

## **IN-VITRO PROPAGATION OF LOCALLY SELECTED BLACK PEPPER (*Piper nigrum* L.)**

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This experiment was designed to establish a protocol for *in-vitro* propagation of black pepper local selections to fulfill the high demand of planting materials. About 3-4 cm long shoot tips of MB12 were collected from bamboo rapid multiplication system and cultured on 1/3 of MS solid medium containing 2.0 mg/l BA, 0.2 mg/l IAA, 0.2 mg/l GA<sub>3</sub> and 500 mg/l PVP. After 6-8 weeks, properly established (55%) shoot tips were cultured on solid MS medium supplemented with concentrations of BA at 0.5, 1.0, 1.5, 2.0 mg/l and 1.5 BA (mg/l) with 3.0 IBA (mg/l). Highest shoot multiplication (1.6) was observed in medium containing 1.5 mg/l BA after 4 weeks ( $P = 0.9341$ ). Rooting of shoots were then tested in 1/2 MS and full MS basal media with three levels of IBA (0.0, 0.5 and 1.0 mg/l) with and without AC (0.2%). Results showed that root number in full MS was higher (5.4) than the 1/2 MS (5.0). Root number was significantly reduced with charcoal (4.9), while 1.0 mg/l IBA showed highest number of roots (6.2). Root length was significantly increased with the application of IBA. *In-vitro* rooted shoots from rooting media; cultured on MS medium, without AC or IBA, with IBA and without AC, with AC and without IBA, with IBA and AC and un-rooted micro shoots were treated with and without "Root-on" powder were established in net house. Higher plant survival and rooting (82.5-100%) were observed in all treatments. *In-vitro* un-rooted cuttings showed 93.3% survival after 8 weeks. Leaf explants of *in-vitro* grown plants (GK49) were cultured on MS medium containing three levels of BA and IAA and incubated in complete dark and 8 hours light. After 8 weeks, callus development from the leaf explants were measured using 1, 3, 5, 7 and 9 scale. Callus development was significantly higher when the leaf pieces incubated in the dark (3.5 score) and significantly higher on media containing 0.5 mg/l and 1.5 mg/l BA with 0.2 mg/l IAA (4.6 and 3.6 score respectively). Leaf explants were also tested in another experiment with two concentrations each of BA and NAA. Higher concentration of NAA significantly increased the callus development irrespectively to BA concentration. Finally, Leaf callus of both experiments turned dark and died after about 8-10 weeks without shoot regeneration.

**Key words:** Acclimatization, Leaf culture, *Piper nigrum* L., Rooting, Shoot multiplication