

Intisari

Kosmos (*Cosmos sulphureus* Cav.) merupakan tanaman tropis mengandung metabolit sekunder sebagai herbisida nabati dan kemikalia. Pemuliaan tanaman dengan peningkatan konsentrasi metabolit sekunder dengan pendekatan bioteknologi melalui transformasi genetik. *SoSPS1* merupakan enzim kunci yang berperan dalam sintesis sukrosa dari tanaman tebu. Akumulasi sukrosa diharapkan dapat meningkatkan konsentrasi hasil metabolit pada tanaman kosmos. *Floral dip* menjadi salah satu metode transformasi genetik melalui *Agrobacterium tumefaciens* yang efisien dan simpel. Penambahan surfaktan silwet L-77 merupakan faktor penting untuk meningkatkan efisiensi transformasi genetik. Pengembangan metode transformasi *floral dip* dan karakteristik morfologi tanaman transgenik kosmos belum pernah dilaporkan sebelumnya. Penelitian ini bertujuan untuk memperoleh konsentrasi surfaktan silwet L-77 yang efektif dan peningkatan fenotipe tanaman kosmos transgenik *SoSPS1*.

Penelitian ini telah dilaksanakan pada bulan Mei 2021 hingga Juni 2022 di sub-lab. Kultur jaringan, sub-lab. Genetika dan pemuliaan tanaman dan rumah kawat, Fakultas Pertanian, Universitas Gadjah Mada. Penelitian transformasi *floral dip* dengan mencelupkan kuncup bunga kosmos ke dalam suspensi *Agrobacterium tumefaciens* strain GV3101 pembawa vektor rekombinan *pRI101AN-SoSPS1* ditambah sukrosa 5% dan perlakuan berbagai konsentrasi silwet L-77 (0; 0,05; dan 0,1%) selama 30 detik dengan 15 sampel per perlakuan. Benih T1 diseleksi menggunakan kanamisin 50 ppm pada kertas saring. Dua puluh sampel DNA tanaman dari setiap perlakuan diekstraksi dari daun kemudian dianalisis molekuler menggunakan metode PCR deteksi gen *npII* ukuran produk 550 bp dan *SoSPS1* ukuran produk 1 kb. Fenotiping tanaman transgenik menggunakan penanda morfologi pada karakter kualitatif dan kuantitatif pertumbuhan, daun dan bunga. Data dianalisis menggunakan beda nyata jujur 95% pada rata-rata jumlah benih per bunga, Uji T pada karakter kuantitatif dan analisis *principal coordinate* berdasar karakter kualitatif.

Hasil penelitian diperoleh bahwa konsentrasi silwet L-77 0,1% memperoleh persentase bunga nekrosis paling tinggi 20%, tetapi memperoleh hasil paling tinggi pada rata-rata jumlah biji per bunga 13,1 biji, jumlah biji 131 biji dan persentase resisten kanamisin 36,64%. Hasil deteksi molekuler perlakuan silwet L-77 0,1% memperoleh tanaman putatif transforman dengan penanda *npII* 100% pita DNA berukuran 550 bp dan penanda *npII-SoSPS1* 75% dengan pita berukuran 400 dan 700 bp. Fenotipe tanaman putatif transforman *SoSPS1* mengalami peningkatan dibanding tipe liarnya pada karakter tinggi tanaman 26%, jumlah cabang 36,58%, panjang trikoma 33,87%. Peningkatan karakter daun: panjang daun 20,45%, lebar daun 27%, panjang terminal 27%, lebar terminal 85,21%, panjang tangkai daun 10% dan lebar lob daun 57%, panjang stomata 17%, lebar stomata 11,48%. Peningkatan karakter bunga seperti panjang tangkai 10%, diameter bunga 18,97%, diameter piringan 13%, jumlah pita 8,6%, panjang pita 12%, lebar pita 33,33%. Pada karakter kuantitatif proporsi keberadaan antosianin 66,67% bentuk ujung daun oval 55,55%, keberadaan antosianin daun 11,11%. *Collar segment* 55,56%, Pita bunga bertipe campuran antara *ligulate* dan *tubular* 44,44%. Hasil analisis PCoA menunjukkan tanaman putatif transforman membentuk kelompok yang terpisah dari tipe liarnya.

Kata kunci: *Floral dip*, Kosmos, *SoSPS1*, Transgenik,

Abstract

Cosmos (*Cosmos sulphureus* Cav.) is a tropical plant that contains secondary metabolites as vegetable and chemical herbicides. Plant breeding by increasing the concentration of secondary metabolites with a biotechnology approach through genetic transformation. SoSPS1 is a key enzyme that plays a role in the biosynthesis of sucrose from sugarcane. Sucrose accumulation is expected to increase the concentration of metabolites in cosmos plants. Floral dip is an efficient and simple method of genetic transformation through *Agrobacterium*. The addition of silwet L-77 surfactant was important factors to increase the transformation efficiency. The development of the flower dip transformation method and the morphological characteristics of cosmos transgenic plants have been previously reported. This study aimed to obtain effective silwet L-77 concentrations, and phenotypes of cosmos transgenic plant SoSPS1.

This research has been carried out from May 2021 to June 2022 in a sub-lab. Tissue culture, sub-lab. Genetics and plant breeding and wire house, Faculty of Agriculture, Universitas Gadjah Mada. Research on floral dip transformation by dipping cosmos flower buds into suspension of *Agrobacterium tumefaciens* strain GV3101 carrying pRI101AN-SoSPS1 recombinant vector plus 5% sucrose and treatment with various concentrations of L-77 (0; 0.05 and 0.1%) for 30 seconds with 15 samples per treatment. T1 seeds were selected using 50 ppm kanamycin on filter paper. Twenty samples of plant DNA from each treatment were extracted from the leaves and then analyzed molecularly using the PCR method to detect the *nptII* gene with a product size of 550 bp and SoSPS1 with a product size of 1 kb. Phenotyping of transgenic plants used morphological markers on qualitative and quantitative characters of growth, leaves and flowers. Data were analyzed using a 95% honest significant difference on the average number of seeds per flower, T-test on quantitative characters and principal coordinate analysis based on qualitative characters.

The results showed that the concentration of silwet L-77 0.1% obtained the highest percentage of flower necrosis 20%, but obtained the highest yield on the average number of seeds per flower 13.1 seeds, the number of seeds 131 seeds and the percentage of kanamycin resistance 36, 64%. The results of molecular detection of 0.1% L-77 silwet treatment obtained a putative transformant plant with *nptII* marker 100% DNA band measuring 550 bp and *nptII*-SoSPS1 marker 75% with band measuring 400 and 700 bp. SoSPS1 transformant putative plant phenotype increased compared to the wild type in the characters of plant height 26%, number of branches 36.58%, trichome length 33.97%. Increased leaf character: leaf length 20.45%, leaf width 27%, terminal length 27%, terminal width 85.21%, petiole length 10% and leaf lobe width 57%, stomata length 17%, stomata width 11.48%. Improved flower characters such as 10% stalk length, 18.97% flower diameter, 13% disk diameter, 8.6% band number, 12% band length, 33.33% band width. In quantitative character, the proportion of anthocyanin is 66.67%, oval leaf tip is 55.55%, leaf anthocyanin is 11.11%. Collar segment 55.56%, floral band mixed type between ligulate and tubular.44,44%. The results of PCoA analysis showed that the transformant putative plants formed a separate group from the wild type.

Key words: Cosmos, Floral dip, SoSPS1, Transgenic