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IN VITRO STUDIES ON *LUFFA ACUTANGULA* LIN. VAR. *AMARA*. ROXB - AN IMPORTANT MEDICINAL PLANT.

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ABSTRACT

Luffa amara is an important wild medicinal plant belongs to the family Cucurbitaceae. All parts of the plants are crystalline bitter compound with cucurbitacin B and Amarinin. The whole plant is used to cure many diseases, especially in anti-cancer, anti-diabetic, anti-jaundice and many ailments. The present study, deals with the callus induction, multiplication of luffa from the nodal, leaf and petiole explants. Different concentrations of auxins and cytokinins were used for callus induction and their fresh and dry weights were measured. Best callus induction was observed in the concentration of 2, 4 – D + TDZ – 1.5 mg/l. higher percentage of fresh and also dry weight callus was obtained in the medium fortified with 1.5 mg/l (2, 4 – D + TDZ). mg/l.

KEYWORDS: **Callus, *Luffa amara*, Fresh and Dry Weight, Cucurbitaceae.**

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INTRODUCTION

An Indian system of medicines has been recommended for different kind of biological activity for their health care needs (Al-Mustafa and Al-Thunibat, 2008., Junaid *et al*, 2011.,). The products obtained from the plants source were clinically used for health benefits, without any chemical modification (Neenah and Ahmed, 2011). According to World Health Organization (WHO), more than 21,000 plants have been used as medicinal around the world (Joseph and Raj, 2011). Medicinal plants continue to be an important resources material for therapeutic agents both in developed and developing countries (Kumar, 2000). There is a tremendous genetic diversity within the Cucurbitaceae, and the adaptation of cucurbit species ranges from arid deserts to the tropical – subtropical regions and finally spreading out to the temperate zone (Whitaker and Davis, 1962). Micropropagation has proven to be the most successful technique for large scale production of plants. Conventional methods of propagation (both sexual and vegetative) are associated with problems, like seasonal seed production, pest problems and lower percentage of seed germination etc. This disadvantage can be overcome by using *in vitro* propagation methods for rapid clonel production of plants (Ajithkumar and Seeni, 1978 and Bhuyan *et al.,* 1997). Micropropagation is a suitable method to obtain a large quantity of genetically homogenous and healthy plant material which can be used for planting (Pierik, 1987). The *Luffa acutangula* Lin. Var. *amara*. Roxb. is an important wild medicinal plant. Plants have been used in traditional medicine for several years (Abu Rabia, 2005). This whole plant is used to cure many diseases, especially in anti-cancer, anti-diabetic, anti-jaundice and many ailments. The seed oil is also known for curing serious skin diseases and prevention of other skin ailments. All parts of the plants are a crystalline bitter principal compound with cucurbitacin B and amarinin. In addition of the luffin and colocynthin is also present. The plant possesses laxative and purgative property. The fruit shows presence of cucurbitacin B and E and Oleanalic acid. It is tonic to intestine cures vata, kapha and anemia. In addition, the cucurbitacins have received great deal of attention because of their cytotoxic and anti-cancer activities (Chan *et al*. ,2005; Atta-ur-Rahman, 2005). Scientifically it is proved as CNS Depressant activity. In India, liquid from the leaves and fruit of *L. acutangula* Var. *amara*. (Roxb) a wild variety, were used to treat jaundice and it is also possess in anti-oxidant and larvicidal activity (Samvatsar and Diwanji, 2000). The plant contains β-carotenes, flavonoid acutosides A-G, Oleanane type of triterpene, saponins, acutosides H and I, oleanalic acid saponins. In the present study, callus induction of the *Luffa* from the leaf, petiole and inter nodal explants has been investigated with different concentrations of auxin and its combination were evaluated.

ABBREVIATIONS

- **MS -** Murashige and Skoog (1962).
- **TDZ** - Thidiazuron.
- **BAP** - Benzyl amino purine.
- **IBA** - Indole butyric acid.
- **2, 4 - D -** 2, 4 - Dichlorophenoxcyacetic acid.

MATERIALS AND METHODS

Young leaf, Petiole, and inter node of *Luffa acutangula* Lin. Var. *amara*. Roxb. were collected from the experimental garden department of plant science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

SURFACE STERILIZATION

Surface sterilization process is to be followed in various steps so as to avoid contamination in the culture conditions explants were kept under running tap water for 5 min to remove any soil particles adhering and then surface sterilized with 1-2 drops of 0.2% Teepol solution (Central Drug House India) and kept under running water for 10-15 min and rinsed three to four times with distilled water. After washing procedures, explants were surface sterilized with mercuric chloride ($HgCl_2$) aqueous (w/v) for 1 - 2 min, followed by (0.1%) Bavistein ($C_9H_9N_3O_2$) aqueous solution (w/v) for 1 - 2 min, subsequently with 70% ethanol for 30 sec and then rinsed 3 - 4 times with sterile distilled water. The surface sterilized explants were then inoculated on solid Murashige and Skoog Media (MS) (1962). Supplemented with various concentrations of plant growth regulators.

MEDIA AND CULTURE CONDITION

The Basal medium consist of MS salts with 0.5% of sucrose (w/v) and solidifying agents with 0.8 % (w/v) (Himedia India) agar for the standardization of the callus.

The basal medium consists of MS salts with 0.5% of sucrose (w/v) and solidifying agents with 0.8 % (w/v) Agar. Thidiazuron (TDZ) is used for the standardization of the callus.

The basal medium consists of MS salts with 0.5% of sucrose (w/v) and solidifying agents with 0.8 % agar (w/v). BAP + IBA – 0.5 – 2.5 mg/L. The media was adjusted to pH 5.7 (5.6-5.8) with 1.0 N NaOH (or) 1.0 N Hcl before adding agar and then sterilized by autoclaving at 120^0 C for 15 min.

The basal medium salts with 0.5 % of sucrose (w/v) and solidifying agents with 0.8% agar (w/v) and 2, 4 – D + TDZ – 0.5 – 2.5 mg/L. The media was adjusted to pH 5.7 (5.6-5.8) with 1.0 N NaOH (or) 1.0 N Hcl before adding agar and then sterilized by autoclaving at 120^0 C for 15 min.

CALLUS INDUCTION

The leaf, petiole and internodal explants measuring 2 – 4 mm were inoculated to the culture tubes containing MS Basal medium supplemented with hormones like 2, 4 – D and TDZ. Well grown callus induced from the explants was transferred to the MS hormonal media and subcultured for every 20 days.

DATA COLLECTION

Data's were taken 10 - 45 days by visual observation of the culture. At the end of the observation period the percentage of response, the day of callus initiation and the nature as well as colour of the callus to different concentration of plant growth regulators were recorded.

RESULTS AND DISCUSSION

After Sterilization explants were cultured on Murashige and Skooge's (1962) basal medium supplemented with 0.7% agar (w/v) and 0.5% Sucrose (w/v). In basal medium, callus growth was very slow and approximately 20% response was observed after **55** days. Explants like node, leaf petiole and leaves were collected from the field. MS Basal medium and different concentrations of TDZ were used for callus induction. A growth regulator TDZ was used in different concentration of the MS Basal medium showed callusing from edges of the explants. TDZ 0.5 – 2.5 mg/L was used but the rate of the callus production was decreased at lower concentration to higher concentrations. The higher concentration of TDZ 2.5 mg/L showed approximately 45% of response after 35 days of callusing. The fresh weight of the callus was 5.235 g (Table. 6) and also dry weight was 0.230 g and it was whitish yellow colour and fragile in nature. Explants like node, leaf and petioles were used in the hormone concentration. The basal medium consists of MS salts with 0.5% of sucrose (w/v) and solidifying agents with 0.7 % agar (w/v). The concentration of the MS, BAP + IBA – 0.5 – 2.5 mg/L was used for the callus production. Lower concentrations to higher concentrations were used for callus production. The higher concentration of BAP + IBA - 2.5 mg/L showed 60 % response after 25 days of callusing. The Fresh weight of the callus was 4.900g (Table. 7) and Dry weight was 0.215g of compact callus and whitish yellow colour was produced. Growth regulator - 2, 4 – Dichlorophenoxy acetic acid (2, 4 – D: 0.5 – 2.5 mg/L) either separately or in

combination with TDZ – Thidiazuron (0.5 – 2.5 mg/L) were tested for the induction of organogenic callus (callus induction medium CIM). The callus occurred at the cut ends of the proximal half of the explants after 10 – 15 days of culture initiation. In individual auxin treatment with 2, 4 – D + TDZ at 1.5 mg/L evoked maximum callus induction (80 %) and produced yellowish green friable callus. Auxin: cytokinin ratio plays a vital role in determining the *in vitro* response of most of the cucurbits (Trulson and Shahin, 1986). Among the combinations, the highest explant response was observed in MS Basal medium with 2, 4 – D + TDZ 1.5 mg/L combinations with high level of 2, 4 – D + TDZ (1.5 mg/L) induced profuse callus formation. Similar results but with callus from nodal explants, were reported in *Stevia rebaudiana* were also maximum callus production was obtained from Nodal explants culture in MS medium with 13.56µM 2, 4 – D (Uddin *et al*., 2006). The higher concentration of 2, 4 – D + TDZ (1.5 mg/L) showed 90 % response after 25 days of callusing. Fresh weight of the callus was 5.984g (Table. 8) and Dry weight was 0.250g of friable callus and whitish green colour was produced. The highest percentage fresh and also dry weight of callus was obtained from this concentration.

Table 1
Callus induction of Luffa amara on basal media

Media	Explant	Observation	Results
MS basal Medium	Node, Inter node and Leaf	No Results	----
	Node, Inter node and Leaf	No Results	----
	Node, Inter node and Leaf	No Results	----
	Node, Inter node and Leaf	No Results	----
	Node, Inter node and Leaf	No Results	----

Table 2
Callus induction of Luffa amara on using TDZ hormone

Hormones		Explant	Observation	Results
TDZ	0.5	Node, Inter node and Leaf	35 - days	+
	1.0	Node, Inter node and Leaf	35 - days	++
	1.5	Node, Inter node and Leaf	35 - days	+++
	2.0	Node, Inter node and Leaf	35 - days	+++
	2.5	Node, Inter node and Leaf	35 - days	+++

Table 3
Callus induction of Luffa amara on 2, 4 – D + TDZ hormones

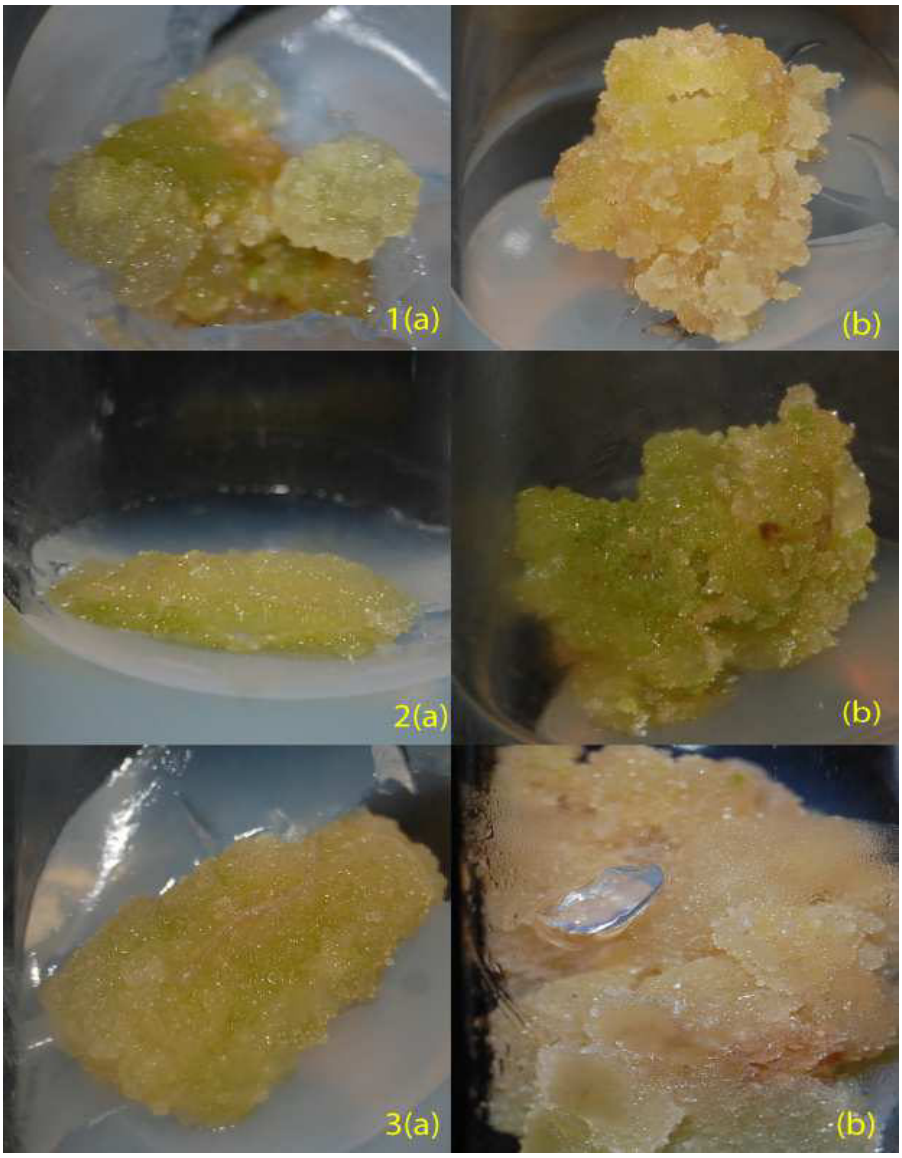
Hormones		Explant	Observation	Results
2, 4 - D + TDZ	0.5+0.5	Node, Inter node and Leaf	25 - days	+
	1.0+1.0	Node, Inter node and Leaf	25 - days	++
	1.5+1.5	Node, Inter node and Leaf	25 - days	+++
	2.0+2.0	Node, Inter node and Leaf	25 - days	+++
	2.5+2.5	Node, Inter node and Leaf	25 - days	+++

Table 4
Callus induction of Luffa amara on BAP + IBA hormones

Hormones		Explant	Observation	Results
BAP + IBA	0.5+0.5	Node, Inter node and Leaf	25 - days	+
	1.0+1.0	Node, Inter node and Leaf	25 - days	++
	1.5+1.5	Node, Inter node and Leaf	25 - days	+++
	2.0+2.0	Node, Inter node and Leaf	25 - days	+++
	2.5+2.5	Node, Inter node and Leaf	25 - days	+++

+ - Slight Callus
++ - Medium Callus
+++ - High Callus

Figure 1



1(a) Leaf Callus initiation ***(b) Leaf Callus Proliferation***
2(a) Petiole Callus initiation ***(b) Petiole Callus Proliferation***
3(a) Internodal Callus initiation ***(b) Internodal Callus Proliferation***

Table 5
Effect of Basal Media using explants from Luffa acutangula Lin. Var. amara. Roxb. For Callus Development.

S. No	Concentration of Media (MS) Mg/mL	Callus Type	Nature of Color's	Plant Materials Callus (gram)	
				Fresh Weight	Dry Weight
1.	Basal	------	------	------	

Table 6
Effect of Auxin and Cytokinin on different explants of Luffa acutangula Lin. Var. amara. Roxb. For Callus Induction.

S. No	Concentration of Media (MS) mg/mL	Callus Type	Nature of Color's	Plant Materials Callus (gram)	
				Fresh Weight	Dry Weight
1.	TDZ – 0.5	Friable Callus	Whitish yellow colour	2.200 g	0.070 g
2.	TDZ – 0.5	Friable Callus	Whitish yellow colour	2.345 g	0.110g
3.	TDZ – 0.5	Friable Callus	Whitish yellow colour	1.880 g	0.065 g
4.	TDZ – 0.5	Friable Callus	Whitish yellow colour	1.685 g	0.020 g
5.	TDZ – 1.0	Fragile Callus	Whitish yellow colour	2.276 g	0.100 g
6.	TDZ – 1.0	Fragile Callus	Whitish colour	1.750 g	0.045g
7.	TDZ – 1.0	Friable Callus	Whitish colour	1.766 g	0.043 g
8.	TDZ – 1.0	Friable Callus	Whitish yellow colour	2.100 g	0.160 g
9.	TDZ – 1.5	Friable Callus	Whitish yellow colour	2.800 g	0.100 g
10.	TDZ – 1.5	Friable Callus	Whitish yellow colour	2.120 g	0.090 g
11.	TDZ – 1.5	Friable Callus	Whitish colour	3.395 g	0.190 g
12.	TDZ – 2.0	Friable Callus	Whitish yellow colour	3.608 g	3.675 g
13.	TDZ – 2.0	Fragile Callus	Whitish colour	3.150 g	0.150 g
14.	TDZ – 2.0	Fragile Callus	Whitish yellow colour	3.675 g	0.170 g
15.	TDZ – 2.5	Fragile Callus	Whitish yellow colour	5.235 g	0.230 g
16.	TDZ – 2.5	Friable Callus	Whitish colour	2.083 g	0.140 g
17.	TDZ – 2.5	Friable Callus	Whitish yellow colour	2.230 g	0.120 g
18.	TDZ – 2.5	Friable Callus	Whitish yellow colour	3.578 g	0.175 g

Table 7

Effect of Auxin and Cytokinin on explants of Luffa acutangula Lin. Var. amara. Roxb. For Callus development.

S. No	Concentration of Media (MS) Mg/mL	Callus Type	Nature of Color's	Plant Materials Callus (gram)	
				Fresh Weight	Dry Weight
1.	BAP + IBA – 0.5	Fragile Callus	Whitish yellow colour	3.230 g	0.150 g
2.	BAP + IBA – 0.5	Friable Callus	Whitish yellow colour	2.600 g	0.170 g
3.	BAP + IBA -0.5	Compact Callus	Whitish yellow colour	0.880 g	0.040 g
4.	BAP + IBA – 0.5	Fragile Callus	Whitish yellow colour	3.190 g	0.190 g
5.	BAP + IBA – 0.5	Compact Callus	Whitish yellow colour	2.000 g	0.150 g
6.	BAP + IBA – 0.5	Compact Callus	Whitish colour	0.160 g	0.106 g
7.	BAP + IBA – 0.5	Fragile Callus	Whitish yellow colour	4.300 g	0.215 g
8.	BAP + IBA – 1.0	Friable Callus	Whitish yellow colour	1.000 g	0.070 g
9.	BAP + IBA – 1.0	Compact Callus	Whitish green colour	0.500 g	0.050 g
10.	BAP + IBA – 1.0	Friable Callus	Whitish yellow colour	4.000 g	0.210 g
11.	BAP + IBA – 1.0	Friable Callus	Whitish yellow colour	0.600 g	0.060 g
12.	BAP + IBA – 1.0	Fragile Callus	Whitish green colour	1.000 g	0.075 g
13.	BAP + IBA – 1.0	Fragile Callus	Whitish green colour	3.520 g	0.170 g
14.	BAP + IBA – 1.0	Compact Callus	Whitish yellow colour	0.900 g	0.050 g
15.	BAP + IBA – 1.5	Compact Callus	Whitish yellow colour	1.000 g	0.075 g
16.	BAP + IBA – 1.5	Friable Callus	Whitish green colour	2.700 g	0.140 g
17.	BAP + IBA – 1.5	Friable Callus	Whitish green colour	0.750 g	0.060 g
18.	BAP + IBA – 1.5	Fragile Callus	Whitish green colour	0.900 g	0.060 g
19.	BAP + IBA – 1.5	Fragile Callus	Whitish yellow colour	1.200 g	0.070 g
20.	BAP + IBA – 1.5	Compact Callus	Whitish yellow colour	0.600 g	0.035 g
21.	BAP + IBA – 2.0	Compact Callus	Whitish green colour	0.300 g	0.045
22.	BAP + IBA – 2.0	Fragile Callus	Whitish green colour	4.500 g	0.185 g
23.	BAP + IBA – 2.0	Fragile Callus	Whitish yellow colour	1.500 g	0.065 g
24.	BAP + IBA – 2.0	Fragile Callus	Whitish yellow colour	1.000 g	0.065 g
25.	BAP + IBA – 2.0	Compact Callus	Whitish yellow colour	3.500 g	0.165 g
26.	BAP + IBA – 2.0	Compact Callus	Whitish yellow colour	0.750 g	0.050 g
27.	BAP + IBA – 2.0	Compact Callus	Whitish green colour	1.000 g	0.085 g
28.	BAP + IBA – 2.5	Compact Callus	Whitish yellow colour	3.000 g	0.100 g
29.	BAP + IBA – 2.5	Compact Callus	Whitish yellow colour	1.500 g	0.110 g
30.	BAP + IBA – 2.5	Compact Callus	Whitish yellow colour	4.900 g	0.215 g
31.	BAP + IBA – 2.5	Fragile Callus	Whitish green colour	4.200 g	0.150 g
32.	BAP + IBA – 2.5	Fragile Callus	Whitish green colour	3.250 g	0.170 g
33.	BAP + IBA – 2.5	Friable Callus	Whitish green colour	0.800 g	0.090 g

Table 8
Effect of Auxin and Cytokinin on explants of Luffa acutangula Lin. Var. amara. Roxb. For Callus development.

S. No	Concentration of Media (MS) Mg/mL	Callus Type	Nature of Color's	Plant Materials Callus (gram)	
				Fresh Weight	Dry Weight
1.	2, 4 – D + TDZ - 0.5	Friable Callus	Whitish colour	4.160 g	0.115 g
2.	2, 4 – D + TDZ - 0.5	Fragile Callus	Whitish colour	4.340 g	0.165 g
3.	2, 4 – D + TDZ - 0.5	Fragile Callus	Whitish yellow colour	4.160 g	0.150 g
4.	2, 4 – D + TDZ - 0.5	Friable Callus	Whitish green colour	3.988 g	0.160 g
5.	2, 4 – D + TDZ - 0.5	Fragile Callus	Whitish yellow colour	4.000 g	0.165 g
6.	2, 4 – D + TDZ - 0.5	Friable Callus	Whitish colour	3.845 g	0.150 g
7.	2, 4 – D + TDZ – 1.0	Friable Callus	Whitish green colour	3.374 g	0.160 g
8.	2, 4 – D + TDZ - 1.0	Friable Callus	Whitish green colour	3.490 g	0.150 g
9.	2, 4 – D + TDZ - 1.0	Friable Callus	Whitish green colour	3.745 g	0.160 g
10.	2, 4 – D + TDZ - 1.0	Fragile Callus	Whitish yellow colour	4.328 g	0.145 g
11.	2, 4 – D + TDZ - 1.0	Fragile Callus	Whitish yellow colour	4.000 g	0.155 g
12.	2, 4 – D + TDZ - 1.0	Friable Callus	Whitish green colour	2.752 g	0.150 g
13.	2, 4 – D + TDZ – 1.5	Friable Callus	Whitish green colour	5.027 g	0.190 g
14.	2, 4 – D + TDZ - 1.5	Friable Callus	Whitish green colour	5.984 g	0.250 g
15.	2, 4 – D + TDZ - 1.5	Fragile Callus	Whitish colour	5.270 g	0.190 g
16.	2, 4 – D + TDZ - 1.5	Friable Callus	Whitish colour	3.670 g	0.205 g
17.	2, 4 – D + TDZ - 1.5	Fragile Callus	Whitish yellow colour	4.517 g	0.165 g
18.	2, 4 – D + TDZ – 2.0	Fragile Callus	Whitish yellow colour	5.240 g	0.090 g
19.	2, 4 – D + TDZ - 2.0	Fragile Callus	Whitish green colour	4.053 g	0.150 g
20.	2, 4 – D + TDZ - 2.0	Friable Callus	Whitish green colour	3.717 g	0.155 g
21.	2, 4 – D + TDZ - 2.0	Friable Callus	Whitish yellow colour	0.688 g	0.002 g
22.	2, 4 – D + TDZ - 2.0	Fragile Callus	Whitish yellow colour	2.530 g	0.140 g
23.	2, 4 – D + TDZ – 2.5	Friable Callus	Whitish green colour	4.950 g	0.180 g
24.	2, 4 – D + TDZ - 2.5	Friable Callus	Whitish green colour	4.260 g	0.150 g
25.	2, 4 – D + TDZ - 2.5	Friable Callus	Whitish green colour	4.960 g	0.140 g

CONCLUSION

In the present study, we have established a high frequency callus initiation and proliferation protocols using leaf, node and leaf petiole explants. Thus, this approach will be an initiative for the mass propagation of a valuable medicinal plant *Luffa amara*.

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