Karakter Fenotipik dan Molekuler Padi Hitam Transgenik (*Oryza Sativa* L. "Cempo Ireng") Pembawa Gen *OsRKD4*

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Abstrak

Penyisipan gen *OsRKD4* untuk menginduksi pembentukan embrio somatik dengan konstruk 35S::GAL4::OsRKD4::GR pada kalus padi hitam telah berhasil dilakukan untuk mengatasi kegagalan regenerasi pada transformasi gen *Hd3a.* Saat ini terdapat 3 galur tanaman transgenik pembawa gen *OsRKD4* generasi T0 yaitu OS1, OS2, dan OS3. Tujuan penelitian ini adalah mengetahui pola segegrasi dan ekspresi gen sisipan *OsRKD4* pada padi hitam transgenik tersebut dalam menginduksi embriogenesis somatik. Pada penelitian ini, resistensi biji terhadap agen penyeleksi berupa antibiotik higromisin 20 ppm pada medium induksi kalus digunakan untuk mengetahui pola segregasi gen sisipan. Induksi ekspresi gen sisipan dilakukan dengan subkultur kalus pada medium dengan 20 µM/L dexamethasone. Setelah dua hari perlakuan dexamethasone 20 µM/L, kalus disubkultur pada *dex free medium* (MS0) dan dilakukan isolasi RNA untuk sintesis cDNA. Keberadaan gen sisipan diamati dengan amplifikasi serta elektroforesis gen *HPT* (455 bp) sedangkan kuantifikasi ekspresi gen *OsRKD4* (191 bp) menggunakan *Real-Time* PCR. Fenotip berupa pembentukan embrio somatik diamati dengan pewarnaan menggunakan Sudan Red 7B serta preparat irisan kalus berumur 10 hari. Hasilnya, OS1 diketahui mengikuti pola segregasi 3:1 dengan nilai X^2 dF 1 pada 1% sebesar 4,267 berdasarkan inokulasi yang dilakukan terhadap 500 biji padi, sedangkan gen *HPT* berukuran 455 bp pada terekspresi pada semua tanaman transgenik. Gen *OsRKD4* berukuran 191 bp terdeteksi pada tanaman transgenik maupun nontransgenik. OS1-D mengekspresikan ge*n OsRKD4* paling tinggi dengan nilai 2,9 *fold.* Fenotip multi embrio somatik juga teramati berdasarkan pewarnaan Sudan Red 7B serta pengamatan preparat irisan.

Kata kunci: Padi Hitam, *OsRKD4*, Transgenik, Embrio Somatik

Phenotypic and Molecular Characters of Transgenic Black Rice (*Oryza Sativa* L. "Cempo Ireng") Harboring *OsRKD4* Gene

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Abstract

The insertion of the *OsRKD4* gene to induce somatic embryo formation with construct of 35S::GAL4::OsRKD4::GR to black rice callus has been successfully conducted to overcome the constraint of regeneration after *Hd3a* gene transformation. Currently there are 3 lines of transgenic plant harboring the gene, which are OS1, OS2, and OS3. The aims of this research are to know the pattern of segregation and expression of *OsRKD4* gene on the transgenic black rice in inducing somatic embryogenesis. In this study, seed resistance to hygromycine antibiotic 20 ppm on the callus induction medium was used to determine the pattern of segregation of insertion genes. Induction of the gene expression was performed by callus subculture on medium with 20 μM / L dexamethasone. After two days of dexamethasone 20 μM / L treatment, callus was subcultured to free medium (MS0) and RNA isolation was conducted for cDNA synthesis. The presence of the inserted gene was observed by amplification and electrophoresis of the selectable marker *HPT* gene (455 bp) while the quantification of *OsRKD4* gene expression (191 bp) is detected by using Real-Time PCR. The phenotype of somatic embryo formation was observed with Sudan Red 7B staining while callus slice were conducted by using 10 days old callus. As a result, OS1 is known to follow 3: 1 segregation pattern with a value of X^2 dF 1 at 1% is 4,267 after inoculation of 500 rice seeds, while the transgenic plants showed 455 bp *HPT* gene. The 191 bp *OsRKD4* gene is detected in both transgenic and non-transgenic plants. OS1-D has the highest expression level with a value of 2.9 fold. The multi somatic embryos phenotype was also observed based on the Sudan Red 7B staining and also sliced preparations.

Keywords: Black Rice, *OsRKD4*, Transgenic, Somatic Embryo