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STABILITY OF MORPHOPHYSIOLOGICAL CHARACTERS, TUBER YIELD, AND FBPase GENE EXPRESSION IN TRANSGENIC POTATO CULTIVAR IPB-CP3

STABILITAS KARAKTER MORFOFISIOLOGI, HASIL UMBI, DAN EKSPRESI GEN FBPase TANAMAN KENTANG KULTIVAR IPB-CP3 TRANSGENIK

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Abstract

Potatoes are an essential agricultural commodity that needs to be genetically improved to increase productivity and meet industrial needs. The research aimed to evaluate the stability of the morpho-physiological characters, tuber yield, and transgene expression of transgenic potato cultivar IPB-CP3 harboring the FBPase gene in the G_0 and G_1 generations. The study was conducted by growing transgenic plants in a greenhouse and analyzing plant morpho-physiological characters, tuber yield, and gene expression. The results showed that transgenic plants had higher values in stem diameter (32.7–50.8%), number of leaves (33.9–41.2%), biomass dry weight (14.4–36.2%), photosynthetic rate (25.2–28.25%), and tuber weight (42.6–196.0%) than non-transgenic plants. However, there was no significant difference in plant height characters between the transgenic and non-transgenic plants. Transgenic plants consistently expressed the FBPase gene at higher level than the non-transgenic plants in the G_0 and G_1 generations. The research results suggest that overexpression of the FBPase gene increases plant growth and tuber yield. This finding implies agricultural practices, particularly in the context of crop improvement through genetic engineering.

Keywords: FBPase gene; Gene expression; IPB-CP3; Photosynthetic

Abstrak

Kentang merupakan komoditas pertanian penting yang harus terus diperbaiki sifat genetiknya untuk meningkatkan produktivitas dan memenuhi kebutuhan industri. Penelitian ini bertujuan untuk mengevaluasi stabilitas karakter morfo-fisiologi, hasil umbi, dan ekspresi transgen pada kentang transgenik kultivar IPB-CP3 yang mengandung gen FBPase pada generasi G_0 *dan* G_1*. Penelitian dilakukan dengan menanam tanaman transgenik di rumah kaca dan menganalisis karakter morfo-fisiologi dan hasil umbi, serta menganalisis ekspresi transgen di laboratorium. Hasil penelitian menunjukkan bahwa tanaman transgenik memiliki nilai lebih tinggi pada karakter diameter batang (32.7–50.8%), jumlah daun (33.9–41.2%), bobot kering biomassa (14.4–36.2%), laju fotosintesis (25.2–28.25%), serta bobot umbi (42.6–196.0%) dibandingkan tanaman non-transgenik. Namun tidak terdapat perbedaan nyata pada karakter tinggi tanaman antara tanaman transgenik dan non transgenik. Tanaman transgenik secara konsisten mengekspresikan gen FBPase lebih tinggi dibandingkan tanaman non-transgenik pada generasi* G_0 *dan* G_1*. Hasil penelitian menunjukkan bahwa ekspresi berlebih dari gen FBPase meningkatkan pertumbuhan tanaman dan hasil umbi. Temuan ini berimplikasi pada praktik pertanian, khususnya dalam konteks perbaikan tanaman melalui rekayasa genetika.*

Kata Kunci*: Ekspresi gen; Fotosintesis; Gen FBPase; IPB-CP3*

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INTRODUCTION

Potatoes, the third most important crop after rice and wheat in the world, were increasingly recognized as an important commodity in the food and horticulture industry in Indonesia since 2010s (Kiloes et al., 2015). The demand for potatoes is rising, driven by the increasing population, changing lifestyles, and the need for an alternative carbohydrate source, especially for people who want a healthy diet or who have special needs, such as diabetics. However, the area of potato cultivation has decreased by 17.10% since 2022, and 2023's BPS data shows a decrease in potato production from 1.3 tons in 2021 to 1.2 tons in 2023 (The Central Bureau of Statistics of Indonesia (BPS), 2024). The production of potatoes is still facing challenges due to factors like groundwater availability, fertilization systems, irrigation, and planting season constraints (Minda et al., 2019; Larkin et al., 2021). Enhancing potato productivity through genetic engineering for high-yielding cultivars is one of the approaches to increase production and fulfill consumer and industrial demand. The approach could target several plant characteristics, such as improving photosynthesis efficiency, agronomic traits, and tuber yields (Foyer et al., 2017; Guo et al., 2018; Hameed et al., 2018).

The improvement of yield-related traits, such as morpho-physiological and agronomic characteristics of the plants, could be achieved through the increase of photosynthetic activity. One of the enzymes that plays a crucial role in photosynthesis is fructose 1,6-bisphosphatase (FBPase), which involves the conversion of fructose 1,6-bisphosphate to fructose 6-phosphate. The conversion could be part of the carbon reduction pathway of the Calvin cycle in the chloroplasts, leading to the starch biosynthesis and the sucrose biosynthesis pathway in the cytosol (Daie, 1993; Li et al., 2020). Previous research showed that overexpression of the FBPase gene originated from *Arabidopsis* increased sucrose synthesis in the cytosol and growth of transgenic *Arabidopsis* (Cho et al., 2012) and *Oryza coarctata* (Mukherjee et al., 2021). Additionally, the expression of the FBPase gene from cyanobacteria increased tuber yields in potatoes (Fatahillah et al., 2016; Olivah, 2019).

The plant growth and yield will be in maximum condition when supported by good root characteristics. Roots are vital for water and nutrient absorption and plant growth. Most vegetable plants, such as potatoes, are sensitive to drought when soil moisture drops below 20%, leading to disrupted photosynthesis, smaller leaves, stunted growth, reduced tuber yield, and quality (Gervais et al., 2021). The root characteristics supporting water absorption under a limited water supply are essential for better plant growth and production. The *Citrullus lanatus*-Root Activating Number 1 (ClRan1) gene from xerophyte wildtype watermelon encodes the Ran1-GTPase enzyme related to root growth. Overexpression of the gene in *Arabidopsis* increased root meristem activity (Wang et al., 2006; Akashi et al., 2011). In addition, according to Xu and Cai (2014), Ran-GTPase protein plays a role in plant development and mediates plant responses to the environment. Research conducted by Akashi et al. (2016) found that the expression of the ClRan1 gene at the root tip increased primary root growth in *Arabidopsis* plants at the seedling phase.

Previous research on introducing FBPase and ClRan1 genes into potato cv IPB-CP3 mediated by *Agrobacterium tumefaciens* strain EHA105 has been conducted by Wijayanti (2022). The expression of the gene under the control of a strong promoter affects the growth and development of the plants in the G_0 clonal generation. However, the stability analysis of the morpho-physiological characters, tuber yields, and gene expression of FBPase in the G_1 generation has yet to be carried out. Therefore, this study aims to study the stability of morphophysiological characters, tuber yields, and gene expression of FBPase in the G_0 and G_1 generations of transgenic potato cv IPB CP3.

MATERIALS AND METHODS

Young plants of a non-transgenic IPB-CP3 potato clone (C3NT) and four transgenic IPB-CP3 clones (C3FB1, C3FB2, C3FB3, C3FB4) containing the FBPase/ClRan1 gene were used in this research (Wijayanti, 2022). The primers used for transgene integration and expression analyses are listed in Table 1.

Table 1. List of primers used in transgene integration and expression analysis

Purpose	Primer names	Primer sequences	Annealing temperature (°C)	Expected size of PCR bands (bp)_
Transgene integration analysis	Act-F	ATGGCAGATGCCGAGGATAT	55	550
	Act-R	CAGTTGTGCGACCACTTGCA		
	rbcS-F	5'-TGAGATAAGGACGAGTGAGG-3'		581
	FBPase_65R	5'-TGCCCACTTCTTCACCGATA-3'		
Transgene expression analysis	Tact-qF	ACATCGTCCT TAGTGGTGGA	58	226
	Tact-qR	GTGGACAATGGAAGGACCAG		
	FBPase-SKqF	5’-GTCCGTCTGATCAGCGATG-3’		161
	FBPase-SKqR	5’-TAGATCAGCTGGCCTTGGAA G-3’		

Propagation and Planting of IPB-CP3 Lines

Potato IPB-CP3 lines from G_0 generation were propagated in MS media according to Murashige and Skoog (1962) at 24 to 25 °C for 4 weeks. Four-week-old G_0 plantlets (WAP) were moved and placed in a greenhouse for one week for acclimatization. Plantlets were then planted in cocopeat media to produce mother stock plants. After 4 WAP, the top branches of the mother plants were cut and planted on cocopeat media for rooting. After two weeks, the rooted seedlings of one non-transgenic and four transgenic potato cv. IPB-CP3 were moved to polybags containing cocopeat media for the experiment that was carried out in the greenhouse, which was located in CV Bumi Agro Technology Field Station, Cisarua, Bandung, at 1,350 masl with an average daily temperature of 14–21 °C. The single-factor experiment was arranged in a completely random design with 15 replications. Each replication consisted of 5 plants. The tubers produced from G_0 plants, called G_1 seeds, were planted in the greenhouse, the same as the G_0 plants. The grown plants from G_1 seeds were observed using the same procedure as the G_0 plants.

Morpho-physiological Characters and Tuber Yield Observation

Morphological characters of non-transgenic and transgenic plants, including the height and diameter of the main stem, the number of nodes, and the number and width of leaves, were observed every 2 weeks from the age of 5–9 WAP. Physiological characteristics, namely photosynthetic rate, stomatal conductance, and the CO_2 intercellular were measured 40 days after planting (DAP) using the Portable Photosynthesis System LICOR type LI-6400XT (LI-COR, USA). Root length and dry weight were observed at 47 DAP. The weight and number of tubers were observed on the day of harvesting.

FBPase Transgene Integration Analysis

Total DNA was isolated from plantlets (3 DAP) using the modified Suharsono (2002) method, which included an additional heat block step and 2% CTAB in the extraction buffer. DNA quantity was measured using a Jenway 7315 UV-Vis Spectrophotometer (Fisher Scientific, UK). The DNA was then used asa PCR template to analyze DNA integrity using primer pairs Act-F and Act-R from the Actin gene (Table 1). Analysis of transgene integration stability in the potato plant genome using specific primers rbcS-F and FBPase_65R for FBPase. PCR was carried out under the following condition: genomic DNA was denatured at 94 °C for 5 min, followed by 35 cycles of amplification (95 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min), and finally 5 min at 72 °C.

FBPase Gene Expression Analysis

Gene expression analysis was performed by isolating total RNA using Trizol (Invitrogen, USA). cDNA was amplified by PCR using SensiFAST™ cDNA synthesis kit (Bioline, UK), procedure with a two-step method. Real-time PCR was performed using the Quant Studio 5 (Thermo-Fisher Sci, USA) RT-PCR machine with a modified SensiFAST™ SYBR® Lo-ROX Kit (Bioline CAT: BIO-94020, UK), where the primer volume was adjusted to 0.3 µL, along with pairs of primers as listed in Table 1. The thermal program was set as follows: 55 cycles of amplification (95 °C for 10 s, 58 °C for 30 s, and 72 °C for 40 s) and finally 20 s at 60 °C. Actin Ct value was used as the internal standard and reference gene for expression analysis. The relative expression of FBPase genes across genotypes was calculated using the Livak $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Data Analysis

Morpho-physiological data, tuber yields, and gene expression were tabulated and graphed using Microsoft Office Excel 2013 and then analyzed with One-way ANOVA using the IBM SPSS Statistics version 25. If the genotypes significantly affected the observed variables, it was then post-hoc tested with the Duncan Multiple Range Test (DMRT) at a= 0.05.

RESULTS

Integration of the FBPase Genes on Transgenic Plants

The total DNA of plants was successfully isolated with exemplary integrity, characterized by the successful Actin gene PCR amplification using the total DNA as a template (Figure 1a). The amplicon formation from the amplified Actin genes indicates that the total DNA of plants was not degraded. Integration of the FPBase gene was verified with the PCR amplification of the transgene in the plant DNA using a pair of primers rbcS-F and FBPase_65R. The rbcS promoter was used in the T-DNA recombinant construct introduced to the potato genome. The rbcS-F primer was designed from the rbcS promoter sequence, and the FBPase_65R primer was designed from the FBpase gene sequence. Amplification using both primers will amplify the FBPase gene inserted in the plant genome. The results showed that the transgene could be amplified in all transgenic potatoes. Still, it was not amplified in non-transgenic potatoes (Figure 1b), indicating the successful integration of the FBPase gene into the plant genome. This integration confirms the stability of the transgene in the G_1 generation of transgenic potato plants.

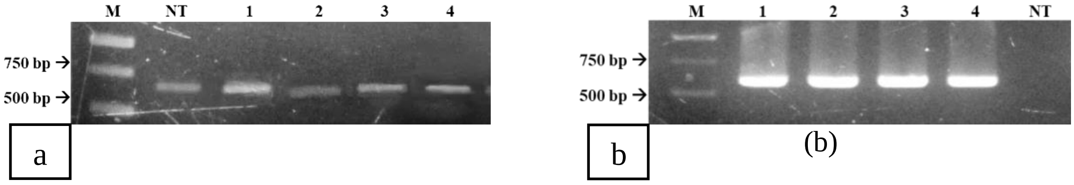

Figure 1. Amplification of Actin (a) and FBPase genes (b) in transgenic potato IPB-CP3 G_1 generation. M= 1kb DNA ladder, NT= non-transgenic potato cultivar IPB-CP3 and 1–4= transgenic potato cultivar IPB-CP3, namely C3FB1, C3FB2, C3FB3, C3FB4, respectively

Morphological Characters

Transgenic and non-transgenic plants were grown in polybags containing cocopeat media. Morphological characteristics were then observed as described in the methods. The results showed that the transgenic plants had significantly higher stem diameter, leaf number, and main stem node number than those of the non-transgenic plants. However, the plant height of the transgenics was not significantly different from the non-transgenic plants in both G_0 and G_1 generations (Table 2). Interestingly, plant height and other morphological characters, such as stem diameter, leaf number, and main stem node number, were higher in G_1 compared to the G_0 generation.

Table 2. Morphological characteristics of transgenic potato cv. IPB-CP3 overexpressing the FBPase/ClRan1 gene at 9 weeks after planting

Lines	Plant height (cm)		Stem diameter (cm)		Number of nodes		Number of leaves	
	G_0	G_1	G_0	G_1	G_0	G_1	G_0	G_1
C3NT	34.56	76.76	0.33^a	0.62^a	10.00^a	10.40^a	9.60^a	10.20^a
C3FB1	39.89	80.44	0.47^b	0.60^a	12.00^{ab}	13.60^b	12.00^{ab}	13.60^b
C3FB2	43.03	80.37	0.55^b	0.83^b	14.20^b	15.40^c	14.20^b	15.40^c
C3FB3	40.53	78.40	0.47^b	0.91^b	11.60^{ab}	13.00^b	11.60^{ab}	13.00^b
C3FB4	39.58	76.14	0.50^b	0.95^b	13.60^b	15.60^c	13.60^b	15.60^c

Note: Data labelled with the same letter in the same column are not significantly different in Duncan's test with α= 0.05; C3NT= non-transgenic plants; C3FB1–4= transgenic plants

Root length and biomass weight were observed at harvest time. Transgenic plants had longer root lengths and higher biomass weights than controls (Table 3). Transgenic G_0 generation plants had a higher fresh weight compared to non-transgenic with an average of 114.65 g, while the fresh weight of the G_1 generation was 197.74 g.

Table 3. Fresh and dry weight biomass and root length of transgenic potato cv. IPB-CP3 overexpressing the FBPase/ClRan1 gene at 11 weeks after planting

Lines	Biomass fresh weight (g)		Biomass dry weight (g)		Root length (cm)	
	G_0	G_1	G_0	G_1	G_0	G_1
C3NT	97.64^a	135.84^a	8.69^a	10.32^a	10.47^a	21.08^a
C3FB1	110.74^{ab}	183.23^b	9.70^a	14.69^{ab}	14.61^a	28.08^b
C3FB2	111.59^{ab}	186.49^b	10.77^a	13.01^{ab}	19.57^b	29.68^b
C3FB3	116.99^b	215.68^b	9.43^a	15.24^b	20.40^b	28.91^b
C3FB4	119.26^b	205.55^b	9.87^a	13.30^b	19.29^b	24.28^{ab}

Note: Data labelled with the same letter in the same column are not significantly different in Duncan's test with $\alpha = 0.05$; C3NT= non-transgenic plants; C3FB1–4= transgenic plants

Physiological Characters

The photosynthesis rate in all transgenic plants was significantly higher than that of non-transgenic plants in both generations (Table 4). However, the higher photosynthesis rate in transgenic plants compared to the control plants was not in line with the response pattern of intercellular CO_2, which was not significantly different among genotypes in both generations. Stomatal conductance of the transgenic plants showed a higher value than that of non-transgenic plants, but only a significant difference in the G_1 generation.

Table 4. Physiological characteristics of transgenic IPB CP3 potato plants overexpressing the FBPase/ClRan1 gene 40 days after planting

Lines	Photosynthesis rates (μmol $H_2Om^{-2}s^{-1}$)		Stomatal conductance (mol $H_2Om^{-2}s^{-1}$)		CO_2 intercellular (μmol CO_2mol^{-1})	
	G_0	G_1	G_0	G_1	G_0	G_1
C3NT	22.86^a	19.31^a	1.38	1.30^a	340.27	347.69
C3FB1	28.57^b	24.66^{bc}	1.82	2.01^b	334.02	346.25
C3FB2	28.90^b	25.42^c	2.02	2.20^b	337.31	347.28
C3FB3	28.23^b	24.47^b	1.90	2.31^b	337.26	349.86
C3FB4	28.78^b	24.44^b	1.81	2.44^b	334.49	348.13

Note: Data labelled with the same letter in the same column are not significantly different in Duncan's test, $\alpha = 0.05$; NT= non-transgenic plants; FB= transgenic plants

Tuber Yield

The number of tubers and tuber weights of transgenic plants were significantly higher than those of non-transgenic plants, showing the impressive potential of transgenic plants. Transgenic plants produced a two to threefold increase in tuber weight compared to non-transgenic plants (Table 5). The tubers of transgenic and non-transgenic lines had similar oval shapes (Figure 2). However, transgenic potatoes were larger than non-transgenic potatoes, further highlighting transgenic plants' potential.

Table 5. Tuber yield of non-transgenic and transgenic potato IPB-CP3 potato overexpressing the FBPase gene

Lines	Number of tubers per plant		Tubers weight per plant (g)	
	G_0	G_1	G_0	G_1
C3NT	3.0^a	5.0^a	13.70^a	59.12^a
C3FB1	6.7^b	6.9^b	39.72^{bc}	70.02^{ab}
C3FB2	7.9^b	8.1^b	29.60^b	101.98^b
C3FB3	6.4^b	8.6^b	39.70^{bc}	94.42^{ab}
C3FB4	9.0^b	7.5^b	53.20^c	70.76^{ab}

Note: Data labelled with the same letter in the same column are not significantly different in Duncan's test, $\alpha = 0.05$; C3NT= non-transgenic plants; C3FB1–4= transgenic plants

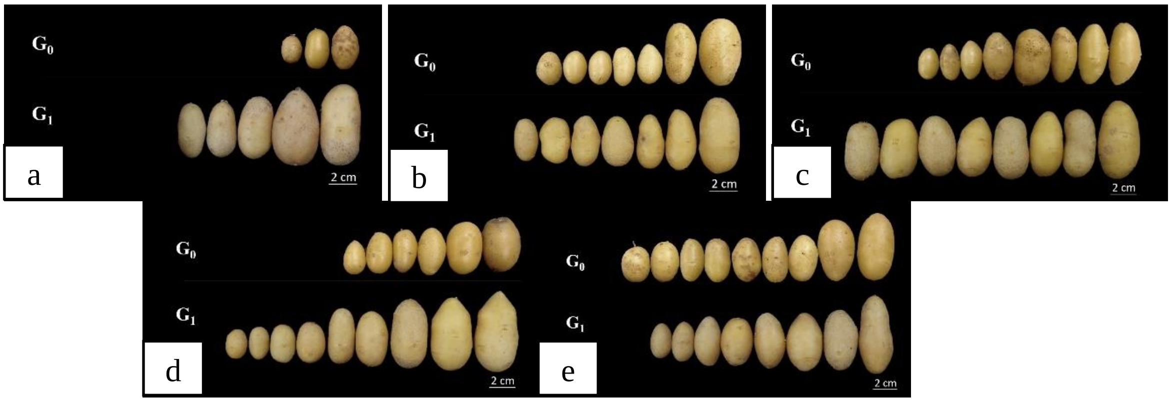

Figure 2. Tuber shape and size of non-transgenic and transgenic potato IPB-CP3, non-transgenic (C3NT) (a), C3FB1 (b), C3FB2 (c), C3FB3 (d), and C3FB4 (e)

Gene Expression Analysis

The expression of the FBPase gene in all G_0 and G_1 plants of the transgenic plants was higher than in non-transgenic plants (Figure 3). The gene expression in G_0 and G_1 of the C3FB4 transgenic line showed a significant 23- and 3.04-fold higher over the non-transgenic plant. The gene expression level varied among transgenic lines, but it had a similar expression pattern between G_0 and G_1 generations.

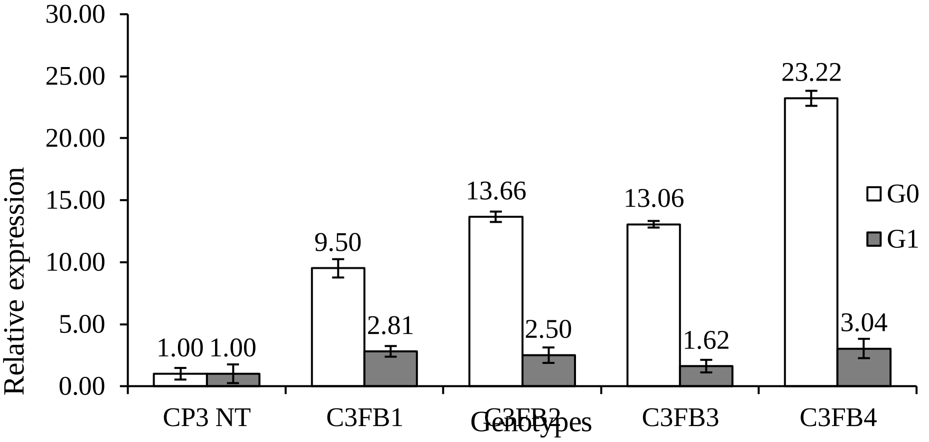

Figure 3. The relative expression of the FBPase gene in the transgenic potato cultivar IPB-CP3. C3NT= non-transgenic plant; C3B1–4= transgenic plants. The error bar indicates standard error

DISCUSSION

The molecular confirmation of putative transgenic plants was achieved using the PCR technique. Actin gene, as a housekeeping gene, is present in every developmental stage of plants and almost all plant tissues. This ubiquity of the Actin gene makes it an invaluable marker for the presence and integrity of genomic DNA. The primers used to amplify the Actin gene of potato plants were explicitly designed to amplify the potato DNA genome and to be absent in the DNA plasmids when used as the DNA template, as the plasmid did not contain the Actin gene. The result showed that the Actin primer pair successfully amplified the potato DNA genome both in non-transgenic and transgenic plants (Figure 1a), indicating that the isolated DNA that will be used to verify the integration of the FBPase gene in the potato DNA genome is in good integrity. The DNA was then used as a template for PCR amplification using the specific rbcS-F and FBPase_65R primer pairs. The rbcS-F primer was designed from the rbcS promoter sequence upstream of the FBPase gene, while the FBPase_65R primer was designed from the FBPase gene sequence. Therefore, successfully amplifying the DNA segment between the two primers will indicate the successful integration of the FBPase gene in the potato genome. The PCR results showed that all plant DNA samples were amplified at the transgene target, except for the non-transgenic DNA (Figure 2b), confirming that the FBPase gene was stably inserted in the potato DNA genome.

Introducing the FBPase gene in the potato cultivar IPB-CP3 affects several morpho-physiological characters observed in this study. The measurement of photosynthesis parameters of non-transgenic and transgenic plants using Licor LI-6400XT (LI-COR, USA) in a greenhouse showed that the photosynthesis rates of transgenic plants were 23.5–26.4% higher than those of non-transgenic plants (Table 4). The research by Simkin et al. (2017) demonstrated that increased fructose 1,6-bisphosphate aldolase (FBPA) expression in *Arabidopsis* led to a higher plant photosynthesis rate, stimulating higher biomass production. As photosynthesis increases, the CO_2 demand of plants also increases, which is facilitated by the wide stomatal openings for gas diffusion. In this research, the average stomatal conductance of transgenic plants was 1.37 and 1.72 times higher than that of non-transgenic plants in the G_0 and G_1 generations, respectively; this indicates that the high stomatal conductance in transgenic plants facilitates the diffusion of CO_2 into the chloroplasts. The effect of FBPase overexpression on the photosynthesis rate in potatoes has also been reported by Fatahillah et al. (2016), Susilawati (2020), and Wijayanti (2022), showing a positive correlation between FBPase activity and enhanced photosynthetic efficiency.

Intercellular CO_2 increases with increasing photosynthesis rate and stomatal conductance (Taylor & Long, 2017). However, this study obtained higher intercellular CO_2 in plants with relatively low photosynthesis rates. According to Long and Bernacchi (2003), some factors cause the photosynthesis rate to have a lower value even with high intercellular CO_2 values, including Rubisco activity, RuBP regeneration, and triose-phosphate (TP) utilization. Khamis et al. (2020) also reported that a higher photosynthesis rate occurs in corn plants despite low intercellular CO_2 values.

Carbohydrates produced during photosynthesis (mainly glucose) are used in various metabolic processes to produce energy, form sucrose, and build carbohydrate-based plant structures. With increased FBPase activity, more carbohydrates can be synthesized and used for plant growth, increasing tissue mass (e.g., fresh weight) and forming physical plant structures (e.g., stem diameter). Photosynthesis rate is one of the factors that significantly influences plant biomass (Conner, 2007) and plant growth. In this study, we reported that transgenic plants overexpressing FBPase have higher stem diameter, number of leaves, number of nodes, fresh and dry biomass, and tuber yields. Previous studies on potato cultivar Nooksack and IPB-CP3 also reported that potatoes with FBPase gene overexpression showed higher plant biomass, two times heavier than control plants (Fatahillah et al., 2016; Wijayanti, 2022). Research by Yan-yan et al. (2022) on canola and tobacco obtained an increase in photosynthesis rate, stem diameter, and root length in transgenic plants expressing cyFBPase. From morpho-physiological data collected from the G_0 and G_1 plants in this research, we conclude that the morpho-physiological characters are stable between the two generations.

The research analyzed the stability of the FBPase gene expression in both G_0 and G_1 plants. All transgenic plants expressed the gene higher level than that in transgenic plants. The relative expression of the gene in G_0 transgenic plants varied from 9.50 to 23.22 fold in the non-transgenic plant, whereas the relative expression in the G_1 generation varied from 1.62 to 3.04 fold. The variation of the transgene expression among the transgenic lines is influenced by promoter selection, insertion position, and environmental factors. Iglesias et al. (1997) reported that gene stability can be influenced by the gene's position relative to the telomere. Insertions near the telomere tend to be more stable in their expression, while insertions in the intercalary or paracentromeric regions show more unstable expression. In this research, the expression of the FBPase gene is controlled by the rbcS promoter, a promoter from a small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RbcS). The mode of action of the rbcS promoter is light-dependent and tissue-specific, primarily regulating gene expression in green tissues such as leaves and stems. It achieves this through light-responsive cis-acting elements, including the I-box and G-box, which interact with specific transcription factors to activate transcription in response to light (Tanabe et al., 2015).

The variation in FBPase gene relative expression between G_0 and G_1 generations of the transgenic lines may also be related to differences in light intensity received by plants during the experiment in the greenhouse. The RbcS gene exhibits a light-dependent expression mechanism that is vital in photosynthetic tissues. According to Tanabe et al. (2015), the expression of IbRbcS1 is induced within three hours of light exposure. It continues to increase throughout the photoperiod, indicating that light is a key regulatory signal for activating the RbcS gene. Similarly, Cui et al. (2015) observed that the soybean rbcS promoter regulates expression levels light-dependently, with increased activity in younger leaves under light exposure and a significant reduction in the absence of light. Kudo et al. (2020) also provided similar findings. Collectively, these studies highlight the importance of light as a critical factor in modulating the expression of RbcS genes across different plant systems.

CONCLUSION

The integration of the FBPase gene in transgenic IPB-CP3 potato plants is stable but shows a variation in the relative expression among transgenic lines and between two generations. However, the expression of the FBPase gene remains higher in transgenic IPB-CP3 potato plants across both generations, supporting their enhanced performance. Transgenic plants exhibit improved growth and physiological characteristics and higher tuber yields in the G_0 and G_1 generations than non-transgenic plants. It is recommended that the number of roots and stolons during the vegetative and generative phases be analyzed, and total root RNA should be isolated to better understand the effects of ClRan1 gene expression on the growth characteristics of transgenic IPB-CP3 potato plants. Further studies on the integration and expression of the FBPase and ClRan1 genes should also be conducted in the G_2 and G_3 generations to assess their stability and impact across subsequent generations.

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