

## ABSTRACT

*Silybum marianum* (L.) Gaertn. is an important medicinal herb due to its active ingredient silymarin, so the present study mainly focused on the silymarin analysis in *in vitro* cultures of *S. marianum*. For seed germination different factors including effect of moisture content, temperature, number of seeds per petriplate, different concentrations of gibberellic acid were tested and 100% seed germination was recorded with alternating 16h cool period of 17°C and 8h warm period of 37°C on aseptic cotton pads soaked with 20 ml distilled water containing 5 seeds per petriplate, the same germination rate was achieved with MS media containing 2.0 mg/L gibberellic acid. These *in vitro* grown plantlets were used as explants for callus induction and regeneration purpose. All of the explants (leaf, root, stem, and hypocotyl) responded well to different growth regulators for callus induction. Seeds and hypocotyls were found to be most suitable explants for callus induction. Best callus induction from hypocotyl explant was recorded in MS medium supplemented with 1.0 mg/L 2,4-D (90%) while seeds responded best at 0.5 mg/L 2,4-D with callus induction frequency of 55% and 1.0 mg/L BAP with callus induction frequency of 79%. Regeneration (shoot and root formation) from hypocotyl derived callus and hypocotyl explants was observed on MS medium supplemented with 1.0 mg/L 2,4-D.

Well grown calluses from different explants were put to HPLC analysis (20µL injection of each sample into C18 column) to determine silymarin content (under the influence of UV detector) in each callus. As silymarin is a mixture of different components all the explant calluses were same with respect to composition of silymarin but varied in their amounts as seed callus has highest silymarin contents (99.9%) while the leaf callus has 95.5%, hypocotyl callus has 95.4% and root callus was found to have only 14.9% silymarin contents.