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Article

The role of Indole-3-butyric acid, folic acid, yeast, and some extracted organic in stimulating the rooting of stevia cuttings

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ABSTRACT
To stimulate the rooting of the cuttings of the stevia plant, the experiment was conducted in pots on 1/3/2020. An apical cutting has been taken from the stems of one-year-old plants containing three nodes while keeping one pair of leaves on the cuttings, and in a CRD design and the arrangement of Split-Plots Design, The first factor included two levels. They are both wounding and non-wounding. The second factor included seven treatments: indolebutyric acid, folic acid, bread yeast, cinnamon, aloe vera gel, garlic extract, and comparison. And five observations for each treatment and four recurrences. The results showed that the wounding treatment was significantly superior to all rooting traits. The percentage increase
The non-wounding treatment was 72.12, 28.27, 31.09, 23.74, and 31.26% for rooting percentage, root length, number of roots, surface area, and dry weight, respectively. Indolebutyric acid and aloe vera gel were significantly superior to all other treatments in rooting percentage and without significant difference, with an increase of 213.00 and 170.88%, respectively, in comparison treatment. In root length, aloe vera gel, garlic extract, yeast and indole butyric treatments were superior. The garlic extract, indole butyric, aloe vera and yeast treatments were significantly superior in the number of roots. The treatments of yeast and aloe vera gel were significantly superior to the surface area of the roots, while in the dry weight of the roots, the treatments of indole butyric and yeast were significantly superior to all other treatments. There was no significant interaction between the study factors in all traits.

Keywords: aloe vera gel, cinnamon, garlic extract

INTRODUCTION

Stevia rebaudiana Bertiona is a small shrub native to Paraguay whose leaves contain approximately 10% stevioside compounds, which are intensely sweet compounds estimated to be 150 to 300 times sweeter than sugar [1]. Stevia has become a major source of highly effective sweeteners, as it produces a sweet taste but has no calories. Steviol glycosides are superior to artificial sweeteners

due to their unique features, such as being non-carcinogenic, non-toxic, hypoallergenic, antimicrobial and containing antioxidants [2,3] Vegetative reproduction is a form of asexual reproduction, Which depends on the presence of multicellular parts (organs) in the plant that can lose differentiation after separating them or cutting them from the original and forming new genetically similar individuals of selected plant origin [4,5] Such organs may include cuttings, bulbs, tubercles, rhizomes, chromes, stems, and others. The ability to regenerate Various plant organs has been invested through several techniques used in agricultural practices to multiply the parent plants (mothers). One of these methods is) cutting), which can also be resorted to when there is a problem in the seed germination percentage. Wounding aims to break the sclerenchyma ring at the sites of the wounding, thus facilitating the emergence of roots from these sites, as the sclerenchyma ring consists of durable (solid) fibrous cells in the cortex and has thick walls outside the root formation area [6] ،Garlic Allium sativum L. It contains a lot of active substances, volatile oils, 25%, containing amino acids with a high percentage of sulfur, such as Cysteins and Methionine, 46% protein and 47% salts, and contains vitamins such as vitamin C as well as mineral salts such as sulfur, phosphorous and manganese. Iodine, calcium, and potassium Aloe vera Aloe belong to the family Asphodelaceae [7,8,9]. The parts used are the thick, lubricated leaves from which the gel containing the anthraquinone derivatives is extracted [10]. Aloe vera gel contains polysaccharides, glucose and mannose, as well as tannins, enzymes, plant hormones (gibberellin, salicylic, and methionine, which is the initiator of ethylene synthesis), amino acids and vitamins such as C [11], and minerals such as calcium [12,13,14,15,16,17] Yeast is microbes from the kingdom of single-celled fungi. Yeast extracts contain proteins, amino acids, nucleic acids, vitamin B and other scarce elements. They are used industrially in the fermentation of pastries [18]. Yeast produces a large number of vitamins, amino acids, and plant hormones such as auxin, cytokinin, gibberellin [19,20,21,22] and Auxin is known to stimulate cuttings to roots, as well as cytokines and gibberellins in newly formed roots [23]. Cinnamomum Zeylanicum, whose bark contains calcium and coumarin [24], which are important factors in rooting the cuttings [25]; as for the other ingredients, they contain fatty acids and amino acids, as well as minerals including magnesium, phosphorous, potassium, iron, copper, and zinc, in addition to containing sugar. Folic acid, or the so-called folacin or pteroylglutamic acid with the molecular formula C19H19N7O6, is a yellow crystalline substance that is insoluble in organic solvents with a chemical composition consisting of two heterogeneous rings known as Pteridine linked with a methylene bridge to P-amino benzoic acid with glutamic acid [26], discovered by Kogel and Haagen-smith 1934The use of auxins is one of the factors that push the cuttings towards rooting and one of the oldest and fastest methods. Indolebutyric is considered the best growth regulator because its decomposition is relatively slow in the plant and has slow transmission. Most of it remains in the treated area, and it is non-toxic to the plant and does not cause collateral damage[27]. Indolebutyric acid promotes the formation, growth, and development of adventitious roots and increases the rate of root formation [28,29].

Materials and methods

Stevia cuttings were prepared from one-year-old plants and the apical cuttings containing three [30,31,32,33.34] With one pair of leaves remaining on the cutting [30] the cuttings were cut at the bottom diagonally and dipped [35,36] in the extracts for 15 minutes [31,33] ,The cuttings were planted directly after the procedures were carried out in plastic containers containing the agricultural medium, which consisted of a mixture of river sand washed several times to ensure that it was free of suspended salts and peat moss in a ratio of 1:1, it was

found that its Electrical conduction EC is equal to 0.6, and its irrigation was taken into account before planting the cuttings to moisten the middle with the pressure of the agricultural medium by hand to stabilize the cuttings well to prevent them from moving and after the completion of planting, the pots were arranged according to the design followed by the CRD design in with a scientific experiment in four recurrence and included the following transactions:

The first factor in the two levels

1- Wounding the lower end of the cutting 2- without wounding

The ends of the cuttings are flooded, each according to his treatment, and they are:-

1. Indolebutyric acid (IBA) at a concentration of 500 mg L^{-1} [30] IBA was obtained from the British company Sigma with a weight of 500 mg and dissolved in a certain amount of distilled water using a Magnetic Stirrer Hotplate device and after making sure that the substance is completely dissolved, the volume was completed to one liter.

2-Folic Asad: was obtained from the Indian Marine Drive Company; 250 mg liter of 1 was taken from it and dissolved in distilled water. A Magnetic Stirrer Hotplate was used for the purpose of dissolving the substance, and the volume was completed with 1 liter of distilled water

3- Yeast: The dry yeast produced by the Turkish company Lesaffre, available in the local markets, was prepared. The cuttings were moistened with distilled water and dipped in dry yeast powder

4- Cinnamon: dry cinnamon bark was taken and then ground into a grinder device, and a fine powder was obtained. A 1:1 solution was prepared; the cuttings were dipped, extracted, and planted in the pots.

5- Aloe Vera Gel: Leaves were taken from the aloe vera plant and the gel was extracted from them and then used by rooting the cuttings by immersing their ends in it.

6-Garlic extract: 250 gm of garlic cloves collected from local markets were fetched, placed with 250 ml of distilled water, and mixed with a Blender. The resulting solution was filtered with two pieces of muslin cloth, so we have a 100% effective solution, 2001) and the required concentration was prepared from it. 10 ml of the solution was added to one liter of distilled water.

7- Comparison treatment

Traits of the measured root growth

The cuttings were extracted from each treatment and placed on a plastic sieve under the tap to remove the soil stuck to the roots, then the roots were dried by air after placing them on blotting paper [37] The following measurements were taken

Percentage of rooting

Calculated from the number of rooted cuttings / the number of transplanted total cuttings x 100

Root length (cm

The root length was measured by using the graduated ruler from the root growth area on the cutting to the root end of each plant of the measured experimental unit, and the average was calculated

The average number of total roots of the plant (cutting root)

Calculate the average according to the number of total roots for each cutting of the measured experimental unit cuttings.

The surface area of plant roots (cm^2 cutting cm^2)

The surface area of the roots of the cuttings was calculated using the Digitizer program. The extracted roots were photographed after placing them on a whiteboard, and a sign was used to indicate a distance of a known length (10 cm) with a colored line drawn next to the root. The image was transferred to the Digimizer program on the computer, and the areas occupied by the root were determined after Marking them [38].

The Average dry weight of the root system of the plant (GM plant

The roots of the cuttings were carefully extracted from each experimental unit, air-dried, and placed on the cardboard with continuous stirring at a ventilated room temperature until the weight was stable. Then, the dry weight of the plant was calculated, and the average was extracted.

Results

Rooting percentage

Table 1 shows that the wounding treatment significantly increased the rooting rate than the treatment without wounding, with an increase rate of 72.12%. This shows the great effect of wounding in stimulating the cuttings to form roots, as it works on three sidesThe first is mechanical, and it is represented in tearing the strong sclerenchyma bands surrounding the area of root formation, which hinders their emergence even if they are emerging. Thus, it is easy to penetrate the detected roots of the sclerenchyma ring and then through the cortex and epidermis to reveal the roots. As for the second aspect, it is vital. The wounding produces the required callus to produce root tissues that differ in their specialization from the original tissues (leg tissues). And stimulating cell division to form root initiators along the edge of the wound, and this stimulation may be due to the accumulation of important hormones in rooting, namely Auxin, ethylene and jasmonic, as well as the accumulation of energy represented by carbohydrates in the wounding area and an increase in the respiratory rate in the New metabolic sink area that was created. This aspect provided hormonal stimulation and an energy source for forming adventitious roots. The third aspect concerns the water balance. The wounded cuttings absorb more water from the growth medium than the nonwounded cuttings, allowing them to remain alive and physiologically active until they can form roots specialized in the absorption process. Wounding also allows for a higher uptake of growth regulators or other compounds prepared for the base of the cutting.

The treatments of indole butyric and aloe vera gel were significantly superior to all other treatments without a significant difference between them, and the treatments of folic, yeast and garlic extract were significantly superior to the comparison and cinnamon treatments, which did not differ significantly from each other.

Folic has an Auxinic action that encourages the formation of methionine and stimulates cell division and the formation of nucleic acids. These roles played by Folic are all in the interest of encouraging rooting in the cuttings. Yeast contains plant hormones such as oxino, cytokinin, gibberellin, amino acid, methionine, and carbohydrates, whose importance in rooting has already been explained. There was no significant interaction between the study factors.

Middle	Compari-son	Garlic extract	Alovera gel	Cinna-mon	Bread yeast	Folic acid	IBA	
59.9	27.5	65.0	75.0	25.0	66.7	66.7	93.3	**Wounding**
34.8	20.0	30.0	53.3	20.0	30.0	35.0	55.0	**Non-woundi ng**
6.25		Non						**AFM**
	23.7	47.5	64.2	22.5	48.3	50.8	74.2	**Average**
	11.69							**AFM**

Table 1. The effect of the study factors on the percentage of surface area of the roots ($cm^2.plant^{-1}$)

Root length

Table 2 shows that the wounding treatment significantly increased the length of the roots with a rate of 28.27% compared to the treatment without wounding.

Yeast and aloe vera treatments were significantly superior to the folic acid, cinnamon, and comparison treatments. They did not differ from the indolebutyric acid and garlic treatments. Indolebutyric acid was significantly superior to the comparison and cinnamon treatments and without a significant difference to the folic acid and garlic treatments. The latter two were significantly superior to the comparison and cinnamon treatments, without significant difference on indole-butyric treatment.

Yeast contains protein, mineral elements, and amino acids, and its production of toxins, gibberellin, and cytokinin indicates that they encourage plant growth, and the root may be the first beneficiary. Aloe vera gel contains methionine (the initiator of ethylene synthesis), which is required for root growth and disclosure. Early and active root growth may be important in increasing their length. It is known that auxin has an important role in developing transverse roots and improving their quality, and IBA improves root growth characteristics. This may be due to the polarization of carbohydrates and compounds involved in rooting to the base of the cuttings where the roots originate or their role in stimulating enzymes that play an important role in the emergence of transverse roots on the cutting, or the content of rooting inhibitors may be high in the cuttings and when using synthetic auxins, rooting increases due to the high percentage of auxin to the extent effective of rooting.

Middle	Compari-son	Garlic extract	Alovera gel	Cinna-mon	Bread yeast	Folic acid	IBA	
4.28	1,48	4.64	6.41	1.76	6.04	4,84	4.77	**Wounding**
3.07	1.52	3,97	3.66	1.08	4.33	3.05	3.86	**Non-woundi ng**
0.529		Non						**AFM**
	1.50	4.30	5.03	1.42	5.18	3.95	4.32	**Average**
	0.989							**AFM**

Table 2. The effect of the study factors on the length of the roots (cm).

Number of roots

Table 3 shows that the wounding treatment significantly increased the number of roots than the treatment without wounding, with a percentage of 31.09%. The number of roots followed the rooting rate in an increase, which may be due to the similarity of the requirements for obtaining both. We can find that the mechanical effect has provided more space for the emergence of root initiators along the wound line, In addition to the effect of reducing the obstruction caused by the sclerenchyma ring, and this applies to stimulating the wounding to form a callus, which is a source of unspecialized cells needed by the initiators of roots, and collecting the important hormones in increasing the number of roots (auxin, ethylene and jasmonic). The collecting of energy represented by carbohydrates in the wounding area and an increase in the respiratory rate represents a hormonal stimulus to increase the number of roots and provide the necessary energy for this. We do not neglect the role of water balance; the wounded cuttings absorb more water from the growth medium than the non-wounding cuttings, making it physiologically active and able to increase the number of root initiators.

The treatment of garlic and indole was significantly superior to that of Folic, cinnamon, and comparison. However, there was no significant superiority among the treatments of garlic extract, indole, aloe vera and yeast, and the treatment of indole did not differ significantly from yeast and aloe vera gel and the treatment of aloe vera was superior to the cinnamon and the comparison and did not differ from the treatment of folic and yeast. The latter did not differ significantly from the folic and it was superior to the treatment of cinnamon and the comparison. The treatment of Folic was significantly superior to the comparison and did not differ significantly from cinnamon, and the latter was superior to the comparison.

These plant hormones act as factors to attract processed foodstuffs and lead to a balance of physiological and biological processes and an increase in the process of carbon metabolism in the area of their addition to the cutting. As for aloe vera gel, it contains polysaccharides, glucose, and mannose As well as plant hormones (gibberellin, salicylic, and amino acid methionine, which is the initiator of ethylene synthesis), vitamins such as C, and minerals such as calcium, it also contains cinnamic acid which goes through multiple steps of the synthetic chains to form auxin IAA, the latter of which is known to stimulate rooting. There was no significant interaction between the two study factors.

Middle	Comparison	Garlic ex-tract	Alovera gel	Cinna-mon	Bread yeast	Folic acid	IBA	
9.55	4.25	12.14	11.38	5.81	11.33	8.53	13.39	**Wounding**
6.58	1.46	10.48	8.65	5.16	7.03	6.09	7.15	**Non-woundi ng**
1.403		Non						**AFM**
	2.86	11.31	10.01	5.49	9.18	7.31	10.27	**Average**
	2.625							**AFM**

Table 3. The effect of the study factors on the number of roots (cutting root)

The surface area of plant roots ($cm^2.plant^{-1}$)

Table 4 shows that the wounding treatment increased the surface area of the roots significantly more than the treatment without wounding, at a rate of 23.74%. The increase in root length(table 2) and their numbers (table 3), a significant increase in wounding treatment reflected in the increase in the surface area of the roots.

Table 4 shows a significant difference between the treatments of the secondary factor, as the yeast treatment was significantly superior to all treatments except for the aloe vera gel treatment. It was followed by the aloe vera gel treatment, which was significantly superior to folic acid, garlic, cinnamon and the comparison and did not differ significantly from indole butyric and the latter was significantly superior to the comparison treatment and did not differ significantly from the treatments of folic acid, garlic extract, cinnamon and the comparison, no significant difference was shown between the folic acid treatment.

Returning to Table 2, we find that the first three treatments that are superior in root length (yeast, aloe vera and indole) are the same superior in surface area (Table 4), and to some extent in the number of roots (Table 3), as they entered the garlic treatment without a significant difference among the four treatments (Garlic - Indole - Aloe Vera – Yeast). Yeast contains many vitamins, amino acids, and plant hormones (auxin, cytokinin, and gibberellin), which encourage plant cell growth and division. These plant hormones attract processed nutrients and lead to a balance of physiological and biological processes and an increase in the process of carbon metabolism in the area they are added to the cuttings. Hence, the roots' surface area increases due to increasing their lengths or diameters. Aloe vera gel, which contains polysaccharides, glucose and mannose, which are carbon sources for building root tissues, as well as plant hormones (gibberellin, salicylic and amino acid, methionine, which is the initiator for the manufacture of ethylene) as well as cinnamic acid that goes through multiple steps of the synthetic chains is to form auxin IAA. The latter is known By stimulating the growth of roots. Auxin is one of the stimuli of root growth and emergence and affects several genes that lead to an increase in the growth of the transverse roots of the plant. There was no significant interaction between the study factors.

Middle	Comparison	Garlic extract	Alovera gel	Cinna-mon	Bread yeast	Folic acid	IBA	
2.786	2.143	2.870	3.180	2.332	3.547	2.777	2.652	**Wounding**
2.125	1.305	1.775	2.815	1.612	2.955	1.880	2.535	**Non-woundi ng**
0,1182		Non						**AFM**
	1.724	2.322	2.997	1.972	3.251	2.329	2.594	**Average**
	0.2211							**AFM**

Table 4. The effect of the study factors on the surface area of the roots ($cm^2.plant^{-1}$)

Root dry weight

Table 5 shows that the wounding treatment increased the dry weight of the roots significantly more than the treatment without wounding by a rate of 31.26% and that the increase in the rooting percentage (Table 1) and the number of roots (Table 3) was followed by an increase in the dry weight of the roots of the stevia plant. Wounded tissues stimulate cell division, and this is due to the natural accumulation of auxins, carbohydrates and other substances in the wound areas, which leads to an increase in the rate of respiration and the production of ethylene, which has an important role in stimulating the formation of transverse roots. Returning to the characteristics of roots, such as rooting percentage, root length, number, and surface area (Tables 1, 2, 3 and 4), we find that wounding has increased all of these characteristics, and as a result, the dry weight of the roots has increased.

The table shows the significant superiority of indolebutyric and yeast treatments over all other treatments and the significant superiority of the treatments of Folic, garlic, and aloe vera over the cinnamon and comparison treatments. Indolebutyric acid is known to be one of the best growth regulators for rooting because its decomposition and transmission are slow in plants, and most of it remains in the treated area. It is low toxicity in high concentrations, effective for a long time, and does not break down if exposed to light. In addition, it encourages the formation, growth and development of the initiators of transverse roots and an increase in the rate of formed roots. Yeast produces auxin, gibberellin and cytokinin. These hormones have a role in cell division and expansion and thus increase living mass. And yeast has an important role in releasing CO2 gas, which affects carbon metabolism, leading to an increase in the rate of carbohydrates. Carbohydrates are a main source of energy and carbon structures during Rooting for their presence in high concentrations in most cuttings. Folic acid has an auxin action and is capable of forming methionine (the initiator of ethylene) because the action of auxin causes cell division, and the subsequent cellular breakdown is caused by ethylene, both of which are necessary for the formation of roots. It is known that the dry mass is the outcome of the growth process that occurs in plant tissues and their ability to retain this mass. There is no significant interaction between the two factors of the study.

Middle	Comparison	Garlic extract	Alovera Gel	Cinna-mon	Bread yeast	Folic acid	IBA	
3.550	1.525	3.475	3.125	1.900	5.532	3.525	5.76	**Wounding**
2.443	1.200	2.300	1.850	1.100	4.000	2.600	4.050	**Non-wounding**
0.3809		Non						**AFM**
	1.363	2.888	2.488	1.500	4.766	3.062	4.908	**Average**
								AFM

Table 5. The effect of the two study factors on the dry weight of the roots*(mg root)*

DISCUSSION

The percentage increase in rooting can be seen, Which amounted to 213.00 and 170.88% in the treatments of indole and aloe vera gel, respectively, over the comparison treatment, auxin is one of the most important catalysts for the rooting process, and it exhibits multiple ways in its effect on plants, such as its effect on several genes that lead to an increase in the growth of adventitious roots of plants, as well as an increase in the activity of the vascular cambium and a high level of RNA in the cuttings. Thus, increased cell division at the height of the rooting process of the cutting[39]. This is in line with what was found by [30]. The Auxin treatment was significantly superior in the rooting percentage of stevia cuttings. Aloe vera gel contains polysaccharides, glucose, mannose, andones (gibberellin, salicylic acid, and methionine, which initiates ethylene production). Among the accompanying and stimulating factors for rooting, such as vitamin C, calcium, and cinnamic acid, which go through the tryptophan synthesis chain, from which the Auxin IAA is formed, the latter is known to stimulate rooting [40] stated that gibberellins stimulate root formation. Therefore this multifaceted composition stimulates rooting at multiple levels.

The concentration of hydrogen peroxide (H_2O_2) increased by double rates after hours of cutting, (H_2O_2) is one of the simulators for the emergence of transverse roots when it is present with auxin and plays an important role in releasing chemical signals that increase the plant's tolerance to biotic and abiotic stresses [41]. in addition to an increase in the level of total soluble sugars in the areas of cuts or wounds [42]. These sugars supply the cells with the energy compounds necessary for divisions and the formation of transverse roots, as well as the gathering of important hormones in increasing the growth and length of roots, thyroxin, and ethylene, as well as increasing the respiratory rate in the wounding area. These factors may contribute to an increase in the length of the roots in the wounding treatment than in the non-wounding treatment. Or the wounding may be from the emergence of roots on the cuttings, which gave them a longer period for the roots to grow and increase their length.

The result agreed with the findings of [43] and [30] that IBA increased the root length of the stevia plant significantly more than the comparison treatment. Garlic contains methionine, the initiator of the production of phytohormone ethylene, known to stimulate the growth and formation of roots, in addition to containing tryptophan, the initiator of Auxin production. It has a major role in the formation of transverse roots, and it affects the biological processes associated with the formation of roots and the transport of carbohydrates to the root growth area. It may be the enzymes related to carbohydrates. The DNA synthesis and RNA

transcription are affected by auxin. There was no significant interaction between the study factors.

Garlic extract contains methionine, which initiates the production of the plant hormone ethylene, stimulating the growth and formation of roots. Forty-four states ethylene is important in early cell regulation to rooting initiators in bean cuttings. Also, the Auxin act that stimulates cell division is irregular in the absence of ethylene. Also, the preparation of the plant with ethylene in the absence of external auxin makes the divisions very little, and thus, there is a lack of rooting[22]. Since garlic contains the amino acid Tryptophan [45] and it is known to be the initiator of auxin production that is known to affect rooting, calcium is also one of the factors accompanying the rooting process[22]. It is expected that the factor affecting rooting may withdraw its effect in increasing the number of roots due to the similarity of the requirements of their occurrence. Indolebutyric acid encourages the formation, growth, and development of Transverse root initiators and increases the rate of roots formed [28,29], This is in agreed with[46] who found an increase in the number of roots of the stevia plant cutting when using indolebutyric acid. Yeast produces a large number of vitamins and amino acids, the most important of them all being phytohormones (oxino, cytokinin, and gibberellin), which are stimulators of plant cell growth and division, and yeast extract contains calcium, and treatment with yeast enhances the ability of the plant to retain water and enhances antioxidants as well as enhancing the metabolism process inside the plant [47].

It is known that the surface area of rooting increases by increasing the numbers of the root, their lengths, and their diameters, or altogether. The damaged tissues due to the wounding may stimulate the production of the hormone ethylene, which is known to encourage the formation of roots, or it may be since the wounded tissues may be stimulated and divided, forming the initiators of the roots and then roots appeared densely along the length of the wound, or it may be due to the accumulation of auxins and carbohydrates and increased breathing in the area of the wound [48] all of these things lead to an increase in the number of roots and consequently the surface area of them increases accordingly.

Yeast treatment increases the plant's ability to retain water[48], which means an increase in the resulting cells' filling, increasing their size and surface area, and enhancing the plant's metabolism process.

Moreover, it is highly effective in encouraging the cuttings to form roots (Table 1) and works to release the free IAA that the cuttings need in the Rooting process [48] This is agreed with what[46] and [50] indicated that indolebutyric gave the highest average root weight in stevia cuttings[51].

CONCLUSIONS

It can be said that wounding is a very important factor in increasing the indicators of root growth of the stevia cuttings and that the treatment of this cutting with indolebutyric acid, aloe vera, garlic extract, and yeast has increased the indicators of root growth.

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