ANALISIS INTEGRASI T-DNA PEMBAWA GEN *AtRKD4* PADA TANAMAN TRANSFORMAN *Coffea arabica* L. KLON AS2K

Gerald Dafa Rhizardy
20/454745/BI/10440

Dosen Pembimbing : Prof. Dr. Endang Semiarti, M.S., M.Sc.

INTISARI

Kopi Arabika (*Coffea arabica* L.) klon AS2K merupakan salah satu komoditas perkebunan di Indonesia yang memiliki nilai ekonomi tinggi dan banyak diminati oleh banyak masyarakat. Perbanyakan kopi Arabika di Indonesia masih belum optimal dibandingkan kopi Robusta. Tujuan peneliti adalah untuk menghasilkan tanaman kopi Arabika unggul melalui rekayasa genetik dengan penyisipan gen kunci embriogenesis *Arabidopsis thaliana RWP-RK Domain 4* (*AtRKD4*). Dilakukan metode transfer gen *AtRKD4* ke sel-sel kalus melalui *Agrobacterium tumefaciens* strain EHA105 pembawa T-DNA dengan gen *AtRKD4* dalam plasmid *pta7002* dengan perendaman larutan agro OD 0.8 selama 1 jam. Konfirmasi integrasi T-DNA pembawa gen *AtRKD4* pada genom *C. arabica* dilakukan dengan amplifikasi gen *RKD4* menggunakan *Polymerase Chain Reaction* (PCR). PCR dilakukan dengan primer *AtRKD4*, *HPT* dan *ACTIN* sebagai kontrol positif serta *DegRKD4* untuk mengetahui adanya gen homolog *AtRKD4*. Hasil penelitian menunjukkan sebanyak 44 dari 342 kalus hasil transformasi dapat bertahan pada medium seleksi dengan nilai frekuensi transformasi 11.26%. Morfologi kalus transforman yang diperoleh menunjukkan tekstur *friable* dan berwarna kuning serta karakterisitk anatomi yaitu sel-sel kecil, nukleus besar, dan sitoplasma tipis. Analisis molekuler dibuktikan dengan hasil amplifikasi DNA genom kopi yang ditandai munculnya fragmen DNA sepanjang 188 bp pada primer *AtRKD4*, 114 bp pada primer *ACTIN*, 545 bp pada primer *HPT*, dan 384 bp pada primer *DegRKD4*.

Kata kunci: *Coffea arabica* L. klon AS2K, embriogenesis somatik, *Agrobaterium tumefaciens*, *RKD4*.

ANALYSIS OF T-DNA INTEGRATION THAT CARRYING THE *AtRKD4* GENE IN *Coffea arabica* L. AS2K CLONE TRANSFORMANT PLANT

Gerald Dafa Rhizardy
20/454745/BI/10440

Supervisor : Prof. Dr. Endang Semiarti, M.S., M.Sc.

ABSTRACT

Arabica coffee (*Coffea arabica* L.) AS2K clone is one of the plantation commodities in Indonesia that has high economic value and is in great demand by many people. The propagation of Arabica coffee in Indonesia is still not optimal compared to Robusta coffee. The researcher's goal is to produce superior Arabica coffee plants through genetic engineering by inserting the key gene for *Arabidopsis thaliana embryogenesis RWP-RK Domain 4* (*AtRKD4*). The *AtRKD4* gene transfer method was carried out to callus cells through T-DNA carrying *Agrobacterium tumefaciens* EHA105 strain with the *AtRKD4* gene in the *pta7002* plasmid by soaking in agro OD 0.8 solution for 1 hour. Confirmation of the integration of the T-DNA carrying the *AtRKD4* gene into the *C. arabica* genome was carried out by amplifying the *RKD4* gene using Polymerase Chain Reaction (PCR). PCR was carried out with the primers *AtRKD4*, *HPT* and *ACTIN* as positive controls and *DegRKD4* to determine the presence of the *AtRKD4* homologous gene. The results showed that 43 out of 382 calli from the transformation could survive on the selection medium with a transformation frequency value of 11.26%. The morphology of the transformed calli obtained showed a friable texture and yellow, as well as anatomical characteristics, namely small cells, large nuclei, and thin cytoplasm. Molecular analysis was proven by the results of coffee genome DNA amplification which was marked by the emergence of DNA fragments of 188 bp in the *AtRKD4* primer, 114 bp in the *ACTIN* primer, 545 bp in the *HPT* primer, and 384 bp in the *DegRKD4* primer.

Keywords: *Coffea arabica* L. AS2K clone, somatic embryogenesis, *Agrobacterium tumefaciens*, *RKD4*.