

## ABSTRACT

*Silybum marianum* (L.) Gaertn. is the most important medicinal plant for the treatment of liver disease. Although this species is extensively used in medicines but the availability of this plant locally is limited. The present study was designed to investigate the response of different explants of *S. marianum* *in vitro* under influence of different combinations and concentrations of plant growth regulators for callus induction and plant regeneration. For seed germination different conditions including soaking time, temperature and different concentrations of GA<sub>3</sub> were applied. Hundred percent seed germination was recorded when seeds were soaked for 12 hours and incubated at 25±2°C, placed on MS medium containing GA<sub>3</sub> (2 mg/L). *In vitro* method for callus induction was successfully established using different explants i.e. hypocotyl, cotyledon and root. MS medium was supplemented with different combinations and concentrations of auxins and cytokinins. It was found that plant growth regulators have obvious effects on callus induction and callus morphology. In present study significant callus induction from hypocotyl explant was observed in MS medium supplemented with combination of IAA (1.0 mg/L) with BAP (1.0 mg/L) while cotyledon explant responded to considerably significant callus induction under the influence of NAA (1.0 mg/L) with Kin (1.5 mg/L). For organogenesis (shoot/root) cotyledon as well as hypocotyl derived calli were transferred to regeneration medium in which MS medium was supplemented with different combinations of PGRs. Different combinations and concentrations of BAP, IBA, NAA, Kin and IAA were tried. Shoot and root induction in hypocotyl derived callus ensued efficiently in MS medium supplemented with IBA (2.0 mg/L) with BAP (4.0 mg/L) while in cotyledon derived callus shoots and roots appeared under PGRs, IAA (1.0 mg/L) with BAP (1.0 mg/L). For direct *in vitro* multiple shoot and root induction from hypocotyl and cotyledon explants, different combinations of 2, 4-D, Kin, IBA, IAA and NAA at different concentrations were applied in MS medium. Multiple shoot induction from hypocotyl explant was observed on MS medium supplemented with IAA (1.0 mg/L), IBA (1.0 mg/L), 2, 4-D (1.0 mg/L) and Kin (1.0 mg/L) while cotyledon explants responded to *in vitro* multiple shoot induction under the influence of 2, 4-D (0.5 mg/L) with Kin (0.5 mg/L) in MS medium. *In vitro* grown plantlets were acclimatized. Plantlets were shifted in pots containing peat moss used as planting material. It was observed that *in vitro* grown plants were morphologically identical to those grown in fields.