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Application of Salicylic Acid and Gibberellic Acid Increase Stem Cutting Growth of *Pennisetum purpureum* cv. Mahasarakham and *Pennisetum purpureum* x *Pennisetum americanum*

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ABSTRACT

The Napier grass continues to be a popular forage crop in Nakhonsawan Province and other areas in the northern and east-northern parts of Thailand. Increasing the growth and biomass of Napier grass growing in low-quality sandy soil will increase the economic value of this plant. The stem cutting of two cultivars of Napier grass, cv. Pakchong 1 (*Pennisetum purpureum* x *Pennisetum americanum*) and sweet grass (*Pennisetum purpureum* cv. Mahasarakham), were exposed to two plant growth regulators, gibberellic acid and salicylic acid, and two application methods, soaking for 24 hr and watering after inoculation into the soil, were studied. For one plant growth regulator test, the most appropriate concentration of gibberellic acid and salicylic acid were 0.01 and 100 mg/L, respectively, for the soaking and watering method. There was an interaction between the combination of plant growth regulator type and application method for both cultivars. The best stimulation effect for sweet grass was soaking with 100 mg/L salicylic acids only. The response of Napier grass cv. Pakchong 1 was different. Soaking with 0.01 mg/L gibberellic acid and watering with 0.01 mg/L gibberellic acid was suitable for increasing shoot and root length while soaking with 0.01 mg/L gibberellic acid and watering with 100 mg/L salicylic acid was suitable for increasing fresh and dry weight. This information will be useful for stimulating the growth of Napier grass in agricultural purposes.

Keywords: Cutting, gibberellin, Napier grass, salicylic acid, soaking

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INTRODUCTION

Napier grass (*Pennisetum purpureum*) is a popular forage crop for economic ruminants in Thailand's central and northeastern parts. Napier grass has several advantages, such as high crude protein, high non-fiber carbohydrate, and low acid detergent fiber content, even though it grows in low soil quality. Also, feeding with sweet grass, the cultivar of Napier grass, decreases methane production from cows compared to feeding with rice straw (Mapato & Wanapat, 2018; Thongruang et al., 2021). Moreover, there are other effective applications of this grass. Napier grass was reported to be an effective choice for phytoremediation. Dwarf Napier grass has been reported to phytostabilize arsenic in soil (Chouychai & Somtrakoon, 2022; Kowitwiwat & Sampanpanish, 2020). This grass species is also reportedly used in the phytoremediation of petroleum hydrocarbon-contaminated soil (Ologidi et al., 2022). The fiber of Napier grass has been reported to be suitable for the paper industry (Daud et al., 2014; Reddy et al., 2014). Also, the lignocellulosic biomass of Napier grass is suitable for bioethanol production because of its low lignin content and relatively high mass per year and per area (Liong et al., 2013; Yasuda et al., 2014). Increasing Napier grass biomass in low soil quality will increase economic worth for all purposes.

The suitable cultivars and planting methods have been selected in many countries in Asia and Africa, but most of them study the number of nodes per stem cuttings (J∅rgensen et al., 2010; Ramadhan et al., 2015). However, using plant growth regulators (PGRs) to induce stem cuttings growth is another interesting method to increase biomass and tolerate unsuitable conditions for growth. Many PGRs have been used to induce plant growth in this condition. The foliar application of 100 mg/L salicylic acid (SA) to three flax cultivars growing in sandy soil could increase plant growth, photosynthetic pigment, phenolic content, and seed yield (Dawood et al., 2019). Also, mung bean plants growing in sandy soil and grown from seed soaking with 50 mg/L gibberellic acid (GA_3) for 12 hr contained a high amount of photosynthetic pigment and indigenous auxin (El Karamany et al., 2019). The application method is also an important step toward success. For example, spraying 100 mg/L salicylic acid on tomato leaves before exposing them to salinity was better than spraying leaves after salinity exposure for enhancing root growth, leaf proline, and soluble sugar content, and this pretreatment in leaves was also better than immersing the tomato seedling root in the same concentration for 30 min before exposing to salinity (Souri & Tohidloo, 2019). For these reasons, the plant growth regulator's appropriate type, concentration, and application method should be studied first in each condition.

Napier grass cultivation in Nakhonsawan Province, Thailand, is increasing, especially in some districts that often suffer from drought conditions, such as Nongbua and Phaisali districts. However, the soil in some areas of the Nongbua district is sandy and low in nutrient elements, making it difficult to cultivate economic crops. If Napier grass could grow well in this area, this would increase farmers' income. The possibility of using plant growth regulators to increase the growth of Napier grass growing in sandy soil was studied. The different concentrations and application methods of salicylic acid and gibberellic acid to different Napier grass cultivars growing in sandy soil were compared. There were two cultivars of Napier grass used in this study, Napier grass cv. Pakchong 1 (*Pennisetum purpureum* x *Pennisetum americanum*) and sweet grass (*Pennisetum purpureum* cv. Mahasarakham), a cultivar of dwarf Napier grass.

MATERIALS AND METHODS

Plant and Soil Preparation

Napier grass cv. Pakchong 1 (*Pennisetum purpureum* x *Pennisetum americanum*) and sweet grass (*Pennisetum purpureum cv*. Mahasarakham*)* were commercial stem cuttings from Nong Bo sub-district, Muang Ubon Ratchathani district, Ubon Ratchathani Province, Thailand. The five-month-old stem was cut to be similar to 24–26 cm/piece for Napier grass cv. Pakchong 1 and 6–8 cm/ piece for sweet grass. These stem cuttings were soaked in water for seven days after being received before being used. The soil used in this experiment was collected from the Nongklab sub-district, Nongbua district, Nakhonsawan Province, Thailand. This soil was air-dried at 28–31°C for at least 24 hr to a constant weight and sent to analyze the chemical and physical properties at the Central Laboratory (Thailand) Co. Ltd., Khonkean, Thailand. This soil texture is sand (87.79% sand, 9.39% silt, and 2.82% clay) with low nitrogen (6.1 mg/kg dry soil) and organic matter (15.1 mg/kg dry soil). The soil pH was 5.67, and available phosphorus and total potassium were 13.65 and 19.66 mg/kg dry soil, respectively.

Experimental Design

Single Plant Growth Regulator. There were two PGRs used in this study, salicylic acid (SA, purity 99.5%, KEMAUS, Australia) and gibberellic acid (GA_3, purity 90%, Sigma-Aldrich, Germany), and two application methods, immersion, and watering, were tested in this study. The experimental design was completely randomized (CRD) with one factor, the different concentrations of each plant growth regulator. After rooting, stem cuttings of Napier grass cv. Pakchong 1 and sweet grass were soaked in 0, 10, 50, and 100 mg/L SA or 0, 0.01, 0.1, and 1.0 mg/L GA_3 for 24 hr before being transferred to soil. The pots used in this experiment were 11.43 cm in diameter, each containing 1 kg of dry soil. The cuttings were inoculated vertically into the soil. There was one stem cutting per pot, 5 pots per treatment, and each pot was watered daily at 20 ml/pot. Another experiment was done with stem cutting watered with 0, 10, 50, and 100 mg/L SA or 0, 0.01, 0.1, and 1.0 mg/L GA_3 as 20 ml/pot on day 0 of the experiment instead of immersion. All pots were cultured in the nursery and received natural sunlight in shaded conditions until the end of the experiment. The plants from each treatment were collected on day 20th after planting to determine the plant growth parameters, including the number of leaves and stems per plant, shoot length, root length, shoot fresh weight, shoot dry weight, fresh root weight, and root dry weight. The ruler measured the length of the plant, and all plant weights were measured by digital balance. The root length/root dry weight and root dry weight/shoot dry weight ratios were calculated using the formulas described in Calvelo-Pereira et al. (2010) and Xu et al. (2018), respectively.

Combine Plant Growth Regulator. The experimental design was 3 x 3 factorial in CRD with 2 factors that were (1) soaking and (2) watering with different plant growth regulators. The plant growth regulators used in this experiment were 100 mg/L SA, 0.01 mg/L GA_3, and distilled water were used as controls. Each stems cutting of Napier grass cv. Pakchong 1 and sweet grass were soaked with 100 mg/L SA, 0.01 mg/L GA_3, or distilled water for 24 hr and combined with watering with 100 mg/L SA, 0.01 mg/L GA_3, or distilled water as 20 ml/pot on starting of the experiment. The details of the experiment are shown in Table 1. The cuttings were inoculated vertically into the soil. There was one stem cutting per pot, 5 pots per treatment, and each pot was watered daily at 20 ml/pot. The plants from each treatment were collected on day 30th after planting to determine the parameter described in the above experiment. The detail of the experimental design is shown in Table 1.

Table 1
The detail of the experimental design

Treatment	Soaking	Watering
1	Distilled water	Distilled water
2	Distilled water	100 mg/L SA
3	Distilled water	0.01 mg/L GA_3
4	100 mg/L SA	Distilled water
5	100 mg/L SA	100 mg/L SA
6	100 mg/L SA	0.01 mg/L GA_3
7	0.01 mg/L GA_3	Distilled water
8	0.01 mg/L GA_3	100 mg/L SA
9	0.01 mg/L GA_3	0.01 mg/L GA_3

Note. SA = Salicylic acid; GA_3 = Gibberellic acid

Statistical Analysis

One-way analysis of variance (ANOVA), two-way ANOVA, and least square difference (LSD) were used for variance analysis and pairwise comparison. One-way ANOVA was used for each plant growth regulator and method application in a single plant growth regulator experiment, while two-way ANOVA was used for combining plant growth regulator experiments.

RESULTS AND DISCUSSION

Effect of Single Plant Growth Regulator on Sweet Grass and Napier Grass cv. Pakchong 1

Exogenous salicylic acid soaking positively affected sweet grass's shoot and root growth. For example, the shoot length, shoot fresh weight, and shoot dry weight of cutting soaked in water were 13.7±0.25 cm, 6.3±0.28 g, and 3.3±0.13 g, respectively, while those of cutting soaked in 100 mg/L salicylic acid were 37.5±20.13 cm, 11.1±0.47 g, and 4.4±0.22 g, respectively (Table 2). Soaking with salicylic acid dramatically increases the shoot length of sweet grass, and the highest shoot length was seen at a cut soaked in 100 mg/L salicylic acid. However, 10 mg/L salicylic acid could statistically induce more shoot fresh and dry weight of sweet grass than other treatments. The response of sweet grass root to exogenous salicylic soaking was as same as shoot, but the highest induction was found in treatment soaking with 100 mg/L salicylic acid for root length and fresh weight, while the highest induction for dry root weight was 50 mg/L salicylic acid. Watering with salicylic acid also increases sweet grass's shoot and root growth. The shoot length, shoot fresh weight, and shoot dry weight of cutting watered with water were 13.7±0.25 cm, 6.3±0.28 g, and 3.3±0.13 g, respectively, while those of cutting watered with 100 mg/L salicylic acid were 21.5±0.28 cm, 11.5±0.32 g, and 5.2±0.34 g, respectively (Table 2). Watering with 100 mg/L salicylic acid is the best concentration for shoot fresh and dry weight, root length, and root dry weight, while 50 mg/L salicylic acid is the best for shoot length and root fresh weight. The number of shoots and leaves per stem cutting of sweet grass soaked with salicylic acid was 1.0–1.4 shoots/cutting and 7–8 leaves/cutting, respectively. Slightly watering with salicylic acid increased the number of shoots per stem cutting to 1.2–2.0 shoots/cutting, but the leaves per cutting were the same number from cutting soaked with salicylic acid (Figure 1).

Exogenous gibberellic acid soaking also increases sweet grass's shoot and root growth. For example, the shoot length, shoot fresh weight, and shoot dry weight of cutting soaked in water were 20.8±0.26 cm, 8.3±0.37 g, and 3.9±0.35 g, respectively, while those of cutting soaked in 0.01 mg/L gibberellic acid were 24.6±0.52 cm, 13.7±0.23 g, and 6.6±0.22 g, respectively (Table 2). However, the low concentration of gibberellic acid (0.01 mg/L) was better than other concentrations for all growth of sweet grass. Increasing gibberellic acid concentration trended to decrease sweet grass growth statistically, especially for root growth. This trend of sweet grass responding to gibberellic acid was seen when watering to cutting. For example, the shoot length, shoot fresh weight, and shoot dry weight of cutting watered with 0.01 mg/L gibberellic acid were 27.4±0.29 cm, 14.0±0.50 g, and 6.1±0.51 g, respectively, while those of cutting soaked in 1.0 mg/L gibberellic acid were 24.9±0.28 cm, 12.8 ±0.36 g, and 5.7±0.38 g, respectively (Table 2). Soaking or watering with gibberellic acid to sweet grass cutting was the same trend as salicylic acid. However, the shoots and roots of cuttings receiving salicylic or gibberellic acid were more robust than those receiving only water (Figure 1). This trend was correlated with decreasing specific root length after exposure to a plant growth regulator that indicated a thicker root than the control (Table 2).

Table 2

Growth of sweet grass soaked or watered with various concentrations of plant growth regulators

	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
Salicylic acid-soaking								
Distilled water	13.7±0.25c	6.3±0.28c	3.3±0.13c	20.3 ±0.27d	1.7±0.16c	0.2±0.10c	0.07±0.03c	1.24±0.24a
10 mg/L	17.7±0.44b	11.7±0.50a	5.4±0.14a	26.4±0.43c	2.6±0.12b	1.3±0.01b	0.24±0.01b	0.21±0.01b
50 mg/L	31.9±0.47a	10.0±0.53b	4.5±0.23b	31.9±0.36b	2.8±0.19b	1.7±0.04a	0.39±0.02a	0.19±0.00b
100 mg/L	37.5±20.13a	11.1±0.47a	4.4±0.22b	36.6±0.38a	4.5±0.18a	1.3±0.05b	0.30±0.02b	0.28±0.01b
Salicylic acid-watering								
Distilled water	13.7±0.25c	6.3±0.28c	3.3±0.13c	20.3 ±0.27d	1.7±0.16c	0.2±0.10c	0.07±0.03b	1.24±0.24a
10 mg/L	20.6±0.21b	7.6±0.50b	3.5±0.24bc	22.1±0.54c	2.9±0.23b	1.5±0.03b	0.44±0.04a	0.15±0.01b
50 mg/L	25.2±0.97a	7.7±0.41b	4.0±0.16b	33.9±0.08b	4.6±0.42a	1.6±0.05b	0.40±0.03a	0.21±0.01b
100 mg/L	21.5±0.28b	11.5±0.32a	5.2±0.34a	38.7±0.48a	5.1±0.28a	2.1±0.16a	0.41±0.03a	0.19±0.02b
Gibberellic acid-soaking								
Distilled water	20.8±0.26c	8.3±0.37c	3.9±0.35c	28.2±0.30b	2.0±0.12c	0.2±0.07d	0.06±0.02c	1.73±0.41a
0.01 mg/L	24.6±0.52a	13.7±0.23a	6.6±0.40a	30.4±0.36a	4.7±0.21a	2.6±0.16a	0.40±0.04a	0.12±0.01b
0.1 mg/L	25.3±0.40a	13.1±0.60a	5.0±0.26b	28.0±0.26b	2.4±0.22c	1.4±0.09c	0.28±0.02b	0.21±0.01b
1.0 mg/L	22.4±0.56b	10.3±0.42b	4.9±0.31b	28.2±0.51b	3.4±0.13b	2.1±0.08b	0.44±0.04a	0.14±0.01b
Gibberellic acid-watering								
Distilled water	20.8±0.26d	8.3±0.37d	3.9±0.35b	28.2±0.30b	2.0±0.12c	0.2±0.07b	0.06±0.024c	1.73±0.41a
0.01 mg/L	27.4±0.29a	14.0±0.50a	6.1±0.51a	31.7±0.51a	4.1±0.26a	2.4±0.06a	0.39±0.034a	0.13±0.00b
0.1 mg/L	22.9±0.55c	10.2±0.43c	5.2±0.30a	27.8±0.38b	2.7±0.13b	1.5±0.09b	0.29±0.021b	0.18±0.01b
1.0 mg/L	24.9±0.28b	12.8±0.36b	5.7±0.38a	28.9±0.29b	3.7±0.20a	2.2±0.10a	0.39±0.015a	0.13±0.01b

Note. The different lower-case letters showed significant differences (P<0.05) between different plant growth regulator concentration

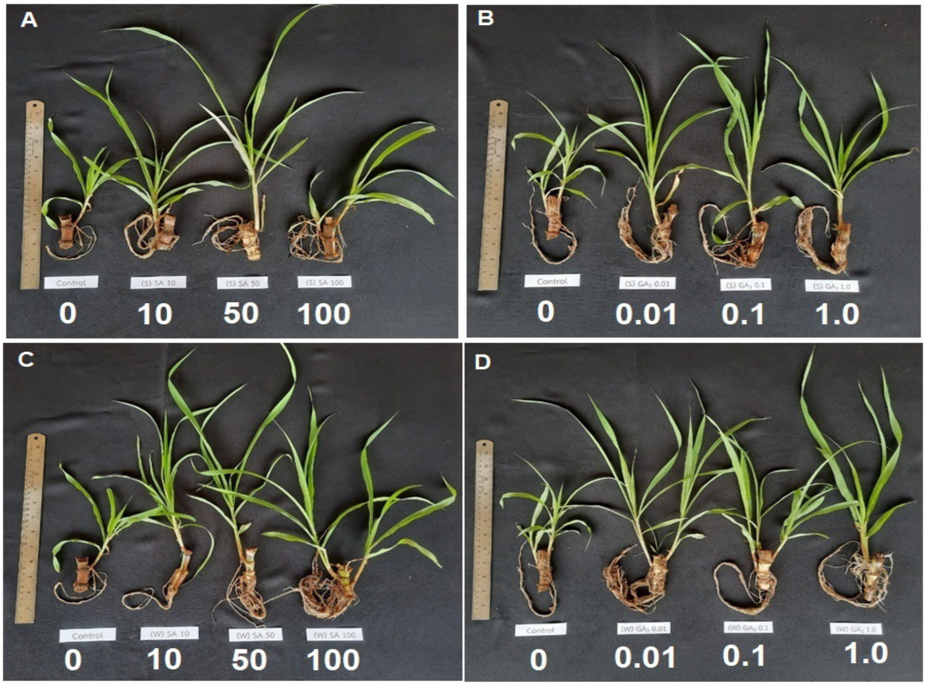

Figure 1. Shoot and root of Sweet grass soaking with various concentrations of SA (A) and GA_3 (B) or watering with various concentrations of SA (C) and GA_3 (D)
Note. SA = Salicylic acid; GA_3 = Gibberellic acid

Exogenous salicylic acid also soaking positively affected both shoot and root growth of Napier grass cv. Pakchong 1. For example, the shoot length, shoot fresh weight, and shoot dry weight of cutting soaked in water were 50.0±0.71 cm, 25.0±0.44 g, and 18.0±0.49 g, respectively, while those of cutting soaked in 100 mg/L salicylic acid were 51.8±0.49 cm, 27.5±1.91 g, and 23.3±0.38 g, respectively (Table 3). Soaking with 50 mg/L salicylic acid could increase shoot length and shoot dry weight of sweet grass, while only 100 mg/L salicylic acid could increase root length and weight of Napier grass cv. Pakchong 1. However, soaking with any concentration of salicylic acid could not induce the shoot fresh weight of Napier grass cv. Pakchong 1. Watering with salicylic acid did not stimulate Napier grass cv's shoot and root dry weight. Pakchong 1. Watering with 100 mg/L salicylic acid is the best concentration for shoot length and fresh weight, root length, and fresh weight. The shoot length, shoot fresh weight, root length, and root fresh weight of cutting watered with water were 50.0±0.71 cm, 25.0±0.44 g, 17.7±0.30 cm, and 1.73±0.03 g, respectively, while those of cutting watered with 100 mg/L salicylic acid were 59.6±0.63 cm, 29.0±0.95 g, 22.3±0.51 cm, and 2.48±0.06 g, respectively (Table 3). The number of shoots and leaves per stem cutting of Napier grass cv. Pakchong 1 soaked with salicylic acid were 1.0 shoots/cutting and 7–8 leaves/cutting, respectively. The shoots and leaves per cutting of cutting watered with salicylic acid were the same as those from soaked salicylic acid (Figure 2).

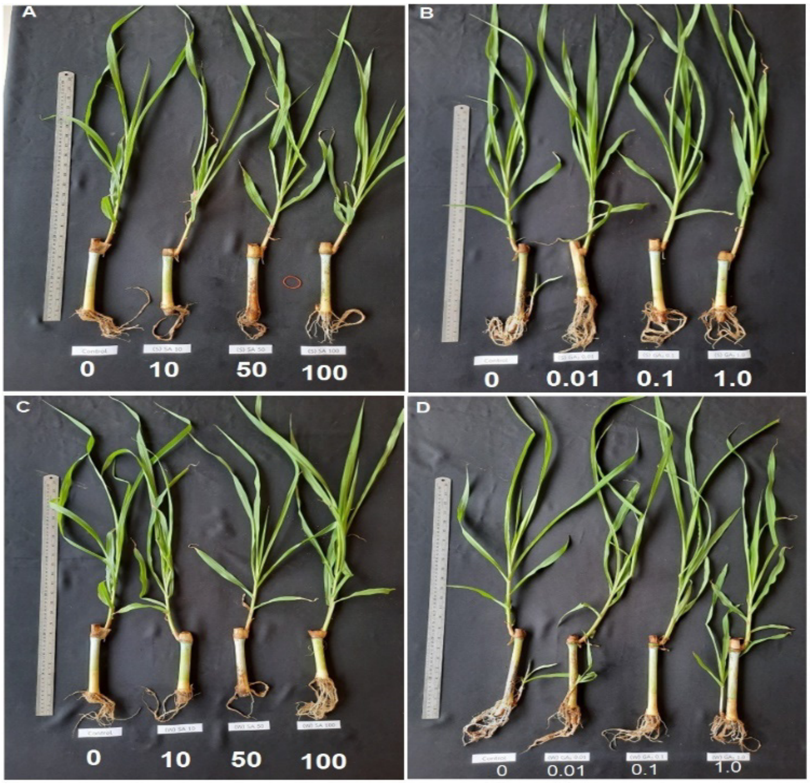

Figure 2. Shoot and root of Napier grass cv. Pakchong 1 soaking with various concentrations of SA (A) and GA_3 (B) or watering with various concentrations of SA (C) and GA_3 (D)

Note. SA = Salicylic acid; GA_3 = Gibberellic acid

Exogenous gibberellic acid soaking increases shoot and root growth of Napier grass cv. Pakchong 1. For example, the shoot length, shoot fresh weight, and shoot dry weight of cutting soaked in water were 52.9±0.64 cm, 24.4±0.92 g, and 20.1±0.28 g, respectively, while those of cutting soaked in 1.0 mg/L gibberellic acid were 66.6±1.50 cm, 29.5±0.79 g, and 23.6±0.47 g, respectively (Table 3). The high concentration of gibberellic acid (0.1-1.0 mg/L) could induce shoot growth, but the low concentration of gibberellic acid (0.01 mg/L) was better than other concentrations for root growth of Napier grass cv. Pakchong 1. The increase of gibberellic acid concentration trended to statistically decrease root growth of Napier grass cv. Pakchong 1. This trend of Napier grass cv. Pakchong 1 root responding to gibberellic acid was seen when watering to cuttings but watering with gibberellic acid did not induce shoot length and root fresh and dry weight. For example, the shoot fresh weight, shoot dry weight, and root length of cutting watered with water were 24.4±0.92 g, 20.1±0.28 g, and 17.7±0.17 cm, respectively, while those of cutting watered with 0.01 mg/L gibberellic acid were 31.7±0.71 g, 25.1±0.44 g, and 18.2±0.42 cm, respectively (Table 3). Soaking or watering with gibberellic acid to Napier grass cv. Pakchong 1 cutting was the same trend as salicylic acid. However, the shoots and roots of cuttings receiving salicylic acid or gibberellic acid were the same as those receiving only water (Figure 2). This trend correlated with the non-significantly different specific root lengths after exposure to a plant growth regulator that indicated the same root size in all treatments (Table 3).

Salicylic acid has been reported to enhance plant growth under water-limit conditions, including plants in the Poaceae family. Bermuda grass (*Cynodon dactylon*) exposed to 1 mM salicylic acid ameliorate adverse effects of water deficit (40% available water) condition on plant growth (Taheri et al., 2017). Also, 0.75–1.50 mM salicylic acid increases the growth of *Lolium perenne* cv. "Numan" exposed to drought stress (Hosseini et al., 2015). However, 200 mg/L salicylic acid increased the growth of Zoysia grass (*Zoysia* sp.) in normal conditions (Beiraghdar et al., 2014) (or in normal conditions, 200 mg/L salicylic acid increased the growth of Zoysia grass [*Zoysia* sp.]) (Beiraghdar et al., 2014). The concentration of salicylic acid and gibberellic acid used in this study has been reported to enhance plant growth in pollution-contaminated soil. For example, foliar spray with 0.5 mM salicylic acid could enhance the growth of *Sorghum bicolor* in cromium (Cr)-contaminated soil (Sihag et al., 2019). Also, 1.0 mg/L gibberellic acid could increase the root fresh weight of *Luffa acutangular* growing in polycyclic aromatic hydrocarbon (PAH)-contaminated soil (Somtrakoon & Chouychai, 2022b), but the concentration used in this study was lower than the concentration appropriate to increase okra seedling growth (5–10 μM) in the presence of 100 mM sodium chloride (NaCl) (Yakoubi et al., 2019).

In this study, both types of grass were not exposed to drought conditions but only planted in sandy soil with low water-holding capacity and nutrient content. The results showed that the appropriate concentration of salicylic acid and gibberellic acid to enhance Sweet grass growth, especially root growth, was 100 and 0.01 mg/L, respectively. The best response of the root was the main point for plant growth regulator selection in this experiment because the robust root is necessary for water and nutrient uptake in low-quality soil. The application via soaking seemed to be better than watering sweet grass in this study. The application method often affects plant response to the plant growth regulator, but the best method trend to depend on plant species, type of plant growth regulator, and environmental conditions. For example, corn seed soaking in 0.1 M GA_3 or IAA

Table 3

Growth of Napier grass cv. Pakchong 1 soaked or watered with various concentrations of plant growth regulators

	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
Salicylic acid-soaking Distilled water	50.0±0.71b	25.0±0.44a	18.0±0.49b	17.7 ±0.30b	1.73±0.03b	0.60±0.02b	0.03±0.001b	0.30±0.01b
10 mg/L	53.4±0.51ab	25.6±0.33a	16.7±0.22c	17.6±0.19b	1.62±0.01c	0.38±0.01c	0.02±0.001b	0.46±0.021a
50 mg/L	60.4±1.29a	28.5±0.54a	22.2±0.44a	17.0±0.42b	1.77±0.02b	0.68±0.04b	0.03±0.002b	0.25±0.019b
100 mg/L	51.8±0.49ab	27.5±1.91a	23.3±0.38a	24.9±0.19a	2.14±0.05a	1.01±0.06a	0.04±0.003a	0.25±0.016b
Salicylic acid-watering Distilled water	50.0±0.71b	25.0±0.44b	18.0±0.49a	17.7 ±0.30bc	1.73±0.03b	0.60±0.02a	0.03±0.001a	0.30±0.01a
10 mg/L	53.6±0.24ab	26.2±0.52b	16.4±0.93a	18.7±0.34b	1.75±0.04b	0.67±0.03a	0.04±0.003a	0.29±0.017a
50 mg/L	52.6±0.93ab	27.2±0.63ab	17.2±0.25a	17.1±0.29c	1.63±0.02b	0.40±0.03a	0.02±0.002a	0.44±0.046a
100 mg/L	59.6±0.63a	29.0±0.95a	17.2±0.35a	22.3±0.51a	2.48±0.06a	0.70±0.17a	0.04±0.010a	0.69±0.422a
Gibberellic acid-soaking Distilled water	52.9±0.64b	24.4±0.92b	20.1±0.28c	17.7±0.17b	1.75±0.03b	0.66±0.03b	0.03±0.002b	0.27±0.014b
0.01 mg/L	61.4±1.53b	25.9±0.59b	19.5±0.36c	24.9±0.66a	2.49±0.09a	0.96±0.02a	0.05±0.002a	0.26±0.008b
0.1 mg/L	70.1±1.82a	28.8±0.40a	21.8±0.47b	18.0±0.42b	1.78±0.04b	0.56±0.04c	0.02±0.002c	0.33±0.023a
1.0 mg/L	66.6±1.50a	29.5±0.79a	23.6±0.47a	17.1±0.26b	1.68±0.03b	0.48±0.02d	0.02±0.001c	0.36±0.019a
Gibberellic acid-watering Distilled water	52.9±0.64a	24.4±0.92c	20.1±0.28c	17.7±0.17a	1.75±0.03a	0.66±0.03a	0.03±0.001a	0.27±0.014b
0.01 mg/L	50.0±5.96a	31.7±0.71b	25.1±0.44a	18.2±0.42a	1.78±0.03a	0.46±0.02b	0.02±0.000b	0.42±0.009a
0.1 mg/L	65.0±0.69a	32.6±0.67b	23.8±0.38b	16.5±0.58b	1.64±0.03b	0.36±0.02c	0.02±0.001b	0.45±0.039a
1.0 mg/L	56.1±1.76a	35.9±0.82a	25.9±0.62a	15.7±0.38b	1.65±0.04b	0.38±0.02c	0.02±0.001b	0.41±0.030a

Note. The different lower-case letters showed significant differences ($P<0.05$) between different plant growth regulator concentration decreases Pb accumulation in plant tissue compared with 0.1 M GA_3 or IAA foliar spray when growing in 800 mg/kg lead (Pb) contaminated soil (Hadi et al., 2010). Seed soaking in 1 mg/L indole-3-butyric acid (IBA) increased shoot and root fresh weights of ridge gourd more than watering with 1 mg/L IBA when growing in 100 mg/kg phenanthrene and 100 mg/kg pyrene contaminated soil (Somtrakoon et al., 2022b). On the other hand, seed soaking with 0.01 mg/kg thidiazuron (TDZ) decreased shoot and root dry weight of corn growing in 46.8 mg/L hexachlorocyclohexane (HCH) contaminated soil when compared with corn watering with 0.01 mg/kg TDZ (Chouychai et al., 2015). The appropriate concentration of salicylic acid and gibberellic acid for the growth of Napier grass cv. Pakchong 1 was the same as sweet grass, 100 and 0.01 mg/L, respectively. This judgment was based on the best of plant root response also. There was more similarity between different application methods than sweet grass. However, the combination of plant growth regulator type and application method was studied further in the next experiment, with only the most appropriate concentration selected from this study.

Effect of Combination of Plant Growth Regulators on Sweet Grass and Napier Grass cv. Pakchong 1

Two factors of plant growth regulation application, soaking and watering, affected all Sweet grass growth traits. There was a significant interaction between soaking and watering with different plant growth regulators on all the growth traits of sweet grass. For the soaking factor, soaking with salicylic acid increases shoot length and shoot fresh weight, shoot dry weight, and root dry weight more than other plant growth regulators while soaking with gibberellic acid increases root-to-shoot ratio more than other factor levels. Also, watering with salicylic acid increased all growth traits of sweet grass more than other factor levels (Table 4).

The combination of plant growth regulators showed that the growth of sweet grass cutting soaked with salicylic acid is better than soaking with gibberellic acid or water. For all soakings with any plant growth regulator, sweet grass watering with salicylic acid or gibberellic acid increases root growth more than watering with water (Figure 3). Soaking with salicylic acid and watering with water increases sweet grass's shoot and root growth more than watering with other plant growth regulators. For example, the shoot and root length of sweet grass soaking with salicylic acid and watering with water were 48.8±0.41 and 33.8±0.41 cm, respectively, while the shoot and root length of sweet grass soaking with salicylic acid and watering with gibberellic acid were 42.4±0.70 and 30.8±0.70 cm, respectively (Table 5).

Two factors of plant growth regulation application, soaking and watering, affected all Napier grass cv. Pakchong 1 growth traits. Only specific root length did not affect by the soaking factor (or the soaking factor did not affect specific root length). There was a significant interaction between soaking and watering with different plant growth regulators on all growth traits of Napier grass cv. Pakchong 1. For the soaking factor, soaking with salicylic acid increases shoot length, shoot fresh weight, shoot dry weight, and root dry weight more than other plant growth regulators, while soaking with gibberellic acid increases root fresh weight more than other factor levels. Also, watering with salicylic acid increased all growth traits of Napier grass cv. Pakchong 1 more than other factor levels (Table 6).

The response of Napier grass cv. Pakchong 1 to plant growth regulator application differed from sweet grass's application. The combination of plant growth regulators showed that the growth of Napier grass cv. Pakchong 1 cutting soaked with gibberellic acid, although watered with any plant growth regulator, is better than soaking with salicylic acid or water (Figure 4). The root's efficiency in producing shoot biomass increases when soaking with gibberellic acid, considering

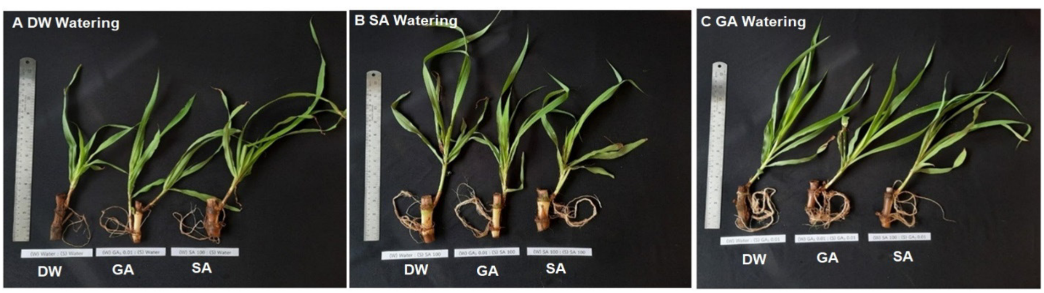

Figure 3. Shoot and root of Sweet grass watering with water (A) or 100 mg/L SA (B) and 0.01 mg/L GA_3 (C) in combination with water, 100 mg/L SA, and 0.01 mg/L GA_3 soaking

Note. SA = Salicylic acid; GA_3 = Gibberellic acid

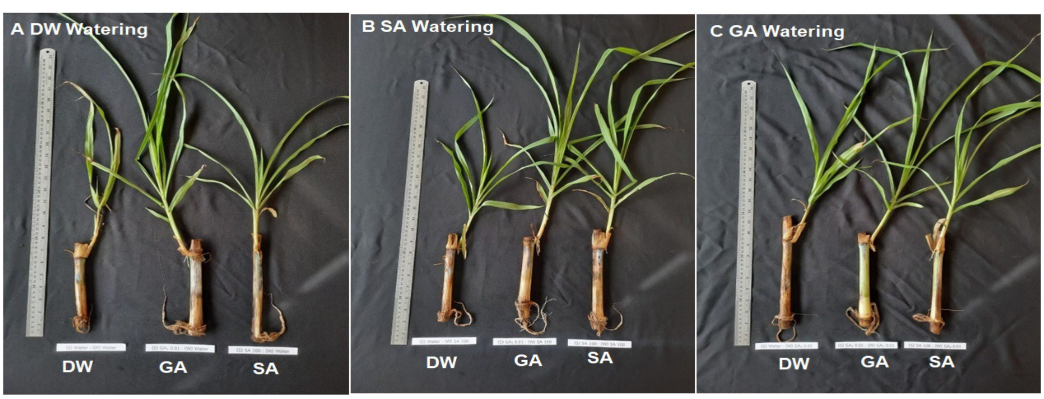

Figure 4. Shoot and root of Napier grass cv. Pakchong 1 watering with water (A) or 100 mg/L SA (B) and 0.01 mg/L GA_3 (C) in combination with water, 100 mg/L SA, and 0.01 mg/L GA_3 soaking

Note. SA = Salicylic acid; GA_3 = Gibberellic acid

Table 4

The main effect of plant growth regulators on sweet grass growth traits

	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
Soaked (factor 1)								
with water	40.3±0.38c	16.4±0.26b	10.0±0.32b	27.2±0.36b	2.0±0.10b	1.1±0.35b	0.13±0.01b	0.28±0.01a
with SA	45.7±0.38a	22.1±0.26a	16.4±0.32a	32.4±0.36a	2.7±0.10a	1.5±0.35a	0.10±0.01c	0.22±0.01b
with GA_3	43.3±0.38b	14.2±0.26c	6.5±0.32c	31.4±0.36a	2.1±0.10b	1.2±0.35b	0.19±0.01a	0.26±0.01a
F-test	**	**	**	**	**	**	**	**
Watered (factor 2)								
with water	41.9±0.38b	16.3±0.26c	10.6±0.32b	31.3±0.36a	2.4±0.10a	1.2±0.35b	0.15±0.01a	0.30±0.01a
with SA	45.7±0.38a	19.2±0.26a	12.7±0.32a	30.5±0.36a	2.2±0.10a	1.4±0.35a	0.12±0.01b	0.23±0.01b
with GA_3	41.7±0.38b	17.2±0.26b	9.6±0.32c	29.1±0.36b	2.2±0.10a	1.3±0.35b	0.15±0.01a	0.23±0.01b
F-test	**	**	**	**	ns	*	*	**
F-test Soaked x Watered	**	**	**	**	**	**	**	**

Note. Different lower-case letters show significant differences within each factor; ns, *, ** = Non-significance (P>0.05), statistical significance (P<0.05), and high statistical significance (P<0.01) of each factor, respectively; SA = Salicylic acid; GA_3 = Gibberellic acid

Table 5

Shoot and root growth of Sweet grass stimulated with various application methods of plant growth regulators (PGRs)

	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
Immerse with water								
Watered with water	32.2±0.73cC	7.5±0.16cC	3.52±0.26bC	24.0 ±0.35cC	1.29±0.13aC	0.57±0.04cC	0.09±0.018bB	0.19±0.04bB
Watered with SA	51.5±0.79aA	19.9±0.43bB	12.64±0.68aB	26.2±0.46bC	2.23±0.08aAB	1.52±0.08aA	0.12±0.009aB	0.17±0.010cB
Watered with GA_3	37.2±0.56bC	21.7±0.30aA	13.75±0.59aA	31.3±0.51aA	2.34±0.09aA	1.33±0.10bA	0.10±0.006aC	0.24±0.017aA
Immerse with SA								
Watered with water	48.8±0.41aA	26.7±0.75aA	21.58±0.94aA	33.8±0.41aB	3.44±0.29aA	1.85±0.04aA	0.09±0.005bB	0.18±0.003bC
Watered with SA	46.0±0.42bB	24.6±0.34bA	18.94±0.52bA	31.3±0.51bB	2.68±0.19aA	1.52±0.04bA	0.08±0.003bC	0.21±0.007bB
Watered with GA_3	42.4±0.70cB	15.0±0.56cB	8.57±0.47cB	30.8±0.70bA	2.04±0.20aA	1.20±0.04cA	0.14±0.008aB	0.27±0.017aA
Immerse with GA_3								
Watered with water	44.8±0.37aB	14.7±0.42aB	6.60±0.24aB	36.2 ±0.41aA	2.34±0.12aB	1.28±0.06aB	0.20±0.013aA	0.28±0.012aA
Watered with SA	39.5±1.14bC	13.0±0.40bC	6.46±0.49aC	34.1±0.33bA	1.81±0.25aB	1.08±0.02bB	0.17±0.014aA	0.32±0.005aA
Watered with GA_3	45.5±0.39aA	14.9±0.45aB	6.57±0.46aC	23.9±0.48cB	2.18±0.19aA	1.28±0.08aA	0.20±0.022aA	0.19±0.013bB

Note. The different lower-case letters showed a significant difference between different PGR watering within the same PGR immersion; Different capital letters showed significant differences between different PGR immersions within the same PGR watering; SA = Salicylic acid; GA_3 = Gibberellic acid

Table 6

The main effect of plant growth regulators on Napier grass cv. Pakchong 1 growth traits

	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
Soaked (factor 1)								
with water	40.3±0.38c	16.4±0.26b	10.0±0.32b	27.2±0.36b	1.4±0.02c	1.1±0.35b	0.049±0.001a	0.24±0.01a
with SA	45.7±0.38a	22.1±0.26a	16.4±0.32a	32.4±0.36a	1.7±0.02b	1.5±0.35a	0.036±0.001b	0.24±0.01a
with GA_3	43.3±0.38b	14.2±0.26c	6.5±0.32c	31.4±0.36a	1.8±0.02a	1.2±0.35b	0.031±0.001c	0.25±0.01a
F-test	**	**	**	**	**	**	**	ns
Watered (factor 2)								
with water	41.9±0.38b	16.3±0.26c	10.6±0.32b	31.3±0.36a	1.4±0.02b	1.2±0.35b	0.040±0.001a	0.24±0.01b
with SA	45.7±0.38a	19.2±0.26a	12.7±0.32a	30.5±0.36a	1.8±0.02a	1.4±0.35a	0.039±0.001a	0.26±0.01a
with GA_3	41.7±0.38b	17.2±0.26b	9.6±0.32c	29.1±0.36b	1.8±0.02a	1.3±0.35b	0.036±0.001b	0.24±0.01b
F-test	**	**	**	**	**	**	**	*
F-test Soaked x Watered	**	**	**	**	**	**	**	**

Note. Different lower-case letters show significant differences within each factor; ns, *, ** = Non-significance ($P>0.05$), statistical significance ($P<0.05$) and high statistical significance ($P<0.01$) of each factor, respectively; SA = Salicylic acid; GA_3 = Gibberellic acid

Table 7

Shoot and root growth of Napier grass Pakchong 1 stimulated with various application methods of plant growth regulators

	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Root-to-shoot ratio	Specific root length (m/g)
Immerse with water								
Watered with water	55.8±0.37bC	18.6±0.36cC	11.4±0.65cC	13.2 ±0.94cC	1.1±0.03cC	0.54±0.03cB	0.05±0.00aA	0.24±0.02aA
Watered with SA	66.3±0.54aC	21.5±0.45bC	14.7±0.36bC	21.1±0.43aA	1.5±0.04bB	0.76±0.02bB	0.05±0.00aA	0.28±0.10aA
Watered with GA_3	66.4±0.60aC	24.1±0.70aC	18.0±0.32aB	17.1±0.48bB	1.8±0.04aA	0.84±0.02aA	0.05±0.00aA	0.20±0.01bA
Immerse with SA								
Watered with water	61.8±0.20cB	20.9±0.36cB	16.0±0.23cB	17.1±0.29bA	1.4±0.03cB	0.70±0.02bA	0.04±0.00aA	0.24±0.01aA
Watered with SA	71.2±0.58bB	30.5±0.86bB	24.2±0.35bB	21.5±0.27aA	1.9±0.05aA	0.82±0.02aAB	0.03±0.00bB	0.26±0.01aA
Watered with GA_3	74.0±1.09aB	34.5±1.16aB	25.4±0.22aA	17.9±0.29bB	1.7±0.04bB	0.76±0.02abB	0.03±0.00bB	0.23±0.01aA
Immerse with GA_3								
Watered with water	86.0±0.89bA	35.1±0.99cA	23.6±0.24cA	15.5 ±0.50cB	1.7±0.01bA	0.70±0.02bA	0.03±0.00aB	0.22±0.01bA
Watered with SA	85.6±0.40bA	42.9±0.83aA	27.0±0.30aA	20.8±0.46bA	1.9±0.02aA	0.85±0.06aA	0.03±0.00aB	0.25±0.02abA
Watered with GA_3	88.2±0.97aA	37.3±0.52bA	25.5±0.37bA	23.1±0.24aA	1.8±0.04bA	0.82±0.02aAB	0.03±0.00aB	0.28±0.01aA

Note. The different lower-case letters showed a significant difference between different PGR watering within the same PGR immersion; Different capital letters showed significant differences between different PGR immersions within the same PGR watering the root-to-shoot ratio (Table 7). Also, soaking with gibberellic acid and watering with gibberellic acid increased the shoot and root length of Napier grass cv. Pakchong 1 more than watering with other plant growth regulators. For example, the shoot and root length of Napier grass cv. Pakchong 1 soaked with gibberellic acid and watered with gibberellic acid were 88.2±0.97 and 23.1±0.24 cm, respectively, during the shoot and root length of Napier grass cv. Pakchong 1 soaked with gibberellic acid and watered with salicylic acid were 85.6±0.40 and 20.8±0.46 cm, respectively (Table 7). However, soaking with gibberellic acid and watering with salicylic acid increase the fresh and dry weight of Napier grass cv. Pakchong 1 more than watering with water and gibberellic acid. For example, the shoot and root fresh weight of Napier grass cv. Pakchong 1 soaked with gibberellic acid and watered with gibberellic acid were 37.3±0.52 and 1.8±0.04 g, respectively, during the shoot and root fresh weight of Napier grass cv. Pakchong 1 soaking with gibberellic acid and watering with salicylic were 42.9±0.83 and 1.9±0.02 g, respectively (Table 7).

The two cultivars of Napier grass respond to a combination of plant growth regulators in different ways. Salicylic acid soaking is appropriate to enhance the growth of sweet grass, but watering with salicylic acid or gibberellic acid after planting decreases the growth of sweet grass except at fresh root weight. The appropriate method for sweet grass increased the 513% of dry shoot weight when compared with treatment without plant growth regulator application. Napier grass cv. Pakchong 1 responds positively to gibberellic acid after soaking and watering with gibberellic acid again, increasing shoot and root length more than other treatments. However, watering with salicylic acid enhanced the fresh and dry weight of Napier grass cv. Pakchong 1 more than soaking with gibberellic acid only. The appropriate method for Napier grass cv. Pakchong 1 increased 137% of shoot dry weight and 58% of shoot length compared to treatment without plant growth regulator application. It is normally of gibberellic acid activity to increase the plant's internode length and shoot length (Graebe, 1987). Also, salicylic acid could increase the fresh weight of the plant. A Foliar spray of 0.01 mM salicylic acid on corn growing in acidic soil could increase the shoot fresh weight of corn (Somtrakoon & Chouychai, 2022a). Salicylic acid also increased the photosynthesis capacity of wheat exposed to heat stress via interaction with proline and ethylene metabolism (Khan et al., 2013). This result showed that it is possible to increase the biomass of Napier grass growing in sandy soil via soaking and watering with a plant growth regulator after planting. However, the physiological and biochemical responses within plant tissue should be studied further to explain the activity and interaction of salicylic acid and gibberellic acid in different cultivars.

CONCLUSION

The appropriate plant growth regulator to stimulate the growth of Napier grass depends on its cultivar. Soaking the stem cutting with 100 mg/L salicylic acid is the only method appropriate to stimulate the length and weight of sweet grass. Soaking with 0.01 mg/L gibberellic acid and watering with 100 mg/L salicylic acid increases the weight of Napier grass cv. Pakchong 1, while soaking with 0.01 mg/L gibberellic acid and watering with 0.01 mg/L gibberellic acid, increases the length of this cultivar.

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