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Assessment of morphological variability and diversity analysis in *Abrus precatorius* (L.) germplasm conserved at National Gene Bank, NBPGR

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Abstract
Abrus precatorius (L.) commonly known as Ratti was a perennial herb which was native to Indian sub-continent. Indian National Gene Bank (NGB), National Bureau of Plant Genetic Resources (NBPGR) conserves 152 accessions (august, 2022) of Ratti collected from the diverse agro-ecological zones of India. The plant has wide range of usage especially in pharmacological industry. In Indian subcontinent, various parts of this plant were traditionally used in Ayurveda and siddha system of medicines. Various Vedic literatures of ancient India had mentioned about its importance in measuring the weight of gold. The present investigation was carried out to assess the morphological variability and analyse the diversity of Ratti germplasm. Apart from this, the study was also focussed to bring out accessions suitable for sole crop and inter-crop cultivation.

Keywords: *Abrus precatorius* (L.), medicinal plants, variability, ratti, morphology

Introduction
Naturally derived medicinal products have received considerable attention among the public in recent years because of their maximum efficiency and minimum side effects. *Abrus precatorius* (L.) (also known Indian liquorice) was a medicinal plant originated from Southeast Asia and native to India (Tabasum *et al*. 2016) [28]. It belongs to pea family, fabaceae and is the only representative genus of the tribe, Abreae (Prenner 2013) [20]. It was mostly found in the drier areas of tropical and subtropical regions (Paul *et al*. 2013) [8]. It was widely distributed at an altitude of upto 1200 m above sea level in the outer Himalayan region (Garaniya and Bapodra 2014) [8]. The plant was well adapted to a wide range of environmental condtions and commonly found as weeds throughout the plains and forest land. It was known by different names like precatory beans, Ratti, Crab's eye, Jequerity pea, Rosary pea, Lucky bean etc. Nearly, 18 species were found in the genus Abrus. This includes *Abrus aureus*, *Abrus baladensis*, *Abrus canescens*, *Abrus diversifoliatus*, *Abrus fruticulosus*, *Abrus gawenensis*, *Abrus kaokoensis*, *Abrus laevigatus*, *Abrus longibracteatus*, *Abrus lusorius*, *Abrus madagascariensis*, *Abrus parvifolius*, *Abrus precatorius*, *Abrus pulchellus*, *Abrus sambiranensis*, *Abrus schimperi*, *Abrus somalensis* and *Abrus wittei* (Bhatia *et al*. 2013) [3]. The term Abrus was derived from the Latin word which means "beautiful" or "graceful" (Okhale and Nwanosike 2016) [14]. It represents the visual appearance of seed. The chromosome number of *Abrus precatorius* (L.) was 2n=22. The seeds are highly poisonous due to the presence of lethal toxin, abrin (Olsnes and Pihl 1973; Bhakta and Das 2020) [15, 2]. The roots and leaves of Ratti also contain glycyrrhizin, which was major component of liquorice plant. The same bioactive constituent was also responsible for its medicinal properties. The antidote for abrus poisoning were not available. It was a toxalbumin that prevents the protein synthesis resulting in cell death. The Abrus toxicity causes symptoms like diarrhea, vomiting and nausea (Alhamdani *et al*. 2015) [1]. On higher dose, it leads to renal failure, encephalopathy and death of the individual. In the traditional system of medicine, all parts of this plant including the roots, leaves and seeds were used. The fresh leaves were used as nerve tonic and in the formulations for skin cancer. The roots and leaves were traditionally used in the treatment of bronchitis and asthma (Taur *et al*. 2017; Qian *et al*. 2022) [29, 22]. Petroleum ether extract of seeds was known to have anti-cancer activity (Das *et al*. 2016) [6] and the methanol extract of leaves have strong antioxidant activity (Palvai *et al*. 2014; Jain *et al*. 2015) [17, 9]. Along with other components, it was main ingredient in the treatment of rabies, tetanus and leukoderma (Shourie and kalra 2013) [27].

In Nigeria, they were used in the treatment of diseases like cough, hepatitis and respiratory tract infections. The detoxified seeds (by boiling) of *A. precatorius* were eaten as food by the residents of Andaman and Nicobar Islands in India (Rajaram and Janardhanan 1992) [23] and many other African nations. Ratti seeds were rich in essential amino acids (except cysteine and threonine). The antinutritional factors like trypsin inhibitor activity, total free phenols, tannins and haemagglutinating activity have also been reported. These components along with toxic albumin, abrin prevents the raw consumption of Ratti seeds (i.e. without detoxification). In ayurvedha and siddha, the seeds for its usage in various therapies need to be detoxified by a process called Sodhana (Nabar *et al*. 2011; Dhoble and Majumdar 2014; Shelke and Misal 2016) [12, 7, 26]. The traditional media used in detoxification of Ratti seeds were cow's milk by using traditional dola yantra. The history of its importance in weights and as weighing unit dates back to Vedic period of ancient India. In Yajurveda, krsnala has been described. Krsnala indicates the seed of *Abrus precatorius* which has been used in measuring the weight of gold. The different Yajurveda Saṃhitas give a detailed account of Ratti seed as a weighing unit. Few among them were Taittiriya Saṃhita (2-3-2), Kaṭhaka Saṃhita (11-4), Maitrayaṇi Saṃhita (2-2-20), Taittiriya Brahmaṇa (1-3-6) and also in Manusmṛti (8-134c and 8-135c) (Boddupalli 2020) [4].

Materials and Methods

Seed material

A total of 99 accessions were chosen based on germination % and availability of sufficient amount of seeds in the Indian National Gene Bank, National Bureau of Plant Genetic Resources (NBPGR). These accessions represent the different agro-ecological zones of India with the exception of north east India (Fig.1 and 2). Though the north east India was reported to have the variability of *Abrus precatorius*, not even a single collection was there in the Genebank. The seeds were taken sowing in two locations *viz*. NBPGR Experimental centre, Issapur and NBPGR regional station, Ranchi on 6th june and 12th july, 2019-20.

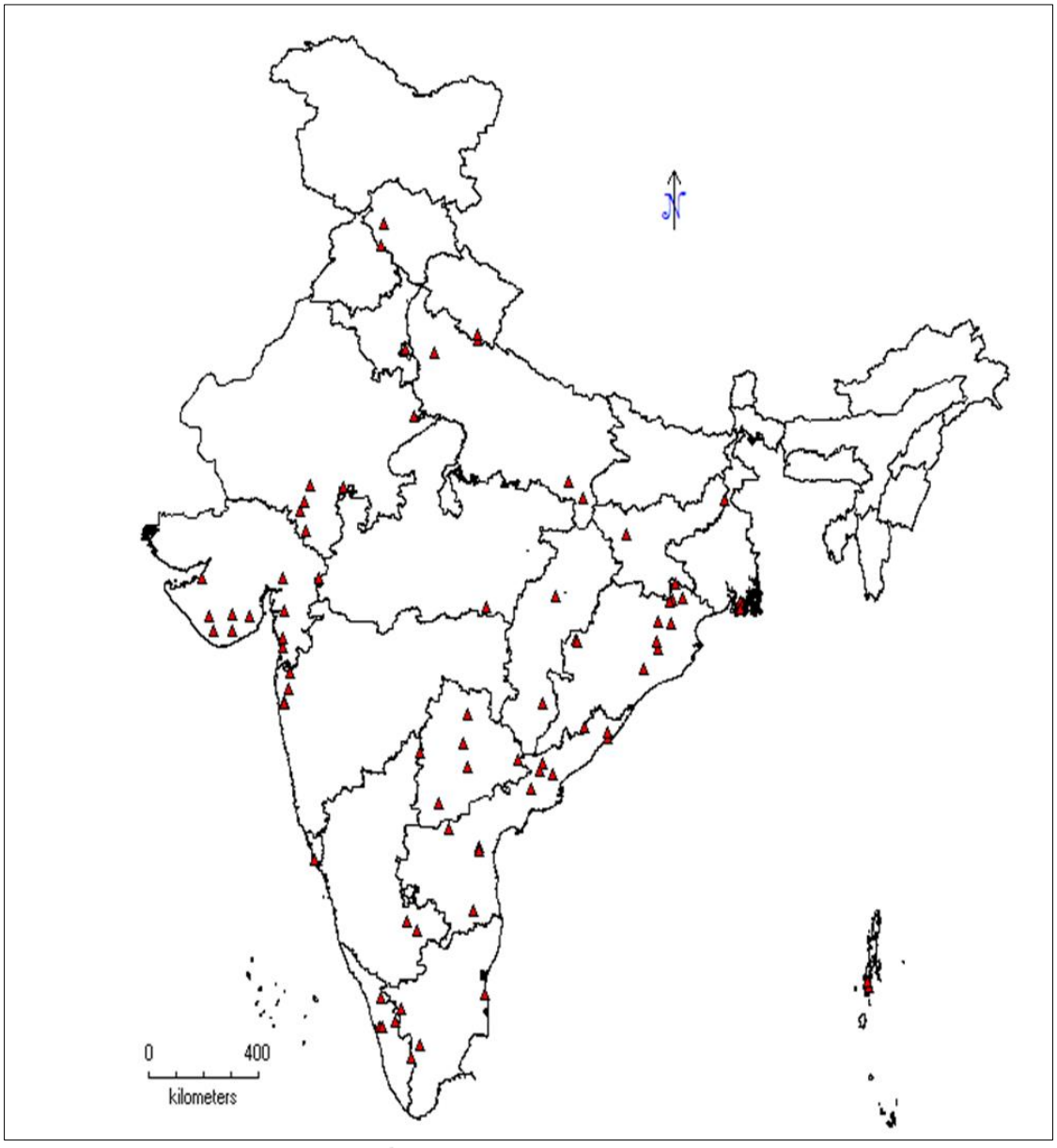

Fig 1: Geographical distribution of Ratti accessions

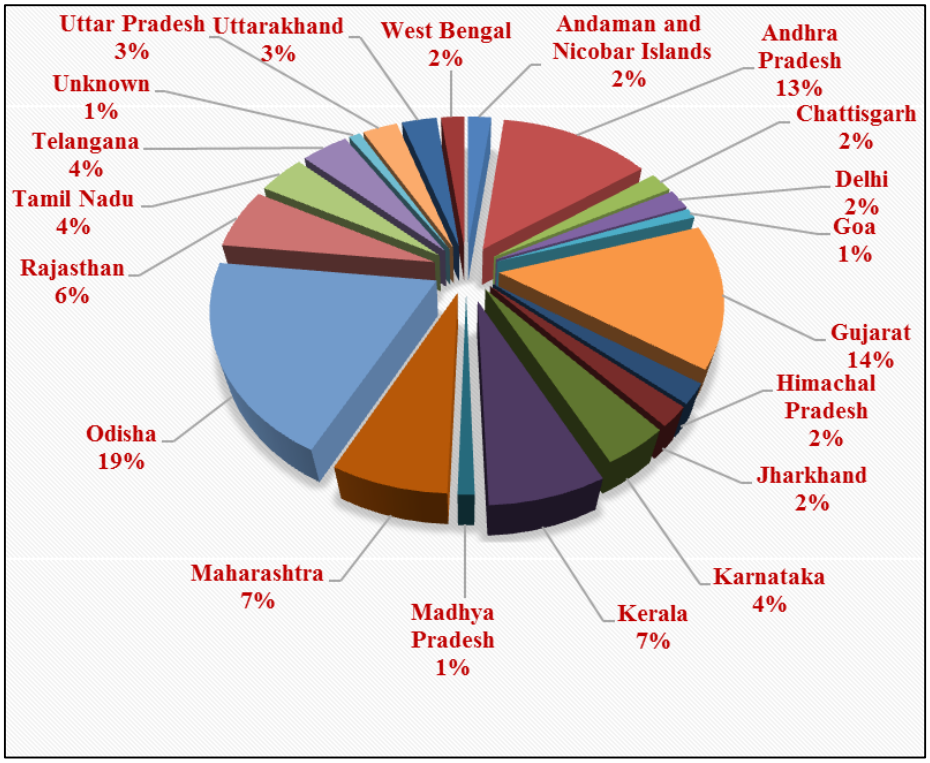

Fig 2: State-wise share of Ratti accessions used in the study

Experimental Design

The seeds were sown as main crop at Issapur whereas it was intercropped with Bael (*Aegle marmelos*) at Ranchi. The experimental design used was augmented block design in which the checks were replicated in each block while the entries were not replicated. Totally, 95 entries and 4 checks were used. Commercial varieties were not available in this crop and therefore accessions itself was used as checks. These checks were IC0306236, IC376080, IC0469931 and IC0418097.

Breaking dormancy

The seeds were highly dormant because of its leathery testa and hard seed coat (Fig. 3). This makes the seed impermeable to water and oxygen (Pallavi *et al*. 2014; Qadir *et al*. 2012) [16, 21]. In order to break the dormancy, two techniques were used (Vyas and Agarwal 1973; Mudasir *et al*. 2012; Prakash *et al*. 2013; Nautiyal *et al*. 2014; Rekha and Santhosh 2017; Malammanavar *et al*. 2019; Sharma 2021) [30, 11, 19, 13, 24, 10, 25]. In the first technique, the seeds were mechanically scarified over sand paper. The seed was held between the index finger and thumb finger and the hilum region was scarified over the rough surface of the sand paper. The other technique used was acid scarification. The seeds were treated with 25% concentrated sulphuric acid for one hour. This had made the minute punctures in the leathery testa making them permeable to water and oxygen.

Fig 3: *Abrus* seeds showing poor germination due to dormancy in IC0400492 placed over the germination paper

Data Recording and analysis

A total of 19 morphological traits (qualitative traits-5 and quantitative traits-14) have been recorded which was based on the descriptor developed by NBPGR. Average of ten random plants/seeds per accession was taken for recording observations. Data of flower colour and seed colour was recorded using Royal horticultural society (RHS) colour chart. Measuring scale was used to record data like pod length (cm), pod width (cm), inflorescence length and vernier caliper was used to record data like seed length (mm) and seed width (mm). Digital weighing balance was used to record the data like seed yield per plant (g) and 1000 seed weight (g). Visual appearance based on naked eye was used record data like plant growth habit, pod shape and seed shape.

The data has been analysed using the software R studio. The Analysis of variance (ANOVA) was done by mixed model analysis based on BLUP values (Best Linear Unbiased Prediction).

Results and Discussion

Qualitative traits

There were mainly five qualitative traits that were used in the characterization of Ratti accessions (Fig. 4, 5, 6, 7 and 8). These traits were as follows:

Fig 4: Variability in plant growth habit

Fig 5: Variability in flower colour

Fig 6: Seed colour variability

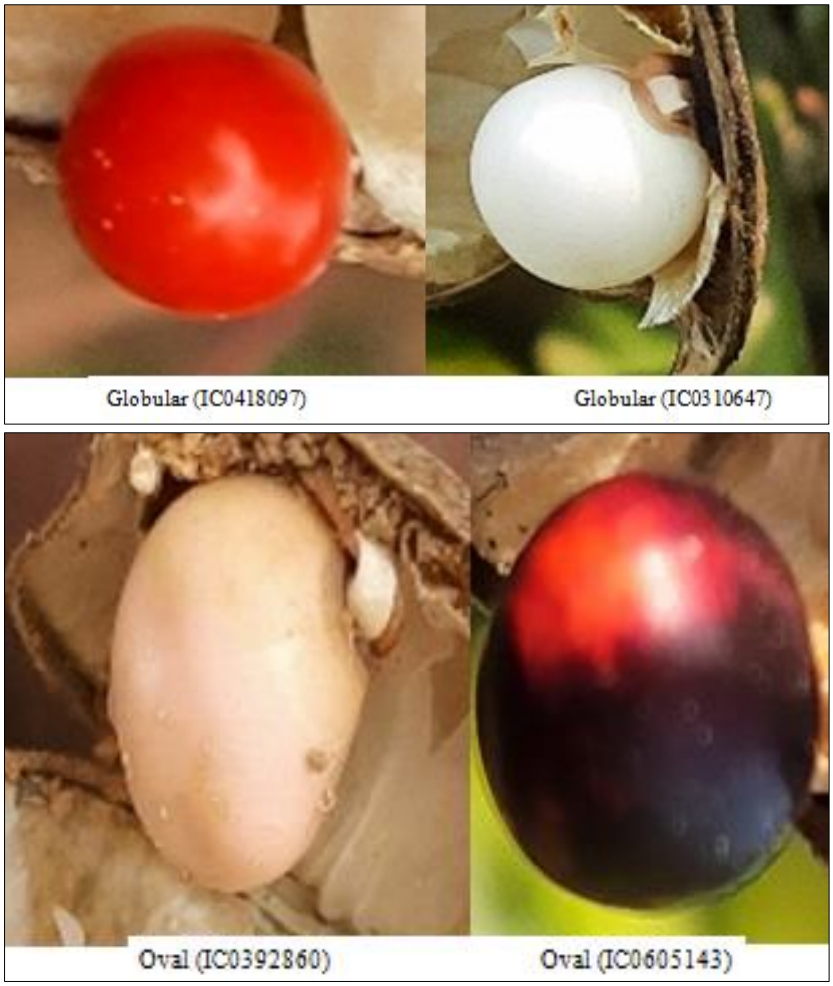

Fig 7: Variability in seed shape

Fig 8: Variability in pod shape

Quantitative traits

There were mainly fourteen quantitative traits that were used in the characterization of Ratti accessions (Table 1).

Table 1: Code used for various quantitative traits in the data analysis

Code used	Quantitative trait
DFI	Days to flower initiation
IL	Inflorescence length (cm)
NIF_P	No. of inflorescence/plant
NF_IF	No. of Flowers/inflorescence
DM	Days to complete pod maturity
NP_C	No. of pods/cluster
NPC_P	No. of pod cluster/plant
PL	Pod length (cm)
PW	Pod width (cm)
NS_P	No. of seeds/pod
SL	Seed length (mm)
SW	Seed width (mm)
1000SW	1000 seed weight (g)
SYP	Seed yield per plant (g)

Table 2: ANOVA table

Source	DFI	IL	NIF_P	NF_IF	DM	NP_C	NPC_P
Genotype	226.08**	1.44**	15.35**	37.66**	229.12**	7.62**	18.39**
Genotype × Environment	4.22*	0.617**	3.85**	6.24e-01ns	7.40**	2.31**	0.24 ns
Environment	12.39**	0.00017ns	5.28**	1.60e-13ns	10.67**	0.29ns	1.74**

Source	PL	PW	NS_P	SL	SW	1000SW	SYP
Genotype	3.22**	0.13**	3.54**	0.23**	0.18**	245.12**	76.37**
Genotype × Environment	2.39e-18ns	4.51e-18ns	0.00021ns	2.38e-03ns	1.71e-03ns	7.919e-06ns	5.82e-12ns
Environment	7.17e-03*	6.62e-05ns	0.00004ns	4.27e-13ns	5.99e-05ns	4.21 e-05ns	4.08ns

Ns–non n significant; * - denotes significant at 5% LOS; * * - significant at 1% LOS

Table 3: Mean performance of Ratti accessions at Issapur and Ranchi

Quantitative traits	Mean performance at Issapur	Mean performance at Ranchi
DFI (days)	440.46	435.84
IL (cm)	8.28	8.17
NIF_P	22.75	19.52
NF_IF	19.20	19.22
DM (days)	483.20	479.15
NP_C	10.70	9.89
NPC_P	13.58	11.76
PL (cm)	4.27	4.38
PW (cm)	1.24	1.26
NS_P	3.49	3.39
SL (mm)	6.21	6.22
SW (mm)	4.63	4.65
1000SW (g)	108.80	108.89
SYP (g)	13.02	10.13

Days to flower initiation (DFI)

Highly significant variation at 1% level of significance was observed among genotypes and also between two locations. Genotype × environment (G×E) interaction was significant at 5% level of significance (Table 2). The average days to flower initiation of Ratti accessions as a sole crop (at Issapur) and intercrop (at Ranchi) were 440.46 days and 435.84 days respectively (Table 3). The top three early flowering accessions at Issapur were IC0526819 (420.3 days), IC0418096 (420.6 days) and IC0261408 (420.9 days) and at Ranchi were IC0261408 (412.7 days), IC0564539 (414.2 days) and IC0469939 (415.3 days).

Inflorescence length (IL)

The genotype and genotype × environment (G×E) interaction have shown highly significant variation at 1% level of significance. The variation among the different environment was statistically non-significant (Table 2). The mean inflorescence length of Ratti accessions as a sole crop (at Issapur) and intercrop (at Ranchi) were 8.28 cm and 8.17 cm respectively (Table 3). The top three accessions with longest inflorescence length at Issapur were IC261421 (11.9 cm), IC0401666 (11.4 cm) and IC397936 (11.3 cm) and at Ranchi were IC0310855 (11.9 cm), IC0310646 (11.5 cm) and IC0306198 (11.3 cm).

No. of inflorescence/plant (NIF_P)

It has shown highly significant variation at 1% level of significance among all the three sources i.e. genotypes, environment and genotype × environment (G×E) interaction (Table 2). The average no. of inflorescence/plant among Ratti accessions as a sole crop (at Issapur) and intercrop (at Ranchi) were 22.75 and 19.52 respectively (Table 3). The top three accessions with highest no. of inflorescence/plant at Issapur were IC0369145 (31.5), IC261421 (30.8) and IC0385619 (30.7) and at Ranchi were IC0322486 (30.2), IC0391888 (30.2) and IC0400421 (29.8).

No. of Flowers / inflorescence (NF_IF)

Highly significant variation at 1% level of significance was observed among the genotypes. The variations among the environment and genotype × environment (G×E) interaction were statistically non-significant (Table 2).

Thus the average no. of Flowers/inflorescence at Ranchi and Issapur i.e. 19.20 and 19.22 were statistically on par (Table 3). The top three accessions with highest no. of flowers/inflorescence at Issapur were IC0263011 (29.8), IC0392839 (28.5) and IC0469963 (27.7) and at Ranchi were IC0400421 (28.5), IC0280795 (28.5) and IC0385619 (28.3).

Days to complete pod maturity (DM)
It has shown highly significant variation at 1% level of significance among all the three sources i.e. genotypes, environment and genotype × environment (G×E) interaction (Table 2). The average no. of days for complete pod maturity at Issapur and Ranchi were 483.20 days and 479.15 days (Table 3). The top three accessions which were short duration and early maturing at Issapur were IC0564539 (455.6 days), IC0470438 (456 days) and IC0526819 (456.5 days) and at Ranchi were IC0564539 (449.3 days), IC0470438 (449.5 days) and IC0526819 (451.9 days).

No. of pods/cluster (NP_C)
The genotype and genotype × environment (G×E) interaction have shown highly significant variation at 1% level of significance. The variation among the different environment was statistically non-significant (Table 2). The average number of pods/cluster of all the Ratti accessions as a sole crop and inter-crop were 10.70 and 9.89 (Table 3). The top three accessions with highest no. of pods/cluster at Issapur were IC0553765 (18.4), IC0371792 (17.8) and IC0469939 (17.5) and at Ranchi were IC0405305 (18.7), IC0470979 (18.5) and IC0280795 (18.4).

No. of pod cluster/plant (NPC_P)
Genotype and environmental sources of variation have shown highly significant variation at 1% level of significance. The variation of genotype × environment (G×E) interaction was statistically non-significant (Table 2). The average no. of pod cluster/plant at Issapur and Ranchi were 13.58 and 11.76 (Table 3). The top three accessions with highest no. of pod cluster/plant at Issapur were IC261421 (17.9), IC0395270 (17.9) and IC0385644 (17.9) and at Ranchi were IC0391888 (17.8), IC0418120 (17.5) and IC0311747 (17.3).

Pod length (PL)
The genotypic sources of variation have shown highly significant variation at 1% level of significance. The environmental variation was significant at 5% level of significance. The variation of genotype × environment (G×E) interaction was statistically non-significant (Table 2). The mean pod length at Issapur and Ranchi were 4.27 cm and 4.38 cm (Table 3). The top three accessions with highest pod length at Issapur were IC0405311 (6.4 cm), IC0617322 (6.4 cm) and IC0405295 (6.3 cm) and at Ranchi were IC0385619 (6.5 cm), IC0405311 (6.5 cm) and IC0405295 (6.5 cm).

Pod width (PW)
Highly significant variation at 1% level of significance was observed among the genotypes. The variations among the environment and genotype × environment (G×E) interaction were statistically non-significant (Table 2). Thus the average pod width of all accessions at Issapur (1.24 cm) and Ranchi (1.26 cm) were statistically on par (Table 3). The top three accessions with highest pod length at Issapur were IC0553765 (1.8 cm), IC0605146 (1.8 cm) and IC0392859 (1.7 cm) and at Ranchi were IC0392860 (1.8 cm), IC0385619 (1.7 cm) and IC0405311 (1.7 cm).

No. of seeds/pod (NS_P)
The genotypic variation was highly significant at 1% level of significance. Remaining sources of variation i.e. environmental and genotype × environment (G×E) interaction were statistically non-significant (Table 2). Therefore, the average no. of seeds/pod at Issapur (3.49) and Ranchi (3.39) were statistically on par (Table 3). The top three accessions with highest no. of seeds/pod at Issapur were IC0418096 (6.3), IC0385619 (6.1) and IC0418115 (6.1) and at Ranchi were IC0306198 (6.7), IC0385635 (6.5) and IC0418096 (6.5).

Seed length (SL)
Genotypic sources have revealed highly significant variation at 1% level of significance, whereas variations of environment and genotype × environment (G×E) interaction were statistically non-significant (Table 2). Thus, the mean seed length at Issapur (6.21 mm) and Ranchi (6.22 mm) were statistically similar (Table 3). Best performing accessions with respect to seed length at Issapur were IC0322320 (7.43 mm), IC0401666 (7.14 mm) and IC0564539 (6.89 mm) and at Ranchi were IC0322320 (7.46 mm), IC0401666 (7.23 mm) and IC0392860 (6.93 mm).

Seed width (SW)
Highly significant variation at 1% level of significance was observed among the genotypes. The variations among the environment and genotype × environment (G×E) interaction were statistically non-significant (Table 2). Thus the mean seed width of all accessions at Issapur (4.63 mm) and Ranchi (4.65 mm) were statistically on par (Table 3). The top three accessions with highest seed width at Issapur were IC0263011 (5.47 mm), IC0400421 (5.39 mm) and IC0349819 (5.38 mm) and at Ranchi were IC0310855 (5.43 mm), IC0397770 (5.42 mm) and IC0261408 (5.42 mm).

1000 seed weight (1000 SW)
The genotypic variation was highly significant at 1% level of significance. Other sources of variations i.e. environmental and genotype × environment (G×E) interaction were statistically non-significant (Table 2). Therefore, the mean value of 1000 seed weight at Issapur (108.80 g) and Ranchi (108.89 g) were statistically on par (Table 3). The top three accessions with maximum 1000 seed weight at Issapur were IC0469939 (127.225g), IC0418120 (127.015 g) and IC0281057 (125.729 g) and at Ranchi were IC0418120 (127.852g), IC0469939 (126.82 g) and IC0281057 (126.628 g). Previously, Chakradhari *et al*. (2019) had reported the per seed weight of Ratti samples that they studied as 125±3 mg.

Seed yield per plant (SYP)
Genotypic sources have revealed highly significant variation at 1% level of significance, whereas variations of environment and genotype × environment (G×E) interaction were statistically non-significant (Table 2). Thus, the mean seed yield per plant at Issapur (13.02 g) and Ranchi (10.13 g) were statistically similar (Table 3). Best performing accessions with respect to seed yield per plant at Issapur were IC0385619 (32.416 g), IC0371792 (30.27 g) and IC0553727 (29.651 g) and at Ranchi were IC0322486 (27.626 g), IC0280795 (27.187 g) and IC0385619 (26.878 g).

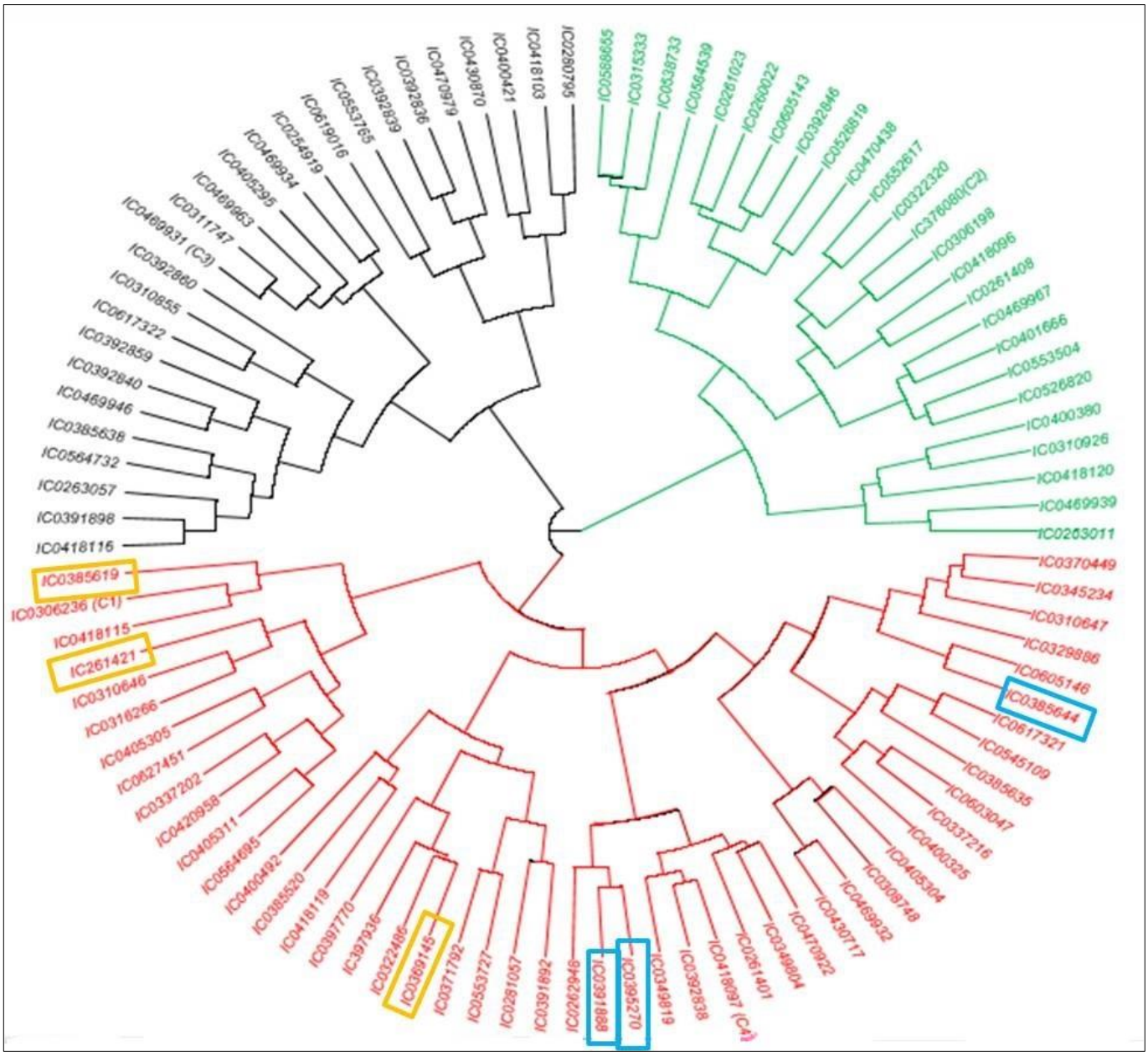

Fig 9: Radial dendrogram based on morphological data of Ratti germplasm.

Cluster analysis

Radial dendrogram was constructed based on the recorded observations and by using the software R studio (Fig. 9). This forms three main clusters which was highlighted with three different colours ie. Main cluster 1 with black colour, main cluster 2 with pink colour and main cluster 3 with light green colour. The main cluster 1, main cluster 2 and main cluster 3 consist of 26 accessions (including 1 check), 48 accessions (including 2 checks) and 25 accessions (including 1 check). All the three main clusters were later divided into 2 sub-clusters each which was further divided into several sub-clusters. In the main cluster 2, accession number highlighted with yellow coloured box depicts the top three best performing accessions with respect to number of inflorescence per plant *viz*. IC0369145 (31.5), IC261421 (30.8) and IC0385619 (30.7). Similarly, accession number highlighted with blue coloured box depicts the top three best performing accessions with respect to number of pod cluster per plant *viz*. IC0385644 (17.9), IC0395270 (17.9) and IC0391888 (17.8).

Conclusion

Despite the Indian sub-continent was the native region of Ratti crop, this work was the first of its kind covering the large number of accessions from different parts of the country. The present investigation revealed wide range of morphological variability among the Ratti accessions in the Indian centre of origin. Diversity of Ratti germplasm from the North east India is yet be explored. The seed yield per plant of Ratti accessions as a sole crop and as an inter-crop were statistically on par. This shows the ability of the plant to adapt to inter-cropping system and give optimum yield. Promising Ratti accessions suitable for main crop (IC0385619, IC0371792 and IC0553727) and intercrop (IC0322486, IC0280795 and IC0385619) cultivation were revealed. These accessions can be utilized by the farmers and pharmacological companies for the large scale commercial cultivation and in the development of drugs.

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Conflict of interest
The authors declare that they have no conflict of interest in the content of the manuscript and study undertaken. The article is original and has not been submitted elsewhere for publication.

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