

Nisachol Khruangchan 2009: Molecular Basis of Imazapyr-Resistant Sugarcane Cell Line.  
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Selection of sugarcane cell line resistant to imazapyr was conducted to investigate the biochemical and molecular basis of herbicide resistance. Selection of sugarcane cells resistant to imazapyr was carried out using callus and cell suspension induced from the tight young furred leaves of sugarcane clone K97-32. The cell suspension was cultured in modified liquid MS medium supplemented with 10% coconut water, 0.5 g/L casein hydrolysate, 0.1 g/L myo-inositol and 3 mg/L 2,4-D. Sugarcane cells resistant to 1  $\mu$ M imazapyr were obtained after 378 days of stepwise selection with increasing concentrations of imazapyr from 0.1 to 1  $\mu$ M. Surviving cells were sub-cultured at 14 days interval. This indicates that the resistant cells were 918-fold more resistant to imazapyr than the normal cells. Moreover, the resistant cell line remained stable for at least 4 months when kept in herbicide-free medium. To establish the biochemical mechanism of resistance to imazapyr, ALS activity was determined in the normal and resistant cells. It was found that ALS activity of the resistant cells at 3, 5, 7, 10 and 14 days after treatment was 2.65, 2.94, 3.36, 2.90 and 2.62-fold higher than that of the normal cells. In addition, to investigate the molecular basis of ALS gene at the target site responsible for the herbicide activity, the partial sequences of ALS gene region covering 455 bp of domain B and E from the normal and resistant cells was amplified and sequenced. The partial sequence of ALS gene of the normal and resistant cells was deposited in the GenBank database with accession number EU243998 and EU243999, respectively. The ALS gene sequence of the resistant cells showed the point mutation of alanine (A) deletion at 666 position. The accumulation of ALS mRNA at transcription level in the resistant cell was not different from that in both non-treated and treated normal cells. The results indicated the amounts of ALS mRNA had no correlation with the levels of ALS activity. This suggested that the imazapyr resistance might be due to an alteration of the ALS activity making it less sensitive to the inhibitory effect of herbicide. Thus, the sugarcane cell was not inhibited by imazapyr.

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Student's signature

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Thesis Advisor's signature