at least two means belong to different populations. Moreover, when different tissues from the same variety are checked for invertase activity, differences in enzyme levels are again encountered (Table 2).

Discussion

Reports are available regarding the differential distribution of acid and alkaline invertases in germinating and mature tissues of sugarcane (Venkataramana and Naidu, 1993; Vorster and Botha, 1998) and *Solanum tuberosum* (Isla *et al.*, 1999). In the present study, changing patterns of appearance of the two invertase isoforms was traced during the early germinative period, which in turn may reflect the differential activity of genes responsible for their synthesis. The increased levels of alkaline invertase detected in day old seedlings and their rapid replacement by acid invertase, dominating the rest of the growth period tested, leads to suggest that during germination, acid invertase is prevalent in all tissues and is required to fulfil the heavy demands of free hexoses in the growing plant. With the synthesis pattern likely to be *denovo,*

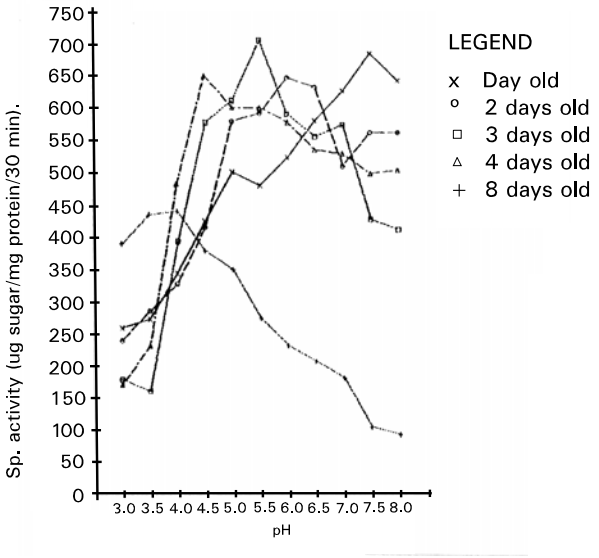

Fig. 1: Invertase activity, pH profile at different days of germination

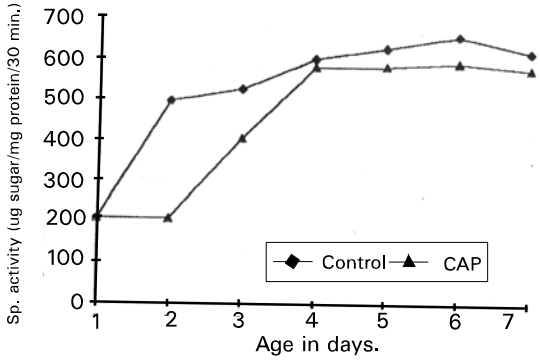

Fig. 2: Invertase activity (ug sugar/mg protein/30 min.) In control and CAP at different days of germintaion

this in turn may be accomplished by turning off or repression of the genes coding for alkaline form followed by switching on or activation of the acid invertase genes, messenger RNA or zymogen. Dey (1986), reports a similar pattern of change in forms of invertase in germinating mung seeds.

In roots and nodules of mung bean, high activities of acid and alkaline invertases were observed at 30 and 50 days after sowing (Chopra *et al.,* 1998). Another aspect of the current work was the intervarietal comparison of invertase activity in different tissues of four cultivated varieties of mung. Differences in enzyme activity were encountered when a given tissue was checked intervarietally while differences were also encountered when the above was measured in different

tissues of the same variety. Manning and Maw (1975), have reported differences in the distribution of acid invertase in different parts of the tomato plant and substantial variation is again found when the latter is compared in species of tomato. In different organs from four different developmental stages of *Arabidopsis thaliana*, cell wall and vacuolar invertase genes are expressed in a development and organ-specific manner (Tymowska-Lalanne and Kreis, 1998). Present results indicate significant differences in the level of invertase among the four cultivars, right from the pregerminative stages i.e. early embryonic tissue which persists through the early post-germinative stages (cotyledons), to the young shoots of sixteen days old plants. Significant differences are also maintained in the reproductive organ, i.e. the flower, of which anthers and petals were taken. Moreover, Duncun's multiple range test applied to the mean values further outlines the varietal differences for respective tissues. This leads us to suggest the possibility of an early selection among different mung varieties performed as early as the embryonic stage for the conversion and efficient utilization of sucrose and the release of monosaccharides. These in turn can be used as a source of energy in the metabolic processes associated with germination and responsible for the survival and vigor of the young plant, a series of events that is directly influenced by this biochemical parameter.

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