**Abstract**

We have developed a new, simple, quick and genotype-independent method for direct regeneration of sugarcane using novel midrib segment explants. Our protocol involves two steps: the pretreatment of starting material on MS (Murashige and Skoog (1962) Physiol Plant 15:473–497) medium containing 3.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) for 8 days under continuous dark and subsequent transfer of the explants to MS medium augmented with 0.1 mg/l benzyladenine (BA) and 0.1 mg/l naphthaleneacetic acid (NAA) under light-dark conditions. On the regeneration medium, numerous globular structures appeared from the explants and subsequently differentiated into shoots. Regenerated shoots attained 2–5 cm height within 30 days of culture initiation and readily rooted on MS basal medium. Hardened plants were successfully established in the greenhouse. The regulation of sugarcane morphogenesis by auxin pretreatment is discussed.