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RESEARCH ARTICLE

Field Evaluation on Growth and Productivity of the Transgenic Sugarcane Lines Overexpressing Sucrose-Phosphate Synthase

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Abstract The development of new sugarcane cultivars using modern agricultural biotechnology is an important strategy in increasing sugar yield, which is directed toward sustainable agriculture. Genetic transformation has been demonstrated to be an effective engineering tool to introduce superior traits into plants. Overexpression of the *SoSPS1* gene in sugarcane resulted in increased sucrose phosphate synthase activity, sucrose content, and biomass in the greenhouse experiment. However, performance evaluation in confined field trials in natural environments is an important step in selecting transgenic sugarcane with novel properties. In this study, transgenic sugarcane lines were cultivated in four experimental locations with different climates and soil types using a randomized block design with five replicates. We observed that transgenic sugarcane lines displayed higher tiller number and plant height than that of non-transgenic sugarcane. The cane yield, and percentage of Brix and Pol in the transgenic lines were also higher than those in non-transgenic sugarcane. Among the transgenic lines, SPS3 exhibited considerable growth and productivity in all field locations. Interestingly, the cane yield was higher in locations supplied with normal irrigation. In contrast, Brix and Pol % were higher in less water-supplied or dry land cultivated canes. In addition, the transgenic sugarcane lines neither affected the bacterial diversity in the soil rhizosphere nor assisted in horizontal gene flow in the soil environment. Therefore, evaluating the growth and productivity of transgenic sugarcane during field trials aids in selecting the best sugarcane line for an appropriate agro-climate.

Keywords Transgenic sugarcane · *SoSPS1* gene · Overexpression · Field performance · Growth and productivity · Bacterial diversity

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Introduction

Sugarcane is the major source material for sugar, accounting for approximately 80% of the global sugar production. Although Indonesia is one among the cane sugar producers, the produced sugar has been unable to meet the national sugar demand. Sugar production can be increased by increasing the sugarcane harvest area, and although this increase enhanced the sugar production by 2.2 million tons, the high consumption rate has led to an annual sugar import of approximately 5.2 million tons (United States Department of Agriculture 2021). Therefore, developing new sugarcane cultivars with high sugar yields can be one of the strategies in fulfilling the sugar demand.

Conventional sugarcane breeding programs have been applied for the development of new sugarcane cultivars. The breeding program is laborious, and includes crosses to produce large progeny populations, phenotypic evaluation and selection based on superior traits, and recombination of selected clones (Yadav et al. 2020). The parent genotype,

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heritability, and the evaluation period affect the success of breeding (Gazaffi et al. 2014). Sugarcane breeding takes more than 13 years to release a new variety (Scortecci et al. 2012). In addition, modern commercial varieties have been developed via interspecific hybridization between *Saccharum* species, and allied genera of *Miscanthus* and *Erianthus* (Meena et al. 2020). However, the species generated from the breeding program differ in their genomic structures, causing meiotic instability, production of aneuploid gametes and sterile seeds in some of their crosses (Butterfield et al. 2001; Rutherford et al. 2014). Sugarcane has a complex genome and allelic variation, high ploidy levels ranging from 5× to 16×, and chromosome numbers ranging between $2n = 99$ and 130 (Scortecci et al. 2012; Thirugnanasambandam et al. 2018), and when crossed produces heterogeneous offspring. Furthermore, vegetative propagation, competition between adjacent plots, environmental interactions, low fertilization and tiny seeds, and poly-aneuploid genomes, makes the breeding progress relatively complex, and prolongs the selection procedure in sugarcane (Milligan et al. 2007; Khan et al. 2013; Yadav et al. 2020).

The development of new sugarcane cultivars using modern biotechnology is an important strategy that improves plant growth and productivity. The established genetic transformation methods are powerful tools that can be applied to introduce superior traits in plants. Among several transformation methods, the *Agrobacterium*-mediated transformation has been widely used in sugarcane (Sugiharto 2018). The advantages of *Agrobacterium*-mediated gene transfer include technical simplicity, low copy number, and minimal genome rearrangement. Therefore, *Agrobacterium*-mediated genetic transformation has been performed in sugarcane to induce viral resistance (Guo et al. 2015; Apriasti et al. 2018; Widyaningrum et al. 2021), insect resistance (Wang et al. 2017; Cristofoletti et al. 2018; Dessoky et al. 2021), fungal resistance (Nayyar et al. 2017), herbicide tolerance (Wang et al. 2017), drought stress tolerance (Sugiharto 2018; Zhu et al. 2020), and to enhance sucrose content and biomass production (Anur et al. 2020).

Sucrose metabolism in plants involves several enzymes, such as sucrose phosphate synthase (SPS; EC 2.4.2.14), sucrose synthase (SuSy; EC 2.4.1.13), and invertase (EC 3.2.1.26). SPS is considered a key enzyme in sucrose synthesis and controlling sucrose accumulation in plants (Huber and Huber 1996a, b). Sucrose accumulation in sugarcane stalks depend on the balance between sucrose synthesis by SPS and their breakdown by invertase (Zhu et al. 1997; Pan et al. 2009). The overexpression of SPS increases sucrose content, plant growth, fiber quality, biomass production, and yield components in several plants (Nguyen-Quoc et al. 1999; Haigler et al. 2007; Park et al. 2008). Recently, SPS overexpression has been reported to enhance sucrose content and biomass production in transgenic sugarcane compared to that of its non-transgenic counterpart (Anur et al. 2020).

Selecting the best transgenic sugarcane line with superior properties as a new variety has not been sufficiently conducted in a greenhouse trial. Large genetic variation often occurs because of the introduction of foreign genes and somaclonal variation formed during the selection stage in the culture medium (Vickers et al. 2005; Sood et al. 2006; Joyce et al. 2014). The establishment of transgenic plants depends on the uniform and stable expression of the desired trait after the gene transfer during field experiments (Joyce et al. 2014; Yao et al. 2017). In addition, sugarcane growth stages, including germination, tillering, stalk elongation, and maturation, are influenced by the environment. Therefore, field experiments should be conducted to evaluate the performance of transgenic sugarcane by considering its adaptability to specific or different environments.

Field experiments on transgenic crops are nonetheless highly controversial owing to concerns over their potential environmental effects. Modifying the sucrose metabolism in sugarcane may alter the bacterial community and mineral content in the soil. Sugar, a major plant root exudate, can serve as energy and carbon sources for microorganisms (Canarini et al. 2019). The alteration in metabolites associated with transgenic plants impacts the soil microbial diversity (Guan et al. 2016). Studies on transgenic sugarcane has revealed that it causes minor changes in the microbial community (Zhou et al. 2016; Wulandari et al. 2021). However, a protein toxin persists for a prolonged period, which affects the microbial community of soil cultivated with transgenic corn expressing the Cry1 gene (Vettori et al. 2003). Therefore, bacterial diversity must be observed to determine the possibility of producing undesirable environmental consequences during the field examination of transgenic plants.

This study aimed to evaluate the growth and productivity performance of transgenic sugarcane lines overexpressing the *SoSPS1* gene in the field, and its evaluation was performed according to the Indonesian Biosafety Commission. The field experiment was performed in East Java, Indonesia, at four different experimental locations with different climates and soil types. The effect on bacterial diversity and the presence of gene flow were also observed during cultivation to avoid unwanted environmental consequences. The results clearly showed that the transgenic cane yield is higher in locations supplied with normal irrigation, but sugar content is higher in dry land. These results did not observe in the previous greenhouse experiment.

Materials and Methods

Plant Materials, Location, and Design of Experimental Trials

The field experiments were evaluated according to the authorization from and supervision of the Indonesian Biosafety Commission (Government Regulation No. B 45/KKH-PRG/05/2018). The experiment was conducted during one growing season (October 2019–September 2020), and included four confined field trials with different climate and soil types (Table 1). In this experiment, the selected transgenic lines (SPS1, SPS3, SPS9) and non-transgenic parent (NT) produced from vegetatively propagated–fourth generation of previous plants (Anur et al. 2020), were germinated and cultivated in the field in a randomized complete block design (RCBD), and occupied 0.5 ha in each experimental field site. The experiment was divided into five blocks (replications), and the transgenic and NT sugarcane were randomly distributed in four experimental plots in each block. Each plot had five rows, 8 m in length with 1.5 m row spacing. Dimension of plot and block were 72 and 360 m square, respectively. The water supply for Jubung Jember (JJ) and Genetri Lumajang (GL) trial locations were provided with a normal irrigation system, while Lombok Bondowoso (LB) and Wringin Situbondo (WS) were not irrigated and depended on rainfall. The GL and WS experimental locations were provided by PT. Perkebunan Nusantara XI, LB from PT. Dwi Cahaya Tembakau, and JJ from Jember University Research Station. The sugarcane was cultivated for 12 months and harvested during the dry season when the crop was entirely matured.

To confirm transgene stability, genomic DNA isolated from transgenic leaves that randomly taken from the trial locations and used for PCR amplification of the *npt*II gene, using the method described previously (Anur et al. 2020). Furthermore, the effect of transgenic sugarcane cultivation on bacterial diversity and gene flow was observed by collecting rhizosphere soil from the four sites at 3, 6, and 9 months after planting (MAP).

Growth Evaluation of Transgenic Sugarcane

The growth of sugarcane in the field was periodically evaluated by measuring the number of tillers (stalk) and plant height at 3, 6, and 9 MAP, representing the phases of tillering, elongation, and maturation, respectively. The tiller number was calculated by counting the tillers at 3 MAP, and the number of stalks at 6 and 9 MAP. Plant height was measured from the base of the stalk to the top of the leaves. The tiller number and plant height were randomly measured using 15 plants per plot in five replicates.

Sugarcane Yield and Sucrose Analysis

Sugarcane cultivated in the four locations was manually harvested at 12 MAP. Stalks, in three rows, were cut at the base and top of the first visible dewlap to remove fresh leaves. The stalks were collected and weighed per row to estimate sugarcane yield per plot or ha, and the weight and diameter were measured for 10 stalks in each row. The stalk from the three rows was crushed and pressed using a small three-roller mill to produce juice and separate the bagasse. Total juice was weighed, and analyzed for Brix and Pol. The Brix was determined using a digital refractometer (MA871, Milwaukee, USA) to measure the soluble solid content of the sucrose-containing solution. Then, the Pol was tested using a saccharimeter to measure the apparent sucrose content in the solution (Gilbert et al. 2009).

Biodiversity of Soil Bacteria

Bacterial diversity in the rhizosphere soil of the cultivated sugarcane was investigated by randomly collecting the soil at a depth of 30 cm below sugarcane row at a distance of 5 cm from the plants. The collected soil was immediately transported to the laboratory, and bacterial diversity was determined according to a previously described method (Wulandari et al. 2021). Serial dilutions of the soil bacterial solution were cultured in the media of Jensen (Das and De 2018), Pikovskaya (Suleman et al. 2018), and nutrient agar in three replicates to determine the number of nitrogen-

Table 1 Climate and soil types of the confined field experimental locations

Confined field trials	Soil type	Climate type (Oldeman)	Wet month number	Location/coordinates
Wringin–Situbondo (WS)	Entisol	E3	< 3	Situbondo/7° 43′ 19.6″ South and 114° 13′ 48.4″ West
Lombok–Bondowoso (LB)	Podsol	D2	3–4	Bondowoso/7° 56′ 48″ South and 113° 55′ 26″ West
Jubung–Jember (JJ)	Regosol	C3	5–6	Jember/8° 11′ 22.7″ South and 113° 38′ 6.389″ West
Genitri Lumajang (GL)	Aluvial	B1	7–9	Lumajang/8° 08′ 21.1″ South and 113′ 22′ 1.6″ West

fixing, phosphate-solubilizing, and total bacteria, respectively, using a plate counting method. The number of culturable bacteria was counted as colony-forming units (CFU) per gram of dry soil.

Detection of Gene Flow into Soil Bacteria by PCR

Polymerase chain reaction (PCR) was conducted to ensure the presence of gene flow into the soil bacterial genome. A single bacterial colony from nitrogen-fixing, phosphate-solubilizing, and total bacteria was selected and transferred into the master mix containing all PCR reagents (Promega, Madison, USA). The PCR reaction was performed using a set of primers to detect the *npt*II gene (Anur et al. 2020) and a set of primers to amplify the gene for 16 rRNA (Wulandari et al. 2021). PCR-amplified DNA was separated by 1% agarose gel electrophoresis and documented using GelDoc (Major Science, CA, USA).

Statistical Analysis

Independent statistical analysis was conducted for each of the locations by comparing transgenic lines to NT sugarcane using Dunnett's test. Statistical significance was calculated using the SPSS 22 software, and was determined at a p-value of ≤ 0.05.

Results

Field Evaluation of Transgenic Sugarcane

The growth and productivity of transgenic sugarcane overexpressing the *SoSPS1* gene was evaluated in four confined field trials with different soil and climate types (Table 1). Water for two locations (JJ and GL) was supplied using an irrigation system, but the other two locations (LB and WS) did not use an irrigation system and depended only on rainfall. Sugarcane seed germination was ensured by cutting the sugarcane stem containing two budded setts and soaking in water overnight, which was planted during the early rainy season (October 2019). To maintain 60 shoots per row (8 m), the ungerminated buds were replaced with separately prepared germinated buds. According to the germination rates, majority of the sugarcane seeds germinated, whereas the WS location observed 80% germination because of the late rainy season. To confirm transgene stability, genomic DNA was isolated from one-month old transgenic leaves and used for PCR analysis (Supplementary Fig 1). The corresponding *npt*II DNA was observed at molecular size 550 bp in all leaves of transgenic lines, but not in leaves of NT sugarcane.

The life cycle of sugarcane consists of four phases: germination, tillering, stem elongation (grand growth), and maturation. The rainy season induced sugarcane tillering and maximum tiller formed at 3 MAP. The tiller number increased at 9 MAP in the GL and JJ locations, which were well-supplied with irrigation water. In contrast, only a slight increase in tiller formation in LB and WS after 6 MAP may be attributed to less water supply. According to the climate type, LB and WS were classified as D2 and E3, with approximately 3 months of rainfall and more than 6 months of dry season (Table 1). Among the four trial locations, the average tiller number was lower at JJ, but was the highest at GL, with approximately 318.13 tiller number per row in transgenic lines at 9 MAP. Compared to the NT parent, majority of the transgenic lines showed a higher tiller number at 3, 6, and 9 MAP (Fig. 1). The tiller number of the SPS3 line significantly increased by approximately 23.7% in all the trial locations compared to that of NT sugarcane, although the increase was only observed at 9 MAP in JJ (Fig. 1d). This result indicates that the SPS3 line produced a higher tiller number, followed by the SPS9 and SPS1 lines compared to that of the NT sugarcane.

Growth rate was compared by measuring plant height at 6 and 9 MAP, but not at 3 MAP, because sugarcane growth is directed toward tiller formation. A comparison of the growth rate, which was observed as plant height, showed that the transgenic lines grew rapidly than that of the NT sugarcane (Fig. 2). Among the four locations, the plant height at GL was higher than that at other locations, and reached 310 cm and 381 cm at 6 MAP and 9 MAP, respectively. The transgenic lines at JJ location also showed a vigorous growth rate and reached a height of 352.9 cm, which was comparable with that at GL location at 9 MAP. This rapid growth rate is likely due to the water supplied in the GL and JJ locations. The SPS3 transgenic line showed significantly higher plant height than that of NT sugarcane at all locations, especially at the maximum growth stage of 9 MAP. Under less water supply, the SPS3 line still displayed a significant increase in growth rate compared to that of the other lines.

The productivity of field-grown transgenic lines were determined by measuring stalk weight and diameter, cane yield, juice weight, Brix, and Pol during harvest. All sugarcane grown in the field were harvested at 12 MAP, coinciding with the NT parent sugarcane, which is categorized as a late-maturing sugarcane. During harvest, the stalk weight and diameter of the transgenic lines were higher compared to that of NT sugarcane in all trial locations (Table 2). Therefore, cane yield expressed as ton/ha, increased in transgenic lines compared to that of NT-sugarcane in all trial locations. The SPS3 line produced the highest cane yield than that of NT sugarcane and reached productivity at 179.74 ton/ha. The harvested cane was

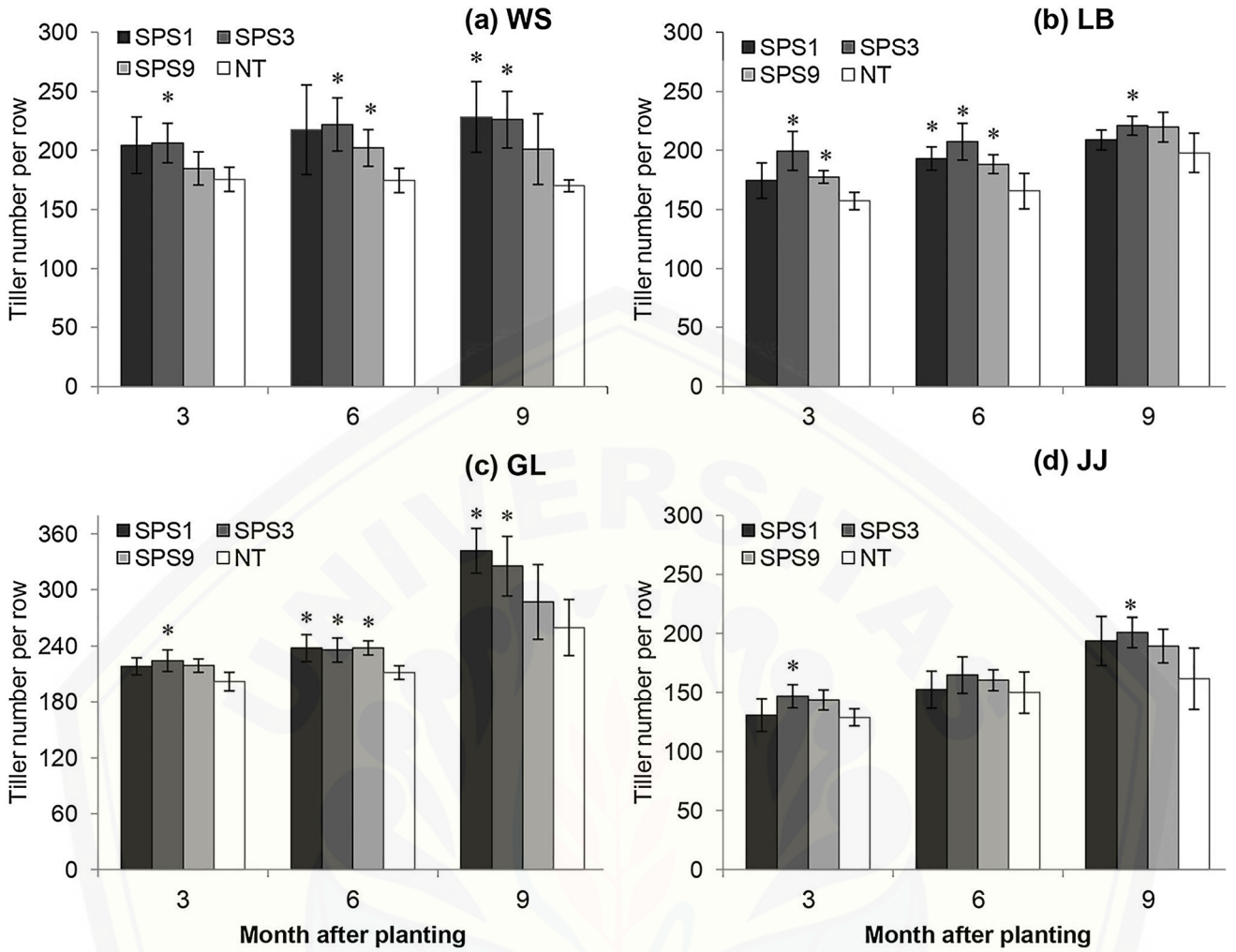

Fig. 1 Tiller number per row (8 m) of transgenic and NT sugarcane at 3,6, and 9 months after planting (MAP) on four field trial locations. **a** Wringin–Situbondo (WS); **b** Lombok–Bondowoso (LB); **c** Genitri–Lumajang (GL); **d** Jubung–Jember (JJ). Values are means ± SD for five replication. Asterisks (*) denote statistically significant differences (Dunnett's test: $p \leq 0.05$)

immediately crushed and pressed in a small three-roller mill to produce sugarcane juice. The Brix (%) determined in the juice was higher in the transgenic lines, and was considerably higher in SPS3 in all locations. Sucrose content expressed in Pol (%) was positively associated with the Brix content of the juice. The Brix and Pol contents were higher in LB, followed by WS, which received less water than that in the GL and JJ locations provided with the irrigation water. However, the GL location produced the highest cane (biomass) yield compared to that of the locations supplied with less water.

Effect of Transgenic Sugarcane on Bacterial Diversity and Gene Flow

Field-grown transgenic sugarcane was presumed to affect bacterial diversity of the rhizosphere soil. The effect of transgenic lines may occur temporarily at a certain growth rate. Therefore, the bacterial population was observed at 3, 6, and 9 MAP, which represent the tillering, elongation, and maturing stages, respectively. Overall, the plate counting analysis showed that the transgenic lines did not decrease the population number of rhizosphere soil bacteria, but considerably increased several bacterial populations compared to that of the NT sugarcane (Fig. 3). The maturing stage observed a decrease in N-fixing and P-solubilizing bacterial population compared to that of the tillering and elongation stages, but not in total bacteria. The bacterial population was higher during the early growth stage, as previously reported (Wulandari et al. 2021). The number of culturable total bacteria was higher and reached a maximum of 2.41×10^8 CFU g^{-1} dry soil, compared to that of N-fixing and P-solubilizing bacteria. In contrast, the sum of culturable N-fixing and P-solubilizing bacteria

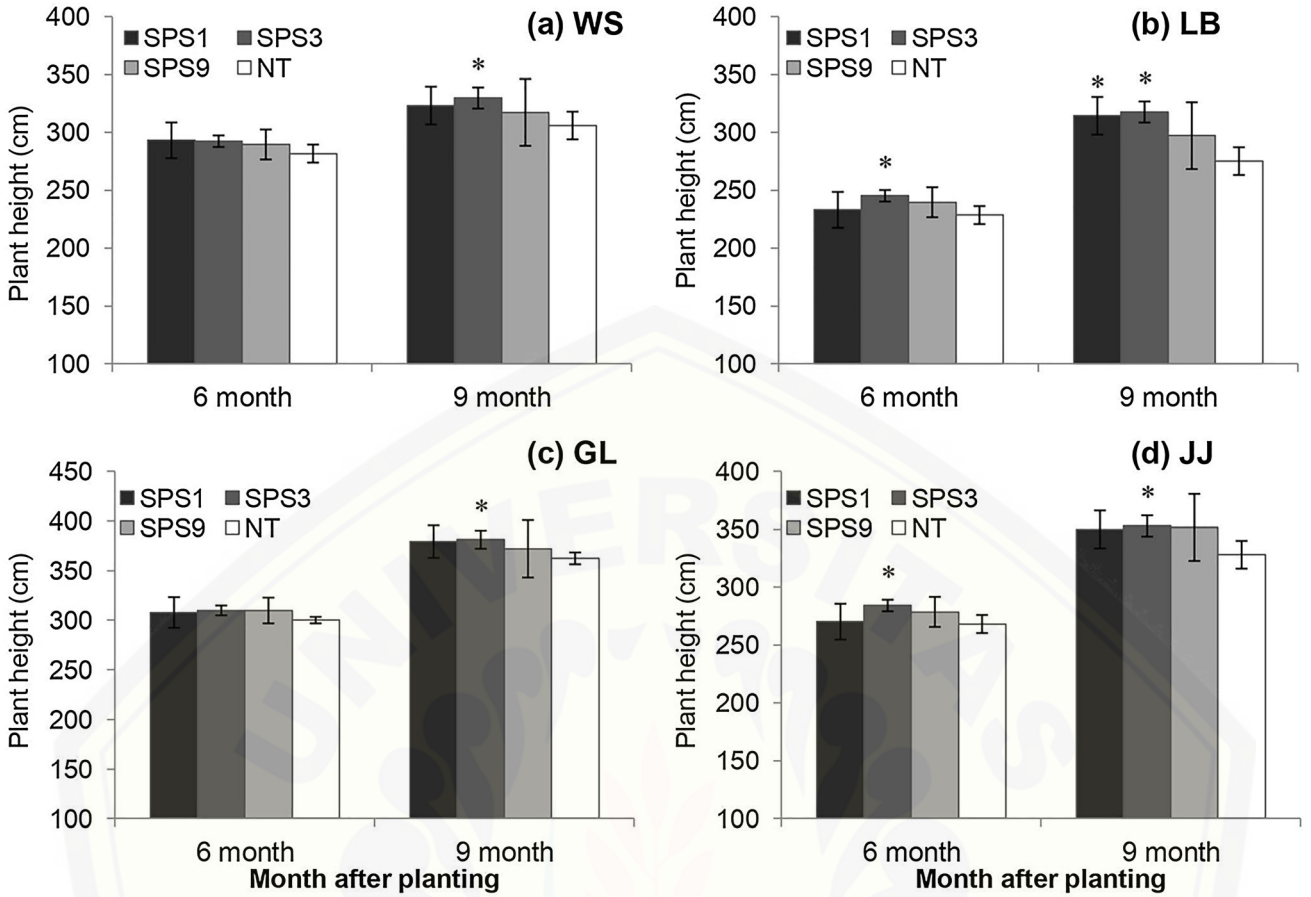

Fig. 2 Plant height of transgenic and non-transgenic sugarcane at 6 and 9 months after planting (MAP) on four field trial locations: **a** Wringin–Situbondo (WS); **b** Lombok–Bondowoso (LB); **c** Genitri–Lumajang (GL); **d** Jubung–Jember (JJ). Values are means ± SD for five replication. Asterisks (*) denote statistically significant differences (Dunnett's test: $p \leq 0.05$)

exceeded the population number of total bacteria because the bacteria were cultured using different culture media. These results indicate that the rhizosphere soil bacteria that were regularly observed during the tillering, elongation, and maturation phases remained unaffected by transgenic lines.

The possibility of gene flow from transgenic lines to related wild species, such as microorganisms, is a major ecological concern. The likelihood of gene flow was observed by conducting PCR using culturable total bacteria, N-fixing and P-solubilizing bacteria with primers specific for *npt*II. To ensure that PCR was conducted using the bacterial DNA genome, a control test was performed using primers for 16S rDNA. The results showed that the *npt*II gene was not amplified in the N-fixing, P-solubilizing, and total bacterial colonies. The *npt*II DNA band was only observed in the positive control bacterial cells of recombinant *E. coli*. However, the corresponding 16S rDNA with a molecular size of 1465 bp was clearly amplified in each of the bacterial colonies (Supplementary Fig. 2). This indicated that gene flow to the bacterial community was not observed in the field evaluation of transgenic sugarcane.

Discussion

The overexpression of the SPS gene increased sucrose content, plant height, stalk number, and weight of transgenic sugarcane in a greenhouse trial (Anur et al. 2020). The selection of transgenic lines cannot be sufficiently based on a greenhouse trial, but requires field evaluation. This study was conducted to evaluate the performance of transgenic line in four confined field trials under the guidance of the Indonesian Biosafety Commission. The results demonstrated that the transgenic lines exhibited higher growth and productivity than that of NT sugarcane, which is consistent with the previous greenhouse trials. The SPS3 line significantly expressed a higher tiller number and plant height in four locations compared to that of NT sugarcane (Figs. 1, 2). In addition, the tiller number of the SPS3 line reached the highest in the GL location, which

Table 2 Yield characteristics of transgenic and non-transgenic sugarcane during harvest (12 MAP) at four field trial locations

Location	Line	Stalk weight (kg/cane)	Stalk diameter cm)	Cane Yield (ton/Ha)	Juice weight (kg)	Brix (%)	Pol (%)
WS	SPS1	1.71 a	2.82 a	81.82 a	1.04 a	16.46 b	11.86 b
	SPS3	1.77 a	2.78 a	82.30 a	1.03 a	16.84 a	12.39 a
	SPS9	1.45 b	2.76 ab	81.25 ab	0.81 b	16.41 b	12.55 a
	NT	1.21 b	2.65 b	69.79 b	0.74 b	16.11 b	11.27 b
LB	SPS1	1.61 a	2.82 a	87.02 ab	0.88 ab	19.33 a	17.08 a
	SPS3	1.63 a	2.83 a	89.35 a	0.92 a	19.26 a	16.75 a
	SPS9	1.46 ab	2.72 ab	84.86 ab	0.87 ab	18.61 ab	16.14 ab
	NT	1.31 b	2.69 b	70.80 b	0.71 b	18.09 b	15.27 b
GL	SPS1	1.72 ab	2.52 a	176.30 a	1.07 a	15.08 ab	10.72 ab
	SPS3	1.82 a	2.54 a	179.74 a	1.09 a	15.66 a	11.58 a
	SPS9	1.66 ab	2.49 a	164.99 a	1.06 a	14.44 b	10.69 ab
	NT	1.55 b	2.43 a	140.23 b	1.01 a	14.03 b	9.72 b
JJ	SPS1	1.86 a	2.66 a	84.28 ab	1.12 ab	15.49 a	11.57 a
	SPS3	1.87 a	2.64 a	89.25 a	1.18 a	15.47 a	11.56 a
	SPS9	1.85 a	2.62 a	80.45 ab	1.09 b	15.03 ab	11.37 a
	NT	1.74 a	2.55 a	72.11 b	1.07 b	14.17 b	10.87 a

Wringin–Situbondo (WS), Lombok–Bondowoso (LB), Genitri–Lumajang (GL), and Jubung–Jember (JJ). Different lowercase letters denote significant differences (ANOVA, Dunnett's test, $p < 0.05$)

was supplied with irrigation water. Although sugarcane in the JJ location was supplied with water, the tiller number was lower compared to that of the other locations. This discrepancy may be due to the oversupply of water with bad drainage in the JJ location (Supplemental Fig. 3). Waterlogging decreases cane height and stalk diameter, and reduces sugarcane productivity (Misra et al. 2020). Furthermore, the cane yield in the SPS3 transgenic line in GL was the highest and practically twice that of the other locations (Table 2). This result confirmed that the overexpression of the sucrose-phosphate synthase gene enhanced the growth and sugar productivity of transgenic sugarcane in field trial experiments.

Water deficit induces the accumulation of small molecules referred to as compatible solutes, such as sugars, amino acids, and betaine glycine, which help in plant growth under conditions of stress (Chen and Murata 2002). Sugar productivity showed that the transgenic lines increased the percentage of Brix and Pol compared to that of NT sugarcane. Brix and Pol considerably increased in the SPS3 line in all trial locations. Furthermore, sugar productivity was higher in LB and WS locations with less water supply than that in GL and JJ locations (Table 2). It was previously reported that the overexpression of SPS gene increased SPS activity and sucrose content in transgenic sugarcane (Anur et al. 2020). The lower water availability induced SPS activity, resulting in higher sugar productivity in transgenic sugarcane cultivated in LB and WS locations. SPS activity is enhanced by water insufficiency due to covalent modification of the enzyme, which is caused by protein phosphorylation of the serine residue at position 424 (Huber and Huber 1996a, b). In addition, water shortage during sugarcane maturation reduces growth, but increases sucrose accumulation in the stem (Inman-Bamber and Smith 2005). Therefore, the sucrose content increased in transgenic lines cultivated in locations with low water availability. Sucrose acts as an osmoregulator and helps transgenic sugarcane lines adapt to water deficit conditions.

The growth and productivity performances of transgenic lines cultivated in four locations showed that the transgenic lines produced a higher cane yield in GL, but higher Brix and Pol in LB and WS (Table 2). The LB and WS were water deficit locations, and according to the climate type E3 and D2 (Table 1), were categorized as dry land. During moderate drought stress at the maturity stage, carbon partitioning is directed toward sucrose yield, which inhibits energy utilization for the growth of sugarcane (Inman-Bamber 2004). This indicates that plants regulate the utilization of carbon partitioning under certain environmental conditions to obtain optimal growth and yield. Redistribution of sucrose, as mobile carbon, by sucrose transporter may be one of the systems for efficient use of assimilated carbon to increase crop yield (Aluko et al. 2021). Sugar production in sugarcane is determined by cane yield and sucrose content in the stalk. The transgenic lines produced

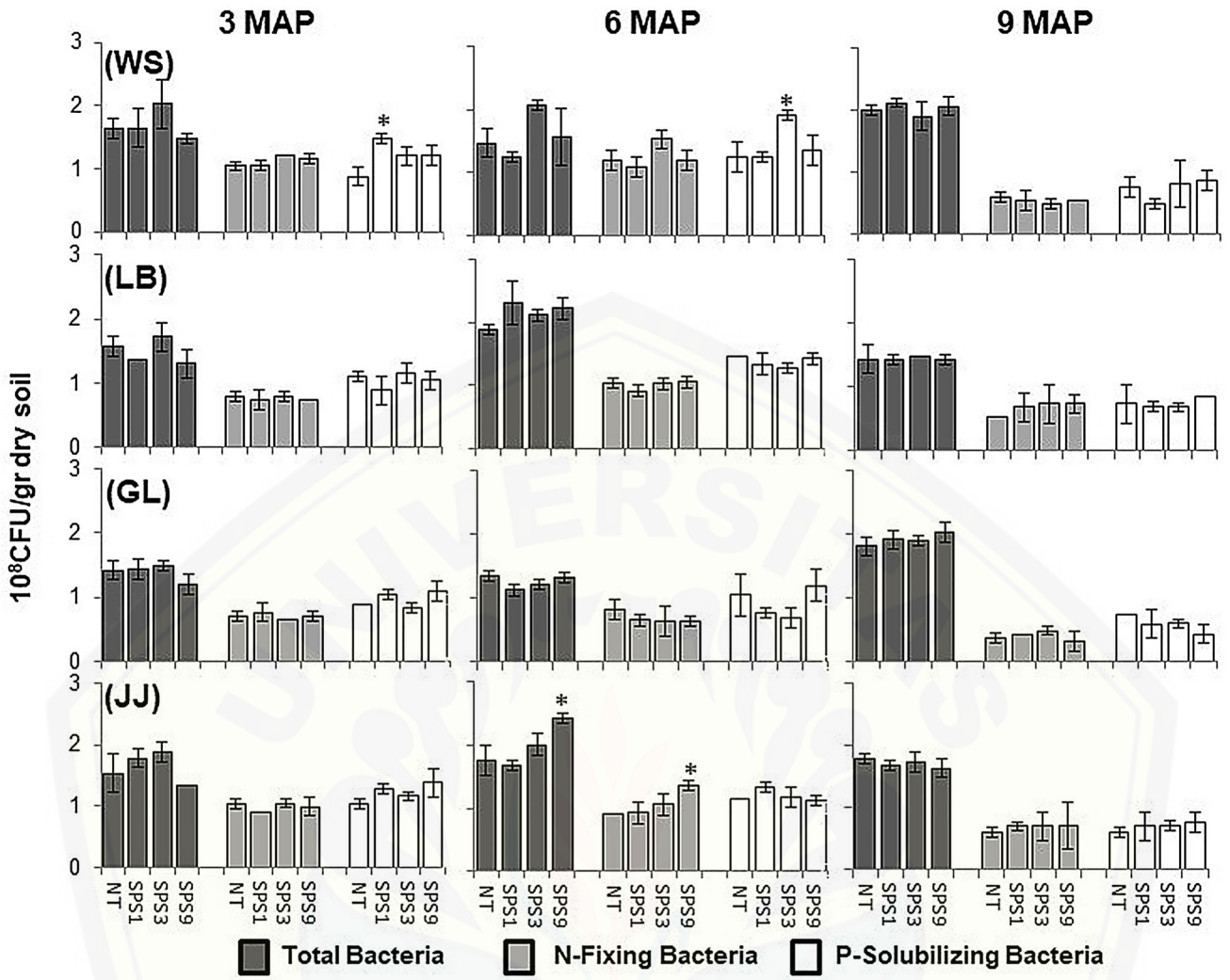

Fig. 3 Population of culturable bacteria isolated from four confined field trials during growth stages of sugarcane. The bacteria population is presented in 10^8 CFU g^{-1} dry soil ± SD (n = 3). Asterisks (*) denote statistically significant differences (Dunnett's test: $p \leq 0.05$)

a higher cane yield in locations with normal water, and a higher sugar content under water-deficit conditions. Therefore, the evaluation of growth and productivity in four locations with different agro-climates provides an important strategy for evaluating the performance of transgenic sugarcane.

The cultivation of field-grown transgenic crops has raised public concern regarding its effect on soil microbial diversity. The effect of transgenic crops on the soil microbial population is an important factor to evaluate in terms of biosafety risks. Among the many essential functions of soil bacteria are organic matter decomposition, biological N fixation, and mineral solubilization. Considering the potential risk, the total, N-fixing, and P-solubilizing bacterial populations were continuously observed in the rhizosphere soil during transgenic sugarcane cultivation at 3, 6, and 9 MAP. The results showed that the transgenic lines did not decrease the soil bacterial populations compared to that of NT-sugarcane at the four locations (Fig. 3). In addition, the concern regarding gene flow has been verified in that the transgenic lines did not transmit the gene of interest to soil rhizosphere bacteria (Supplement Fig. 3). These results confirm a previously reported result that transgenic sugarcane overexpressing the SPS gene does not affect the rhizosphere soil bacterial population, enzyme activity, nutrient content, or gene flow (Wulandari et al. 2021). Most studies suggest that transgenic crops cause minor changes in the microbial community, including transgenic sugarcane (Zhou et al. 2016). Thus, this study validates that no significant differences were observed while evaluating the soil environment of transgenic and NT sugarcane.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12355-022-01121-7.

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Authors' Contribution All authors have read and agree to the final version of manuscript. Conception and designed the study, B.S, and S.; carried out the field experimental works, S, S.I.W, R.M.A, I.R.N and P.D; carried out the laboratory works, S.I.W and I.R.N.; validation, data curation, S, R.M.A and I.R.N.; writing-review, and editing manuscript, R.M.A and B.S.; project administration, P.D.; funding acquisition, B.S.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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