ARTICLE

The phenotypical and biochemical characteristics of the flower *Cosmos sulphureus* T1 overexpressing *SoSPS1* (*Sucrose Phosphate Synthase*)

Crop Breeding and Applied Biotechnology
25(3): e51402533, 2025
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332025v25n3a33

Tantri Swandari[1,2*], Dyah Weny Respatie[1], Endang Sulistyaningsih[1], Widhi Dyah Sawitri[1] and Aziz Purwantoro[1]

Abstract: *Yellow cosmos (Cosmos sulphureus Cav.) is an ornamental plant with abundant secondary metabolites that can be used as natural herbicides. The present study evaluated the influence of overexpressing the Sucrose Phosphate Synthase (SoSPS1) gene on phenotypic features and accumulation of biochemical component in transgenic cosmos flowers. This genetic transformation was carried out using the Agrobacterium tumefaciens-mediated floral dip method. The quantitative characteristics of wild-type and transgenic cosmos flowers differed significantly in the number of flowers per plant and ray florets, flower diameter, ray floret width, pedicle length, fresh weight per flower, and seed number. Sucrose accumulation and total phenol content in transgenic plant flowers were higher than in the wild-type. These findings support the utilization of yellow cosmos as an ornamental plant for bioherbicide production.*

Keywords: *Bioherbicide, floral dip, sucrose, total phenol, transgenic*

INTRODUCTION

Cosmos sulphureus Cav., or yellow cosmos, is an ornamental plant of the Asteraceae family with a dominant phenolic content (Ortega-Medrano et al. 2023). Research confirms that yellow cosmos flower extract can reduce the number of buds, roots, leaf length, and rhizome size of the purple nutsedge weed (*Cyperus rotundus* L.) (Respatie et al. 2019). This result indicates that it can function as a natural herbicide. Natural herbicides, or bioherbicides, are sustainable and environmentally friendly options for weed control. Furthermore, producing bioherbicides that are provided in ready-to-use form remains challenging and is often considered financially unviable for large-scale weed control. At specific bioactive concentrations, bioherbicide production requires large quantities of plant biomass (Roberts et al. 2022). Thus, it is necessary to apply biotechnology, namely genetic transformation, to enhance the content of plant bioactive compounds.

The floral dip transformation strategy provides an option for plant genetic engineering by increasing transformation frequency. In *C. sulphureus* Cav., floral dip transformation has been carried out by inserting the neomycin phosphotransferase II (*npt*II) genes and green fluorescent protein (GFP). The results demonstrated that this transformation method increases efficiency by

***Corresponding author:**
E-mail: tantri14swandari@instiperjogja.ac.id

Received: 13 November 2024
Accepted: 09 April 2025
Published: 27 June 2025

[1] Gadjah Mada University Faculty of Agriculture, Jl Flora 1 Bulaksumur, Yogyakarta, Daerah Istimewa Yogyakart 55281, Indonesia
[2] Institut Pertanian STIPER, Agrotechnology, Jl Nangka II, Maguwoharjo, Depok Sleman, Yogyakarta 55283, Indonesia

12.78 ±1.53% (Purwantoro et al. 2023). Molecular investigations and phenotypic evaluations of the transgenic *npt*II yellow cosmos in generations T1 and T2 indicated morphological changes in the mixed ray, floret, ligulate, and tubular forms (Irsyadi et al. 2022, Muchyiddin et al. 2024). The results of this study became the basis for transforming the *Sucrose Phosphate Synthase* (SPS) gene in yellow cosmos.

Sucrose Phosphate Synthase is an essential enzyme involved in synthesizing sucrose from uridine diphosphate-glucose (UDPG) and fructose-6-phosphate. The identification of the *SPS* gene from sugarcane cDNA provides information on the specific amino acid sequence called the *SoSPS*1 gene (*SPS Saccharum officinarum* L. clone 1). Overexpression of *SoSPS*1 in sugarcane increases the sugar content of leaf organs while also significantly enhancing plant height. These findings suggest that inserting the *SoSPS*1 gene into sugarcane helps to increase yield-related traits (Anur et al. 2020). The overexpression of the *SoSPS*1 gene has been investigated in rice (Mulyatama et al. 2022, Shidiqi et al. 2024), citrus (Suputri et al. 2020), tomato (Afidah et al. 2022), and yellow cosmos (Purwantoro et al. 2024). In the T1 generation of yellow cosmos, the overexpression of the *SoSPS*1 gene causes segregation in growth-type characteristics, stem anthocyanins, branch density, increased internode length, and stem diameter, as well as increased total phenol (17.52%), reduced sugar (28.77%), and total sugar (14.71%) in the leaves.

Despite these findings, most research has focused on the effects of *SoSPS*1 overexpression on vegetative organs, such as leaves, while its impact on reproductive structures, particularly flowers, remains largely unexplored. This is noteworthy because *C. sulphureus* flowers contain higher levels of secondary metabolites, primarily phenolic compounds, which may act as allelochemicals for weed suppression. Understanding how *SoSPS*1 overexpression affects secondary metabolite accumulation in flowers could unlock new opportunities for utilizing yellow cosmos as both an ornamental plant and a source of bioherbicides. To address this gap, the present study investigates the influence of *SoSPS*1 gene overexpression on flower characteristics, focusing on secondary metabolite content to support its potential application as a bioherbicide resource.

MATERIAL AND METHODS

Plant materials

The research used yellow cosmos seeds obtained through floral dip transformation with *Agrobacterium tumefaciens* strain GV3101 containing recombinant plasmids pRI101AN-*SoSPS*1, paired with kanamycin antibiotic selection markers, sourced from Prof. Bambang Sugiharto at the University of Jember, Indonesia (Irsyadi et al. 2022, Purwantoro et al. 2024). The altered T1 yellow cosmos plants were selected from the flowers that produced numerous seeds. For this research, 21 seeds were obtained from two flower buds (the first blossom produced 11 seeds, while the second, 10 seeds). The wild yellow cosmos plants, with 20 seeds, served as the control group.

Selection and confirmation of SoSPS1 transgene in T1 yellow cosmos

T1 yellow cosmos seeds were germinated for one week in a petri dish filled with filter paper soaked in a 50-ppm kanamycin antibiotic solution. The kanamycin-resistant germinating seeds were then transplanted into polybags containing planting media mixed with soil and manure. The plants were treated in greenhouses until they completed the generative phase, during which phenotypic, molecular, and biochemical analysis were performed. Confirmation of the *SoSPS1* transgene in T1 yellow cosmos was conducted through molecular analysis, which began with DNA extraction from plant leaves aged 2–3 weeks after transplantation. Based on previous studies, DNA extraction was performed using 2% cetyltrimethylammonium bromide (CTAB). Plant DNA was amplified using specific primers for detecting *SoSPS1* genes: *CaMV-35S* 5'-GAAGACGCGTTCCAACCACG-3' and *SPS-P9* 5'ACCACGGTATGCCACAATGTA-3', along with *NPT*II F 5'-GTCATCTCACCTTGCTCCTGCC-3, *NPT*II R 5'-GTCGCTTGGTCGGTCATTTCG-3' (Purwantoro et al. 2024). The PCR cocktail 10 µL mixture consisted of 5 µL Master Mix Powerpol®, 2 µL nuclease-free water, 1 µL for each forward and reverse primers (10 mM), and 1 µL of DNA samples. The PCR reaction was as follows: pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 1 min, repeated for 40 cycles (PCR T100™ thermal cycler, Bio-Rad, USA). The presence of the *SoSPS*1 and *npt*II genes was detected by electrophoresis (Bio-Rad, USA) in 1% agarose gel added DNA staining and 5 µL ladder Smobio®. The DNA band was visualized under a UV Transilluminator (Muchyiddin et al. 2024).

Characterization of flower phenotype

The characterization of yellow cosmos flowers was carried out when the plant entered the generative phase. Flowers were observed weekly over a four-week period, with five flowers observed per week for each sample plant. The qualitative and quantitative parameters observed followed the yellow cosmos characterization guidelines provided by the International Union for the Protection of New Varieties of Plants (UPOV). The observed quantitative traits included the number of flowers and petals, flower and disc diameter, petal length and width, flower stalk length, flower fresh and dry weight, number of seeds per flower, and average seed weight. Qualitative traits included flower type, disc type, flower segmentation, flower crown type, longitudinal axis, crown curvature, crown length and width ratio, crown color, disc color, and flowering time. Qualitative traits included flower type, disc type, flower segmentation, ray floret type, longitudinal axis, ray floret curve, ratio length and width of ray floret, disc and ray floret color, and flowering time.

Biochemical observation

The total phenol content was evaluated using a sample of dried yellow cosmos flowers weighing approximately 2 g. These flowers comprised a combination of those collected during the previous phenotypic flower observation, conducted weekly over four consecutive weeks, with five flowers collected each week. The flowers were pulverized using a blender or mortar and suspended in aquades. The extract was then filtered into a 100 mL measuring flask and filled to the total capacity with the extraction solvent. This solution was used as a stock solution to determine the total phenol content, which was quantified using the Folin–Ciocalteu assay. Each biochemical analysis was performed in duplicate.

The reduced sugar was measured using the Nelson–Somogy spectrophotometry technique (Purwantoro et al. 2024). Total sugar was measured using a 1-gram sample of dried flowers that were crushed and dissolved in 50 mL of distilled water in an Erlenmeyer flask, then mixed with 5 mL of 25% HCl and heated in a water bath at 100 °C for 10 minutes. After the cooling process, aquades were added to a volume of 100 mL in a measuring flask. The solution was mixed and then filtered, which produced filtrates. Filtrate was taken up to 1 mL, diluted with Nelson's C reagent, and then heated in a water bath at 100 °C for 30 minutes. After the solution had cooled, 1 mL of arsenomolybdate was added and mixed using a vortex before adding aquades until the volume reached 10 mL. The solution was homogenized, and the absorbance was measured using a spectrophotometer at 540 nm. The absorbance values were recorded and computed using the standard curve that had been previously produced. The sucrose content was measured by entering the total sugar and reduced sugar values into the following formula: % Sugar content = % Total sugar content − % Reduced sugar content. The concentrations of phenolic compounds, reducing sugars, and total sugars was determined using a Shiminzu UV-1202 UV/Vis spectrophotometer.

Data analysis

Excel was used to compile and evaluate categorical floral data as percentages, which were then converted into ordinal ratings based on UPOV's description of *C. sulphureus*. This ensured consistency in measuring plant traits and improved statistical analysis. IBM SPSS Statistics 22 was used for ANOVA, Tukey's HSD test ($\alpha = 0.05$), standard deviation, and normality assessment of quantitative data. A logarithmic transformation (LOG 10 (x)) was applied to correct for positive skewness and meet normality requirements for parametric tests. Both qualitative and quantitative data were analyzed using OriginPro 2024 to examine variability and clustering of yellow cosmos plants, differentiating between *SoSPS*1-negative, *SoSPS*1-positive, and wild-type plants.

RESULTS AND DISCUSSION

Selection and confirmation of *SoSPS*1 transgene in T1 yellow cosmos

Selection with 50 ppm kanamycin immersion revealed that 20 out of 21 yellow cosmos seeds (95.24%) generated from floral dip transformation could germinate. DNA extraction and validation of the *SoSPS*1 gene occurred when the plant was three weeks old after transplantation. The results of molecular confirmation revealed that 14 out of 20 germinated seeds (70%) tested positive for the *SoSPS*1 gene. The transgenic-positive cosmos plants individuals were identified by amplification of the target DNA segment of 700bp (supplementary data). The percentage of individuals confirmed positive for *SoSPS*1 transgene was exceptionally high. However, not all plants survived through the flowering

stage. Previous research on in planta transformation of the *SoSPS*1 gene in citrus plants (*Citrus nobilis* L.) reported a lower transformation efficiency of 16.7% (Suputri et al. 2020), while the efficiency of in planta transformation in tomato plants was approximately 19.8%. In contrast, floral dip transformation in yellow cosmos plants using *A. tumefaciens*, with the *SoSPS*1 gene as the target and the *npt*II gene as a marker, demonstrated relatively high results, with 70% of plants confirmed positive for *SoSPS*1 and 65% confirmed positive for *npt*II. Moreover, the transformation efficiency of the *npt*II gene in yellow cosmos observed in this study (73.3%) was consistent with findings from a previous study (Irsyadi et al. 2022). These results highlight that in planta transformation using the floral dip method is highly suitable for yellow cosmos due to its relatively high transformation efficiency. Similarly, high transformation efficiency has been observed in pigeon pea plants carrying the *cryAb* gene, as confirmed by PCR analysis using specific primers, with successful inheritance of the gene in the T1 generation (Singh et al. 2023).

Characterization of flower phenotype

The data revealed that insertion of the *SoSPS*1 gene in the yellow cosmos showed different traits compared to wild-type plants. The observed changes included variations, either increasing or decreasing, in the proportion of individuals displaying each phenotypic trait. All observed proportions in wild-type plants had an upward-facing flower position, but transgenic plants exhibited two blossom positions: upward and outward. The flower types were significantly different, with the wild-type plants producing single flowers and transgenic plants producing exclusively semi-double blooms (Table 1). Collar segmentation (paracorolla) was observed only in *SoSPS*1 transgenic plants, while all wild-type plants lacked this trait. All wild-type plants investigated in this study had ligulate-type ray florets. Besides ligulate types, transgenic plans exhibited a mixed ray floret type, combining ligulate and tubular forms. The proportion of moderately incurved longitudinal axes in wild-type and transgenic plants was more remarkable than the strengthened and weakened incurved types. Both wild-type and transgenic plants displayed medium-type apical incisions. However, a shallow-type ray floret was also detected. The curvature rate of ray florets in wild-type plants had the highest proportion in the distal three-quarters curvature criterion (73.33%). In contrast, transgenic plants exhibited a balanced proportion in the entire length and distal three-quarters type. The length and width ratio of ray florets in wild and transgenic plants was 2:1 (medium-type). The ray florets and discs colors in wild-type and transgenic plants were nearly identical. Ray florets in wild-type plants were characterized as brilliant greenish yellow color (80%), and the same was observed in transgenic plants (*npt*II+*SoSPS*1+, *npt*II+*SoSPS*1-, and *npt*II-*SoSPS*1-), except for *npt*II-*SoSPS*1- plants, which were vivid greenish yellow. The disc color was divided into brilliant yellow and vivid yellow. Both wild-type and transgenic plants showed the highest proportion in the brilliant yellow group (60%). Flowering time varied significantly between wild-type and transgenic plants. Wild-type plants tended to flower on time, 8–10 weeks after transplanting, while transgenic plants tended to flower later/slower, more than 12 weeks after transplanting.

The quantitative characteristics of wild-type and transgenic cosmos flowers differed significantly in the number of flowers per plant, the number of ray florets, flower diameter, ray floret width, pedicle length, fresh weight per flower, and seed number. Transgenic *npt*II+*SoSPS*1+ plants produced a higher number of flowers per plant compared to wild-type plants. Wild-type yellow cosmos plants produced the same flower number as *transgenic plants npt*II+*SoSPS*1- and *npt*II-*SoSPS*1+. Transgenic *npt*II and *SoSPS*1 plants produced considerably more ray florets than wild-type plants. The flower diameter and ray floret width of *npt*II+*SoSPS*1+ transgenic plants were significantly different than those of wild-type plants, but there was no significant difference between *npt*II+*SoSPS*1-, *npt*II-*SoSPS*1+, and *npt*II-*SoSPS*1. Transgenic yellow cosmos plants had longer pedicles than wild-type plants. The confirmed *npt*II+ *SoSPS*1+, *npt*II-*SoSPS*1+, *npt*II-*SoSPS*1-, and *npt*II+*SoSPS*1- plants were shown in descending order by flower pedicle length. The flowers fresh weight on the *npt*II+*SoSPS*1+ transgene plants was similar to that of wild-type plants as well as to *npt*II+*SoSPS*1-, *npt*II-*SoSPS*1+. Wild-type plants produced more seeds per flower, while transgenic plants harboring *npt*II+*SoSPS*1+ produced the fewest. The quantitative parameters of flowers that did not alter significantly between transgenic and wild-type plants, namely disc diameter, ray floret length, dry weight per flower, and seed weight. The transgenic yellow cosmos flower *SoSPS*1 showed a considerable increase in the number of ray florets, flower diameter, ray floret width, and pedicle length (Table 2).

According to Figure 1, the flower position of wild-type and transgenic plants was consistently upward. Transgenic plants had semi-double flowers (large, dense petals), but wild-type plants had solitary flowers. Transgenic plants had a larger habitus than wild-type plants (data not shown). The distribution of wild-type and transgenic yellow cosmos

Table 1. The comparison of qualitative flower characteristics between T1 generation transgenic yellow cosmos phenotypes (*npt*II and *SoSPS*1) and wild-type yellow cosmos

Trait	Sub trait	Proportion (%)				
		Wild-type	*npt*II+ *SoSPS1*+	*npt*II+ *SoSPS*1-	*npt*II- *SoSPS*1+	*npt*II- *SoSPS*1-
Flower head: attitude	Upward	100	53	6	0	24
	Outward	0	6	0	6	6
	Downward	0	0	0	0	0
Flower head: disc type	Daisy	100	59	6	6	29
	Anemone	0	0	0	0	0
Flower head: type	Single	100	0	0	0	0
	Semi double	0	59	6	6	29
	Double	0	0	0	0	0
Collar segments	Absent	100	18	0	0	18
	Present	0	41	6	6	12
Ray floret type	Ligulate	100	53	0	6	12
	Tubular	0	0	0	0	0
	Ligulate & tubular	0	6	6	0	18
Longitudinal axis	Strongly incurved	0	18	0	0	6
	Moderately incurved	53.33	35	6	6	24
	Weakly incurved	46.67	6	0	0	0
Ray floret: incisions of apex	Shallow	20	24	0	0	6
	Medium	80	35	6	6	24
	Deep	0	0	0	0	0
Ray floret: curve part of axis	Distal half	13.33	12	6	0	0
	Distal three-quarter	73.33	24	0	0	6
	Entire length	13.33	24	0	6	24
Ray floret: ratio length/ width	Low	0	0	0	0	0
	Medium	100	59	6	6	29
	High	0	0	0	0	0
Ray floret color	Brilliant greenish yellow (Yellow Group 3A)	80	35	6	6	0
	Vivid greenish yellow (Yellow Group 2A)	20	24	0	0	29
Disc floret color	Brilliant yellow (Yellow Orange Group 8A)	40	12	0	0	12
	Vivid yellow (Yellow Orange Group 9A)	60	47	6	6	18
Time of flowering beginning	Early	20	18	0	0	0
	Medium	80	24	6	6	24
	Late	0	18	0	0	6

was considerably different (Figure 2). Wild-type plants were located on the left, while transgenic plants (*npt*II+*SoSPS*1+, *npt*II+*SoSPS*1-, *npt*II-*SoSPS*1+) and non-transgenic plants (*npt*II-*SoSPS*1-) appeared on the right. The distribution of confirmed transgenic and non-transgenic plants was difficult to identify. This indicates that the phenotype characteristics of flowers in transgenic and non-transgenic plants are substantially similar. Transgenic plants containing the *npt*II and *SoSPS*1 genes were distributed in quadrants I and II, while non-transgenic plants were in quadrant I. Variations in flower characteristics were also observed in plants with positive *npt*II and negative *SoSPS*1, and negative *npt*II and positive *SoSPS*1. Quadrant II included plants with positive *npt*II and negative *SoSPS*1, whereas quadrant I includes plants with negative *npt*II and positive *SoSPS*1.

Plant breeding aims to change the structure of ornamental plants, particularly through genetic transformation procedures that create numerous new lines with distinctive traits. This study also demonstrated how plant genetic modification has altered flower characteristics. Plants expressing the *SoSPS*1 and *npt*II genes showed both qualitative and quantitative alterations in their yellow cosmos flowers. The most visible qualitative change was the type of flower head, where wild-type plants had single-type flowers (12-13 ray florets), while transgenic plants showed a semi-double

Table 2. The comparison of quantitative characteristics between T1 generation transgenic yellow cosmos (*npt*II and *SoSPS*1) and wild-type yellow cosmos

Traits	Wild-type	*npt*II+ *SoSPS*1+	*npt*II+ *SoSPS*1-	*npt*II-*SoSPS1*+	*npt*II- *SoSPS*1-	F value	P value
Number of flowers per plant (unit)	127.90ab	147.90a	125.00ab	129.00ab	112.00b	3.833	0.013
Number of ray florets (unit)	12.51b	15.82a	16.17a	16.00a	16.21a	16.174	0.000
Flower diameter (cm)	4.97b	5.68a	5.12ab	5.11ab	5.02ab	5.183	0.003
Disc diameter (cm)	1.09a	1.32a	1.17a	1.31a	1.27a	2.33	0.080
Ray floret length (cm)	1.89a	2.23a	2.26a	2.19a	2.03a	1.882	0.140
Ray floret width (cm)	1.08b	1.43a	1.17ab	1.31ab	1.21ab	4.713	0.005
Length of pedicle (cm)	11.24c	15.19a	13.53b	14.93a	14.66a	59.125	0.000
Fresh weight per flower (g)	0.554ab	0.594a	0.503ab	0.571ab	0.481b	2.971	0.036
Dry weight per flower (g)	0.107a	0.139a	0.124a	0.120a	0.119a	1.537	0.218
Number of seeds per flower (unit)	9a	6b	8ab	8ab	7ab	6.257	0.001
Seed weight (g)	0.011a	0.012a	0.012a	0.013a	0.014a	0.446	0.775

According to the results of ANOVA and Tukey's HSD test, mean values for each floral phenotypic characteristic of cosmos flowers followed by the same letter in the column do not differ significantly at the 5% significance level.

type (15-16 ray florets), resulting in the later showing a more compact floral appearance. Previous research results indicated that the phenotypic characteristics of T1 transgenic yellow cosmos (*Cosmos sulphureus*) carrying neomycin phosphotransferase II gene led to changes in the type of ray floret to a mixed ligulate and tubular form. This suggests that the *npt*II gene affected the phenotype of yellow cosmos. The result revealed that the *npt*II gene was randomly inserted into the plant genome (Irsyadi et al. 2022). These results are consistent with our study, but the proportion of plants that developed the mixed-type ray florets trait was relatively low (6%). Significant changes in quantitative flower traits among *SoSPS*1 and *npt*II-positive plants included the number of flowers per plant, number of ray florets, flower diameter, ray floret width, pedicle length, and fresh weight per flower.

Biochemical observation

This publication investigated the biochemical content of dried yellow cosmos petals. The result shows that the flowers of transgenic and non-transgenic plants harboring *npt*II and *SoSPS*1 accumulated more total sugar than wild-type plants (13.2%), whereas non-transgenic plants (15.9%) have more total sugar than transgenic plants (15.2%). The reduced sugar content in flowers of wild-type, transgenic, and non-transgenic plants harboring *npt*II and *SoSPS*1 was identical (12.7–13%). The accumulation of sucrose in transgenic plant flowers was the highest, as well as the total phenol content (Figure 3). Transgenic yellow cosmos plants showed higher sucrose accumulation than wild-type and non-transgenic plants. The analysis results align with the expectation that overexpression of the *SoSPS*1 gene enhances *SPS* enzyme activity, resulting in increased sucrose

Figure 1. Morphological characters of the transgenic and wild-type yellow cosmos flower. A. Growth trait of upward-facing flowers on a transgenic plant. B. Growth trait of upward-facing flowers on wild-type plants. C. Transgenic flower with ligulate ray florets. D. Wild-type flower with ligulate ray floret. E. Medium incision of the apex on a transgenic plant. F. Medium incision of apex on wild-type plant. G. Plant habitus of transgenic plant. H. Plant habitus of wild-type plant.

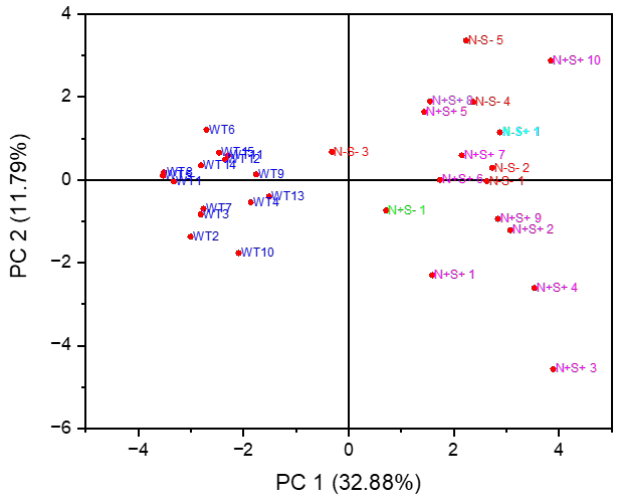

Figure 2. Distribution of transgenic and wild-type plants based on phenotype characteristics under principal component analysis. WT: wild-type plant; N+S+ (positive *npt*II and *SoSPS*1); N+S- (positive *npt*II, negative *SoSPS*1); N-S+ (negative *npt*II, positive *SoSPS*1); N-S-(negative *npt*II, negative *SoSPS*1).

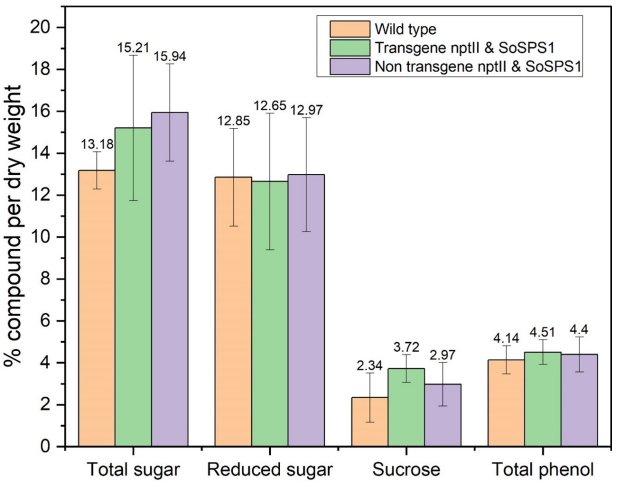

Figure 3. Biochemical character (total sugar, reduced sugar, sucrose, and total phenol) of yellow cosmos flower in wild-type, transgenic *npt*II and *SoSPS*1, also non-transgene plants.

accumulation. Transformation of the *SoSPS*1 gene in sugarcane plants (*Saccharum officinarum* L.) with *A tumefaciens* increased sucrose production in leaf organs. A large accumulation of sucrose and starch content caused overexpression of the gene that enhanced *SPS* activity. Increasing *SPS* activity in transgenic plants was coupled with invertase activity. Indica rice plants transformed with the *SoSPS*1 gene showed that overexpression of the *SoSPS*1 gene significantly increases transcript and protein levels, followed by an increase in *SPS* activity and sucrose content in the leaves of transgenic rice strains (Mulyatama et al. 2022, Shidiqi et al. 2024). Similar results are shown in transgenic sugarcane plants: double overexpression of *SoSPS*1 and *SoSUT*1 first-generation plants showed higher expression at the translational level (SUT and *SPS* enzymes) and higher average sucrose content in stem and leaf compared to non-transformed sugarcane plants (Anur et al. 2020). *SoSPS*1 transgenic tomato plants showed a twofold increase in *SPS* activity compared to non-transgenic plants (Afidah et al. 2022). No significant difference in glucose levels (a reducing sugar) was observed in this study. This is likely due to glucose serving as the predominant soluble sugar during the early stages of flower development. In contrast, sucrose is the primary sugar for long-distance transport and accumulates during flower maturation (Iftikhar et al. 2020). The optimization of sucrose production via *SoSPS*1 gene expression increases sucrose accumulation, which is then used as a substrate for synthesizing phenols-secondary metabolites that enhance flower quality, visual appeal, and potential bioactivity. Without this modification, glucose is more frequently utilized as an energy source, but it is less effective for improving carbohydrate storage and secondary metabolite production in the flower. According to these results, the *SoSPS*1 gene enhances sucrose accumulation. It indirectly affects the synthesis of phenolic compounds, which causes transgenic plants to have higher total phenols levels compared to wild-type plants. In conclusion, the overexpression of the *SoSPS*1 gene significantly affects the phenotypic traits and biochemical composition of yellow cosmos flowers, particularly in flower morphology and sucrose accumulation. These findings highlight the potential of yellow cosmos as a source of bioherbicide material. Further research is needed to explore the long-term stability of the transgene, its effects on subsequent generations, and its practical application in bioherbicidal development.

ACKNOWLEDGEMENTS

This study was supported by Indonesian Education Scholarship, Center for Higher Education Funding and Assessment, and Indonesian Endowment Fund for Education. The binary vector, primer set, and *A. tumefaciens* strains were provided by Prof. Bambang Sugiharto at the Center of Development of Advanced Science and Technology, University of Jember. We also express our gratitude to the Laboratory of Plant Breeding, Department of Agronomy, Faculty of Agriculture, UGM, for providing research facilities. DATA AVAILABILITY

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

REFERENCES

Afidah SN, Agustien ID, Dewanti P and Sugiharto B (2022) Increased activity of sugarcane sucrose-phosphate synthase in transgenic tomato in response to N-terminal truncation. **Indonesian Journal of Biotechnology 27**: 43-50.

Anur RM, Mufithah N, Sawitri WD, Sakakibara H and Sugiharto B (2020) Overexpression of sucrose phosphate synthase enhanced sucrose content and biomass production in transgenic sugarcane. **Plants 9**: 1-11.

Iftikhar J, Lyu M, Liu Z, Mehmood N, Munir N, Ahmed MAA, Batool W, Aslam MM, Yuan Y and Wu B (2020) Sugar and hormone dynamics and the expression profiles of sut/suc and sweet sugar transporters during flower development in petunia axillaris. **Plants 9**: 1-19.

Irsyadi MB, Sawitri WD and Purwantoro A (2022) Molecular and phenotypic characteristics of T1 transgenic yellow cosmos (*Cosmos sulphureus*) carrying neomycin phosphotransferase II gene. **Biodiversitas 23**: 6097-6105.

Muchyiddin MGAA, Sawitri WD, Irsyadi MB, Swandari T and Purwantoro A (2024) Segregation analysis and floral phenotype of T2 transgenic yellow cosmos (*Cosmos sulphureus* Cav.) carrying neomycin phosphotransferase II gene in second generation. **Biodiversitas 25**: 1711-1717.

Mulyatama RA, Neliana IR, Sawitri WD, Sakakibara H, Kim KM and Sugiharto B (2022) Increasing the activity of sugarcane sucrose phosphate synthase enhanced growth and grain yields in transgenic indica rice. **Agronomy 12**: 1-12.

Ortega-Medrano RJ, Ceja-Torres LF, Vázquez-Sánchez M, Martínez-Ávila GCG and Medina-Medrano JR (2023) Characterization of *Cosmos sulphureus* Cav. (Asteraceae): Phytochemical screening, antioxidant activity and chromatography analysis. **Plants 12**: 896.

Purwantoro A, Irsyadi MB, Sawitri WD, Fatumi NC and Fajrina SN (2023) Efficient floral dip transformation method using *Agrobacterium tumefaciens* on *Cosmos sulphureus* Cav. **Saudi Journal of Biological Sciences 30**: 103702.

Purwantoro A, Swandari T, Respatie DW, Sawitri WD and Murti RH (2024) Evaluation of phenotypic characters and total phenol content in T1 putative transgenic yellow cosmos (*Cosmos sulphureus* Cav.) with *SoSPS*1 transgene. **Indian Journal of Biochemistry & Biophysics 61**: 704-713.

Respatie DW, Yudono P, Purwantoro A and Trisyono YA (2019) The potential of *Cosmos sulphureus* flower extract as a bioherbicide for *Cyperus rotundus*. **Biodiversitas 20**: 3568-3574.

Roberts J, Florentine S, Fernando WGD and Tennakoon KU (2022) Achievements, developments and future challenges in the field of bioherbicides for weed control: A global review. **Plants 11**: 1-18.

Shidiqi MHA, Hariyanto F, Sugiharto B, Ermawati N and Handoyo T (2024) Phenotypic characterization and evaluation of transgenic indica rice overexpressing *SoSPS*1 gene in greenhouse trials. **Journal of Crop Science and Biotechnology**: 1-8.

Singh M, Kaur A, Taggar GK, Sandhu JS, Malik P, Rani M, Singh I, Singh R and Singh S (2023) Inheritance and expression analyses of cry1Ab gene in transgenic pigeon pea tolerant to Maruca pod borer. **Crop Breeding and Applied Biotechnology 23**: 1-8.

Suputri NPAEO, Dwiyani R, Darmawanti IAP and Sugiharto B (2020) *Agrobacterium tumefaciens*-mediated in planta transformation method for the *SoSPS*1 gene in citrus plants (*Citrus nobilis* L.). **International Journal of Biosciences and Biotechnology 7**: 31.