ABSTRACT

THESIS TITLE- **Betalain production *in vitro* and DOPA 4, 5-dioxygenase mediated biotransformation in *Amaranthus tricolor* L. (submitted by-Mousumi Biswas)**

Amaranthus tricolor L. is a leafy amaranth from the Amaranthaceae family producing red-violet betacyanin. Betalains are a class of indole-derived pigments found in plants of the order Caryophyllales. Betalains have not been investigated well as opposed to other plant pigments. In spite of use of betalains as natural colorants in food industries and biological activities reported so far, the *in vitro* culture of this plant has been mentioned in a few articles but no report exist on its pigment production. The present study was therefore undertaken to explore such possibilities.

The best amaranthin-producing callus was induced on Murashige and Skoog (MS) medium supplemented with NAA (0.25 mg/L) and BA (2 mg/L) using stem segment as explants. Stable red *A. tricolor* callus line (IIT BT/PBT *At* 11) was maintained upto 24 subcultures. *A. tricolor* suspension culture establishment shifted the production to betalamic acid than betacyanin in the spent medium as a result of osmotic and shear stress. Optimization of the critical MS media components led to a 2-fold increase in the betacyanin production of the *A. tricolor* callus cultures. Amaranthin was the major betacyanin pigment found to be present in both *A. tricolor* leaves and callus culture. Methylated arginine-betaxanthin was isolated both from *A.tricolor* leaves and callus culture for the first time in this species. Betalamic acid was found to possess antioxidant, antiproliferative and alpha-amylase inhibitory activities.

Pathway specific precursor such as L-DOPA (1 mM) feeding led to 6-fold increase in amaranthin content with the simultaneous accumulation of betaxanthin in *A. tricolor* callus. A melanin-like pigment (84.33 mg/g FW) was synthesized by 5-hydroxyindole fed *A. tricolor* callus cultures. The DOPA 4, 5-dioxygenase enzyme was isolated from *A. tricolor* callus with a specific activity of approximately 467 pkat/mg protein. The strong iron chelator, o-phenanthroline inactivated the enzyme markedly and thus lowered the amaranthin production in *A. tricolor* callus. Calcium chloride and methyl jasmonate were found to be the most effective elicitors for enhancement of amaranthin production in *A. tricolor* callus. Effect of salt stress (NaCl) revealed that a high salinity environment is unfavorable for amaranthin production in *A. tricolor* callus.

Keywords: *Amaranthus tricolor* L.; Betalains; Betalamic acid; Biological activities; Biotransformation; DOPA 4, 5-dioxygenase