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Effect of paclobutrazol on growth and root morphology of 12 crossed stevia *in vitro*

Abstract. Stevia (*Stevia rebaudiana* Bertoni) is a low-calorie alternative sweetener. The superior varieties of Stevia in Indonesia are limited. Availability of seeds plays an important role in the Stevia plant breeding program for both the selection stage and the production of high-yielding varieties. Tissue culture is the best method of Stevia propagation, but the low adaptability of plantlets causes death in the acclimatization phase. The growth and root morphology of 12 crossbreed Stevia were evaluated. A Completely Randomized Design was used with factorial consisting of two factors were Stevia genotypes (STG1,7,8,10, SBG3,4,5,7,10, SGB2,3, and SBT11) and the plant growth regulator (PGR) concentration of Paclobutrazol (PBZ) (0.1, 0.5, and 1.0 ppm) and indole acetic acid (IAA) control (0.5 and 1 ppm). The results showed that the fastest shoot emergence time was SBT11 at 0.1 and 0.5 ppm PBZ media at 4 days after culture (DAC). The fastest root emergence time was SBG7 at 0.1 and 1 ppm PBZ media (8 DAC). The highest shoots were at 1 ppm IAA media (18 cm). The highest number of shoots was STG10 at 1 ppm PBZ and SBG3 at 0.5 ppm IAA (4 shoots). The highest number of internodes was SBG5 at 0.5 ppm IAA (25 internodes). The highest number of leaves was SBG3 at 0.5 ppm IAA (57 leaves). The highest number of roots was SGB2 at 0.5 ppm PBZ (5 roots). The greenest leaf color was SBG7 at 1 ppm PBZ media. The PBZ accelerated the emergence of shoots and roots and the number of roots.

Keywords: Genotype · Interaction · *In vitro* · Paclobutrazol · Stevia

Pengaruh paclobutrazol terhadap pertumbuhan dan morfologi akar 12 stevia hasil persilangan *in vitro*

Sari Stevia (*Stevia rebaudiana* Bertoni) merupakan sumber pemanis alternatif berkalori rendah. Varietas unggul Stevia di Indonesia terbatas. Ketersediaan bibit menjadi kunci dalam program pemuliaan tanaman Stevia baik untuk pada tahap seleksi maupun produksi varietas unggul. Kultur jaringan adalah metode perbanyakan Stevia terbaik, tetapi kemampuan adaptasi planlet yang rendah menyebabkan kematian pada fase aklimatisasi. Tujuan penelitian ini untuk mengevaluasi pertumbuhan dan morfologi akar dari 12 Stevia hasil persilangan. Metode percobaan yang digunakan adalah Rancangan Acak Lengkap faktorial dua faktor, yaitu genotipe Stevia (STG1,7,8,10, SBG3,4,5,7,10, SGB2,3, and SBT11) dan konsentrasi zat pengatur tumbuh paklobutrazol (PBZ) (0,1; 0,5; dan 1,0 ppm) serta *indole acetic acid* (IAA) sebagai kontrol (0,5 dan 1 ppm). Hasil penelitian menunjukkan waktu muncul tunas tercepat adalah SBT11 pada 0,1 dan 0,5 ppm PBZ pada 4 hari setelah tanam (HST). Waktu muncul akar tercepat adalah SBG7 pada 0,1 dan 1 ppm PBZ (8 HST). Tunas tertinggi diperoleh pada media 1 ppm IAA (18 cm). Jumlah tunas terbanyak diperoleh STG10 pada 1 ppm PBZ dan SBG3 pada 0,5 ppm IAA (4 tunas). Jumlah ruas terbanyak diperoleh SBG5 pada 0,5 ppm IAA (25 ruas). Jumlah daun terbanyak diperoleh SBG3 pada 0,5 ppm IAA (57 daun). Jumlah akar terbanyak diperoleh SGB2 pada 0,5 ppm PBZ (5 akar). Warna daun terhijau diperoleh SBG7 pada 1 ppm PBZ.

Kata kunci: Genotipe · Interaksi · *In vitro* · Paclobutrazol · Stevia

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Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a potential sweetener commodity that can be developed in Indonesia and has become the government's attention as a commodity to support sugar self-sufficiency (Detik Finance, 2021). Stevia is known as a low-calorie alternative sweetener and is 300 times sweeter than sucrose. It is easily digested in the body and has many benefits such as anti-hyperglycemic, anti-hypertensive, anti-caries, and immunomodulatory (Samuel *et al.*, 2018). Stevia is also regulated by Food and Drug Administration/FDA (Dwyer *et al.*, 2015).

Tissue culture is one of the most effective methods for Stevia propagation. Plant tissue culture can produce plants vegetatively in large quantities in a shorter time, produce genetically and physiologically uniform plants, and pathogens free (Sinta and Amanah, 2019). Other methods of stevia propagation can use seeds and cuttings. Stevia seeds have high uniformity, low fertility (Raina *et al.*, 2013), and low germination ability, viability, and vigor (Khalil *et al.*, 2014). Meanwhile, cutting propagation is required a large area (Djajadi, 2014). The limitations of the stevia mother plant cause high pathogenic (Chotikadachanarong and Dheeranupattana, 2013), the viability and vigor of seedlings are easily decreased due to repeated cutting (Sinta and Amanah, 2019).

In tissue culture, plantlets generally have low environmental adaptation resistance. It is caused by an underdeveloped cuticle, epicuticular wax, and functional stomata. It caused high transpiration of stomata and cuticles during acclimatization (El-Fadl, 2017) and plantlet death during acclimatization. Before acclimatization, explants were generally grown in root media. The growth regulators that play the most role in root induction are auxins, especially IAA (Indole-3-Acetic Acid) because they are most abundant in plant tissues (Zhao, 2010). In the research of Merindasya *et al.* (2013) the best concentration of IAA in stevia tissue culture was 0.5 mg/L and in the study of Seyis *et al.* (2017) 1 mg/L IAA was also used as a root induction medium for the treatment of the Stevia genotype.

Plant growth inhibitory hormones or retardants are also widely used to produce plants with better adaptation to heterotrophy during acclimatization (Sumaryono and Sinta, 2011). Retardants generally caused shortening of plant segments, reduction of leaf size, increasing the concentration of green leaf color, thickening of roots, and reducing damage due to wilting (Hassanen and Khalil, 2013). Paclobutrazol (PBZ) is one of the most widely used retardants to improve plant adaptation, including stevia (El-Fadl, 2017). Paclobutrazol research on stevia showed that 0.1 mg/L increased plant performance (Sumaryono and Sinta, 2011). El-Fadl (2017) also reported that the highest number of shoots, leaves, and roots was obtained at 0.1 mg/L PBZ, while the highest fresh and dry weight of shoots was at 0.5 mg/L ppm. PBZ is also reported to increase the content of chlorophyll-a, chlorophyll-b, carotenoids, and carbohydrates in Stevia. The successful use of PBZ was also reported at 1 mg/L in apples (*Malus domestica*) (Te-chato *et al.*, 2009) and pomelo (*Citrus maxima* (Burm.) Merr.) (Dewi *et al.*, 2015). In addition, PBZ can stimulate the amount and weight of fresh root biomass independently or in combination with other auxins (Wiesman and Riov, 1994). Although these two exogenous growth regulators have different mechanisms of action, the use of PBZ has the potential to stimulate root growth. Gimenes *et al.* (2018) reported that PBZ at rate of 0.5; 1.0; 1.5 mg L^{-1} promoted root thickening and reduced length of aerial parts and roots on *Zygopetalum crinitum*. Medium contained 2 µM IAA or 2 µM of PBZ *Pinus massoniana* Lamb. improved rhizogenesis in vitro shoot culture by combined exogenous NAA, PBZ increased rooting rate and its number (Wang and Yao, (2021).

Explant growth and adaptability during acclimatization are influenced by plant genotypes (Sinta and Amanah, 2019). So, genotype selection needs to be considered. The genotype of crossbreed stevia from Garut, Tawangmangu, and Bogor accessions, is the result of exploration and continues to be developed for the assembly of superior stevia varieties (Amien *et al*., 2020). They have the best seed quality, and quantity of stevioside among accessions of the laboratory collection of the Faculty of Agriculture, Universitas Padjadjaran (Atmojo, 2015). Besides genetic factors, plant growth is also influenced by the interaction of genotype and environment (Poehlman and Sleper, 1995). Each different genetic constitution causes different responses to the same medium

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composition, and conversely (Amien *et al*., 2020). This urged research to be conducted to obtain the best stevia growth, according to the effect of the interactions of 12 crossbreed genotypes and plant growth regulatory (PGR) concentrations of PBZ and IAA. As well as an effort to develop Stevia in increasing genetic diversity with the most effective and efficient method of propagation.

Materials and Method

This research was carried out in the Tissue Culture, Technology Laboratory, Plant Breeding, Department of Agronomy, Agrotechnology Study Program, Faculty of Agriculture, Universitas Padjadjaran in March-June 2021. The main ingredients used were 1 cm shoot explants which had 2 leaves from cultures of 12 crossbreed stevia genotypes (Table 1), Indole-3-acetic acid (IAA), Paclobutrazol (PBZ), and Driver and Kuniyuki Walnut (DKW) media.

A Completely Randomized Design (CRD) was used with factorial consisting of two factors, i.e., stevia genotypes (STG1,7,8,10, SBG3,4,5,7,10, SGB2,3, and SBT11) and the PGR concentration of PBZ (0.1; 0.5; and 1.0 ppm) and IAA control (0.5 and 1 ppm). This experiment was carried out in two replications, with four bottles per replication. Stevia growth data were analyzed using analysis of variance (ANOVA) with the F test at the 5 % significance level and significant differences were tested using the Scott-Knott test at the same level. Analysis was performed using software R-Project 4.1.0 and R-Studio 1.4.1103.

The experiment was started by sterilizing the equipment, making stock media solutions, making plant growth regulator (PGR) solutions, making media, planting explants, maintaining culture, and observing cultures. DKW media were prepared by adding 30g/L of sucrose, and 7g/L of gelling agent. Then the media bottles were sterilized using an autoclave at 121°C with a pressure of 1 ATM for 15-20 minutes (Gamborg and Phillips, 1995). The explants were planted in a Laminar Air Flow Cabinet (LAFC). Culture maintenance at a temperature of ± 25-28 °C, the humidity of ± 60,75%, and 40 watts TL lamp or ± 1000 Lux for 16 hours of the light period.

Table 1. The Genotype of crossbreed stevia's accessions of Garut, Tawangmangu, and Bogor.

Genotype Code	Description	
P1	STG 1	1st Crossbreed Tawangmangu Garut
P2	STG 7	7th Crossbreed Tawangmangu Garut
P3	STG 8	8th Crossbreed Tawangmangu Garut
P4	STG 10	10th Crossbreed Tawangmangu Garut
P5	SBG 3	3rd Crossbreed Bogor Garut
P6	SBG 4	4th Crossbreed Bogor Garut
P7	SBG 5	5th Crossbreed Bogor Garut
P8	SBG 7	7th Crossbreed Bogor Garut
P9	SBG 10	10th Crossbreed Bogor Garut
P10	SGB 2	2nd Crossbreed Garut Bogor
P11	SGB 3	3rd Crossbreed Garut Bogor
P12	SBT 11	11th Crossbreed Bogor Tawangmangu

Observations were made for eight weeks after culture (WAC) including the time of emergence of shoots and roots; shoot height; the number of shoots, internodes, leaves, and roots; and leaf color. Observations of the time of emergence of shoots and roots were carried out every day, for the height and number of shoots, number of internodes, leaves, roots, and leaf color were carried out at the end of observation (8 WAC). Observation of leaf color using the Royal Horticultural Society Color Chart (RHSCC).

Result and Discussion

Analysis of variance for 12 genotypes of crossbreed stevia traits at various concentrations of PGR (Table 2.) showed that there was an interaction between genotypes of crossbreed stevia and concentration of PGR. An interaction between genotypes of crossbreed stevia and concentration of PGR found in the number of shoots, number of internodes, number of leaves, number of roots, except for shoot length.

Table 2. Analysis of Variance for 12 genotypes of crossbreed Stevia Traits at Various Concentrations of PGR.

Source of Variation	Df	MS (Mean of Square)				
		Shoot Length	Num. of Shoots	Num. of Internodes	Num. of Leaves	Num. of Roots
Genotype	11	15,4	5,382***	93,60***	486,30***	1,1702
PGR	4	367,7***	0,771**	170,51***	706,90***	1,4847
Genotype: PGR	44	19	0,707***	19,61*	101,30**	1,6509*
Residuals	60	14	0,19	11,72	48,70	0,8908
CV		28,6	23,1	24,5	21,9	35,1

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

Shoot emergence time. Each treatment gave a different response to the speed of the shoot's emergence day (Table 3). The average results of the fastest shoot emergence days were four days, which were obtained for the SBT11 genotype at 0.1 and 0.5 ppm PBZ. The emergence of shoots that occurred in the first week was also obtained in SBG10 at 1 ppm PBZ and SBG2 at 1 ppm IAA, with the emergence time being the fifth day. While on STG1, STG7, and SBG4 at 0.1 ppm PBZ and SBG10 at 1 ppm IAA and 0.5 ppm PBZ shoots grew on the seventh day.

Table 3. An average stevia shoots emergence time.

Genotype (P)	Time of Shoot Emergence (Days)				
	PGR Concentration (G)				
	G1	G2	G3	G4	G5
P1	24	10	7	-	36
P2	7	-	7	8	14
P3	23	32	8	8	8
P4	15	14	35	31	13
P5	23	17	14	10	24
P6	32	9	7	21	17
P7	16	9	19	15	38
P8	9	37	16	9	19
P9	-	7	9	7	5
P10	9	5	-	30	14
P11	-	50	11	10	-
P12	41	48	4	4	-

Note:
"-" = new shoots did not appear until the end of the observation (8 weeks)
P1 = STG 1, P2 = STG 7, P3 = STG 8, P4 = STG 10, P5 = SBG 3, P6 = SBG 4, P7 = SBG 5, P8 = SBG 7, P9 = SBG 10, P10 = SGB 2, P11 = SGB 3, P12 = SBT 11, G1 = Control IAA 0.5 ppm, G2 = Control IAA 1 ppm, G3 = Paclobutrazol 0.1 ppm, G4 = Paclobutrazol 0.5 ppm, G5= Paclobutrazol 1 ppm.

The emergence of many shoots occured in weeks 1-3. Some treatments did not grow new shoots until the observation ended 12 weeks, i.e., STG1 at 0.5 ppm PBZ, SGB2 at 0.1 ppm PBZ, SGB3 and SBT11 at 1 ppm PBZ. The fastest time of shoot emergence was shown on genotypes STG7, 8, SBG10, and SBT11 compared to other genotypes. The use of PBZ on media, according to data that PBZ accelerated the shoot emergence in several genotypes. In the research by Kepenek and Karoğlu (2011) on apples (*Malus domestica*), the time of shoot emergence was influenced by interactions of genotype and concentrations of PBZ/PP333 and Alar-85.

Root emergence time. The results of observations of root emergence time showed that SBG7 at 0.1 and 1 ppm PBZ showed the fastest root emergence time in eight days, and at 0.5 ppm PBZ only differed by 1 day, the ninth day (Table 4). When compared with the use of IAA, roots appear 1 week later. The emergence of roots that occurred on the ninth day also occurred in SBG3 at 0.1 ppm PBZ; SBG4 at 1 ppm PBZ; and SGB2 at 0.5 ppm PBZ. Root emergence occurred at week 2 except for STG7 with 0.1 ppm PBZ occurring on week 5 or day 32.

Table 4. An average stevia roots emergence time.

Genotype (P)	Time of Root Emergence (Days)				
	PGR Concentration (G)				
	G1	G2	G3	G4	G5
P1	24	27	10	21	11
P2	14	10	32	10	11
P3	18	20	20	22	16
P4	23	18	11	15	13
P5	10	12	9	12	10
P6	18	13	13	10	9
P7	15	21	17	11	10
P8	14	15	8	9	8
P9	22	15	11	25	13
P10	22	18	10	9	18
P11	14	13	10	16	16
P12	21	26	10	14	18

Note: P1 = STG 1, P2 = STG 7, P3 = STG 8, P4 = STG 10, P5 = SBG 3, P6 = SBG 4, P7 = SBG 5, P8 = SBG 7, P9 = SBG 10, P10 = SGB 2, P11 = SGB 3, P12 = SBT 11, G1 = Control IAA 0.5 ppm, G2 = Control IAA 1 ppm, G3 = Paclobutrazol 0.1 ppm, G4 = Paclobutrazol 0.5 ppm, G5= Paclobutrazol 1 ppm.

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STG7, SBG3, SBG4, and SBG7 genotypes gave the best root emergence time compared to other genotypes, and the use of PBZ gave a faster root emergence time than IAA, although not for all genotypes. A concentration of 0.1 ppm PBZ gave the fastest root emergence time. In all genotypes with the fastest root emergence time, arrowroot parents were assumed to have the best ability in root growth.

PBZ promotes root formation due to increased abscisic acid (Soumya *et al.*, 2017) and cytokines (Desta and Amare, 2021). IAA was the main hormone in root formation but it had a complex transport and synthesis process (Saini *et al.*, 2013), so IAA's work as root induction took longer to work on its target function. Thus, the work of PBZ in accelerating the root formation was more effective than IAA.

Shoot length. There was no interaction effect between genotype and PGR concentration on the stevia shoot length character (Table 5 and Figure 1). The longest shots were obtained in the media with the addition of 0.5 and 1 ppm IAA, while the use of PBZ obtained short shoot lengths.

Table 5. An average shoot length of 12 genotypes of crossbreed Stevia at Various Concentrations of PGR.

PGR Concentrations (G)	Shoot Length (cm)
IAA 0.5 ppm (G_1)	16.70 a
IAA 1 ppm (G_2)	18.00 a
0.1 ppm (G_3)	9.83 b
0.5 ppm (G_4)	10.67 b
1.0 ppm (G_5)	10.33 b

Note: Means within a column followed by different letters are different at the P ≤ 0.05 level.

The use of PBZ suppressed the average shoot length of 6.03-8.17 cm. In the study of Sumaryono and Sinta (2011) IAA was reported to increase the shoot length of stevia up to 38 mm, while the use of higher concentrations of PBZ significantly decreased shoot length, except for 0.1 mg/L PBZ. The suppression of stevia shoot length at a concentration of 0.1-2 mg/L PBZ on MS media was also reported in El-Fadl (2017). The suppression of shoot length due to PBZ is caused by inhibition of cell division in the shoot sub-apical meristem (El-Fadl, 20 17). Short shoot length due to PBZ could also occur as a result of the internode shortening effect (Wen *et al.*, 2013).

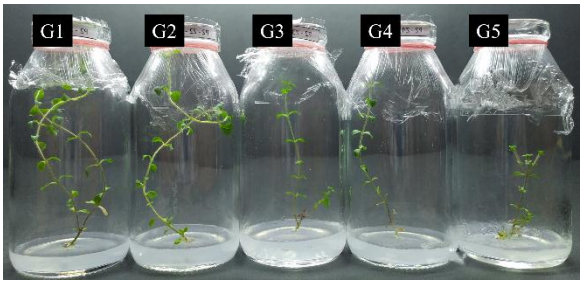

Figure 1. Shoot Length Comparison at Various Concentrations of PGR in STG7 Genotype. Note: Shoot length at 0.5 and 1 ppm IAA media was the highest compared to another PGR concentration.

Number of shoots. On the character of the number of shoots, the interaction effect of 12 genotypes of stevia and various PGR concentrations of PBZ and IAA control was obtained (Table 6). The highest number of shoots was on STG10 at 1 ppm PBZ and 0.5 and 1 ppm IAA compared to other PBZ concentrations. The shoots number of STG10 at 1 ppm PBZ was 4.08 shoots. Genotypes SBG3 and 5 at 0.5 ppm IAA obtained the highest number of shoots compared to other genotypes, i.e., 4.00 and 3.87 shoots. In media containing 1 ppm, IAA genotypes STG10, SBG3, 4, and 5 also obtained the most shoots compared to other genotypes. At 0.1 ppm PBZ, the highest number of shoots was obtained on SBG3 and 4 compared to other genotypes. STG10 at 1 ppm PBZ compared to other eleven genotypes also obtained the highest number of shoots (Figure 2 and 3).

The interaction of genotype and PBZ was also reported by Kepenek and Karoğlu (2011) on apple varieties of Amasya, M9, and Starking Delicious that the M9 variety gave a negative response compared to the other 2 varieties to PBZ. Each genotype had a different effect because each genotype had different abilities depending on the genes it has. The use of 0.1 mg/L PBZ on Stevia was reported to increase the number of shoots up to 11 shots from the control without PBZ but at a concentration of 0.5-2,mg/L PBZ decreased the number of shoots to 15 shoots per explant (El-Fadl, 2017). Soumya *et al.* (2017) explained that PBZ could induce the number of shoots. However, according to Ehirim *et al.* (2014), the addition of retardant to the culture media caused suppression of the number of shoots. This difference could occur due to different genotypes and even species as well as different test concentrations.

Table 6. An average number of shoots of 12 genotypes of crossbreed stevia in various PGR concentrations.

Genotype (P)	Number of Shoots (pcs) PGR Concentrations (G)				
	G1	G2	G3	G4	G5
P1	1.50d A	1.37b A	1.17b A	1.00a A	1.12c A
P2	1.96c A	1.00b A	1.54b A	1.88a A	2.38b A
P3	2.25c A	1.54b A	1.87b A	2.12a A	2.50b A
P4	3.13b A	3.88a A	1.63b B	1.75a B	4.08a A
P5	4.00a A	3.00a A	2.62a A	2.83a A	2.37c A
P6	2.42c A	3.50a A	3.13a A	2.25a A	3.08b A
P7	3.87a A	3.88a A	1.62b A	2.33a A	1.29c A
P8	1.63d A	1.25b A	1.62b A	1.12a A	1.42c A
P9	1.00d A	1.42b A	1.62b A	1.50a A	1.25c A
P10	1.29d A	1.12b A	1.00b A	1.12a A	1.25c A
P11	1.13d A	1.17b A	1.62b A	1.38a A	1.00c A
P12	1.25d A	1.12b A	1.12b A	1.37a A	1.00c A

Note: Means within a column followed by different letters are different (lowercase for vertical comparison and capital letters for horizontal comparison) at the P ≤ 0.05 level.
P1 = STG 1, P2 = STG 7, P3 = STG 8, P4 = STG 10, P5 = SBG 3, P6 = SBG 4, P7 = SBG 5, P8 = SBG 7, P9 = SBG 10, P10 = SGB 2, P11 = SGB 3, P12 = SBT 11, G1 = Control IAA 0.5 ppm, G2 = Control IAA 1 ppm, G3 = Paclobutrazol 0.1 ppm, G4 = Paclobutrazol 0.5 ppm, G5= Paclobutrazol 1 ppm.

Figure 2. Shoot Number Comparison at Various Concentrations of PGR in STG10 Genotype. Note: Shoot length 1 ppm PBZ, 0,5 and 1 ppm IAA media was the highest compared to another PGR concentration.

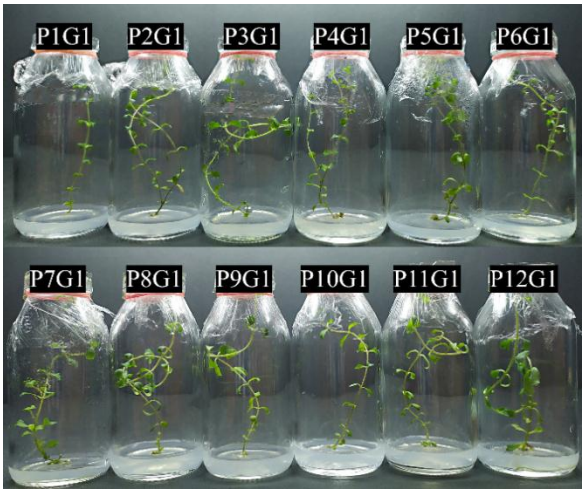

Figure 3. Shoot Number Comparison of 12 Genotypes of Crossbreed Stevia at 0.5 ppm of Paclobutrazol. Note: Shoot number of SBG3 (P5) and SBG5 (P7) were the highest compared to other genotypes.

Number of internodes. The observation results of the number of internodes according to the results of the ANOVA test showed that there was a significant effect on the interaction of the stevia genotype and the PGR concentration (Table 7). The highest number of internodes was obtained by SBG5 at the addition of 0.5 and 1 ppm IAA compared to all PBZ concentrations, which were 25.00 and 21.62 internodes.

The STG10 genotype at 1 ppm IAA, 0.1, and 1 ppm PBZ also obtained the highest number of internodes compared to the use of other concentrations. The number of STG10 internodes at 1 ppm IAA is 24.42 segments. The use of 1 ppm IAA media obtained the highest number of internodes in the STG10, SBG3, 4, and 5 genotypes compared to the other eight genotypes.

The addition of PBZ at all concentrations showed a low number of internodes and was significantly different when compared to IAA controls on several genotypes. The use of Paclobutrazol is reported to be able to shorten the segment (Wen *et al.*, 2013) due to the suppression of kaurene, which inhibits gibberellin biosynthesis so that cell division morphologically decreases. Paclobutrazol could also suppress the effect of IAA in plants (Soumya *et al.*, 2017), while IAA could induce internode elongation (Chen *et al.*, 2018).

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Table 7. An average number of internodes of 12 genotypes of crossbreed stevia in various PGR concentrations

Number of Internodes (pcs)					
Genotype (P)	PGR Concentrations (G)				
	G1	G2	G3	G4	G5
P1	9.00a A	12.25b A	9.42a A	8.62a A	7.25a A
P2	20.33a A	12.87b A	10.83a A	11.62a A	12.75a A
P3	17.25a A	12.79b A	9.87a A	11.62a A	14.87a A
P4	18.62a A	24.42a A	10.37a B	10.88a B	20.62a A
P5	24.87a A	20.71a A	15.12a A	15.79a A	23.87a A
P6	14.42a A	23.33a A	19.25a A	12.63a A	16.92a A
P7	25.00a A	21.62a A	10.92a B	14.67a B	7.79a B
P8	14.87a A	16.37b A	13.25a A	9.08a A	15.04a A
P9	11.50a A	16.21b A	9.62a A	10.50a A	10.58a A
P10	14.37a A	11.50b A	8.75a A	10.38a A	11.75a A
P11	15.21a A	14.79b A	12.50a A	10.13a A	7.75a A
P12	15.21a A	15.62b A	11.42a A	10.25a A	8.25a A

Note: Means within a column followed by different letters are different (lowercase for vertical comparison and capital letters for horizontal comparison) at the P ≤ 0.05 level.
P1 = STG 1, P2 = STG 7, P3 = STG 8, P4 = STG 10, P5 = SBG 3, P6 = SBG 4, P7 = SBG 5, P8 = SBG 7, P9 = SBG 10, P10 = SGB 2, P11 = SGB 3, P12 = SBT 11, G1 = Control IAA 0.5 ppm, G2 = Control IAA 1 ppm, G3 = Paclobutrazol 0.1 ppm, G4 = Paclobutrazol 0.5 ppm, G5= Paclobutrazol 1 ppm.

Number of leaves. There was an interaction effect of 12 Stevia genotypes with PGR concentrations on the number of leaves (Table 8). The number of leaves of Stevia 8 MST on the control medium, 0.5 ppm IAA genotypes STG7, SBG3, and 5 obtained more leaves than the other genotypes. The highest number of leaves was obtained by SBG3 at 0.5 ppm IAA with 56.75 leaves. In 1 ppm IAA media, genotypes STG10, SBG3, 4, and 5 also obtained more leaves than the other genotypes. The STG7 genotype at 0.5 ppm IAA when compared with other PBZ concentrations obtained more leaves. In the STG10 genotype at 1 ppm IAA, 56.13 leaves were obtained, although it was not significantly different at 1 ppm IAA and 1 ppm PBZ. SBG5 and SBT11 compared to all concentrations of PBZ obtained more leaves at 0.5 and 1 ppm IAA.

Table 8. An average number of leaves of 12 genotypes of crossbreed stevia in various PGR concentrations.

Number of Leaves (pcs)					
Genotype (P)	PGR Concentrations (G)				
	G1	G2	G3	G4	G5
P1	21.00b A	27.75b A	21.83a A	20.00a A	18.00a A
P2	48.79a A	27.62b B	25.08a B	28.62a B	30.50a B
P3	38.75b A	29.96b A	25.00a A	28.75a A	34.75a A
P4	41.37b A	56.00a A	23.62a B	27.25a B	48.08a A
P5	56.75a A	46.88a A	36.63a A	38.58a A	51.75a A
P6	31.58b A	52.17a A	44.37a A	28.62a A	36.83a A
P7	56.13a A	47.12a A	23.42a B	33.75a B	18.08a B
P8	32.00b A	35.62b A	30.62a A	21.00a A	34.46a A
P9	24.50b A	35.58b A	24.63a A	24.13a A	24.92a A
P10	33.46b A	27.62b A	20.88a A	23.50a A	28.62a A
P11	33.12b A	33.00b A	28.25a A	22.00a A	19.12a A
P12	32.54b A	33.50b A	22.83a B	23.12a B	17.12a B

Note: Means within a column followed by different letters are different (lowercase for vertical comparison and capital letters for horizontal comparison) at the P ≤ 0.05 level.
P1 = STG 1, P2 = STG 7, P3 = STG 8, P4 = STG 10, P5 = SBG 3, P6 = SBG 4, P7 = SBG 5, P8 = SBG 7, P9 = SBG 10, P10 = SGB 2, P11 = SGB 3, P12 = SBT 11, G1 = Control IAA 0.5 ppm, G2 = Control IAA 1 ppm, G3 = Paclobutrazol 0.1 ppm, G4 = Paclobutrazol 0.5 ppm, G5= Paclobutrazol 1 ppm.

The addition of PBZ at all concentrations showed a lower number of leaves and was significantly different when compared to the IAA control in several genotypes. The suppression of leaf growth due to the influence of Paclobutrazol was also reported in the study of Dewi *et al.* (2015) that the application of PBZ to apple culture suppressed leaf growth and even decreased after the 4th week and completely fell off at the 24th week with a concentration of 5 mg/L PBZ. Another effect of PBZ on leaves was a thickening of the leaf lamina (Wen *et al.*, 2013) caused by the thickening of palisade and spongy cells (El-Leil, 2016).

Number of roots. There was an interaction effect of genotype and PGR concentration of the number of roots (Table 9). The highest number of roots was obtained in 0.5 ppm PBZ media compared with another PGR concentration. 4.75 roots were obtained from SGB2, 4.37 roots for SBG5, 4.12 roots for SBG4, and STG1, STG7, and SBG7 were compared with other genotypes.

Table 9. An average number of roots of 12 genotypes of crossbreed stevia in various PGR concentrations.

Number of Roots (pcs)					
Genotype (P)	PGR Concentrations (G)				
	G1	G2	G3	G4	G5
P1	0.83a A	1.88a A	1.25a A	3.74a A	2.25a A
P2	3.25a A	4.04a A	1.33a A	3.12a A	1.75a A
P3	3.12a A	3.21a A	2.62a A	2.12b A	3.12a A
P4	2.50a A	2.29a A	3.25a A	2.25b A	2.67a A
P5	4.00a A	1.58a A	2.38a A	1.92b A	2.87a A
P6	3.00a A	3.33a A	2.70a A	4.12a A	2.73a A
P7	2.88a A	2.87a A	1.63a A	4.37a A	2.88a A
P8	1.87a A	1.84a A	3.50a A	3.84a A	3.54a A
P9	2.42a A	2.79a A	1.38a A	2.00b A	4.17a A
P10	2.71a B	1.62a B	3.72a A	4.75a A	3.00a B
P11	2.71a A	4.42a A	1.88a A	2.00b A	1.62a A
P12	2.71a A	2.12a A	2.46a A	2.25b A	2.13a A

Note: Means within a column followed by different letters are different (lowercase for vertical comparison and capital letters for horizontal comparison) at the P ≤ 0.05 level.
P1 = STG 1, P2 = STG 7, P3 = STG 8, P4 = STG 10, P5 = SBG 3, P6 = SBG 4, P7 = SBG 5, P8 = SBG 7, P9 = SBG 10, P10 = SGB 2, P11 = SGB 3, P12 = SBT 11, G1 = Control IAA 0.5 ppm, G2 = Control IAA 1 ppm, G3 = Paclobutrazol 0.1 ppm, G4 = Paclobutrazol 0.5 ppm, G5= Paclobutrazol 1 ppm.

Based on the results obtained that PBZ was a growth regulator that supported the growth of the number of roots (Figure 4 and 5). This was in line with the function of PBZ, which had been reported to promote root growth (Soumya *et al.*, 2017). PBZ inhibited gibberellin biosynthesis and increased cytokinin and abscisic acid, which causes an increase in plant root growth (El-Fadl, 2017). The optimum concentration for the number of Stevia roots was also in line according to El-Fadl (2017), which is 0.5 ppm PBZ.

The use of auxin, IAA in culture media could increase the growth of the number of roots due to increased levels of auxin in the media (Ehirim *et al.*, 2014). The use of IAA in Stevia root culture media was reported to be effective in increasing the number of roots (Nower, 2014).

Leaf color. The green color at 8 WAC was dominated by the "yellowish green" group with codes 141 and 143. The addition of PBZ to the media increased the green color of the leaves in the genotypes STG1, 7, 8, and 10, SBG4, 5, and 7, and SGB2 (Table 10). While the use of IAA on the media showed a yellow-green color in the STG10, SBG7, SGB2, and 3 genotypes.

Leaf color is a reflection of the chlorophyll content contained in the leaves (Pérez-Patricio *et al.*, 2018). The number of chloroplasts affects the biosynthesis of steviol glycosides because chloroplasts play a role in the synthesis of precursors (Kumari *et al.*, 2021).

The use of PBZ had been reported to increase chlorophyll levels (Wen *et al.*, 2013) and prevent chlorophyll degradation (Desta and Amare, 2021). This happened because PBZ increased cytokinin levels, which function in increasing chlorophyll biosynthesis (Soumya *et al.*, 2017). The dark green color due to the use of PBZ was caused by an increase in the denser chlorophyll content per unit of leaf area due to the reduction in leaf area due to the inhibition of cell division due to PBZ (Desta and Amare, 2021).

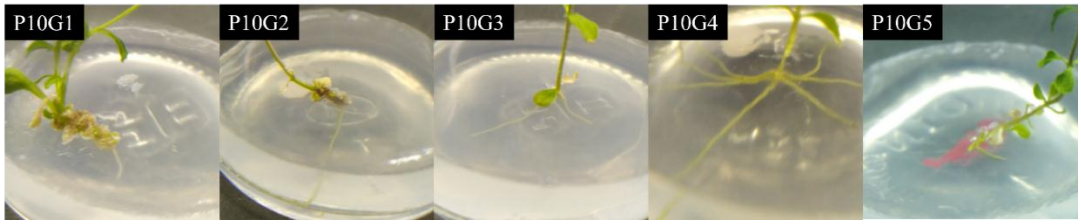

Figure 4. Roots Comparison at Various Concentrations of PGR in SGB2 Genotype. Note: Root number at 0.5 ppm PBZ media was the highest compared to another PGR concentration.

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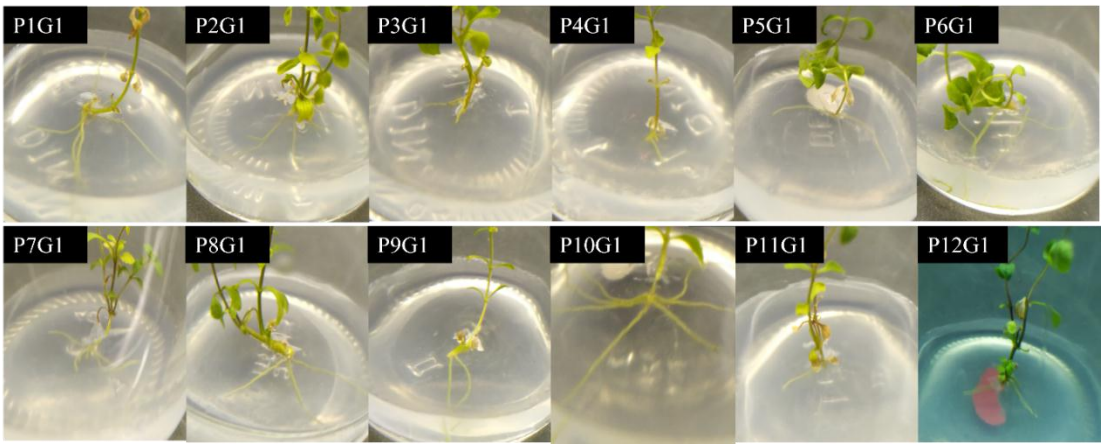

Figure 5. Roots Comparison of 12 Genotypes of Crossbreed Stevia at 0.5 ppm of Paclobutrazol. Note: Root number of SGB2 (P10), SBG5 (P7), SBG4 (P6), STG1 (P1), STG7 (P2), and SBG7 (P8) were the highest compared to other genotypes.

Table 10. Stevia leaf color of 12 genotypes of crossbreed stevia in various PGR concentrations

Genotype (P)	PGR Concentrations (G)									
	G1		G2		G3		G4		G5	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
P1	141A	141A	143A	141A	141A	141A	141A	139A	139A	141A
P2	141A	141A	141A	143A	141A	139A	141A	141B	141A	143A
P3	141A	141B	143A	141A	141A	141A	139A	139B	139B	141B
P4	143A	144A	141A	141B	141B	141B	141A	141B	139A	143A
P5	141B	141A	141B	143A	141A	141A	141A	141A	141A	143A
P6	141A	143A	141A	141A	141A	141B	139A	143A	143A	143A
P7	141A	141A	141A	141A	141B	139B	141A	141A	141A	141B
P8	144A	141A	143A	141B	139B	141A	141B	141A	141A	139A
P9	143A	143A	143A	143A	143A	143A	143A	141B	143A	143A
P10	144A	143B	144A	143A	141B	141A	141A	141B	141A	139A
P11	143A	144A	143A	143A	143A	143A	143A	143A	141B	141B
P12	143A	143A	143A	141A	141B	143A	141B	143A	143A	141B

Note: R= Replication; P1 = STG 1, P2 = STG 7, P3 = STG 8, P4 = STG 10, P5 = SBG 3, P6 = SBG 4, P7 = SBG 5, P8 = SBG 7, P9 = SBG 10, P10 = SGB 2, P11 = SGB 3, P12 = SBT 11; G1 = Control IAA 0.5 ppm, G2 = Control IAA 1 ppm, G3 = Paclobutrazol 0.1 ppm, G4 = Paclobutrazol 0.5 ppm, G5= Paclobutrazol 1 ppm.

The leaf color data in this study had a weakness because of the insignificant difference in the green color of stevia leaves, which can occur due to technical errors of observation, where the accuracy of color matching depends on the observer's eye and perception of color. Color perception refered to the part of the electromagnetic spectrum that was visible to the human eye (Hurlbert and Ling, 2017). Color observations could also become even more wrong if the observer's eyes become tired. In Post and Schlautman's (2020) study, the RHS Color chart could provide an accurate depiction of most petals, but it is difficult to distinguish wide color variations across the petals.

Conclusion

The growth of twelve stevia genotypes was influenced by genotype interactions and PGR concentration on the number of shoots, number of internodes, number of leaves, and number of roots. The shoot length was only affected by the concentration of PBZ. The fastest average shoot emergence time was SBT11 at 0.1 and 0.5 ppm PBZ (4 days). The fastest average root emergence time was SBG7 at 0.1 and 1 ppm PBZ (8 days). The longest shoot average was obtained at 1 ppm IAA (18.0 cm) media. The highest average number of shoots was on STG10 at 1 ppm PBZ and SBG3 at 0.5 ppm IAA (4.00

shoots). The average of the highest number of segments was on SBG5 at 0.5 ppm IAA (25.00 segments). The highest average number of leaves was obtained by SBG3 at 0.5 ppm IAA (56.75 leaves). The highest average number of roots was obtained by SGB2 at 0.5 ppm PBZ (4.75 roots). The greenest leaf color was obtained by SBG7 at 1 ppm PBZ. The PBZ accelerated the emergence of shoots and roots and the number of roots.

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