Penapisan dan analisis molekuler padi (Oryza sativa L.) Ciherang transgenik OsDREB1A generasi BC4F2 dan BC5F1 untuk toleransi terhadap salinitas tinggi = Screening and molecular analysis of rice (Oryza sativa L.) Ciherang transgenic OsDREB1A lines BC4F2 and BC5F1 for high salinity tolerance

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Abstrak

Salinitas merupakan salah satu cekaman abiotik yang mengancam produksi padi di Indonesia. Dalam rangka mendukung program ketahanan pangan, BB-Biogen telah melakukan pengembangan varietas padi Ciherang toleran salinitas hingga generasi BC4F2 dan BC5F1. Tanaman generasi BC4F2 dan BC5F1 Ciherang-OsDREB1A selanjutnya memerlukan serangkaian pengujian. Pertama, penapisan toleransi terhadap salinitas tinggi untuk menyeleksi tanaman yang menunjukkan sifat toleran terhadap salinitas tinggi. Kedua, analisis molekuler yang mencakup analisis integrasi, ekspresi OsDREB1A, dan Southern blot. Hasil penapisan salinitas terhadap BC4F2 dan BC5F1 Ciherang-OsDREB1A selama 26 hari dengan EC akhir berkisar 18 mS/cm telah berhasil menyeleksi 134 individu putatif transgenik dari total 543 tanaman uji.
Hasil analisis integrasi menggunakan primer hptII menujukkan 73 dari 134 tanaman putatif transgenik memiliki hasil positif hptII. Seluruh tanaman yang positif PCR hptII juga terdeteksi memiliki hasil positif PCR menggunakan primer kombinasi 35S-496-F/OsDREB1A-R, mengindikasikan kestabilan integrasi transgen tetap terjaga selama persilangan. Hasil analisis ekspresi OsDREB1A menunjukkan terdapat variasi level ekspresi OsDREB1A antar individu Ciherang transgenik. Hasil analisis Southern blot menunjukkan jumlah salinan T-DNA berjumlah sekitar 6--8 kopi. Galur terbaik berdasarkan hasil analisis molekuler adalah BC5F1-K14-23-3 dan BC4F2-K13-11-3.
.....Salinity is one of the abiotic stresses that threaten rice production in Indonesia. In order to support the food security programs, BB-Biogen has started doing development of salinity tolerant rice varieties Ciherang up to BC4F2 and BC5F1 generations. BC4F2 and BC5F1 generations of Ciherang-OsDREB1A transgenic lines require a series of tests for verifying the salinity tolerance and stability of transgene integration. First, screening for selecting the Ciherang-OsDREB1A transgenic lines that revealed tolerance to high salinity. Second, molecular analysis that includes analysis of integration, OsDREB1A expression, and Southern blot. The screening result of Ciherang-OsDREB1A transgenic lines BC4F2 and BC5F1 for 26 days with final EC approximately 18 mS/cm, had been successfully selected 134 putative transgenic plants of a total 543 tested plants.
PCR analysis results showed that 73 of 134 putative transgenic plants had PCR positive using hptII-F/hptII-R primer. Plants were detected positive in PCR analysis using hptII-F/hptII-R primer were also positive in PCR analysis using specific primer 35S-496-F/OsDREB1A-R, indicating that the stability of the transgene integration is maintained during the crossing. The results of OsDREB1A expression analysis showed that there were variations in expression levels among individuals Ciherang-OsDREB1A transgenic lines. The results of Southern blot analysis showed that the T-DNA copy number around 6--8 copies. Best lines based on the results of molecular analysis is BC5F1-K14-23-3 and BC4F2-K13-11-3.