INTISARI

Bawang merah (*Allium cepa* L. Kelompok Aggregatum) merupakan salah satu komoditas hortikultura di Indonesia yang diusahakan oleh petani secara intensif. Bawang merah yang ditanam secara vegetatif menggunakan umbi dan secara generatif menggunakan biji memiliki perbedaan umur tanam dan hasil panen umbi. Oleh karenanya, pengkajian lebih lanjut mengenai gen-gen yang mengatur pembentukan umbi melalui analisis transkriptomik perlu dilakukan. Untuk melakukan analisis transkriptomik seperti teknik quantitative *Polymerase Chain Reaction* (qPCR), gen referensi (*housekeeping gene*) diperlukan untuk normalisasi data gen ekspresi Penelitian ini bertujuan untuk menentukan metode isolasi mRNA dan sintesis cDNA serta mengidentifikasi beberapa *housekeeping gene* pada bawang merah sebagai studi awal pada level ekspresi gen. Alur penelitian yang dilakukan meliputi persiapan sampel umbi, daun dan bunga bawang merah dengan umur 9 minggu setelah tanam(MST), 11 MST dan 14 MST untuk generasi G1 (bibit dari umbi) dan sampel berumur 16 MST, 18 MST dan 21 MST untuk generasi G0 (bibit dari biji), desain primer spesifik berdasarkan sekuens gen actin, tubulin dan GAPDH, isolasi mRNA, sintesis cDNA, selanjutnya divalidasi oleh teknik *Polymerase Chain Reaction* (PCR), dan DNA sekuensing. Hasil penelitian menunjukkan semua sampel dapat dilakukan isolasi mRNA menggunakan Thermo Scientific GeneJET Plant RNA Purification Mini Kit dengan penggunaan 100 mg sampel dan sintesis cDNA dengan RevertAid First Strand cDNA Synthesis Kit dengan penggunaan 500 ng template RNA. Amplifikasi gen Actin, Tubulin dan GAPDH menghasilkan produk berukuran 142 bp ; 116 bp; dan 136 bp. Gen Actin, GAPDH dan Tubulin terekspresi hampir konstan pada organ tanaman umbi dan bunga dan terekspresi inkonsisten pada organ tanaman daun. Dari ketiga *housekeeping gene* yang diuji, gen tubulin berpotensi menjadi kontrol internal pada organ tanaman umbi dan bunga, tetapi gen potensial untuk kontrol internal di daun masih belum secara konstan diekspresikan. Oleh karenanya, penggunaan lebih dari satu gen referensi dibutuhkan untuk melakukan analisis pada organ daun.

Kata Kunci : Bawang merah, *housekeeping gene*, isolasi mRNA, sintesis cDNA, PCR

ABSTRACT

Shallots (*Allium cepa* L. Aggregatum Group) are one of the horticultural commodities in Indonesia that are intensively cultivated by farmers. Shallots which grow vegetatively using bulb and generatively using seeds have different period and yields. Therefore, the study on the regulation of bulb formation in transcriptomic level is necessary. Transcriptomic analysis approach utilizes the quantitative polymerase chain reaction (qPCR) technique, a suitable reference gene (housekeeping gene) is required to normalize gene expression data. This study aimed to optimize the method of mRNA isolation and cDNA synthesis, also to identified several housekeeping genes in shallots as a preliminary study at the transcriptomic level. The method of this study was including the preparation of samples of bulbs, leaves and flowers from shallots at 9 week after sowing (WAS), 11 WAS and 14 WAS for G1 generation (seedlings from bulb) and samples from shallots at 16 WAS, 18 WAS and 21 WAS for G0 generation (seedlings from seed), then detection of house keeping genes encoding actin, tubulin and GAPDH gene using set of primers designed. Preparation of house keeping genes detection consists of mRNA isolation, cDNA synthesis, validatation through Polymerase Chain Reaction (PCR), and DNA sequencing. The results showed that mRNA isolation could be carried out with a sample of 100 mg using the Thermo Scientific GeneJET Plant RNA Purification Mini Kit and cDNA synthesis using RevertAid First Strand cDNA Synthesis Kit with 500 ng RNA templates. The housekeeping genes including actin, tubulin and GAPDH genes from shallot were amplified with the sizes of 142 bp, 116 bp and 136bp. Actin, GAPDH and Tubulin genes were expressed almost constantly in bulb and flower organs and inconsistently expressed in leaf. Three housekeeping genes have been tested, and based on the results, it showed that the tubulin gene has the potential as an internal control in bulb and flower organs. However, the potential for genes for internal control in leaves has not been constantly expressed, therefore we have to use more than one reference gene for expression analysis in leaves

Keywords: shallot, housekeeping gene , mRNA isolation, sintesis cDNA, PCR