

Indole -3- Butyric Acid Induces Plant Regeneration From Stem Cuttings Of Three Medicinal Plants

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Abstract

Field regeneration of three (3) medicinal plants - *Securidaca longepedunculata* (violet tree), *Ocimum gratissimum* (scent leaf) and *Pterocarpus mildebraedii* by means of rooting of stem cuttings at different lengths of 10 -15 cm and 15 -20 cm were studied. The effect of the plant hormone Indole-3-Butyric acid (IBA) at 5g/l was tested on rooting, bud sprout and leaf formation of the rooted stem cuttings of the plant species as well as the effect of the length of the stem cuttings on their growth and survival rates. The results of the study revealed that stem cuttings of *O. gratissimum* treated with IBA and the control treatment sprouted within 5 - 8 days. The treated *O. gratissimum* stem cuttings produced a slightly higher number of buds (2.58 ± 0.86) when compared to the control (2.00 ± 0.89) with no significant difference at $P \leq 0.05$. Stem cuttings of the treated *P. mildbraedii* cuttings sprouted within 8 -10 days with 1.40 ± 0.37 number of buds, with the control showing no sprouts. Consequently, IBA application produced more leaves (13.00 ± 5.58) and roots (1.33 ± 0.01) than the control. However, *O. gratissimum* stem cuttings had the highest leaf (13.08 ± 4.47) and root numbers (135.00 ± 13.45) followed by the control with values- 13.00 ± 5.58 for leaf development and 61.66 ± 2.34 for root number respectively. Irrespective of the treatments, none of the stem cuttings of *S. longepedunculata* sprouted. This study showed that exogenous application of IBA to stem cuttings of the tested plants, except *S. Longepedunculata*, improved the root number, number of leaves and number of buds.

Keywords: Stem cuttings, Indole-3-Butyric acid (IBA), Rooting

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Introduction

From time immemorial, plants have been an important source of food, fibre and medicine (Nweze *et al.*, 2004; Thangavel *et al.*, 2014). According to the World Health Organization (WHO), about 50% of people still rely primarily on traditional remedies such as herbs for their medicine and 65% of Indian populace domicile in rural areas use traditional form of medicine to

meet their primary health needs (Alice *et al.*, 2014). Of the medicinal plant species, about 10% are cultivated while the larger proportion (90%) are obtained from the wild. The domestic cultivation of medicinal plants is a well-accepted way of producing plant materials both for conservation and other relevant purposes (Tandon *et al.*, 2009). However, harvesting from the wild poses serious challenges as was seen in cases of many plant species that became

threatened and this led to the loss of genetic diversity and habitat destruction, disrupting biodiversity which is the store house of species richness (Afolayan and Adebola, 2004; Kuldeep *et al.*, 2012). A lot of resources have been channelled into curbing the loss of biodiversity and various protocols have been successfully established. Unfortunately, not much improvement has been noticed (Tripathi, 2008). Increased use of forest land for other purposes, over exploitation and unscientific collection of flora of medicinal and aromatic plants have enormously depleted the existence and massive demand of medicinal plants (Alice *et al.*, 2014). Due to improved agronomic and medicinal traits, it has been reported that the development of regeneration methods is of major importance in medicinal plant biotechnology. Plant genetic resources are the major biological basis of world food security which, to a great extent, support every life on the planet earth. Hence, conservation of such a buffer is considered fundamental and provides priority in all sectors of global development (Tandon *et al.*, 2009). Plants are totipotent in nature, hence plant biotechnology takes advantage of this property of plant development to solve the issue of decreasing plant biodiversity (Slater *et al.*, 2003). When living organisms are injured or lose parts of their bodies, many are able to regenerate new tissues or organs to minimize the impact of the local damage (Ikeuchi *et al.*, 2016), but the mode of this regeneration varies markedly among taxa (Birnbaum and Sánchez-Alvarado, 2008). The influence of plant growth regulators on plant regeneration has been reported in several medicinal plants by different researchers (Faridah *et al.*, 2011, Sanoussi *et al.*, 2012, Onyeulo *et al.*, 2018). Regeneration is a widely conserved physiological response in both animals and plants (Pulianmackal *et al.*, 2014) and has long been utilized for clonal propagation in the form of cutting and grafting (Melnyk and Meyerowitz, 2015). The regenerative capacity of plant cells can be enhanced when explants are soaked in solutions of plant hormones (Chupeau *et al.*, 2013) with explants from juvenile plants regenerating shoots more effectively than those from the mature plants (Zhang *et al.*, 2015). Indole-3-Butyric Acid (IBA), a plant hormone in the "auxin" group is naturally abundant in potato tuber peels (Ludwig, 2000). It is applied exogenously and has been used successfully as a

plant growth regulator promoting rooting (Hunt *et al.*, 2011) and exerting different effects on plant growth and development, e.g. regulating responses of plants against biotic and abiotic stresses (Tognetti *et al.*, 2010). Treating cuttings with auxins increases the percentage of rooting, root initiation, number of roots and uniformity of rooting (Elhaak *et al.*, 2015).

Securidaca longipedunculata (Violet tree) of the family *Polygalaceae* is a medicinal herb indigenous to Africa (Alafe *et al.*, 2014) occurring in a broad range of vegetation ; it is resistant to bush fires but frost sensitive (Donald *et al.*, 2011). *S. longipedunculata* have been employed traditionally for the treatment of various diseases like paralysis (Dawit *et al.*, 2003; Soumen *et al.*, 2012), inflammation (with analgesic properties) (Alafe *et al.*, 2014), infectious diseases (Ojewole *et al.*, 2000), among others. On the other hand, *Ocimum gratissimum* (African basil) is a perennial herbaceous, drought-tolerant plant (Aguiyi *et al.*, 2000; Elizabeth *et al.*, 2012). In Nigeria, it is found in the Savannah and coastal areas and it is called with various local names such as 'Efinrin' in Yoruba, 'Diadoyal' in Hausa and 'Nchuanwu' in Igbo (Owulade, 2004). It has been used in the treatment of various ailments including diabetes mellitus (Aguiyi *et al.*, 2000), rheumatism, paralysis, epilepsy, high fever, sunstroke, influenza, gonorrhoea and mental illnesses (Soumen *et al.*, 2012). It has anti-hyperglycemic effects (Simon and James, 2007), so it is transformed to herbal tea and used as anti-diabetic therapy (Mohammed *et al.*, 2007). *Pterocarpus mildbraedii* of the family *Fabaceae* is a green leafy vegetable locally known as "Oha ojii" in eastern Nigeria (Rosemary *et al.*, 2015). According to Alice *et al.*, (2014) and Ngwuli *et al.*, (2019), it is indigenous to Africa. Its leaves are used for food and medicinal purposes. It is endowed with antioxidant, hypocholesterolemic, chemo-protective and anti-bacterial properties (Rosemary *et al.*, 2015).

Reports on the methods of regenerating *S. longipedunculata* Fresen using the stem cuttings are lacking. *P. mildbraedii* Harms and *O. gratissimum* L whose leaves serve as a source of food and medicine have been reported to be difficult to root using loamy soil, hence the need for the current study. This study therefore

is necessary so as to provide an efficient and quick means of regenerating these plant species so as to curb the problem of extinction. Rooting of stem cuttings has proven to be the fastest method of producing large quantities of uniform seedlings, contributing significantly to the conservation of species, future biotechnological exploitation of its medicinal properties and food security, as well as making the species available for future research activities.

Materials and Method

Plant collection and Soil composition

Three medicinal plants – *S. longepedunculata*, *P. mildbraedii* and *O. gratissimum* were used in this study. The branches of *S. longepedunculata* and *P. mildbraedii* were obtained from Nkalagu in Enugu State, Nigeria while that of *O. gratissimum* was obtained from the Botanical Garden of Plant Science and Biotechnology, University of Nigeria. The rooting medium was composed of Loamy soil (LS), Sawdust (SD) and Poultry manure (PM) in the ratio of 2:1:1, respectively, all placed in polythene bags and measured to the weight of 12 kg each. Indole-3-Butyric Acid (5g/l) (Sigma-Aldrich) was prepared according to the manufacturer's instruction.

Sample preparation

The branches of *S. longepedunculata*, *P. mildbraedii* and *O. gratissimum* were defoliated and cut at varying lengths of 10-15cm and 15 – 20cm long, each bearing nodes. A total number of 52 stem-cuttings were used in this study consisting of 32 treated stem cuttings (10 cuttings of *P. mildbraedii*, 12 cuttings of *O. gratissimum* and 10 cuttings of *S. longepedunculata*) and 20 stem cuttings (5 cuttings of *P. mildbraedii*, 5 cuttings of *O. gratissimum* and 10 cuttings of *S. longepedunculata*.) served as control.

Sample Pre-treatment and Plant Procedure

The stem cuttings were treated with the rooting hormone IBA (5g/l) and soaked for 24hrs before planting in an erect position in the rooting medium according to the methods of Sanoussi *et al.*, (2012). The experiment was carried out in a randomized complete block design (RCBD) and set up under a natural partial shade. This was mechanically irrigated daily and the resulting weeds handpicked while observations were made for signs of metabolic activities on the cuttings. The study was carried out during the rainy season (July to September, 2019) under a natural partial shade in the Botanical garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Observations and data collection

The parameters observed include Period of bud sprouting, number of sprouted buds per stem cutting, period of bud development into photosynthesising leaves, the number of leaves produced which were counted three weeks after planting at intervals of 7 days, leaf lengths and leaf diameters, root formation and effect of stem cutting length on seedling growth and survival. The resulting data were collected and documented.

Statistical analysis

Data collected were analyzed using one way Analysis of variance (ANOVA) at 95 percent probability levels ($P \leq 0.05$).

Results and Discussion

The results of the determination of periods of initial and final bud break varied among the stem cuttings of the 3 medicinal plants studied. The stem cuttings of *O. gratissimum* (both treatments and control) had the earliest period of bud sprout (5-8 days after planting) as seen in fig.1 and plate 1. This was followed by the treated stem cuttings of *P. mildbraedii* (8-10days after planting) as seen in fig. 1 and plate 2 with the control showing zero sprout. However, both the treated and the control stem cuttings of *S. longepedunculata* showed zero sprout throughout the study period.

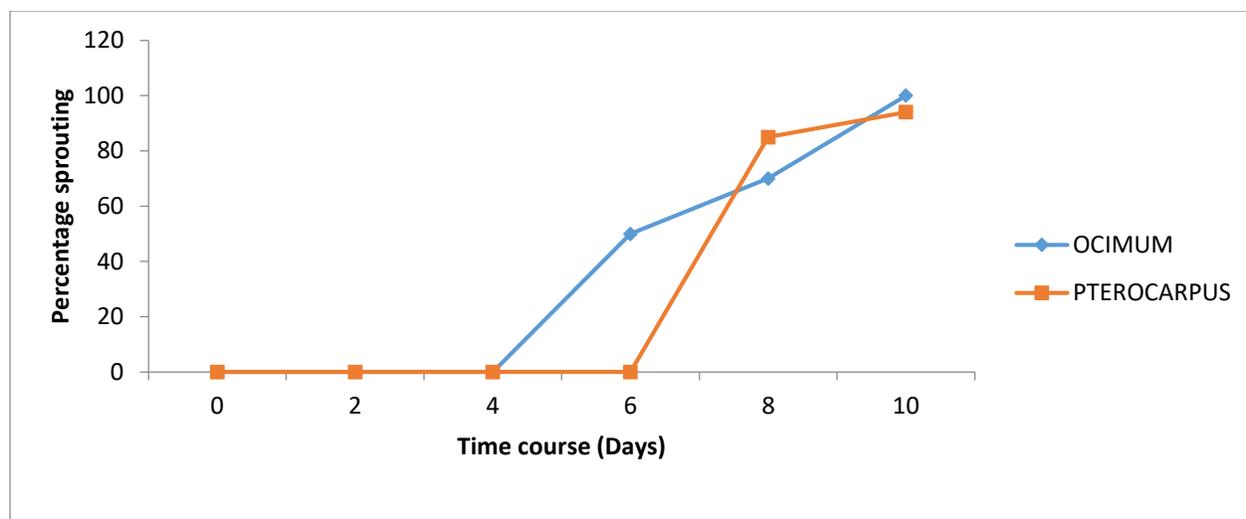


Fig 1: Time course on the percentage sprouting of *O. gratissimum* and *P. mildbraedii* stem cuttings.

From fig.1 above, the time course of sprouting in *O. gratissimum* had 50% bud sprouts on the 6th day which increased to 70% bud sprout on the 8th day. On the other hand, *P. Mildbraedii* had 85% bud sprout on the 8th day and 94% bud sprout on the 10th day.

Bud sprouts on all stem cuttings of *O. gratissimum*, including the control, can be attributed to inherent factors in the species. The results of this study on *O. gratissimum* did not conform to earlier reports of Kouakou *et al.*, 2016; Mahipal and Manokari, 2016; and Fadli *et al.*, 2017 who reported that the application of Indole-3-Butyric acid to the stem cuttings of *Garcinia kola*, *Couroupita guianensis* and *Citrus melo* they studied respectively produced significant differences in the mean number of buds compared to their controls. Nevertheless, in the present study, there is no significant difference in the bud mean number of treated *O. gratissimum* stem cuttings compared to the control. This implies that *Ocimum gratissimum* can be ranked as an easy to root cultivar.

In terms of the number of buds produced, the treated stem cuttings of *O. gratissimum* produced the highest number of buds (Plate 4). In contrast to *O. gratissimum*, a higher number of buds was produced in the untreated stem cuttings of *P. mildbraedii* than the treated ones (Plate 3). The inability of the untreated *P. mildbraedii* stem cuttings to produce buds is related to the effect of insufficient hormone inherent in the cuttings.

This is in agreement to an earlier report of Alagesabooopathi (2012) on *Andrographis macrobotrys*. Alagesabooopathi (2012) stated that the ability of the treated cuttings of *A. macrobotrys* to produce higher numbers of bud sprouts could be as a result of the combined effects of the applied rooting substances and inherent hormones in the plant. On the other hand, *S. longepedunculata* showed zero sprout. Table 1 shows the number of buds produced by the stem cuttings during the study period presented in their mean \pm standard error values. These variations ranged from 0.00 ± 0.00 to 2.58 ± 0.86 .

In table I, there was a significant difference between the treated (T1) and untreated (control-T2) stem-cuttings of *P. mildbraedii* after 28 and 49 days, respectively. However, there was no significant difference when we compared the treated groups at 28 days and 49 days. The same applies to the untreated groups with no sprouted buds. For *O. gratissimum*, there was no significant difference between the treated (T1) and untreated (control-T2) cuttings. Also, the time difference for T1 did not differ at all. The same holds true for T2 where either sprouts at 28th or 49th day did not differ significantly. *S. Longepedunculata* did not produce sprouts throughout the whole experimental period (49 days) whether treated or untreated.

Table 1: Mean number of sprouted buds from stem cuttings of *O. gratissimum*, *P. mildbraedii* and *S. longepedunculata*

	<i>O. gratissimum</i>	<i>P. mildbraedii</i>	<i>S. longepedunculata</i>
After 28 days			
T1	2.50 ± 0.48	1.30 ± 0.26 ^a	0.00 ± 0.00
T2	2.00 ± 0.31	0.00 ± 0.00 ^b	0.00 ± 0.00
	<i>O. gratissimum</i>	<i>P. mildbraedii</i>	<i>S. longepedunculata</i>
After 49 days			
T1	2.58 ± 0.86	1.40 ± 0.37 ^a	0.00 ± 0.00
T2	2.00 ± 0.89	0.00 ± 0.00 ^b	0.00 ± 0.00

T1 = Treated with 5 g/l of IBA, T2 = Control.

As in the case of the period of bud sprout and number of buds produced, table 2 reveals that treated stem cuttings of *O. gratissimum* showed higher leaf development possessing relatively higher number of leaves, longer leaf length, leaf diameter and higher leaf area than the control with no significant difference in the parameters. *P. mildbraedii* stem cuttings produced less number of leaves than *O. gratissimum*. The stem cuttings of *P. mildbraedii* (control) and that of *S. longepedunculata* produced no leaves. However, not all buds that sprouted in the stem cuttings of *O. gratissimum* and *P. mildbraedii* developed

into leaves. The inability of these sprouted buds to develop into leaves is in agreement with the works of Nzekwe (2002) and Sani *et al.*, (2016). They revealed that not all buds that sprouted on the stem cuttings of *Irvingia wombolu* and *Moringa oleifera* persisted till leaf development. Again, there is a significant difference between the treated and the control stem cuttings of *P. mildbraedii*. These variations ranged from 5.00 ± 1.58 to 9.75 ± 2.02 for observations made after 28 days of planting and 9.40 ± 3.05 to 13.08 ± 4.47 for observations made after 49 days of planting.

Table 2: Number of leaves developed by stem cuttings during the course of the study

		NL	LL (cm)	LD (cm)	LA (cm ²)
<i>Ocimum</i>	T1	9.75 ± 2.02	2.53 ± 0.47	1.33 ± 0.26	3.53 ± 0.94
	T2	6.60 ± 1.72	1.94 ± 0.48	1.26 ± 0.33	2.24 ± 0.95
<i>Pterocarpus</i>	T1	5.00 ± 1.58	1.86 ± 0.53 ^a	1.02 ± 0.31 ^a	2.54 ± 0.90 ^a
	T2	0.00 ± 0.00	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b

<i>Securidaca</i>	T1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	T2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
After 49 Days					
		NL	LL (cm)	LD (cm)	LA (cm²)
<i>Ocimum</i>	T1	13.08 ± 4.47	3.51 ± 0.81	2.10 ± 0.47	8.86 ± 2.34
	T2	13.00 ± 5.58	2.62 ± 1.09	1.64 ± 0.68	5.46 ± 2.41
<i>Pterocarpus</i>	T1	9.40 ± 3.05 ^a	1.99 ± 0.82 ^a	1.03 ± 0.46 ^a	4.05 ± 2.71 ^a
	T2	0.00 ± 0.00 ^b			
<i>Securidaca</i>	T1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	T2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

NL = Number of leaves, LL = Leaf length, LD = Leaf diameter, LA = Leaf area, T1 = Treated with 5 g/l of IBA, T2 = Control

The stem-cuttings of *O. gratissimum* and *P. mildbraedii* cut at 15 – 20 cm showed better growth rate compared to the ones cut at 10 -15 cm. This is in agreement with the study carried out by Okafor *et al.* (2015) on *P. mildbraedii* where cuttings of 15 -20 cm length sprouted earliest and had vigorous growth than cuttings of 5 and 10 cm length. The better growth result obtained in stem cuttings lengths of 15 -20 cm in the studied plants can be related to different factors such as level of endogenous auxins and other growth regulators which may be lower in small-sized cuttings thereby resulting to reduced growth percentage, the presence of more nodes on the stem cuttings with longer lengths resulted to more bud sprout and leaf formation (Hamilton *et al.*, 2009). This however, implies that the length of the stem-cuttings contributes to the vegetative growth of the plant.

In determination of the number of roots per stem cutting (Table 3), the treated *O. gratissimum* had

the highest number of roots followed by the control. The treated *P. mildbraedii* stem cuttings produced roots as well. This agrees with the previous reports of Tchoundjeu *et al.*, 2002; Teklehaimanot *et al.*, 2004; Sanoussi *et al.*, 2012 who revealed that treating stem-cuttings by dipping in auxin solutions ranging from concentrations of 2.5 to 5 g/l or when sprinkled with rooting powder stimulate the rhizogenesis of *Prunus africana*, *Osyris Lanceolata* and *Vitex donania* stem cuttings, respectively. This, however, showed that treating stem cuttings with a rooting hormone such as IBA is more adequate for obtaining best responses by the stem cuttings in terms of root production. The inability of *P. mildbraedii* (control) to produce roots can be attributed to season among other factors in line with the work of Ngwuli *et al.* (2019) which revealed that *P. mildbraedii* proved difficult to root during the rainy season in Nigeria. Despite the treatment, none of the stem cuttings of *S. longepedunculata* produced roots.

Table 3: Mean number of roots produced by the stem cuttings of *O. gratissimum*, *P. mildbraedii* and *S. longepedunculata*

	<i>O. gratissimum</i>	<i>P. mildbraedii</i>	<i>S. longepedunculata</i>
T1	135.00 ± 13.45 ^a	1.33 ± 0.01 ^a	0.00 ± 0.00
T2	61.66 ± 2.34 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00

T1 = Treated with 5 g/L of IBA, T2 = Control.

It was also observed that *S. longepedunculata* stem-cuttings did not show any sign of growth in all parameters studied after the experimental period. This can be traced to the fact that it is a woody plant and the recalcitrant nature of most woody plants make regeneration difficult. This

conformed to earlier report of Solomon and Belayneh (2017) on the investigation on *Acacia lahai* which showed that regeneration of woody plants has been a challenge due to their slow growth and unresponsive characteristics of the plant cell to plant growth regulators.

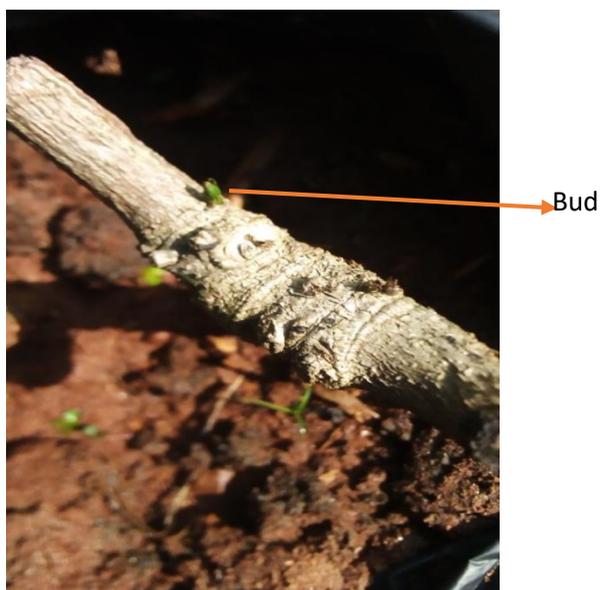


Plate 1: Bud sprout on *P. mildbraedii* stem-cutting



Plate 2: Bud sprout on *O. gratissimum* stem-cutting



Bud



Bud

Plate 3: Multiple bud sprout by *P. mildbraedii* stem cutting.

Plate 4: Multiple bud sprout by *O. gratissimum* stem cutting.



Leaf

Plate 5: Leaf development on stem cutting of *P. mildbraedii*



Leaf

Plate 6: Leaf development on stem cutting of *O. gratissimum*



Plate 7: Stem cutting of *P. mildbraedii* at 15-20 cm length after 28 days of planting.



Plate 8: Stem cutting of *P. mildbraedii* at 10-15 cm length after 28 days of planting.



Plate 9: Stem cutting of *O. gratissimum* at 10-15 cm length after 21 days of planting



Plate 10: Stem cutting of *O. gratissimum* at 15-20 cm length after 21 days of planting



Plate 11: Scanty adventitious root formation on *O. gratissimum* stem-cutting that served as control.



Plate 12: Numerous adventitious root formation on *O. gratissimum* stem cutting that was treated with 5 g/l of IBA.



Plate 13: Root formation on *P. mildbraedii* stem cutting treated with 5 g/l of IBA



Plate 14: *P. mildbraedii* stem-cutting that served as control showed no root formation

Conclusion

The plant *P. mildbraedii* has been proven difficult to cultivate/regenerate during the rainy season. This study has revealed that regeneration can be achieved by treating the stem cuttings with the appropriate concentrations of Indole-3-Butyric

Acid (IBA). The high percentage of rooting responses of the treated *O. gratissimum* stem-cuttings can be relied upon for mass production of the species for future biotechnological exploitation of the species' bio-active compounds. For effective and efficient regeneration and domestication of *P. mildbraedii*

and *O. gratissimum*, stem-cutting lengths of 15-20 cm which showed better growth compared to the cuttings of 10 -15 cm should be utilized. However, further studies are necessary for developing a regeneration method for *S. longepedunculata* using the stem cuttings.

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