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IN VITRO REGENERATION OF GINGER USING SHOOT TIP EXPLANTS

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ABSTRACT

The investigation entitled "*In vitro* regeneration of ginger using shoot tip explants" was carried out during 2012-15 at the Tissue Culture Laboratory, Department of Horticulture, Nagaland University, School of Agricultural Sciences and Rural Development, Medziphema Campus, Nagaland. The plant source was taken from the locally grown disease free ginger farm and the investigation was laid out in Completely Randomized Design (CRD). Shoot tips of 1-2 cm were cultured in modified MS medium supplemented with ±30 g sucrose in various concentrations of cytokinins (BAP @ 0, 1, 1.5, 2, 2.5 and 3 mgl^{-1}) and IBA (@ 0.5 mgl^{-1} and NAA (@ 0.5 mgl^{-1}) for callus formation, shoot proliferation and root formation. After 8 weeks of culture, best recorded medium was used for further multiplication. After fourth sub culture, buds were used for the study of root induction. The days to callus formation ranged from 12.63 days (MS + 3mgl^{-1} BAP) to 17.77 days. The highest (28.87 days) number of days taken for the initiation of shoot was recorded in 2 mgl^{-1} BAP followed by 1 mgl^{-1} BAP + 0.5 mgl^{-1} IBA *i.e.* 28.67 days and the least (20.43 days) number of days was observed in 3 mgl^{-1} BAP followed by 2 mgl^{-1} BAP + 0.5 mgl^{-1} IBA) *i.e.* 22.20. It was observed that the days to root initiation was earliest in MS + 2.0 mgl^{-1} IAA with 11.77 days and maximum number of roots $explants^{-1}$ in MS + 2.0 mgl^{-1} NAA with 8.11 roots $explants^{-1}$ whereas maximum root length (2.18 cm) in MS + 1.5 mgl^{-1} IBA.

(Key words: *in vitro*, ginger, shoot tip, callus, multiplication)

INTRODUCTION

Ginger, botanically known as *Zingiber officinale* Rosc., belonging to the family Zingibereaceae is a rhizomatous medicinal spice. It is a herbaceous perennial grown as an annual crop. Commercially it is grown for its aromaticrhizomes, which are used both as a spice and for medical purposes. Generally, ginger is vegetatively propagated through mature rhizome segments having three to four buds. It has a habit of rare flowering and non-viable seed production (Malamug *et al*., 1991). In addition ginger is heavily attacked by systemic diseases like wilt diseases (*Pseudomonas solanacearum*), yellow disease (*Fusarium oxysporum* f.) and soft rot (*Pythium* sp.) which are difficult to eliminate through conventional methodologies. As conventional propagation has a low rate of multiplication, *in vitro* technology is an alternative for multiplication of ginger for commercial cultivation. Tissue culture is one such technique which can be efficiently and conveniently used to acquire disease free stock of source material. Earlier Hosoki and Sagawa (1977) were the first to report their success in the production of an average of six shoots bud^{-1} from *in vitro* culture. From that time onwards, several workers had succeeded in their *in vitro* culture of ginger following the organogenesis pathway (Balachandran *et al*., 1990; Dekkers *et al*., 1991), while others had used the indirect somatic embryogenesis pathway (Malamug *et al*., 1991; Babu *et al*., 1992; Kackar *et al*., 1993). On the other hand, Sharma *et al*. (1995) had successfully produced disease free ginger micro rhizomes *in vitro*. Others (Dekkers *et al*., 1991) have also used successfully the *in vitro* technique for conservation of germplasm.

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory at Department of Horticulture, SASRD, Medziphema Campus, NU: SASRD-Farm, Nagaland.

Source of plant material: The genotype *Zingiber officinale* cv. Nadia was chosen for the study. Healthy and disease free rhizomes were taken from local collections and were kept in sand and cocopit layer at 1:1 in a bed to get growing buds or sprouts for the use as explants.

Preparation of explants: Shoot tips explants were thoroughly washed in water with few drops of detergent (geepol) and rinsed with distilled water 2 to 3 times and again they were

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immersed with Bavistin for 25 minutes and then rinsed with DW for 2-3 times. Asepsis was administered by immersing for 1 minute in 70% ethanol followed by 15 min immersion in diluted solution of 3% sodium hypochlorite (NaOCl) and washed 3 times with sterile distilled water. Shoot tips approximately 2-3 cm were carefully trimmed to initiate the culture. Light conditions in the growth cabinet were fixed to 2,000 Lux and a photoperiod of 8 hr of dark 16 hr^{-1} of light at 25±2°C.

Culture media: For the induction of callus and regeneration of plantlets in ginger, MS (Murashige & Skoog, 1962) medium was used in present investigation. The following culture media were used in the present investigation depending on specific purposes as mentioned:

A. For shoot initiation

S_1- MS+ 1 mgl^{-1} BAP, S_2- MS+ 1 mgl^{-1} BAP + 0.5 mgl^{-1} IBA, S_3- MS+ 1 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, S_4- MS+ 1.5 mg mgl^{-1} BAP, S_5- MS+ 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} IBA, S_6- MS+ 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, S_7- MS+ 2 mgl^{-1} BAP, S_8- MS+ 2 mgl^{-1} BAP + 0.5 mgl^{-1} IBA, S_9- MS+ 2 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, S_{10}- MS+ 2.5 mgl^{-1} BAP, S_{11}- MS+ 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} IBA, S_{12}- MS+ 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, S_{13}- MS+ 3 mgl^{-1} BAP, S_{14}- MS+ 3 mgl^{-1} BAP + 0.5 mgl^{-1} IBA, S_{15}- MS+ 3 mgl^{-1} BAP + 0.5 mgl^{-1} NAA

B. For root formation

R_1- MS + 0.5 mgl^{-1} IAA, R_2- MS + 1.0 mgl^{-1} IAA, R_3- MS + 1.5 mgl^{-1} IAA, R_4- MS + 2.0 mgl^{-1} IAA, R_5- MS + 0.5 mgl^{-1} IBA, R_6- MS + 1.0 mgl^{-1} IBA, R_7- MS + 1.5 mgl^{-1} IBA, R_8- MS + 2.0 mgl^{-1} IBA, R_9- MS + 0.5 mgl^{-1} NAA, R_{10}- MS + 1.0 mgl^{-1} NAA, R_{11}- MS + 1.5 mgl^{-1} NAA, R_{12}- MS + 2.0 mgl^{-1} NAA

Three pieces of sterilized explants of 1-2 cm were inoculated in each culture bottle containing sterile culture medium with different concentrations and combination of growth regulators. When the shoots grew about 4-5 cm in length, they were separated from each other and again cultured on freshly prepared root induction medium to induce root. Day to day observations were carried out to note the response of growing plantlets. When the plantlets become 6-10 cm in length with sufficient root system, they were taken out from the culture bottles. The roots were gently washed out with running tap water. The plantlets were transplanted to trays containing the potting mixture of sand, FYM and cocopeat 1:1:1. The experimental design was carried out following Completely Randomized Design (CRD) for Analysis of Variance and means using Duncan Multiple Range Test (DMRT), Duncan (1955).

RESULTS AND DISCUSSION

Callus formation

Different concentrations and combination of BAP, IBA, NAA and IAA were used in MS medium using shoot tip as explants in this experiment for shoot initiation and root formation. A total of 15 different media combinations for shoot proliferation and 12 media combinations for rooting was taken. The results of the experiment revealed a wide range of variation in days required for callus initiation. Callus formation and shoot growth is strongly supported by cytokinin concentration and a minimum level is necessary to support growth with an optimum for maximum elongation. However, increased levels of cytokinin inhibited apical dominance and promoted lateral shoot primordial under higher concentration of BAP in MS media. This might be the reason of higher percentage of callus formation and maximum microshoot under the medium MS+ 3 mgl^{-1} BAP + 0.5 mgl^{-1} IBA. Such variation was also found when the performance of per cent callus induction is influenced by the variety and type of explants. Strains of callus differing in appearance, colour, degree of compaction and morphogenetic potential commonly arise from a single experiment. The callus formation in all the treatments was observed to be greenish white in colour. Ilahi and Jabeen (1987) also reported that callus growth stopped on sub culturing to plain basal media but when sub cultured on a medium containing BAP and 2, 4- D it developed into greenish white compact callus masses. This might have happen due to non uniform cell division because cell division does not take place throughout the culture mass but is located primarily in a meristematic layer on the outer periphery of cells. The inner parts of the callus remain as an undividing mass of older tissue and in time may differ colour, physiologically and morphologically from cells of the outer layer. Division in the exterior layer decreases and appearances of the callus may become knobby as cell division becomes restricted to specific island of cells. This variation in cell age and type may produce creamish white to greenish white callus masses. Another reason might be because the inner cells are older with creamish white colour and exterior cells are younger with greenish white as a meristematic region persists around the periphery of the callus masses.

The present study agrees with the findings of Ramachandran and Chandrashekharan (1992). They reported that the callus formation, development of roots and rhizome like structures were noticed from excised ginger anthers cultured on MS medium containing 2,4-D and coconut milk. In the present study, the less number of days for callus initiation (12.63) were observed in 3 mgl^{-1} BAP supplemented media followed by 3 mgl^{-1} BAP + 0.5 mgl^{-1} IBA) *i.e.* 12.73 days.

Shoot characteristics

The results indicate that higher concentration of BAP was responsible for faster callus formation and shoot multiplication and lower level *i.e.* 3 mgl^{-1} BAP influenced to increase the shoot length. The highest (28.87 days) number of days taken for the initiation of shoot was recorded in 2 mgl^{-1} BAP followed by 1 mgl^{-1} BAP + 0.5 mgl^{-1} IBA *i.e.* 28.67 days and the least (20.43 days) number of days was observed in 3 mgl^{-1} BAP followed by 2 mgl^{-1} BAP + 0.5 mgl^{-1} IBA) *i.e.* 22.20 days. S_{13} (3 mgl^{-1} BAP) was found to be statistically at par with S_8 (2 mgl^{-1} BAP + 0.5 mgl^{-1} IBA), S_{11} (2.5 mgl^{-1} BAP + 0.5 mgl^{-1} IBA) and S_{14} (3 mgl^{-1} BAP + 0.5 mgl^{-1} IBA). The

results are comparable to Khatun *et al.* (2003), who reported that the average mean days for the initiation of shoot was 26 days and initiation of shoot in the basic MS media took 27.67 days. However, Hosoki and Sagawa (1977) reported there were no shoots in the basic medium. From the results it is also clear that levels of BAP showed lesser number of days for shoot initiation. The maximum number of shoots explant^{-1} was obtained in the media containing 3 mgl^{-1} BAP+ 0.5 mgl^{-1} IBA (3.66 shoots explant^{-1}) as shown in table 2. This was evident from the earlier findings of Mohammed and Quraishi (1999), who obtained maximum number of shoots (3.33) from single explants when medium contained 3 mgl^{-1} BAP. Similar results were also obtained by Dipti *et al.* (2005), who reported the highest number of multiple shoots in media supplemented with 3 mgl^{-1} BAP in rhizome bud in turmeric. Balachandran *et al*. (1990) also obtained four (4) number of shoot buds on MS medium+ 3 mgl^{-1} BAP. Yusuf *et al.* (2011) found that shoot buds of *Boesenbergia rotunda* (L.) Mansf. cultured on MS medium supplemented with 2 mgl^{-1} BAP and 0.5 mgl^{-1} NAA produced maximum number of multiple shoots (5 shoots explants^{-1}).The MS media in combination with 3 mgl^{-1} BAP enhanced the rate of shoot multiplication within 3 weeks of subculture. Zuraida *et al.* (2016) observed that absence of NAA or increasing to 3 mgl^{-1} in combination with BAP does not imply any significant effects on the number or length of shoots except the latter increased the number of roots (in the range of 10- 11 roots explants^{-1}) they also found that shoot tip of *Curcuma longa* (L.) cultured on MS supplemented with 2 or 3 mgl^{-1} BAP and 1 mgl^{-1} NAA produced highest average number of shoot (2.4 and 2.6 shoots, respectively). The media combination of 1 mgl^{-1} BA with 0.5 mgl^{-1} IBA and NAA showed very less response with high callusing but produced abnormal shoots. Muda and Khalid (2004) indicated that *in vitro* propagation ginger (*Z. officinale*) was successfully established on MS medium supplemented with a range of 1.0–3.0 mgl^{-1} BAP to obtain higher percentage of shoot proliferation. The maximum length of shoot buds was recorded in 2 mgl^{-1}BAP + 0.5 mgl^{-1} NAA (5.17 cm). Abbas *et al.* (2011) found similar results when cultures were transferred to MS media containing 1.5 and 2.5 mgl^{-1} BAP and recorded shoot length 3.18 cm and 2.93 cm respectively. Sharma and Singh (1995) observed that MS medium containing 2.0 mg Kin+ 2.0 mg NAA gave highest shoot length (6.8 cm) response of *Z. officinale*. Similarly, Kambaska and Santilata (2009) reported that the combination of MS media with BAP (2.0 mgl^{-1}) and NAA (0.5 mgl^{-1}) elicited optimal response in shoot length of 6.2 ± 0.37 cm shoot^{-1} in ginger cultures.

Root characteristics

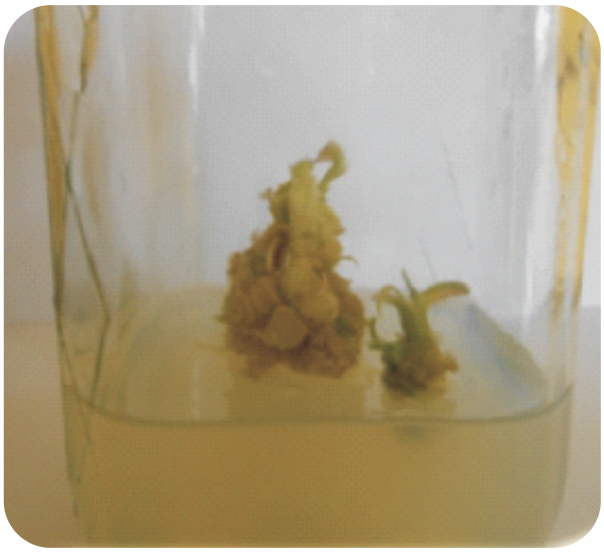
The days to root initiation was earliest in MS + 2.0 mgl^{-1} IAA with 11.77 days and maximum number of roots explant^{-1} in MS + 2.0 mgl^{-1} NAA with 8.44 roots explant^{-1} whereas maximum root length (2.18 cm) in MS + 1.5 mgl^{-1} IBA. Meenakshi *et al.* (2001) mentioned that in turmeric, maximum rooting with maximum root length was observed in NAA 0.3 mgl^{-1}. Sit and Tiwari (1998) reported that shootlets of turmeric were rooted on half strength MS medium with IBA at 0.0 to 0.5 mgl^{-1}and they were concluded that rooting did not occur in the absence of IBA and the number of roots rootlet^{-1} was proportional to IBA concentration. Similarly, Rehman *et al.* (2004) observed that rooting of shoots in turmeric was obtained on ½ MS medium with 0.1 – 1 mg l^{-1} IBA. IBA-derived auxin has strong roles in various aspects of root development, including regulation of root apical meristem size, root hair elongation, lateral root development, and formation of adventitious roots. The root apical meristem is a collection of undifferentiated cells at the root tip region that display indeterminate growth. The balanced cell division and differentiation in this tissue gives rise to new root tissue, while maintaining a small group of cells that undergo occasional cell division. Maintaining proper auxin levels and establishment of an auxin gradient in these tissues is essential to establish root patterning and meristem formation (Pascuzzi and Benfey, 2009). Choi (1991) reported that callusing was best when base or middle portion explants of ginger were cultured on medium containing 0.5 ppm NAA, while shoot and root formation were best on medium containing 0.1 to 1 ppm NAA + 1.0 ppm BA. According to the findings of Abbas *et al.* (2011) MS medium supplemented with NAA at different concentration was more efficient on number of roots formation than those formed on MS medium supplemented with IAA at different concentrations with average 6.90 and 3.05 roots shoot^{-1}, respectively. Nirmal *et al*. (2005) found that the required quantity of auxin is high for better proliferation; thus, further increase in root number would be at the expense of shoot length. Accordingly, in this study, small number of root was observed in cultures on auxin free basal medium and/or at lower level of NAA treatments. This in turn would have a considerable impact on the survival potential of the plantlets at the later stage of growth after field transplanting. Ayenew *et al.* (2012) reported that medium supplemented with 1 mg l^{-1} NAA alone resulted in 8.75 roots with 2.95 cm length on average.

Table 1. Performance of explant and treatment interaction on different callus characters

Hormone concentration	Callus characters			
	Days to induction	Type	Colour	% induction
MS+ 1 mgl^{-1}BAP	16.60 $_{bcd}$	Friable	Greenish white	55.33
MS+ 1 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	17.77 $_{ab}$	Friable	Greenish white	33.00
MS+ 1 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	17.40 $_{abc}$	Friable	Greenish white	55.00
MS+ 1.5 mgl^{-1}BAP	15.37 $_{def}$	Friable	Greenish white	66.00
MS+ 1.5 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	15.17 $_{defg}$	Friable	Greenish white	44.00
MS+ 1.5 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	15.93 $_{cde}$	Friable	Greenish white	77.33
MS+ 2 mgl^{-1}BAP	17.43 $_{abc}$	Friable	Greenish white	77.33
MS+ 2 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	14.10 $_{fgh}$	Friable	Greenish white	77.33
MS+ 2 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	16.50 $_{bcd}$	Friable	Greenish white	68.00
MS+ 2.5 mgl^{-1}BAP	14.27 $_{efgh}$	Friable	Greenish white	65.00
MS+ 2.5 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	14.07 $_{fgh}$	Friable	Greenish white	75.00
MS+ 2.5 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	13.53 $_{gh}$	Friable	Greenish white	88.70
MS+ 3 mgl^{-1}BAP	12.63 $_{h}$	Friable	Greenish white	88.70
MS+ 3 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	12.73 $_{h}$	Friable	Greenish white	88.70
MS+ 3 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	13.73 $_{fgh}$	Friable	Greenish white	88.70
SEm±	0.53			3.74
CD $_{0.05}$	1.54			10.79

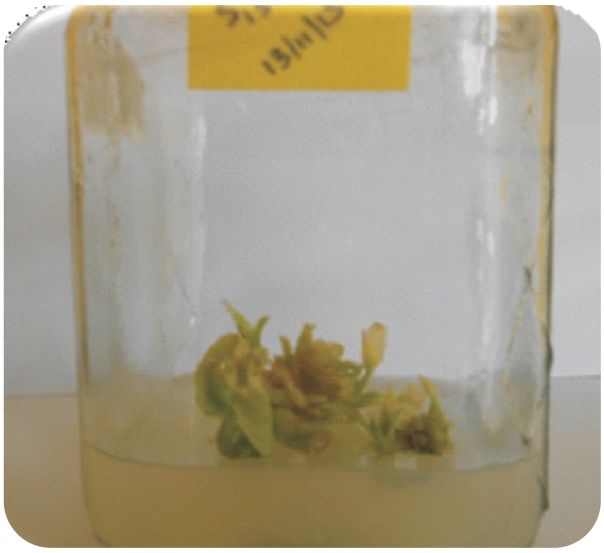
Table 2. Performance of explants and treatment interaction on different shooting characters

Hormone concentration	Shooting characters		
	Days to shoot	No.of microshoot	Length of shoot (cm)
MS+ 1 mgl^{-1}BAP	26.53 $_{abcd}$	1.11 $_{cd}$	2.53 $_{d}$
MS+ 1 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	28.67 $_{a}$	1.33 $_{bcd}$	3.28 $_{bcd}$
MS+ 1 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	28.30 $_{ab}$	2.11 $_{b}$	3.20 $_{bcd}$
MS+ 1.5 mgl^{-1}BAP	25.00 $_{cde}$	1.11 $_{cd}$	3.95 $_{bc}$
MS+ 1.5 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	25.40 $_{cd}$	1.88 $_{bc}$	3.58 $_{bcd}$
MS+ 1.5 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	27.10 $_{abc}$	1.44 $_{bcd}$	3.23 $_{bcd}$
MS+ 2 mgl^{-1}BAP	28.87 $_{a}$	1.44 $_{bcd}$	3.10 $_{bcd}$
MS+ 2 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	22.20 $_{f}$	1.22 $_{cd}$	4.08 $_{ab}$
MS+ 2 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	26.60 $_{abcd}$	1.89 $_{bc}$	5.17 $_{a}$
MS+ 2.5 mgl^{-1}BAP	23.03 $_{ef}$	1.44 $_{bcd}$	4.38 $_{a}$
MS+ 2.5 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	22.37 $_{f}$	1.44 $_{bcd}$	4.07 $_{ab}$
MS+ 2.5 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	22.80 $_{ef}$	2.22 $_{b}$	3.48 $_{bcd}$
MS+ 3 mgl^{-1}BAP	20.43 $_{g}$	3.22 $_{a}$	3.29 $_{bcd}$
MS+ 3 mgl^{-1}BAP + 0.5 mgl^{-1}IBA	22.43 $_{f}$	3.66 $_{a}$	2.83 $_{cd}$
MS+ 3 mgl^{-1}BAP + 0.5 mgl^{-1}NAA	24.30 $_{def}$	3.33 $_{a}$	2.73 $_{d}$
SEm±	0.71	0.26	0.34
CD $_{0.05}$	2.05	0.77	0.99



Callusing observed

Shoot proliferation in MS+ 1.5 mg l^{-1} BAP



Shoot proliferation in MS+ 3 mg l^{-1} BAP

Multiple shoots at MS+ 2 mg l^{-1} BAP

Multiple shoots at MS+ 3 mg l^{-1} BAP

Root elongation at MS+ 2 mg l^{-1} NAA

Table 3. Performance of treatment interaction on different rooting characters

Hormone concentrations	Days to root initiation	No. of roots explant^{-1}	Length of root (cm)
MS +0.5 mgl^{-1}IAA	16.21	2.77	1.43
MS + 1.0 mgl^{-1}IAA	15.55	3.66	1.65
MS + 1.5 mgl^{-1}IAA	13.10	4.44	1.82
MS + 2.0 mgl^{-1}IAA	11.77	6.77	2.01
MS + 0.5 mgl^{-1}IBA	17.10	2.88	1.78
MS + 1.0 mgl^{-1}IBA	16.99	3.66	1.64
MS + 1.5 mgl^{-1}IBA	15.77	5.22	2.18
MS + 2.0 mgl^{-1}IBA	13.88	8.44	1.84
MS + 0.5 mgl^{-1}NAA	17.88	3.44	1.47
MS + 1.0 mgl^{-1}NAA	16.21	6.77	1.24
MS + 1.5 mgl^{-1}NAA	14.55	5.99	1.85
MS + 2.0 mgl^{-1}NAA	12.22	8.11	2.14
SEm±	0.56	0.32	0.15
CD $_{0.05}$	1.64	0.94	0.43

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