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Adaptation Strategy of Stevia in the Lowlands Through Pruning and Eco Enzyme Based on Morphofisiology Characteristics

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Abstract. High solar radiation and temperature in lownlands can accelerate stevia's life cycle, prompting earlier flowering and reducing nutritional content. Pruning is expected to stimulate lateral shoot growth, increasing branch count and consequently plant productivity. Additionally, enzymes in eco enzyme glycosides, which provide wetness. This study aimed to understand the adaptation of stevia grown in lownland areas with shoot pruning and eco enzyme treatments. The research was conducted on agricultural land at USU (3°33' S, 98°39' E, 28 m asl) from July to September 2024, using a randomized block design with two treatment (pruning and eco enzyme) and three replications. Results showed that pruning at 4 WAP (weeks after planting) delayed flowering up to 78 DAP (days after planting), increased total chlorophyll a and b, and tended to increase leaf number, branch-count, and canopy width, though these effects were not significant. Pruning at 8 WAP improved the root-to-shoot ratio. Eco enzyme application at 1 ml/L significantly affected the root-to-shoot ratio, and the best treatment combination was pruning at 4 WAP with eco enzyme at 1 ml/L. In conclusion, pruning and eco enzyme treatment improved stevia's adaptability and productivity under lowland conditions.

1. Introduction

Stevia (*Stevia rebaudiana*) is a in the Asteraceae family native to Paraguay, whose leaves can be used as natural sweetener. The Stevia plant is classified as a medicinal plant because it contains stevioside, rebaudioside A and steviol with a sweetness level 300 times sweeter than sucrose [1]. Stevia can be used as a medicine for diabetes, hypertension and obesity. The use of stevia in Indonesia already exists but is not yet optimal, so the potential for using stevia is still very large. The stevia market in 2021 will reach 675.22 million US dollars with growth of around 10% per year and is projected to reach 1.1 billion US dollars in 2026. This could be an opportunity to develop stevia as a sugar substitute and fill the global stevia market share.

Stevia is a food that has several uses which has certain physiological functions that are beneficial for health. Stevia leaves are known as a sweetener that is low in calories compared to cane sugar, so it is good for diabetes sufferers to consume because it has a zero glycemic index [2]. The sweet taste of stevia comes from stevioside which is a glycoside composed of glucose, supphorose and steviol. Stevioside, rebaudiosides (A, B, C, D, E, F, M, and N), steviol biosides rubusoside, and dulcoside (A-B) are among the steviol glycoside chemicals that are present in the stevia plant [3]. The leaf extract of stevia has been reported to contain higher levels of antioxidants (48.18 µg/ml) compared to ascorbic acid (10.12 µg/ml) [4].

The problem concerning the cultivation of stevia is its rapid flowering. Stevia will tend to experience a decrease in its stevioside content of up to 50% after flowering [5]. Therefore, pruning is carried out on the shoots so that branches form and the production of more leaves. Shoot pruning will maximize lateral shoot growth and balance the formation of new branches, which will likely increase the plant's productivity. Pruning can increase the production of 1.36

tonnes/ha (2010) and 1.59 tonnes/ha (2011) or 13 and 17%, each compared to non stagnation, respectively [6]. Increasing productivity after plants are pruned can be done by administering eco enzyme. Enzymes can act as biocatalysts that can speed up chemical reactions such as metabolism in plants. Use eco enzyme as a liquid organic fertilizer it can be used as a growth stimulant and improves the taste profile and quality of produce. This is due to the presence of enzymes such as trypsin, amylase, organic acids such as acetic acid and several plant nutrients such as N, P and K, as well as containing lactic acid bacteria which play a role in plant growth [7]. Based on the above statement, the author would like to conduct research regarding adaptaion strategy of *Stevia rebaudiana* in the lownlands through pruning and eco enzyme based on morphology characteristics.

2. Research Methods

2.1 Study Area

The study was carried out in the USU agricultural field (3°33 LS, 98°39 BT, 28 M above sea level) from July to September 2024. The study was carried out at the Faculty of Agriculture, University of Sumatra Utara, Jl. Dr. A. Sofian No.3, Padang Bulan, Kec. Medan Baru, Medan, 20155, North Sumatra, Indonesia.

Figure 1. research site and map location

2.1 Procedures

The research was carried out on research land using polybags with a capacity of 10 kg and using paranet with a density of 50%. The production of eco enzyme was carried out before the research was carried out, which required a fermentation time of 3 months. Flowering age, number of leaves, number of branches, root-shoot ratio, and canopy width were among the morphological observations. The physiological characters observed included chlorophyll a, b, and total. Chlorophyll content was calculated based on wavelengths of 649 and 665 nm using a spectrophotometer.

2.3 Data Analysis

A group randomized design with two treatments and three repetitions was used for the experiment. First factor is pruning: Without pruning, pruning in 4 WAP, pruning in 8 WAP. Second factor is eco enzyme consentration: 0,1,2, and 3 ml/l. The analysis results were further tested using DMRT Test.

3. Results and Discussion

3.1 Morphologycal observation of flowering age, number of leaves, number of branches, root shoot ratio, and canopy width

Shoot pruning has no significant effect on the flowering time of stevia plants. The longest flowering was found in the pruning treatment at 8 WAP and the administration of 2 ml/l eco enzyme. Harvest time is related to production and the contents of the plant. The rapid flowering causes Stevia planted in the lowlands to flower more quickly so production is lower compared to the highlands [8]. Stevia is quicker to harvest when the plants are still short and have low

biomass. However, if harvesting is done after the flowering period has passed, it can reduce the amount of sugar in stevia leaves [9].

Table 1. Flowering age, number of leaves and branches, root shoot ratio, canopy width of shoots pruning and eco enzyme treatment

Treatment	Flowering age	Number of leaves	Number of branches	Root shoot ratio	Canopy widht
	--day--	*--pcs--*	*--pcs--*	*--g--*	*--cm--*
Without pruning shoots	56.50	52.14	20.61	1.89b	27.08b
Pruning 4 WAP	71.17	61.72	23.08	4.21a	37.38a
Pruning 8 WAP	71.83	61.00	22.42	6.67a	34.59a
Without eco enzyme	64.11	55.63	21.00	1.98c	33.40
Eco enzyme 1 ml/l	69.67	61.89	21.11	7.84a	37.56
Eco enzyme 2 ml/l	66.89	53.93	23.07	4.37b	30.47
Eco enzyme 3 ml/l	65.33	61.70	22.96	2.82c	30.64
Without pruning + without EO	55.33	43.22	17.22	1.14h	27.00
Without pruning + EO 1 ml/l	59.67	60.89	19.89	1.89df	31.67
Without pruning + EO 2 ml/l	56.00	47.00	22.11	2.90d	24.13
Without pruning + EO 3 ml/	55.00	57.44	23.22	1.62fg	25.53
Pruning 4 WAP + without pruning	66.33	62.44	24.56	2,69d	38.40
Pruning 4 WAP + EO 1 ml/l	77.67	63.44	20.44	3.39cd	44.17
Pruning 4 WAP + EO 2 ml/l	71.00	56.11	25.33	5.11bc	34.73
Pruning 4 WAP + EO 3 ml/l	69.67	64.89	22.00	5.63b	32.20
Pruning 8 WAP + without EO	70.67	61.22	21.00	2.12d	34.80
Pruning 8 WAP + EO 1 ml/l	71.67	61.33	21.11	18.26a	36.83
Pruning 8 WAP + EO 2 ml/l	73.67	58.67	21.78	5.09bc	32.53
Pruning 8 WAP + EO 3 ml/l	71.33	62.78	23.67	1.20gh	34.20

Pruning shoots significantly increases the number of leaves of a stevia plant. The results showed that the shoot pruning treatment aged 4 WAP produced a higher number of leaves. Stevia plants tend to respond by increasing the growth of new shoots which contributes to an increase in the number of leaves. Pruning in the early vegetative phase can stimulate better lateral growth resulting in more leaves [10]. Pruning in the vegetative phase is better than the generative phase. This is because pruning in the generative phase can reduce the plant's ability to produce assimilate [11]. Application eco enzyme with a concentration of 1 ml showed a significant increase in the number of leaves. Eco enzyme has the amylase enzyme which supports plant growth with the help of the amylase enzyme producing the gibberellin hormone which functions to stimulate the leaf organs in plants [12].

Pruning carried out at 4 weeks of age, which is still in the early vegetative phase of plant growth, can stimulate better growth and higher yields. Pruning serves to control the growth of shoot roots and accelerate the growth of lateral shoots, both of which support the development of additional branches on the main stem [13]. Stevia is a type of plant that has the ability to quickly produce shoots or branches [14]. When entering the flowering phase, you will see abundant twig growth, especially on the stem near the top of the plant. Number of branches of stevia plants that were applied *eco enzyme* with a concentration of 3 ml increased significantly. In contrast to the treatment without eco enzyme administration, the grant eco enzyme at a concentration of 10 ml/L also shown a high number of branches [15]. The ability of eco enzymes to promote the synthesis of the hormones auxin and cytokinin, which are crucial for cell elongation and division, is assumed to be the cause of this rise in branch count.

The root shoot ratio showed a significant effect in the treatment without pruning. The root crown ratio reflects the balance between the crown and root parts. Plants with a higher root shoot ratio tend to photosynthesize more efficiently. This research shows that treatment without pruning increases resistance to environmental stress. Stating that in the lowlands with increasing temperatures, the rate of respiration in the shoots and roots exceeds the rate of photosynthesis taking place, resulting in limited carbohydrates in the soil [16]. In this situation, the roots have a lower priority compared to the shoot, so that more carbohydrates accumulate in the shoot organs than in the roots. A large root crown ratio indicates that more of the plant's assimilate results are used by the plant for crown growth than roots. One important factor for plant growth is the root shoot ratio which shows the plant's ability to absorb nutrients [17].

Although the canopy width parameters of stevia plants were larger after trimming at 4 WAP, there was no discernible difference between the pruning treatment at 8 WAP. This shows that the plant growth response is better in the initial phase of pruning. In the 4th week, the stevia plant is still in an active vegetative phase so pruning can stimulate the growth of new shoots and increase the number of leaves, thereby contributing to a larger canopy width. Pruning carried out on plant shoots is useful in reducing rivalry between fruit and leaves for photosynthesis and can trigger the growth of shoots towards the side or lateral shoots [18].

3.2 Physiological observations of chloropyll a, b, and total

Table 2. Chlorphyll a,b, and total of shoots pruning and eco enzyme treatment

Treatment	Cholorpyll a	Chloropyll b	Total Chloropyll
	---mg/g ---	*--mg/g-*	*-mg/g-*
Without pruning shoots	28.44ab	38.36b	66.80b
Pruning 4 WAP	27.51b	44.47a	71.98a
Pruning 8 WAP	28.63a	37.48b	66.11b
Without eco enzyme	28.20	40.21	68.41
Eco enzyme 1 ml/l	27.71	42.51	70.22
Eco enzyme 2 ml/l	28.04	41.42	69.46
Eco enzyme 3 ml/l	28.83	36.27	65.10
Without pruning + without EO	28.91	35.95	64.86
Without pruning + EO 1 ml/l	27.51	43.44	70.95
Without pruning + EO 2 ml/l	28.73	37.22	65.95
Without pruning + EO 3 ml/	28.61	36.83	65.44
Pruning 4 WAP + without pruning	27.68	43.56	71.24
Pruning 4 WAP + EO 1 ml/l	26.95	46.74	73.69
Pruning 4 WAP + EO 2 ml/l	27.42	46.55	73.97
Pruning 4 WAP + EO 3 ml/l	27.99	41.04	69.04
Pruning 8 WAP + without EO	28.01	41.12	69.12
Pruning 8 WAP + EO 1 ml/l	28.67	37.35	66.01
Pruning 8 WAP + EO 2 ml/l	27.97	40.51	68.47
Pruning 8 WAP + EO 3 ml/l	29.88	30.95	60.83

Description: Numbers followed by different letters indicate significant differences according to Duncan's Multiple Range Test at level = 5%, WAP (Weeks after Planting).

Pruning shoots on stevia plants has an effect on increasing the chlorophyll a, b and total content in the leaves. This is because pruning can affect the balance of plant metabolism thereby increasing the chlorophyll content. The pruning treatment at plant age 4 weeks after planting showed higher chlorophyll b and total content than other treatments, while the chlorophyll a content was higher in the pruning treatment at 8 WAP. This shows that pruning time has a significant influence on the chlorophyll content in stevia leaves. Increased photosynthetic

efficiency, positive response to pruning and better growing conditions in the early phase of plant growth. It is known that pruning treatment 14 DAT gives the best results on chlorophyll content. This is because after pruning there is competition for photosynthate between newly formed plant parts such as shoots or branches so that the absorption of photosynthate in the leaves is higher compared to plants that have not undergone pruning [19].

Chlorophyll b and total were higher when pruning at 4 WAP because in the early growth phase the plant was more responsive to pruning. Pruning at this age can stimulate shoot growth and increase the number of leaves which contributes to increasing chlorophyll. The increase in chlorophyll a content at 8 WAP pruning was caused by better chlorophyll accumulation in later growth phases. At this age, plants are more mature and can synthesize chlorophyll a.

4. Conclusions

Pruning shoots could slow down stevia flowering by up to 78 DAP in the 4 WAP pruning treatment. Pruning at 4 WAP increases total chlorophyll a, b and tends to increase the plant canopy's width, number of branches, and number of leaves, although the effect is not significant. In addition, pruning at 8 WAP can increase the ratio of stevia root shoots. The root shoot ratio is significantly impacted by 1 ml/l of eco enzyme. The best combination of pruning and eco enzyme treatment is 4 MST pruning and 1 ml/l eco enzyme.

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