Abstract

An optimized system for indirect and direct regeneration of *Tribulus terrestris* L. was established using different explants derived from *in vitro* seedlings and field grown plants. This study has been carried out at the Department of Botany, Government College University, Lahore during period of years 2013-2014 in Plant Biotechnology Laboratory. Disinfection of seeds was achieved in 5% clorox solution. Two seeds per jar and 28°C incubation temperature on full strength MS and WP media were found effective for seed germination. Mechanically de-coated seeds dipped in 1% hydrogen peroxide solution for 48 hours responded well with 50% germination percentage. Explants were chemically sterilized and culture medium MS was used for callogenesis, regeneration and rhizogenesis stages. Cotyledonary explants produced callus of lush green colour with granular texture under exogenous application of 2,4-D (3 mg/L) with BAP (1 mg/L) and produced callus index of 400 within 21 days after inoculation. Concentration of BAP 1 mg/L with different concentrations of 2,4,5-T supported to callus production from cotyledonary and epicotyl explants being firstly reported. *In vitro* seedling stem initiated light green, compact callus in 19 days in MS medium added with 3 mg/L NAA and 2 mg/L BAP, epicotyl explants produced callus in 17 days with 1 mg/L BAP and 4 mg/L 2,4-D producing 300 callus index. Callus induction in *in vitro* seedling derived hypocotyl explants is being first time reported with granular, light brown callus under the influence of 1 mg/L NAA and 2 mg/L BAP. Shoot tips, nodes, leaves and petioles produced callus with combinations of Zeatin, NAA and 2,4-D. Indirect regeneration was achieved with 1 mg/L 2,4-D and 4 mg/L BAP from callus of *in vitro* nodal explant, on 20% coconut milk and 0.5 mg/L BAP by epicotyl derived calli in 12 days and under exogenous application of 2 mg/L NAA and 4 mg/L BAP from *in vitro* stem derived callus. *Ex vitro* shoot tip derived callus showed significant results on MS medium supplemented with 0.5 mg/L BAP and 150 mg/L casein hydrolysate. Direct regeneration from nodal and shoot tip explants was achieved good with various BAP concentrations as compared to Kin. Casein hydrolysate, tomato extract, sweetlime water and silver nitrate were also used to produce positive results. Indirectly or directly regenerated shoots were used for micropropagation through root induction on PGR free medium or 2 mg/L IBA containing MS medium. Root induction response was observed in 1 week and plantlets were successfully transferred in peat moss