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RESEARCH ARTICLE

Effect of Saline Growth Medium on Temperature Optima of Invertases in Sugarcane (*Saccharum officinarum* L.)

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ABSTRACT

Sugarcane is a major source of sugar production in Pakistan but high levels of salts present in the soil reduce its growth considerably. Therefore, physiological and biochemical basis of yield reduction under saline conditions was studied. Invertases were extracted, purified and characterized from sugarcane grown under salinity to evaluate the effect of saline conditions on various characteristics of these enzymes. An attempt was made to investigate the possible effect of salinity on the stability of soluble acid invertases (SAI) in terms of temperature optima from two local cultivars of sugarcane. The temperature optimum of native SAI of CP-77-400 and COJ-84 was found to be 55 and 50°C respectively. The saline growth medium did not affect temperature optima of COJ-84 invertases, while SAI from CP-77-400 showed a 5 degree decrease at all salinity levels.

INTRODUCTION

The climate of Pakistan is very suitable for growth of sugarcane but soil salinity is threatening its growth and development (Qureshi and Barrett-Lennard, 1998). Soil salinity is a major barrier which adversely affects plant growth and its economic yield (Shannon, 1984; Francois, 1996; Hussain et al., 2010). Most of the enzymes which were isolated from the salt tolerant plants showed similar behaviour as from sensitive species (Hussain et al., 2003). As the NaCl concentration increases in the growth medium, it induces a progressive decrease in specific activity of mitochondria enzymes in roots (Hussain et al., 2010) or in rapidly growing tissues at 50 to 150 mM concentration (Flowers, 1972).

It has been suggested that invertases regulate the accumulation of sucrose in sugarcane stem storage tissue (Gayler and Glasziou, 1972). Soluble acid invertase (SAI) concentrations are low before sucrose accumulates (Pan et al., 2009) but this may be reverse in other cases (Lontom et al., 2008). High sucrose accumulation might be the result of other factors as well (Zrenner et al., 1996). The physiological application of said factors is only possible if we acquire their knowledge in terms of stability and catalysis. Sugarcane is a major source of sugar production in our country but high levels of salts present in the soil reduce its growth considerably (Hussain et al., 2004, Gomathi and Thandapani, 2005). Therefore, physiological and biochemical basis of yield reduction under saline conditions must be investigated. So, an attempt was made to extract, purify and characterize invertases from sugarcane grown under salinity to evaluate the possible effects of saline conditions on various characteristics of these enzymes. In the present study, we investigated the effect of salinity on temperature optima of invertases in two local cultivars of sugarcane.

MATERIALS AND METHODS

Cultivation and harvesting of sugarcane

The local cultivars of sugarcane CP-77-400 and COJ-84 were grown in field plots. The field plots were lined with good quality polythene sheet before they were filled with loam soil. Both cultivars consisted of 20 one-eyed sets with eye position upward. Fifteen plants per treatment were maintained after germination for a period of 180 days. The original level of salinity in the

soil was 25mM (control). The natural salinity of the soil was accounted for, while developing the salinity levels and four different salinity levels i.e., 50, 100, 150 and 200mM NaCl were developed. The plants were harvested after six months. Trash was removed and plants were washed with water before storing in cold room at 4°C.

Extraction of invertases

Sugarcanes of each salinity treatment were crushed and pressed separately to get juice. Juice of all the canes from respective treatments was mixed and dialyzed against distilled water thoroughly at 4°C. Soluble salts and sugars were removed by dialysis. Then the juice was concentrated by freez-drying. Invertase activity and total proteins in the juice were also determined.

Invertase activity

Invertase assay was undertaken by using appropriate amount of enzyme at pH 5.5 in 50mM sodium acetate. 50mM sucrose solution was used as substrate. The test tubes containing reaction mixture were incubated at 50°C for half an hour. The reaction was terminated by placing the test tubes in boiling water for 5 minutes and then cooled in tap water. The amount of reaction product as glucose was determined by adding 100µl of quenched reaction mixture in 1ml of glucose measuring kit (Biocon, Germany) at 37°C for 10 min as described in Biocon's user manual.

"1µmol of glucose equivalent liberated/min under define conditions was equal to one unit of invertase activity".

Purification of invertases

Impurities were removed from crude invertases using a combination of ammonium sulfate precipitation and different types of column chromatography such as Hiload Q.Sepharose, Mono-Q ion exchange, hydrophobic interaction and gel filtration on Pharmacia fast protein liquid chromatography (FPLC) system (Siddiqui et al., 1997). The methodology for enzyme assay after purification and characterization of invertase has been described earlier by the author (Hussain et al., 2009). Invertases were characterized by converting them into apoenzymes. Apoenzymes were prepared by dialyzing invertases against 5 mM EDTA (chelating agent) dissolved in 50 mM MOPS/KOH (pH 7.0) for 14 hours. The EDTA was removed by dialyzing apoenzymes intensively against thirty liters of distilled water for 24 hours (four changes of water). Effect of saline growth medium on temperature optima of sugarcane invertases was determined using the method of Rangaranjan and co-workers (Rangaranjan et al., 2000). Invertase was assayed as described earlier under invertase activity at 2.3 above, different temperatures ranging from 10-80°C. Various time course aliquots were withdrawn and assayed immediately for enzyme activity. Energy of activation was determined at different salinity levels by applying Arrhenius plots and temperature optima of native invertases were also determined (Hussain et al., 2003).

RESULTS AND DISCUSSION

The temperature optimum of native SAI of CP-77-400 and COJ-84 was found to be 55 and 50°C, respectively. The saline growth medium did not affect temperature optima of COJ-84 invertases, while SAI from CP-77-400 showed a 5-degree decrease at all salinity treatments (Fig.1, Table 1).

Table 1: The temperature optima and energy of activation of invertases from sugarcane cultivars CP-77-400 and COJ-84 grown under salinity

Cultivar	NaCl levels				
	(Control)	50 mM	100 mM	150 mM	200 mM
	Temp optimum °C				
CP-77-400	55	50	50	50	50
COJ-84	50	50	50	50	50
	*E*a (kJ mol^{-1})				
CP-77-400	55.29	48.97	38.58	48.22	53.21
COJ-84	36.25	25.11	46.89	32.67	28.27

Where, temperature optimum was determined at 50°C and pH 5.5, respectively.

The energy of activation (*E*a) for sucrose hydrolysis by sugarcane SAI was determined by applying Arrhenius plots. The plots for SAI of native and all saline media grown CP-77-400, as well as of COJ-84 showed a similar pattern (Fig.2).

The *E*a for sucrose hydrolysis by SAI secreted by both cultivars of sugarcane grown under saline conditions gave evidence that under salinity the expressed invertases may be of better quality because *E*a values at the transition temperature for all SAI of CP-77-400 showed a decreasing trend and maximum decrease was found at 100 mM treatment, which showed *E*a of 38.58 kJ mol^{-1} as compared to control having 55.29 kJ mol^{-1} (Table-1). On the other hand, SAI of COJ-84 showed a complicated trend in *E*a and presented an increasing and decreasing trend with the increase in salinity level and maximum decrease was observed at 50 mM NaCl treatment. However, at 100 mM level the *E*a was increased to 46.89, as compared to control having 36.25 kJ mol^{-1} (Table 1).

Thermophilicity, i.e., the ability of enzymes to show activity at high temperatures in the presence of substrate (Flowers, 1972) was evaluated by assaying the SAI at various temperatures ranging up to 80 °C. The saline growth media did not affect temperature optima of COJ-84 invertases, while SAI from CP-77-400 showed a 5-degree decrease at all salinity treatments. The *E*a values for sucrose hydrolysis of all SAI of CP-77-400 showed a decreasing trend, and maximum decrease was found at 100 mM NaCl, while SAI of COJ-84 showed a complicated trend and showed

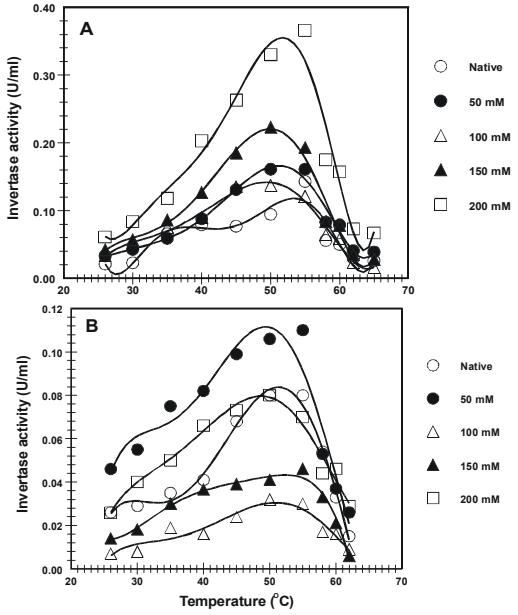

Fig. 1: Effect of saline growth medium on pH optima of sugarcane invertases: CP-77-400 (A), COJ-84 (B).

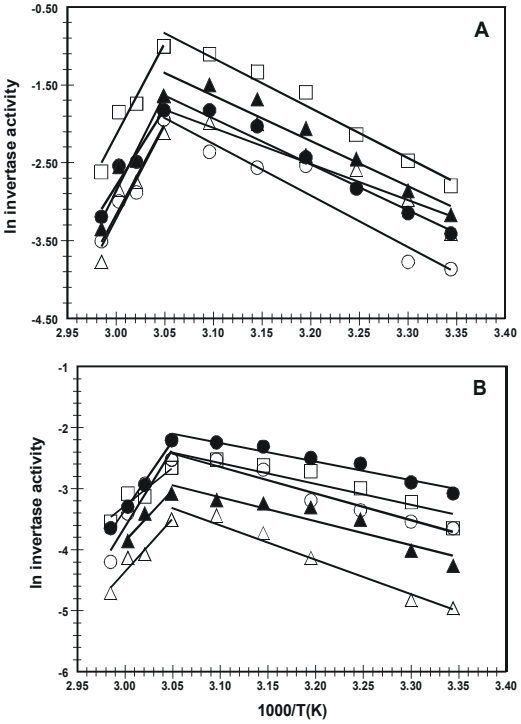

Fig. 2: Arrhenius plots for the determination of activation energy (Ea) for sucrose hydrolysis by sugarcane invertases: CP-77-400 (A), COJ-84 (B).

increasing and decreasing trend with increase in salinity level, which explained that under salinity, the expressed SAI required low energy for transition state formation as compared to control. AitAbdelkader et al. (2000) from bacteria and Cairns et al. (1995) from mold reported similar results. Most of the enzymes isolated from the salt tolerant plants show similar behaviour as from sensitive species. The increasing concentrations of NaCl and Na_2SO_4 in growth medium induce a progressive decrease in specific activity of mitochondrial enzymes in pea roots (Hussain et al., 2009) or rapidly growing tissues at 50 to 150 mM NaCl concentrations (Flowers, 1972).

Conclusion

The ability of enzymes to show activity at high temperatures in the presence of salt (NaCl) was evaluated by assaying the SAI at various temperatures ranging up to 80°C. The saline growth media did not affect temperature optima of COJ-84 invertases, while SAI from CP-77-400 showed a 5-degree decrease at all salinity treatments.

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