

Confirmation of Transgenic Robusta Coffee (*Coffea canephora*) Transformed by Chitinase-encoding Gene and Its Propagation Through Somatic Embryogenesis

Konfirmasi Kopi Robusta (Coffea canephora) Transgenik Hasil Transformasi dengan Gen Chitinase dan Perbanyakannya Melalui Embriogenesis Somatik

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Summary

Genetic engineering of Robusta coffee resistant to fungal diseases might be done by introducing a chitinase-encoding gene into genome of this plant. This research was aimed to confirm transgenic plant of BP 308 clone Robusta coffee transformed by chi gene and to evaluate its ability for the somatic embryogenesis. Confirmation of transgenic was carried out by analysis the presence of NPTII gene as a selectable marker for Canamycin resistant using PCR technique. The somatic embryo initiation and reproduction were evaluated in 11 plant accessions. Three kinds of sucrose concentration, 20%, 30% and 40% were applied in initiation stage of somatic embryo germination. The suitability of 4 medium, namely M1 (without addition by liquid medium), M2 (addition by liquid medium contained 0.25 mg/l kinetin), M3 (addition by liquid medium contained 0.25 mg/l IAA) and M4 (addition by liquid medium contained 0.25 mg/l GA₃) was evaluated for somatic embryo maturation. The result showed that 8 out of 10 plant accessions tested were transgenic and they could be propagated through somatic embryogenesis. The ability of transgenic plant for somatic embryo initiation, reproduction and regeneration were similar with that of non-transgenic one. Germination of somatic embryo could be improved by using 40% sucrose. Maturation of somatic embryo could be improved by addition of fresh liquid medium on the ancient gelled medium that used for somatic embryos reproduction. The best result was obtained on addition of fresh medium contained 0.25 mg/l GA₃ in which 65% of the somatic embryos developed to pre-germinate somatic embryo.

Key words: *Coffea canephora*, transgenic plant, somatic embryogenesis.

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Ringkasan

Rekayasa genetika kopi Robusta untuk ketahanan terhadap fungi dapat dilakukan dengan memasukkan gen kitinase pada genome tanaman tersebut. Penelitian ini ditujukan untuk mengkonfirmasi tanaman transgenik kopi Robusta klon BP 308 hasil transformasi menggunakan konstruk gen chi dan mengevaluasi kemampuan embriogenesis somatiknya. Konfirmasi transgenik dilakukan dengan menganalisis adanya fragmen DNA gen NPTII sebagai penanda seleksi terhadap kanamisin dengan teknik PCR. Inisiasi dan reproduksi embrio somatik terdiri atas 11 perlakuan, yaitu 11 aksesori tanaman. Pengecambahan embrio somatik terdiri atas 33 perlakuan, yaitu kombinasi 11 aksesori tanaman dengan 3 konsentrasi sukrosa, yaitu 20%, 30% dan 40%. Pendewasaan embrio somatik terdiri atas 44 perlakuan, yaitu kombinasi 11 aksesori tanaman dengan 4 jenis media cair yang ditambahkan pada embrio somatik tahap reproduksi embrio somatik, yaitu M1 (tanpa penambahan medium cair), M2 (medium cair diperkaya 0,25 mg/l kinetin), M3 (medium cair diperkaya 0,25 mg/l IAA) dan M4 (medium cair diperkaya 0,25 mg/l GA₃). Hasil penelitian menunjukkan bahwa 8 dari 10 tanaman hasil transformasi terbukti sebagai tanaman transgenik. Kemampuan embriogenesis somatik tanaman transgenik tidak berbeda dengan tanaman non transgenik. Pengecambahan embrio somatik semua aksesori tanaman yang diuji dapat ditingkatkan dengan menggunakan media perakaran yang diperkaya dengan 40% sukrosa. Pendewasaan embrio somatik dapat diperbaiki dengan menambahkan media cair yang diperkaya dengan 0,25 mg/l GA₃. Dengan penambahan media ini 65% embrio somatik dapat mencapai fase prakecambah.