higher rate than the Central India lakes. The Himalayan lakes show a distinguished depositional pattern, i.e., sedimentation rate decreases from near-shore to the deepest portion (central part) of the lakes. In case of Central India lakes, maximum rate of sedimentation is found near the entry point of the main drain, but overall there is no resemblance in sedimentation pattern of these lakes.

The study reveals that $^{210}$Pb and $^{137}$Cs dating techniques are useful to determine recent sediment accumulation rates and patterns in lakes. As the $^{137}$Cs dating technique provides the sedimentation rate based on the depths recorded in 1963–64, this technique is a powerful tool for determining the sedimentation rate and pattern in water bodies, particularly in Himalayan lakes where anthropogenic activities have accelerated the sedimentation rate in the past 50 years.


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Sustained activities of carbon metabolizing enzymes determine seed size in Vigna radiata (mungbean)

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The activities of enzymes involved in the control of carbon flux of two genotypes of Vigna radiata (mungbean) differing in seed weight were compared in order to elucidate the factors controlling seed size and to analyse the relationship between seed development and metabolism. Biomass accumulation in ‘large’ seeds was maintained till maturity. However in ‘small’ seeds, maximum accumulation was achieved approximately five days earlier. Sucrose synthase (SuSy, EC 2.4.1.13) activity for small seeds increased sharply from 10 to 15 days after flowering (DAF) and then declined till maturity. However in large seeds, the activity increased more slowly but rapidly after 15 DAF till maturity. Lesser activity of SuSy in podwall of large genotype indicated more assimilate channelling into the seeds, a much stronger sink for sucrolysis. Enzymes UDP-glucose and ADP-glucose pyrophosphorylase (UGPase, EC 2.7.7.9 and AGPase, EC 2.7.7.27) corresponded to the pattern of SuSy in large seeds, showing coordination between these enzymes regulating carbon

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flux for sustained storage activity. Thus, the seed filling phase is prolonged in large seeds having better sink strength compared to the small seeds. Hexokinase (EC 2.7.1.1) activity follows the pattern of SuSy, UGPase and AGPase in developing seeds. We conclude that a steady sink activity of the enzymes controlling carbon flux entering the seed may be required for the large seed size of the bold seeded genotypes.

**Keywords:** ADP-glucose pyrophosphorylase, hexokinase, seed size, sucrose synthase, UDP-glucose pyrophosphorylase, *Vigna radiata*.

SEEDS represent a well-defined system for analysing sink metabolism and plant development. Seeds accumulate starch, storage proteins and oil in different proportions depending on the species. These products are synthesized in the storage organs, the endosperm or the cotyledons, mainly based on imported sucrose and amino acids. Considering the economic importance and worldwide demand for bold seeded varieties, seed metabolism and accumulation of storage products become a subject of intensive investigation. To analyse how seed metabolism is connected with its development and how biosynthetic pathways are controlled by specific enzymes requires an integrated biochemical approach. Thus, the activities of enzymes controlling carbon flux entering the mungbean pods were determined, viz. sucrose synthase (SuSy), ADP-glucose pyrophosphorylase (AGPase), UDP-glucose pyrophosphorylase (UGPase) in podwall (PW) and seeds of two genotypes differing in weight at different developmental stages. SuSy acts as a major sink activity-related enzyme in potato, chickpea, mungbean and lentils. Possibly a cytosol-located AGPase can allow efficient partitioning of sucrose into ADP-glucose, which is finally utilized for the starch synthesis.

Starch biosynthetic activity within a sink organ is a major determinant of sink strength. AGPase is the rate-limiting step in starch biosynthesis; an increase in AGPase activity within the cotyledons could increase the strength of the developing seeds. AGPase activity in turn depends upon the formation of glucose-1-phosphate by UGPase. When sucrose concentration is built up in the developing cotyledons after the initial mitotic activity during the first few days of seed development, SuSy, UGPase and AGPase may become important in determining sink strength of the developing seeds. The interdependence of these enzymes is evident from the reactions they catalyse as shown below.

\[
\begin{align*}
\text{UDP} + \text{Sucrose} & \xrightarrow{\text{SuSy}} \text{UDP-glucose} + \text{Fructose} \\
\text{UDP-glucose} + \text{PPI} & \xrightarrow{\text{UGPase}} \text{Glucose-1-phosphate} + \text{UTP} \\
\text{Glucose-1-phosphate} + \text{ATP} & \xrightarrow{\text{AGPase}} \text{ADP-glucose} + \text{PPI}.
\end{align*}
\]

Although the above reactions are reversible, continuous utilization of ADP-glucose for starch synthesis makes the above reactions work in the direction shown. Here we test the hypothesis that SuSy, UGPase and AGPase together constitute the sink strength of the developing seeds and possibly are also the determinants of seed size.

Higher mitotic activity in the cotyledons of ‘large’ seeded genotype is expected in comparison with ‘small’ seeded genotype. The final seed size of *Vicia* is reported to be positively correlated with the phase of cell division of the embryo. Cell proliferation requires energy from respiratory or glycolytic activity. Plant hexokinase, an essential glycolytic enzyme, has been shown to be involved in sugar sensing and signalling, and is proposed to be a dual-function enzyme with both catalytic and regulatory functions. The results from this study will allow a more integrated understanding of seed development and metabolism, by increasing our current knowledge about the enzymatic steps exerting a rate-limiting role on storage product synthesis as well as on seed maturation. Such knowledge also has significant potential for applications based on achieving desirable changes that improve seed metabolism and agricultural yield.

Plants of mungbean (*Vigna radiata* L. cv. ML 267 – small seeded genotype and cv. SML 668 – large seeded genotype) were sown in the fields in July following recommended agronomic practices. At flowering, uniformly growing plants were selected and their flowers were tagged daily for 5 days. Plants along with their pods were collected at 5, 10, 15, 20 and 25 days after flowering (DAF) and brought under ice to the laboratory. Enzymes were extracted immediately from PW, and seed and enzyme activities were assayed on the same day.

For extracting SuSy, the required tissue (0.5–1.0 g) was homogenized in cold (3–4°C) 100 mM HEPES buffer (pH 8.2) containing 10 mM EDTA, 15 mM KCl, 5 mM MgCl₂, 2 mM sodium diethyl dithiocarbamate and 5 mM β-mercaptoethanol. Insoluble polyvinylpyrrolidone (Sigma P2806, 100 mg g⁻¹ tissue) was also added while extracting the enzyme. The supernatant, after centrifuging at 10,000 g for 15 min, was passed through sephadex G-25 column using 10 mM HEPES buffer (pH 7.0) to remove small molecular-weight impurities. For extraction of hexokinase and pyrophosphorylases, 20 mM HEPES buffer (pH 8.0) containing 1 mM EDTA, 5 mM MgCl₂ and 5 mM 2-mercaptoethanol was used. Polyvinylpyrrolidone (100 mg/g tissue) was also added during extraction. The extract was centrifuged at 10,000 g for 15 min. The pellet was washed twice with extraction buffer and the pooled supernatant was used immediately for assaying the activities of AGPase and UGPase.

SuSy was assayed at 30°C in the direction of sucrose breakdown in the presence of UDP, as described earlier. Hexokinase activity was measured by coupling the production of glucose-6-phosphate to the reduction of NADP in the presence of excess glucose-6-phosphate dehydro-
genase at 30°C, as described by Copeland and Morell. UGPase and AGPase enzymes were assayed at 30°C by coupling their action with phosphoglucomutase and glucose-6-phosphate dehydrogenase, and measuring the formation of reduced NADP spectrophotometrically. All enzymes were extracted from at least three different samples and assayed in duplicate. Data are the mean ± SD of these values.

Dry-weight accumulation of mungbean seeds of the large-seeded (SML-668) and small-seeded (ML-267) genotypes was compared. Large seeds reached maximum dry weight of 59 mg seed⁻¹ at 25 DAF while small seeds reached maximum dry weight of 39 mg seed⁻¹ at 20 DAF (Figure 1). Thus, the time to gain maximum dry weight in developing seeds was sustained till maturity in the large genotype.

SuSy activity was significantly higher in PW of small-seeded genotype in comparison with large-seeded genotype. Activities of UGPase and AGPase in PW of the two genotypes are comparable. However, hexokinase activity was more in PW of large-seeded genotype (Figure 2).

On comparing the data of SuSy, UGPase and AGPase and hexokinase in developing seeds of the two genotypes, a significant difference in their development pattern was observed. In contrast to small-seeded genotype where activity of these enzymes declined 15 DAF, activities of enzymes continued to increase in developing seeds of large-seeded genotypes till maturity (Figure 3). It appears that small seeds completed their storage earlier than large seeds. Higher and sustained sink-filling of the large genotype till maturity is probably responsible for its larger seed size. SuSy is a marker for storage activity, an increase in SuSy activity is generally accompanied by the onset of starch synthesis. SuSy is responsible mainly for sucrose degradation in legume cotyledons during seed-filling. The carbohydrate status during legume-seed development is likely to play a role in controlling the flux through the breakdown–synthesis cycle. In Vicia faba cotyledons, the affinity of SuSy for sucrose is low and the enzyme is inhibited by free hexoses. During the pre-storage phase, a higher hexose to sucrose ratio at 5 and 10 DAF stages in mungbean seed might prevent SuSy from making any large contribution to sucrose degradation. In Vicia seeds, invertase was responsible for the longer period of high hexose conditions accompanied by a longer cell division phase, increasing the final cell number in the large embryos. With ceasing cell divisions, the legume embryo enters a transition phase and switches from an invertase- and

![Figure 1](image1.png)
**Figure 1.** Dry-weight accumulation curves. ––– Large-seeded var. SML-668; –– Small-seeded var. ML-267.

![Figure 2](image2.png)
**Figure 2.** Comparative activity pattern of SuSy, UGPase, AGPase and hexokinase in PW of the small- and large-seeded genotypes of mungbean during the development of ML-267 (small-seeded) and SML-668 (large-seeded) cultivars. Bars represent ± SD of three replicates. Bars have not been shown where SD was smaller than the symbol. Activity has been expressed as nmol of product formed min⁻¹ g⁻¹ fresh wt.
hexose-based to a sucrose-based metabolism controlled by SuSy\textsuperscript{7}. High sucrose level of 10 DAF (i.e. at the onset of the storage phase) promotes the SuSy pathway in developing mungbean\textsuperscript{15}. SuSy as a major sink activity-related enzyme is reported to be induced by sucrose at the transcriptional level\textsuperscript{16}. In maturing \textit{V. faba}\textsuperscript{17} and pea cotyledons\textsuperscript{18}, dry-weight accumulation \textit{in vitro} is dependent on high sucrose levels. A sharp rise in SuSy activity for small seeds from 10 to 15 DAF marked the onset of the storage phase (Figure 3). However, in large seeds the activity increased more slowly from 10 to 15 DAF, but rapidly after it to attain a maximum at 25 DAF. We conclude that small seeds initiated the storage phase and finished earlier, whereas storage activity was maintained till maturity in large seeds.

Lesser activity of SuSy in PW of large seeds (Figure 2) indicated more assimilate (sucrose) channelling into seeds, which acts as a stronger sink for sucrolysis, wherein the synthesis of storage products is high. A comparison between the large and small genotypes in \textit{V. faba} gave a similar kind of pattern for SuSy and AGPase, wherein sustained enzyme activities in large cotyledons were maintained till maturity and small genotype showed a short storage phase\textsuperscript{8}. Like the present observations in mungbean, the small genotype of \textit{V. faba} also showed a markedly reduced time to reach maturity. AGPase corresponded to the pattern of SuSy activity (Figure 3) showing coordination between these enzymes in regulating carbon flux for sustained storage activity in large seeds. Increased SuSy activity has been correlated with higher transcript levels of AGPase\textsuperscript{19}, starch synthesis\textsuperscript{20} and overall sink strength\textsuperscript{21}. Thus, a steady sink activity throughout the seed-filling period is required for better sink strength. Increase in AGPase activity in sink tissues of maize resulted in its enhanced seed size\textsuperscript{22}. Transgenic wheat showing increased AGPase activity in endosperm produced on average 38% more seed weight per plant\textsuperscript{23}. SuSy has been implicated in maturation and storage of seeds\textsuperscript{24}.

In tissues where sucrose degradation is metabolically important, for example, sink tissues like seeds and tubers, UGPase is believed to have a role in the synthesis of glucose-1-phosphate (the precursor of ADP-glucose synthesis by AGPase reaction) from UDP-glucose produced by SuSy\textsuperscript{8}. The activities of UGPase correlating with that of AGPase both in the PW and developing seeds, thus allow an efficient coupling of the enzymes, viz. SuSy, UGPase and AGPase in controlling carbon flux for effective partitioning of sucrose into ADP-glucose, finally utilized for the starch synthesis\textsuperscript{15}. There are reports that reduced AGPase activity is associated with reduced UGPase activity in developing oil seeds, showing that both these activities are related with each other\textsuperscript{25}.

There is a strong history of plant sink/source-type studies showing that sugars can either induce or repress various enzymes\textsuperscript{26}. For example, sucrose hydrolysis in the cytosol has been identified as part of the signal-controlling glycolysis and respiration in potato tubers\textsuperscript{27}. Hexokinase has been identified being at least part of a sugar-sensing mechanism. Because hexokinase is a cytosolic enzyme, increased hexose concentrations by cleavage of the cytosolic sucrose would be expected to cause sugar-responsive changes in gene expression. It was suggested that either hexose transport or a membrane-associated hexose sensor is involved in sugar sensing\textsuperscript{28}. Higher levels of hexokinase activity in seeds of large genotype indicated its involvement either as a phosphorylating enzyme or as a sugar-sensing agent or both.

**Figure 3.** Activity pattern of SuSy, UGPase, AGPase and hexokinase in developing seeds of large- and small-seeded cultivars of mungbean. □—□. Large-seeded var. SML-668; □—□. Small-seeded var. ML-267. Bars represent ± SD of three replicates. Bars have not been shown where SD was smaller than the symbol. Activity has been expressed as nmol of product formed min\textsuperscript{-1} g\textsuperscript{-1} fresh wt.

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Basis specificity of climate change in western Himalaya, India: Tree-ring evidences

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Tree-ring-width chronologies of Himalayan cedar (Cedrus deodara) from moisture-stressed sites in Alaknanda, Bhagirathii, Tons, Satluj (lower) and Chandra-Bhaga river basins in western Himalaya were studied to understand the basin-specific as well as synoptic-scale features of climate change. In the past 325 years, extreme cool and wet climate during 1734 and 1803 and extreme hot and dry climate during 1705, 1707, 1767, 1774, 1782, 1873, 1887, 1890, 1892 and 1974, common in all the basins, reflect synoptic-scale features. However, in 1816, extreme low growth in trees over all the basins could have resulted due to reduced photosynthesis caused by impaired solar radiation reaching the ground.

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