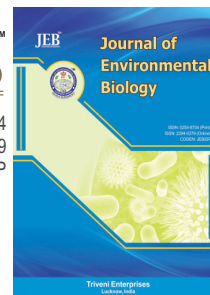




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Purification of sucrose synthase from thermotolerant wheat grains and its characterization



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Abstract

Aim : Wheat is common and popular cereal with wide utilization and acceptability. High temperature stress drastically affects crop productivity and quality by disturbing sucrose metabolism. Hence, the aim of present investigation was to purify and characterize of sucrose synthase from thermotolerant wheat for various physico-chemico-kinetic properties.

Methodology : The immature grains of thermotolerant wheat WH-1021 were used for purification of sucrose synthase by using traditional protein purification techniques viz. $(\text{NH}_4)_2\text{SO}_4$ fractionation, gel filtration through sephadex G-100 and DEAE-cellulose ion exchange chromatography. The purity was checked by native PAGE, while subunit by SDS PAGE.

Results : The enzyme was purified about 27 fold with approximately 37% recovery. The native enzyme had a molecular weight of 269 KDa and subunit molecular weight was found to be 63 KDa, indicating that enzyme is a homotetramer. The purified enzyme exhibited optimum activity at 37°C with thermostability upto 50°C and pH 6.5. Sucrose synthase showed K_m values of 14.28 and 1.18 mM for sucrose and UDP, respectively. Its activity was inhibited by Mn^{2+} (38.5%), while NO_3^- stimulated the activity by 20.8% at 2 mM concentration.

Interpretation : Among the various nucleotides tested, the enzyme was highly specific for UDP as substrate and NADP^+ and G-6-P were found to be the potent inhibitors. The kinetic studies revealed that sucrose synthase catalyzed the sucrose degradation by ping-pong mechanism. The results of the present study would help in planning the strategies for increased wheat productivity without compromising the quality under stress conditions.

