

Article

Identification and Registration of the Novel High-Rhizome-Yielding Variety Bharamputra-1 of *Kaempferia galanga* L.

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Abstract: *Kaempferia galanga* is an endangered plant whose recognition as a flavoring agent and perfumery ingredient has increased its demand greatly. Therefore, the present investigation aimed at the identification of high-rhizome-yielding varieties of *K. galanga*. A total of forty-nine germplasms were collected from different parts of India and planted at CSIR-NEIST, Jorhat experimental farm, during 2013. The two-year evaluation of essential morphological and chemical data was recorded for the selection of superior rhizomes with a high rhizome yield during 2014 and 2015. Subsequently, multi-location field trials were conducted with the selected elite germplasm along with controls using a randomized complete block design, and relevant morphological traits as well as essential oil quality data were recorded for all the lines for three consecutive years during 2016, 2017 and 2018. The essential oil quality was analyzed by using GC/MS. The data obtained were statistically analyzed for stability based on rhizome yield, essential oil yield and days to maturity. A high-rhizome-yielding variety of *K. galanga* was identified and named Bharamputra-1. It was found to be stable in multi-locational trials conducted in Northeast India. The variety showed a mean rhizome yield of 10.01 tones/ha. Stability parameters, namely, $\beta_i = 1.13$ and $\sigma^2_{di} = -0.07$ were recorded and found to be superior to those of the other examined varieties. The chemical profiling of the rhizome essential oil of the selected germplasm was also performed using GC/MS, which revealed ethyl p-methoxycinnamate (37.25%), trans-ethyl cinnamate (28.35%), endo-borneol (8.91%), eucalyptol (6.83%), (-)-camphor (3.98%) and 3-carene (3.77%) as the main components. The cultivation of this identified variety could help in the successful commercial cultivation of the crop.

Keywords: Medicinal plant; multilocation; stability; variety development; high rhizome yield; essential oil; *Kaempferia galanga*



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1. Introduction

Kaempferia galanga L. is an indigenous rhizomatous perennial herb belonging to the Zingiberaceae family and is also known as aromatic ginger, sandy ginger, chandramula, kencur, cekur and kacholam [1–4]. It is aromatic in nature and widely available in the tropics and subtropics of Bangladesh, China, India, Indonesia, Japan, Java, Laos, Malaysia, Myanmar, Nigeria, South Africa, Sudan, Sri Lanka, Thailand and Vietnam [5]. In India, it is distributed in the states of Andhra Pradesh, Arunachal Pradesh, Assam, Karnataka, Kerala, Manipur, Meghalaya, Odisha and West Bengal [5,6].

It is a potent aromatic medicinal plant suitable for cultivation in shady areas. Its growth, yield and quality are dependent on the planting time and the type of seed material. Mother rhizomes can be planted in the spring season and requires 7–8 months for maturing. Thereafter, the rhizome can be harvested, and the essential oil can be extracted from both the rhizome as well as dried aerial portion of the plant on maturity [2].

The ethnomedicinal applications of the rhizomes of this plant include 59 different Ayurvedic combinations used mainly for curing wounds, skin diseases, epilepsy, asthma,

fever, cough, headache, splenic disorders, rheumatism and for stimulating digestion [2]. Additionally, the pharmacological applications are based on its antimicrobial, anti-inflammatory, analgesic, antidiarrheal, anthelmintic, antineoplastic, cytotoxic, mosquito repellent, larvicidal and sedative activities [1,7,8]. The decoction of the rhizome powder with honey cures cough and pectoral infections [9,10]. *Kaempferia galanga* is famous for its medicinal and edible uses due to the presence of many important bioactive compounds [11]. The importance of the rhizome is enhanced by its nutritious properties, as it contains high levels of carbohydrates and proteins, aromatic essential oil, starch and alkaloids [12,13]. The rhizomes also contain beneficial metals such as Co, K, Fe, Mg, Mn, Ni, P and Zn [14]. Because of the presence of high levels of carbohydrates, proteins and beneficial elements, *K. galanga* may act as a healthy supplement for human consumption and may also be processed further as a phytoceutical food in the flavor industry.

Till now, the cultivation of this economically and medicinally important plant species has not been practiced commercially. However, in India, folklore and ethnic practitioners widely use the rhizomes of *K. galanga* for the preparation of different herbal formulations. This has led to the indiscriminate exploitation of this species due to its transfer from its wild habitat. This plant species has also been declared threatened and it is becoming rare in India and Bangladesh [15,16]. Furthermore, the demand of *K. galanga* is over 100 tons of dried rhizomes [17], and its current market value is INR300–400 per Kg [18]. Moreover, the price of *K. galanga* essential oil varies in the range of USD 600–700 per Kg in the international market. The essential oil of aromatic rhizomatous plants possesses numerous biological activities such as larvicidal, nematocidal, anti-inflammatory, antimicrobial, antidepressant, antioxidant, anthelmintic effects [2,19–21]. The essential oil of *K. galanga* contains several important chemical compounds such as ethylcinnamate, ethyl p-methoxycinnamate, kaemgalangol A, kaempulfonic acids, kaempferide, kaempferol, 3-carene-5-one, cystargamide B and xylose, which are responsible for the biological activity of the plant [5]. Similarly, a report also mentioned the presence of ethyl-p-methoxycinnamate, propanoic acid, and pentadecane as the major chemical constituents [22].

Therefore, since there is a great requirement of this medicinally important plant species, it is highly recommended to develop a variety of *K. galanga*, superior in rhizome yield. A high production will not only minimize its extensive collection but also increase its cultivation owing to its economic and industrial importance. Northeast India, with its unique climate and favorable conditions, could be a good area for the cultivation of this economically important plant species. Till date, various cultivation aspects of this important plant have not been standardized, and no varietal development study has been conducted on this important plant species. Therefore, the present work is the first study on the varietal development of a high-rhizome-yielding strain of *K. galanga*.

2. Materials and Methods

2.1. Plant Material and Experimental Site

During the year 2013, forty-nine diverse germplasms of *K. galanga* were collected from different regions of India, namely, Assam, Arunachal Pradesh, Meghalaya, Manipur, Sikkim and Kerala (Table 1). The planting materials, i.e., the rhizomes, were identified by the plant breeder of the ARD department. Voucher specimens of the same were prepared and deposited in the departmental herbarium of the institute, with code numbers RRLKG1 to RRLKG49. The collected samples were planted first with augmented design during 2014 and later, in 2015, with a randomized complete block design with three replications at the experimental farm of CSIR-NEIST, Jorhat, Assam. The line-to-line and plant-to-plant spacing was maintained at 20 × 20 cm. The fertilizer used in the experiment was NPK (nitrogen/phosphorus/potassium) in the ratio of 150:80:60 kg/ha. The soil type of the experimental area was sandy loam with a pH of around 4.8.

Table 1. GPS (Global Positioning System) location of the collected germplasms of *K. galanga*.

S. No.	Pedigree	Site of Collection			Latitude (N)	Longitude (E)
		Village	District	State		
1.	Kn-1	Sikarighat	Karbi Anglong	Assam	26°18'46.4'' N	93°11'19.1'' E
2.	Kn-2	Manja	Karbi Anglong	Assam	25°58'09.0'' N	93°26'22.0'' E
3.	Kn-3	Teteliguri	Karbi Anglong	Assam	26°18'47.6'' N	93°03'55.1'' E
4.	Kn-28	Latabari	Bokakhat	Assam	26°38'09.5'' N	93°35'23.8'' E
5.	Kn-47	Haibargaon	Nagaon	Assam	26°20'38.6'' N	92°40'11.5'' E
6.	Kn-50	Sandanpur	Nagaon	Assam	26°20'48.9'' N	92°40'07.8'' E
7.	Kn-52	Sonapur	Kamrup	Assam	26°18'00.6'' N	91°39'02.7'' E
8.	Kn-53	Sonapur	Kamrup	Assam	26°18'03.9'' N	91°39'11.3'' E
9.	Kn-59	GhoramaraPothar	Morigaon	Assam	26°15'31.6'' N	92°20'38.1'' E
10.	Kn-63	Rajagaon	Morigaon	Assam	26°14'51.8'' N	92°20'05.2'' E
11.	SG-13	Bakarigaon	Morigaon	Assam	26°15'31.4'' N	92°20'39.2'' E
12.	SG-12	Nongpoh	Ri-Bhoi	Meghalaya	25°51'16.9'' N	91°50'00.1'' E
13.	SG-16	Kunduvura	Thrissur	Kerela	10°32'07.1'' N	76°13'12.1'' E
14.	PG-25	Umiam	Ri-Bhoi	Meghalaya	25°40'21.4'' N	91°55'41.9'' E
15.	PG-4	Nongstoin	West Khasi Hills	Meghalaya	25°31'39.4'' N	91°14'50.2'' E
16.	PG-8	Tura	West Garo Hills	Meghalaya	25°32'09.8'' N	90°12'09.5'' E
17.	GP-1	Mohmaiki	Bokakhat	Assam	26°37'45.7'' N	93°37'03.2'' E
18.	Kn-38-5-1	Tarapur	Silchar	Assam	24°49'53.8'' N	92°46'55.6'' E
19.	Kn-33-4	Sandanpur	Nagaon	Assam	26°20'48.1'' N	92°40'10.8'' E
20.	Kn-33-5	Chirukandi	Silchar	Assam	24°49'46.7'' N	92°46'25.7'' E
21.	Kn-37-6-1	Pasighat	East Siang	Arunachal Pradesh	28°04'56.8'' N	95°18'57.7'' E
22.	Kn-37-3-1	Oyun	East Siang	Arunachal Pradesh	27°53'20.4'' N	95°18'59.1'' E
23.	Kn-37-2-1	Runne	East Siang	Arunachal Pradesh	28°03'01.5'' N	95°15'00.0'' E
24.	Kn-36-5-1	Pangin	Siang	Arunachal Pradesh	28°08'24.3'' N	95°16'27.6'' E
25.	Kn-36-1-1	Roing	Lower Dibang Valley	Arunachal Pradesh	28°07'11.1'' N	95°49'22.6'' E
26.	Kn-34-11-1	Tezu	Lohit	Arunachal Pradesh	27°55'37.0'' N	96°09'13.8'' E
27.	Kn-34-10-1	Diyun	Changlang	Arunachal Pradesh	27°32'36.7'' N	96°05'36.0'' E
28.	Kn-34-8-1	Pangin	East Siang	Arunachal Pradesh	28°12'27.2'' N	94°59'32.5'' E
29.	PG-23	Barfok	North Sikkim	Sikkim	27°29'39.6'' N	88°30'18.3'' E
30.	Kn-15	Japhou	Chandel	Manipur	24°19'53.8'' N	94°00'31.9'' E
31.	GP-5	Senapati	Senapati	Manipur	25°15'55.4'' N	94°00'55.0'' E
32.	GP-4	KhuraiThoudamLeikai	East Imphal	Manipur	24°49'05.3'' N	93°57'40.6'' E
33.	GP-3	Thaoroijam	West Imphal	Manipur	24°48'28.8'' N	93°51'42.2'' E
34.	Kn-38-1	Patturaikkal	Thrissur	Kerela	10°32'18.2'' N	76°12'03.7'' E
35.	Kn-38-2	Mebo	East Siang	Arunachal Pradesh	28°09'55.9'' N	95°25'02.6'' E
36.	Kn-38-3	Tezu	Lohit	Arunachal Pradesh	27°55'30.3'' N	96°09'29.8'' E
37.	Kn-38-4	Ziro	Lower Subansiri	Arunachal Pradesh	27°32'38.5'' N	93°49'18.0'' E
38.	Kn-38-5	Nongpoh	Ri-Bhoi	Meghalaya	25°51'28.5'' N	91°50'51.4'' E
39.	Kn-38-6	Nongstoin	West Khasi Hills	Meghalaya	25°31'48.2'' N	91°16'39.7'' E
40.	Kn-38-7	Chawang	North Sikkim	Sikkim	27°26'15.5'' N	88°35'48.8'' E

Table 1. Cont.

S. No.	Pedigree	Site of Collection			Latitude (N)	Longitude (E)
		Village	District	State		
41.	Kn-38-8	KhuraiThoudamLeikai	Imphal East	Manipur	24°48'53.8" N	93°57'41.5" E
42.	Kn-38-9	Lamjaotongba	Imphal West	Manipur	24°46'22.1" N	93°54'57.5" E
43.	Kn-38-10	Poothole	Thrissur	Kerela	10°31'03.7" N	76°12'24.1" E
44.	Kn-38-11	Umiam	Ri-Bhoi	Meghalaya	25°40'55.2" N	91°55'21.5" E
45.	Kn-35-4	Puthurkkara	Thrissur	Kerela	10°31'30.0" N	76°10'52.7" E
46.	Kn-36-1	Pottammal	Kozhikode	Kerela	11°15'28.2" N	75°48'38.9" E
47.	Kn-36-4	Kottooli	Kozhikode	Kerela	11°15'40.8" N	75°47'51.6" E
48.	Kn-36-6	Koikkal	Kollam	Kerela	8°53'59.3" N	76°36'59.2" E
49.	Kn-37-2	Punkunnam	Thrissur	Kerela	10°32'16.9" N	76°12'01.3" E

2.2. Morphological Data Recording

The morphological and yield data, such as rhizome yield (tons/ha), number of mother rhizomes, number of primary rhizomes, dry rhizome recovery %, days to maturity, essential oil quality, and quantity data were recorded for two years during 2014 and 2015. The two-year evaluation through clonal selection led to the identification of one high-rhizome-yielding line which was named Bharamputra-1. All qualitative and quantitative data were studied and recorded as per standard procedure and experimental needs. All essential agronomic practices were followed to raise good crops [23]. Afterwards, a two-year evaluation of the quantitative and qualitative data of the elite variety Bharamputra-1 was carried out following different agronomic traits such as rhizome yield (tons/ha), number of mother rhizomes and primary rhizomes, dry rhizome recovery (%), essential oil % and days to maturity. This new line was planted in four different locations of Northeast India, namely, Jorhat (Assam), Gorigoan (Sikkim), Imphal (Manipur) and Lakhamijan (Assam) during 2016, 2017 and 2018. The earlier released varieties of *K. galanga*, namely, 'Rajani' and 'Kasthuri', were used as control varieties. The stable nature of the variety was confirmed through multi-locational trials and evaluated by the Eberhart–Russell method and AMMI model.

2.3. Extraction of the Essential Oil

The essential oil was extracted from 300 g of dried rhizome of *K. galanga* by hydro-distillation using a Clevenger apparatus for 6 h. Anhydrous sodium sulphate (Na_2SO_4) was used to absorb the moisture content present in the essential oil after the extraction. A total of three replicates were prepared, and the average yield value was calculated as the final essential oil yield%. The formula used to calculate the essential oil yield% (DWB) is reported below:

$$\text{Essential oil\% (DWB)} = (\text{Amount of essential oil extracted (g)}) / (\text{Amount of dried rhizome distilled (g)}) \times 100$$

2.4. Qualitative Analysis of the Essential Oil

The analysis of the essential oil was performed using the Agilent Technologies Apparatus model No. 6890 for gas chromatography (GC) with a flame ionization detector (FID) and an Agilent technologies gas chromatographer coupled with the mass-selective detector MSD 5975C for mass spectrometry (MS). An HP-5MS capillary column of 60 m $\times$ 0.25 mm i.d and film thickness of 0.25 μm was used; He at 1 mL/min flow rate was used as carrier gas. The components were identified by comparing their mass spectra with spectra in the Willey mass spectral/NIST library and then confirmed by comparison with the Kovat's indexes on the HP5-MS column [24,25]. The standard samples obtained from Hi-media, Sigma-Aldrich, were run in the same GC conditions, and their retention times were compared with those

of the peaks previously eluted to identify the components present in the samples without using a correction factor. The standards injected for gas chromatography were *p*-cymene, *D*-limonene, eucalyptol, (-)-camphor, *endo*-borneol, α -terpineol, (-)-bornyl acetate, thymol, *trans*-ethyl cinnamate, β -copaene, spatulenol, epicubenol, τ -cadinol, pentadecane, ethyl *p*-methoxycinnamate.

2.5. Statistical Analysis

All the morphological and chemical data were subjected to a stability analysis for six yield-related characters. The stability parameters, i.e., the regression coefficient (β_i) and the deviation from the regression coefficient (σ^2_{di}) were analyzed through the Eberhart and Russell (1966) model to check the stability in all four locations [26]. AMMI model analysis was performed to validate the high-yielding genotypes with stable performance through biplot analysis. Furthermore, the mean, standard error and coefficient of variation were also calculated. All the statistical analyses were carried out by using the ‘metan’ package in the open-source R 4.2.0 software.

3. Results and Discussion

The morpho-chemical data evaluation for the ninety-five genotypes of *K. galanga* during the years 2014 and 2015 recorded a range of rhizome yield (RY) from 3.37 to 10.30 tonnes/ha, number of mother rhizomes (NMR) from 4–7, number of primary rhizome (NPR) from 6–15, dry rhizome recovery (DRR) of 20–32%, an essential oil yield (EO) of 0.52–1.00% and number of days to maturity (DOM) from 260–282 days (Table 2).

Table 2. Morpho-chemical traits of the collected *K. galanga* germplasms and the variety named “Bharamputra-1”.

Traits	Rhizome Yield tonnes/ha	No. of Mother Rhizomes	No. of Primary Rhizomes	Dry Rhizome Recovery %	Essential Oil %	Days to Maturity
Range of collected germplasms	3.37–10.30	4–7	6–15	20–32	0.52–1.00	260–282
Brahmaputra-1 identified germplasm	10.30	7	13	26.5	0.94	261

ha = hectare, No = number.

The selection trial led to the recognition of a superior germplasm with high rhizome yield (10.30 tonnes/ha). The other five traits also showed a distinct performance, with seven and thirteen mother and primary rhizomes respectively, a dry rhizome recovery of 26.5%, an essential oil yield of 0.94% and 261 number of days required for maturity (Table 2). This selected superior line was then planted in four different regions of NE India for 3 years; the related data are reported in Table 3. The superior varieties Kasthuri and Rajani of *K. galanga* released by Kerala Agriculture University, India, were used as control varieties. The rhizomes of both the varieties differ in their colour and size, light brown and large rhizome in Kasthuri while creamy white and medium rhizome in Rajani [27]. The present investigation showed that the average rhizome yield of Bharamputra-1 was 10.01 tonnes per ha compared to 5.43 and 5.41 tonnes/ha for the control varieties. Earlier studies reported the yield of the Kasthuri and Rajani varieties to be 2.52 and 2.55 tonnes of dry rhizome per hectare [27]. The traits such as the number of mother rhizomes and primary rhizomes were almost similar in all three varieties, with slight differences. The average NMR was observed to be 4.35 and 4.68 for Rajani and Kasthuri, respectively, and 5.68 for Bharamputra-1. Earlier literature also reported the NMR and NPR for Rajani to be on average 4 and 10, i.e., almost similar to those of the present study [23]. Although the NMR and NPR appeared similar for all the three varieties, but Bharamputra-1 is considered superior in terms of rhizome yield, since the weight of the rhizomes was found to be higher for Bharamputra-1 owing to their large size compared to that of the other two varieties. The days to maturity, dry rhizome recovery and essential oil yield revealed lower values for

Bharamputra-1 (266 days; 24.99%; 0.90%) than for the control varieties Kasthuri (271 days; 26.35%; 0.88%) and Rajani (279.28 days; 29.68%; 0.94%) (Table 3).

Table 3. Average morpho-chemical data of the three-year (2015, 2016 and 2017) multi-locations trial of the *K. galanga* variety “Bharamputra-1” and the controls.

	Name of the Variety	Location	Rhizome Yield Tones/ha	No. of Mother Rhizomes	No. of Primary Rhizomes	Dry Rhizome Recovery %	Essential Oil %	Days to Maturity
1	Brahmaputra-1	Jorhat	10.00	6.42	12.58	25.80	0.90	263.58
		Gorigaon	9.55	6.00	11.25	24.12	0.90	266.00
		Imphal	10.40	5.29	13.67	26.20	0.90	266.13
		Lakhimijan	10.10	5.00	10.33	23.83	0.90	268.33
		Avg.	10.01	5.68	11.96	24.99	0.90	266.01
2	Rajani Check variety-1	Jorhat	5.10	4.67	9.00	29.55	0.90	279.88
		Gorigaon	5.37	4.08	11.67	29.43	0.97	283.00
		Imphal	5.85	4.33	10.33	32.70	0.98	279.92
		Lakhimijan	5.38	4.33	10.29	27.04	0.89	274.33
		Avg.	5.43	4.35	10.32	29.68	0.94	279.28
3	Kasthuri Check variety-2	Jorhat	5.30	4.71	10.67	24.55	0.84	270.63
		Gorigaon	4.92	4.33	10.42	25.83	0.89	261.46
		Imphal	5.55	5.00	10.33	26.33	0.93	276.33
		Lakhimijan	5.88	4.67	12.67	28.70	0.87	275.58
		Avg.	5.41	4.68	11.02	26.35	0.88	271.00
	SE(m)	-	1.53	0.40	0.48	1.39	0.02	3.87
	CV	-	0.31	0.12	0.06	0.07	0.03	0.02

ha = hectare, No = number, Avg = average, SE = standard error, CV = coefficient of variation.

All the recorded data were then subjected to statistical analysis to confirm their stability in all four different locations. The major constraint faced by the plant breeders is the identification of high-yielding and stable genotypes which could survive varied environments [28]. Therefore, plant breeders employ strategies such as multilocation trials and evaluations in different favorable and unfavorable conditions to determine consistent effects [29,30].

Analysis of variance (ANOVA) through the regression method was performed for all the studied economical traits of the three selected genotypes (Bharamputra-1, Kasthuri and Rajani). The rhizome yield and number of mother rhizomes was found to be significantly different, at $p < 0.005$, among the genotypes. Additionally, the dry rhizome recovery and essential oil yield revealed significant differences among the genotypes, at $p < 0.05$. The expression of the traits may vary due to different biotic and abiotic factors such as genotype, soil, climate, environmental fluctuations [31]. The genotype–environment interaction was significant for rhizome yield, essential oil yield and days to maturity (Table 4). No fluctuations due to environmental effects were observed for the genotype Bharamputra-1, while Kasthuri showed significant differences in the traits rhizome yield and number of mother rhizomes, at $p < 0.05$. Similarly, the control variety Rajani demonstrated variation in the traits number of primary rhizomes and number of mother rhizomes.

Table 4. Analysis of variance (ANOVA) through the regression method for the six traits of the three selected genotypes in MLT.

Source of Variation	DF	RY	NMR	NPR	DRR	EO	DOM
Total	11	41.77	3.97	13.87	55.29	0.02	400.33 ***
GEN	2	225.11 ***	15.22 ***	21.55	186.45 *	0.03 *	1437.81
ENV + (GEN $\times$ ENV)	9	1.03	1.47	12.16	26.15	0.01	169.78
ENV (linear)	1	6.35	4.71	5.79	63.36	0.06	212.66
GEN $\times$ ENV (linear)	2	0.12 *	1.46 *	3.43	26.45	0.00 *	421.89 *
Pooled deviation	6	0.44	0.93	16.13	19.84	0.01	78.60
Bharamputra-1	2	0.18	1.67	23.58	7.89	0.02	39.49
Kasthuri	2	0.56 *	0.86 *	14.43	36.12	0.01	81.72
Rajani	2	0.59	0.26 *	10.38 *	15.52	0.01	114.58
Pooled error	56	3.35	12.21	28.54	33.52	0.03	338.67

DF = degree of freedom, RY = rhizome yield, NMR = number of mother rhizomes, NPR = number of primary rhizomes, DRR = dry rhizome recovery, EO = essential oil yield, DOM = date of maturity, GEN = genotype, ENV = environment, MLT = multilocation trial, * = significant at 5%, *** = significant at 0.5%

Lal et al. (2022) reported a significant $G \times E$ interaction in the traits rhizome yield, dry rhizome recovery and essential oil yield, which indicates the importance of studying the performance of genotypes in varied environments [31]. A stability analysis was therefore performed using the Eberhart and Russell method using the regression coefficient (β_i) and the deviation from regression (σ^2_{di}). This clearly indicated that the identified variety, i.e., Bharamputra-1, showed stable performance in all the four locations in relation to all six characters (Table 5).

Table 5. Stability analysis performed using the Eberhart and Russell model of agro-morphological and quality traits of “Bharamputra-1” and the control varieties.

Genotypes	Rhizome Yield Tonnes/ha			No. of Mother Rhizomes			No. of Primary Rhizomes			Dry Rhizome Recovery %			Essential Oil %			Days to Maturity		
	μ	β_i	σ^2_{di}	μ	β_i	σ^2_{di}	μ	β_i	σ^2_{di}	μ	β_i	σ^2_{di}	μ	β_i	σ^2_{di}	μ	β_i	σ^2_{di}
Bharamputra-1	10.01	1.13	−0.07	5.68	2.07	−0.14	11.96	1.52	2.14	24.99	0.92	0.04	0.93	0.76	0.00	266.01	0.40	−4.62
Rajani	5.41	1.15	−0.02	4.68	0.19	−0.24	11.02	−0.51	1.00	26.35	−0.08	3.57	0.88	0.90	0.00	271.00	3.68 *	0.66
Kasthuri	5.42	0.73	−0.02	4.35	0.74	−0.31	10.32	2.00	0.49	29.68	2.16	0.99	0.94	1.34	0.00	279.28	−1.08 *	4.76
Population mean		6.95			4.90			11.1			27.01			0.92			272.10	

μ = mean, β_i = regression coefficient, σ^2_{di} = deviation from regression, * = significant at 5%.

According to the Eberhart and Russell (1966) model, a genotype is said to be stable if it has a mean greater than the population mean, $\beta_i \sim 1$ and $\sigma^2_{di} \sim 0$ [32,33]. The mean values of Bharamputra-1 for all the traits were higher than the population mean and the control varieties except dry rhizome recovery, essential oil yield and days to maturity. From the present study, it can be interpreted that in few cases the lower mean values than the population mean is also a desirable factor. The variety Bharamputra-1 showed the mean values of 10.01 tonnes/ha, $\beta_i = 1.13$ and $\sigma^2_{di} = -0.07$ for rhizome yield. In contrast Rajani and Kasthuri depicted mean value (5.41 and 5.42 tonnes/ha), regression coefficient (1.15 and 0.73) and deviation of regression (-0.07 and -0.07) for rhizome yield making it less desirable than the identified line for selection of this particular trait. Similarly, a significant β_i for the trait, days to maturity in the control varieties makes it unstable in nature. This increases the favorability of Bharamputra-1 compared to the control varieties based on its consistent performance. Additionally, the rhizome yield, essential oil yield and dry rhizome recovery for Bharamputra-1 showed β_i equal to 1.13, 0.76 and 0.92, respectively, and σ^2_{di} equal to -0.07 , 0.00 and 0.04, respectively, indicating high stable nature. The varieties Rajani and Kasthuri showed significant $\beta_i > 1$ for days to maturity interpreting adaptability to unfavourable environment (Table 5). Singh et al. (2020) also reported that

a β_i significantly greater than the unity represents stability below the average, and a β_i significantly lower than unity denote stability above the average [34]. The essential oil yield was found to be better and more stable for the control varieties compared to the identified superior variety. Earlier, a variety named Jor Lab K-1 was released, which was rich in essential oil yield (2.38%) and was stable for four different locations of NE India [23]. The identified germplasm was registered by the Plant Germplasm Registration Committee (PGRC) of ICAR-NBPGR, New Delhi, vide registration number INGR17081 [35] (Figure 1).



Figure 1. Germplasm registration certificate of Bharamputra-1 by the NBPGR, New Delhi.

For the variety Bharamputra-1, the essential oil yield was found to be stable with 0.93%, but the rhizome yield was far superior with 10.01 tonnes/ha compared to only 7.16 tonnes/ha in the variety Jor Lab K-1 [23]. AMMI ANOVA, a widely used stability method, was also used to confirm the uniformity of the trait performance in multiyear MLT for the identified Bharamputra-1 genotype. Highly significant values at $p < 0.005$ were observed for RY, DRR, EO, DOM in the studied environments, demonstrating wide variation in different environmental conditions. The effect of environment on the genotype was observed for the traits NPR, DRR, EO and DOM (Table 6). The environmental parameters can greatly influence the phenotypic characters, making it necessary to study the stability of genotypes. Genotypic variation was observed for all the studied traits in AMMI ANOVA, with p value less than 0.005. The principal component analysis was carried out differentiating two principal components (PC1 and PC2). A strong correlation for PC1 was observed for DOM, followed by DRR and NPR, while PC2 showed a strong correlation with DOM, followed by NPR and DRR. AMMI biplots 1 and 2 were also constructed for all traits (RY, NPR, NMR, DRR, EO, DOM) which indicated that Bharamputra-1 is highly stable for RY, DRR, EO and DOM compared to Rajani and Kasthuri (Table 6).

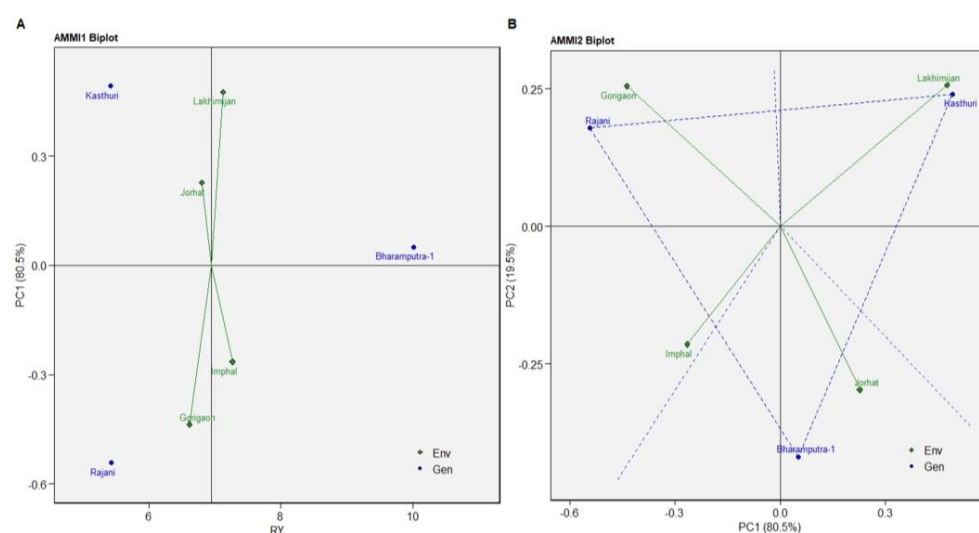
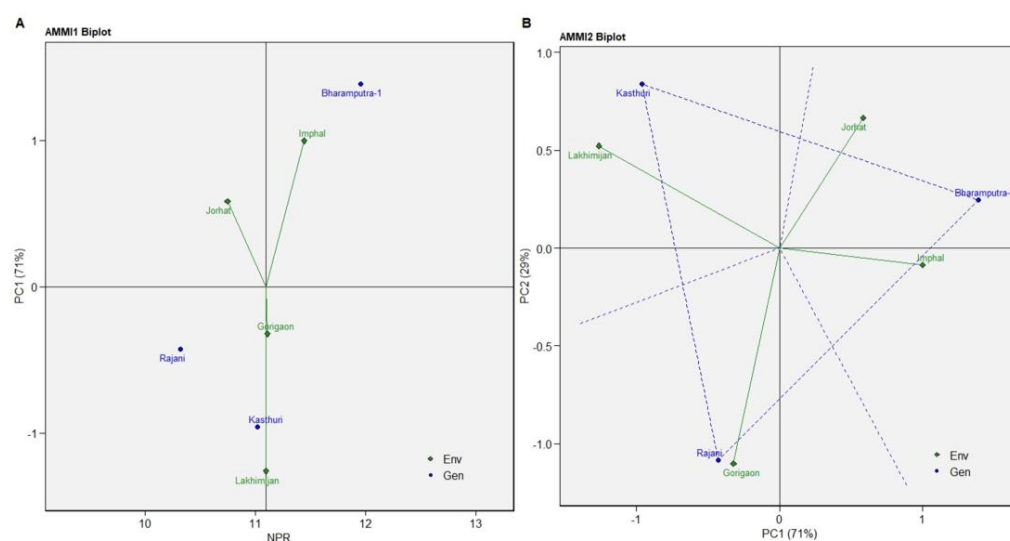
The AMMI1 biplot of rhizome yield revealed Bharamputra-1 as the highest rhizome-yielding genotype, with consistent performance across the studied MLT (Figure 2A). For the trait, number of primary rhizomes, a consistent uniformity in the number was observed for the Rajani and Kasthuri varieties, but a higher number of primary rhizomes was found for Bharamputra-1, with unstable nature (Figure 3A).

All the selected genotypes were placed in the vertices of the AMMI2 biplot triangle of both RY and NPR, indicating the best suitable genotype for that particular location [33]. The favorable genotype suited for the locations Imphal and Jorhat is Bharamputra-1, while Rajani and Kasthuri demonstrated high rhizome yield and number of primary rhizomes in the locations Gorigaon and Lakhimijan, respectively (Figures 2B and 3B).

Table 6. AMMI analysis of variance of the six traits for three selected genotypes in MLT.

Source	DF	RY	NMR	NPR	DRR	EO	DOM
		MS	MS	MS	MS	MS	MS
ENV	3	6.35 ***	4.71	5.79	63.36 ***	0.06 ***	212.66 ***
REP (ENV)	28	0.32	2.08	3.22	2.22	0.01	35.75
GEN	2	675.32 ***	45.67 ***	64.65 ***	559.36 ***	0.09 ***	4313.44 ***
GEN × ENV	6	1.44	4.26	51.83 ***	85.97 ***	0.03 ***	657.68 ***
PC1	4	0.58	2.04	18.39 *	39.00 ***	0.01	293.14 ***
PC2	2	0.28	0.18	15.05	7.97	0.00	71.41
Residuals	248	0.76	2.76	6.44	7.57	0.01	76.47
Total	293	5.39	3.01	7.68	13.43	0.01	117.72

DF = degree of freedom, RY = rhizome yield, NMR = number of mother rhizomes, NPR = number of primary rhizomes, DRR = dry rhizome recovery, EO = essential oil yield, DOM = date of maturity, GEN = genotype, ENV = environment, MLT = multilocation trial, REP = replication, MS = mean squares, PC = principal component, * = significant at 5%, *** = significant at 0.5%.

**Figure 2.** (A) AMMI1 biplot (B) AMMI2 biplot for rhizome yield.**Figure 3.** (A) AMMI1 biplot (B) AMMI2 biplot for number of primary rhizomes.

Again, for NMR, the Rajani and Kasthuri varieties were more stable than Bharamputra-1 (Figure 4A). The variety Kasthuri was best suitable for Imphal, Rajani variety for Lakhimijan, and Bharamputra-1 for Jorhat and Gorigaon, as revealed through AMMI2 biplot analysis of NMR (Figure 4B).

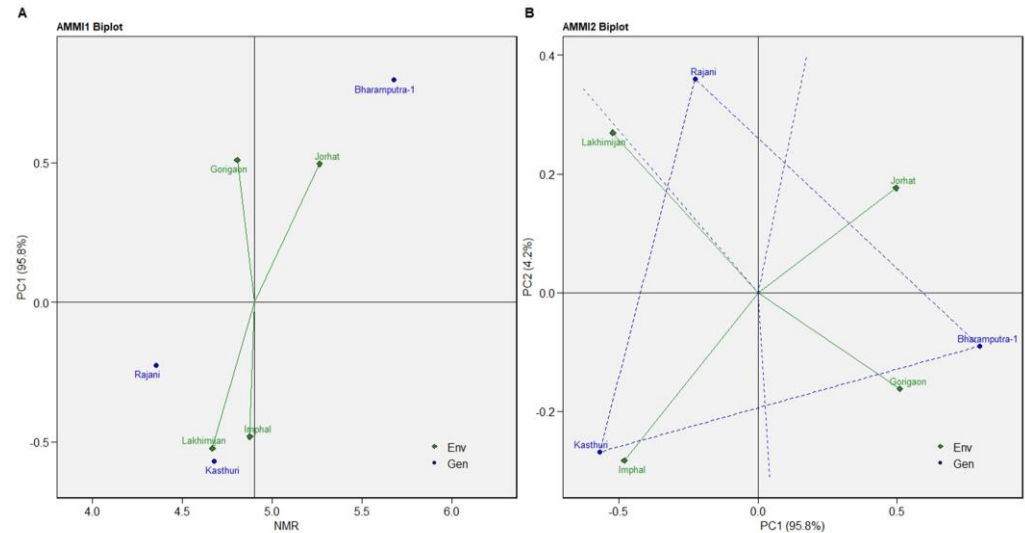


Figure 4. (A) AMMI1 biplot (B) AMMI2 biplot for number of mother rhizomes.

The dry rhizome recovery of Bharamputra-1 was found to be less than those of Kasthuri and Rajani but showed stability in all MLTs (Figure 5A). As predicted in AMMI2 biplot, DRR of Bharamputra-1 was highest in Jorhat while those of Rajani in Imphal and Gorigaon, and Kasthuri in Lakhimijan (Figure 5B).

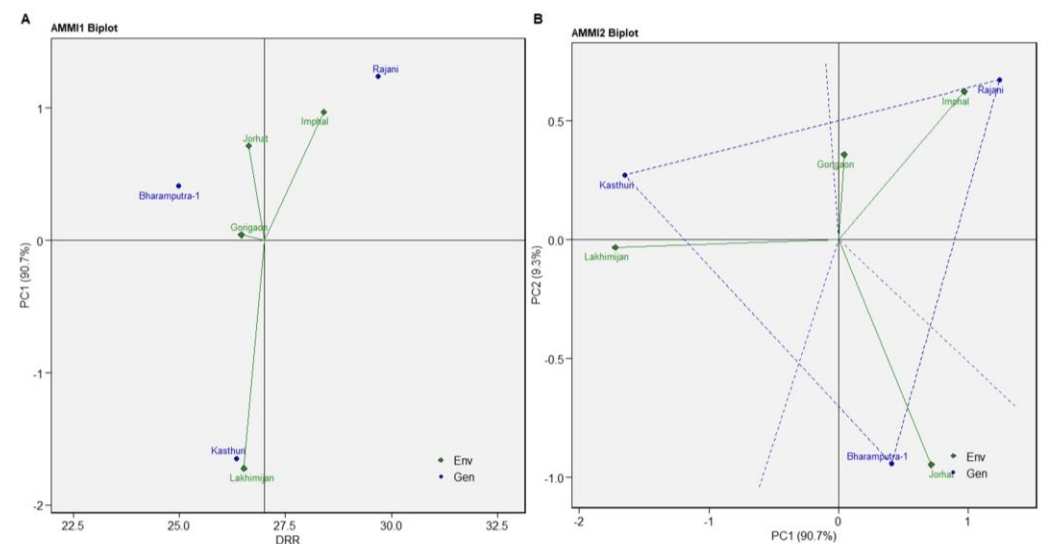


Figure 5. (A) AMMI1 biplot (B) AMMI2 biplot for dry rhizome recovery.

Similarly, the essential oil yield was highest in Rajani variety followed by Bharamputra-1 and Kasthuri varieties but Bharamputra-1 was the most stable genotype compared with the other two varieties (Figure 6A). According to the AMMI2 biplot of EO, Bharamputra-1 appeared as the most suitable high-yielding genotype for the locations. According to the AMMI2 biplot of EO, Bharamputra-1 was the most suitable high yielding genotype for the location Jorhat; Kasthuri for Lakhimijan and Imphal; while Rajani for Gorigaon (Figure 6B).

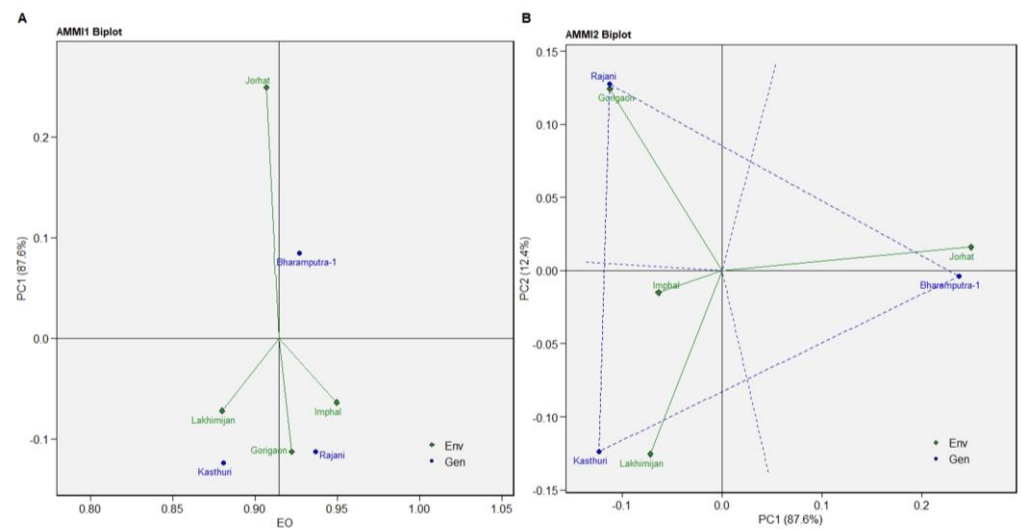


Figure 6. (A) AMMI1 biplot (B) AMMI2 for essential oil yield.

The date of maturity was earlier for Bharamputra-1, followed by Kasthuri and Rajani, as revealed by the AMMI1 biplot (Figure 7A). Bharamputra-1 was indicated as the genotype suitable for Lakhimjan and Gorigaon locations, while Kasthuri and Rajani was favourable for Imphal and Jorhat, respectively (Figure 7B). Both the Eberhart–Russel and the AMMI model analyses clearly demonstrated Bharamputra-1 as a superior stable genotype based on rhizome yield and essential oil yield.

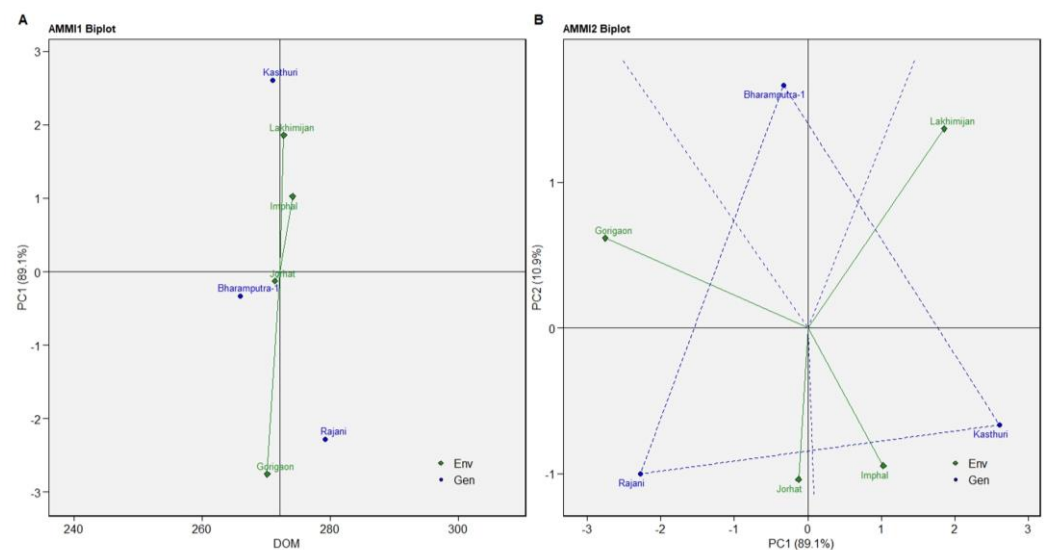


Figure 7. (A) AMMI1 biplot (B) AMMI2 biplot for days to maturity.

The GC/MS analysis of the essential oil extracted from Bharamputra-1 is presented in Table 7 and Figure 8. The analysis showed that the principal component identified were ethyl-p-methoxycinnamate (37.25%), trans-ethyl cinnamate (28.35%), endo-borneol (8.91%), eucalyptol (6.83%), (-)-camphor (3.98%) and 3-carene (3.77%). A total of thirty chemical constituents were identified, which accounted for 98.58% of the total essential oil composition (Table 7, Figure 8).

Table 7. GC/MS analysis of the rhizome essential oil of the registered germplasm “Bharamputra-1”.

Sl. No.	Name of the Compound	RT	Area %	KI*	KI**	Identification Method
1	Benzaldehyde	7.18	1.25	966	961	1,2
2	3-Carene	7.68	3.77	1003	1001	1,2,3
3	Pseudolimonene	8.07	0.64	1004	1005	1,2
4	<i>p</i> -Cymene	8.45	0.41	1026	1027	1,2
5	<i>D</i> -Limonene	8.67	0.87	1030	1028	1,2,3
6	Eucalyptol	9.93	6.83	1033	1032	1,2,3
7	Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl	10.67	0.26	1124	1125	1,2
8	(-)-Camphor	10.79	3.98	1141	1146	1,2,3
9	<i>endo</i> -Borneol	16.62	8.91	1166	1165	1,2
10	3,6-Octadienal, 3,7-dimethyl	17.37	0.41	1182	1183	1,2
11	α -Terpineol	17.76	0.29	1189	1190	1,2,3
12	Carveol	18.57	0.37	1224	1225	1,2
13	(-)-Bornyl acetate	22.04	0.24	1280	1285	1,2
14	Thymol	23.12	0.89	1297	1296	1,2,3
15	Car-3-en-5-one	23.60	0.49	1314	1314	1,2
16	Myrtenyl acetate	24.34	0.16	1328	1326	1,2
17	(+)-Cyclosativene	24.55	0.22	1362	1364	1,2
18	3 <i>H</i> -3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3a <i>R</i> -(3a. α .,4. β .,7. α .)]	24.73	0.27	1401	1398	1,2
19	Alloisolongifolene	25.58	0.35	1409	1409	1,2
20	β -Copaene	26.61	0.47	1428	1430	1,2,3
21	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl	26.92	0.15	1452	1452	1,2
22	<i>trans</i> -Ethyl cinnamate	29.67	28.35	1462	1464	1,2,3
23	<i>tert</i> -Butyl- <i>p</i> -benzoquinone	32.25	0.08	1472	1472	1,2
24	<i>cis</i> -Calamenene	34.04	0.14	1520	1520	1,2,3
25	Spatulenol	34.28	0.17	1574	1571	1,2
26	Epicubenol	35.52	0.29	1622	1627	1,2,3
27	τ -Cadinol	36.52	0.15	1635	1633	1,2
28	Pentadecane	37.00	0.68	1703	1707	1,2,3
29	Ethyl <i>p</i> -methoxycinnamate	40.68	37.25	1781	1785	1,2,3
30	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl-, [4a <i>S</i> -(4a. α .,4b. β .,7. β .,10a. β .)]-	44.57	0.24	1923	1919	1,2
Total = 100%, Identified compounds= 98.58%, Unidentified compounds= 1.42%						

KI* = Kovats Index experimental, KI** = Kovats Index literature (Adams, 2017). 1. Comparison of the retention indices with those in the literature, 2. comparison of the mass spectra with those in mass libraries, 3. comparison of the retention times with those of standards injected in the same GC conditions.

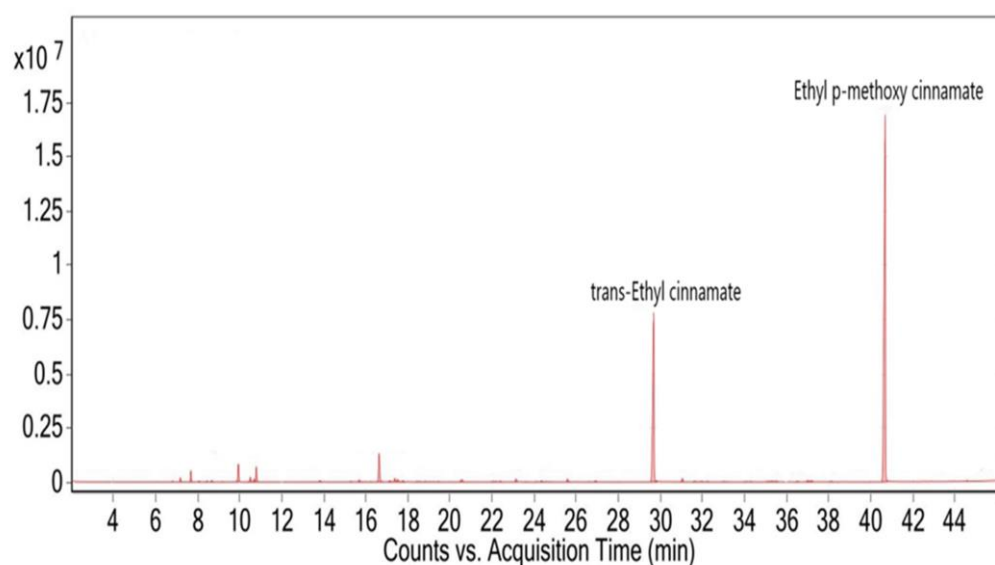


Figure 8. GC/MS chromatogram of Bharamputra-1 essential oil.

A superior variety released earlier, named Jor Lab K-1, contains ethyl-trans-cinnamate (31.12%), ethyl-trans-p-methoxycinnamate (14.3%), 1,8-cineole (10.57%), δ -3-carene (5.12%) and n-pentadecane (4.8%) as the major components of the rhizome essential oil [23]. Similarly, the essential oil extracted from *K. galanga* rhizomes collected from the southern region of India comprises ethyltrans-p-methoxycinnamate (28–70%), trans-ethylcinnamate (11.5–26.6%), pentadecane (6–16.5%), δ -3-carene (0.1–6.5%), 1,8-cineole (0.2–5.2%) and bornenol (1–2.4%) as the principal components [36]. The presence of higher concentrations of ethyl p-methoxycinnamate and trans-ethyl cinnamate in the identified cultivar, which are responsible for several biological activities like anti-inflammatory, anti-tuberculosis, anti-angiogenic, larvicidal activities [5], etc., is an added advantage of the variety. The compound ethyl p-methoxycinnamate also possesses hypopigmentation effects and can act as a skin whitening agent to be utilized in cosmetics [5]. Therefore, this variety will be economically helpful to growers, entrepreneurs and also to the cosmetics, pharmaceuticals, food-flavoring, aromatherapy and perfume industries. This is the first report on a high-rhizome-yielding and stable variety of *K. galanga* from India. The cultivation of this variety will be helpful economically to growers, entrepreneurs and agro-based industries.

4. Conclusions

A detailed multiyear and multilocation study was performed to identify the novel germplasm Bharamputra-1, with high rhizome and essential oil yields, which was shown to maintain its stability in different environmental conditions. The stability of the identified germplasm was compared with those of the two earlier registered varieties Kasthuri and Rajani, released by Kerala Agriculture University, India, through the Eberhart–Russell and AMMI models. The GC/MS analysis of the essential oil also demonstrated a high ethyl p-methoxy cinnamate content, responsible for the bioactivity of the plant. The identification of the Bharamputra-1 variety of *K. galanga* will help in maintaining the sustainable cultivation and conservation of this medicinally important endangered plant. Furthermore, the identification of this superior variety of *K. galanga* will provide the necessary boost to the pharmaceutical industry through higher value essential oil and high rhizome yield. Earlier, no high-yielding rhizome variant of *K. galanga* was reported; therefore, this is the first report on the identification of a superior line (high rhizome yield) of this crop. The germplasm Bharamputra-1 was registered with the NBPGR (National Bureau of Plant Genetic Resources), New Delhi, vide registration number INGR17081.

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Abbreviations

AMMI = Additive Main effects and Multiplicative Interaction; GC/MS = Gas Chromatography/Mass spectrometry; RY = rhizome yield; NMR = number of mother rhizomes; NPR = number of primary rhizomes; DRR = dry rhizome recovery; EO = essential oil yield; DOM = date of maturity; ha = hectare; ANOVA = Analysis of Variance; MLT = multilocation trial.

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