



Research Article

Study of morphological diversity of traditional aromatic rice landraces (*Oryza sativa* L.) collected from Assam, India

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Received: 2017-10-25; Accepted: 2017-11-16

Abstract: Rice is one of the major cereal grain consumed more than half of the world's population. Aromatic rice is one of the most widely accepted rice due its pleasant aroma. Traditionally, many varieties of aromatic rice are grown by the farmers of Assam maintaining a diverse gene pool. In the present study, morphological variation was studied in 22 aromatic rice landraces using qualitative and quantitative traits. Keteki Joha is very popular indigenous aromatic rice grown in a small pocket of Assam for its high yield. Though Kola Kunkuni Joha is relatively smaller grain size and low yield but have high demand due to its high aroma as compared to other landraces. Significant positive correlations was occurred at 0.05 level between kernel length and seed weight with seed width ($r = 0.6734^*$) and (0.5881^*) as well as seed weight with kernel width ($r = 0.5433^*$). The correlation between seed width and kernel width (0.9663^{**}) showed significantly positive relationship which shown at 0.01 level. Characterization of aromatic rice landraces of Assam would be a boon for the breeders for designing further rice improvement programme.

Keywords: Aromatic rice, diversity, landraces, seed morphology

Introduction

Nutritionally rice is one of the major cereal grain consumed more than half of the world's population. Scented or aromatic rice is an important commodity worldwide and command premium prices in local and international market over non-scented varieties because of their superior grain quality and pleasant aroma (Nayak *et al.*, 2002). Aromatic rice emits a special aroma with cooking. Aroma is rated the highest desired trait followed by taste and elongation after cooking by Indian consumers (Bhattacharjee *et al.*, 2002). Indian sub-continent is traditionally rich in the diversity of rice including the wild progenitors of cultivated rice (Singh *et al.*, 2001). It is a home for aromatic rice diversity (Bisne and Sarawgi, 2008) and grown in almost all the states, covering more than 30 per cent of the total cultivated area (Adhikari *et al.*, 2012; Chakravorty *et al.*, 2013; Sarma *et al.*, 2016). From the agro horticultural view point North east is the richest reservoir for genetic variability in aromatic rice crops (Hore 2005). Because of the special flavour and economic value, aromatic rice bears special significance in the present globalized era. Traditionally, many varieties of aromatic rice are grown by the farmers of Assam maintaining a diverse gene pool of aromatic rice which differs in various aspects such as aroma intensity, durability, grain shape and size, production potentialities etc. Assam is particularly rich in rice germplasm (Das *et al.*, 2010). The aromatic rice landraces of Assam is a superior class under *salis* or winter rice especially known as Joha rice. The Joha rice cultivars are favoured for its unique aroma, good cooking qualities and outstanding palatability (Das *et al.*

2010). Agro-morphological characterization of germplasm accessions is fundamental criteria in order to provide information of plants (Lin, 1991). Diversity studies in rice was done on improved and ancestral rice varieties of Philippines using morphological characters (Caldo *et al.*, 1997; Juliano *et al.*, 1998; Nahar *et al.*, 2016) and on Asian wild cultivated indigenous rice in Yunnan, China (Zeng *et al.*, 2003). Agro-morphological traits, both qualitative and quantitative have been commonly and traditionally used to estimate relationships between genotypes (Goodman, 1972). Though a large number of aromatic rice is existed suiting agro-climatic conditions of Assam but its characterization is not sufficient. The goal of the present study was to characterize morphological characters of native aromatic rice landraces of Assam.

Materials and Methods

Collection of rice landraces

22 aromatic landraces were collected from twelve districts viz., Nagaon, Karbi-anglong, Kamrup, Goalpara, Baksa, North lakhimpur, Jorhat, Cachar, Udalguri, Darrang, Dhemaji of Assam during 2015 - 2016. Areas of these regions were surveyed to get information from farmers about their preference of rice landraces. A diverse collection of short and long kernel, traditional aromatic rice landraces was made (Table 1). The exploration area lies between latitude $24^{\circ}68'52''N$ and longitude $90^{\circ}62'E$ to latitude $27^{\circ}01'69''N$ and longitude $95^{\circ}22'14'E$. It is surrounded on the north by Bhutan and Arunachal Pradesh, on the east by Arunachal

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Pradesh, Nagaland and Manipur, on the west by Meghalaya, Bangladesh and West Bengal, on the south by Tripura and Mizoram.

Methodology adopted and observations recorded

Random sampling procedure was followed for collection of different landraces. During collection, informal interaction was made with farmers of Assam. Kernel colours were recorded using code number mentioned in colour chart developed by RHS (The Royal Horticultural Society), London, United Kingdom (Royal Horticultural Society, 2015). Some visual observations like Seed coat colours and presence of awn were recorded for 22 rice landraces along with presence or absence of aroma. Rice were measured in grams by weighing 1000 seeds, length and breadth of kernel was also measured with the help of centimeter scale; length and breadth ratio was calculated; presence or absence of awn was observed and different qualitative characters were recorded on the basis of feedback from farmers.

Morphological character analysis

The morphological descriptors of various morphological characters are presented in Table 1. Observations were recorded on five randomly selected plants for different morphological descriptors viz., 1. Leaf Length (LL) in cm at heading stage; 2. Leaf Width (LW) in cm at heading stage; 3. Leaf Blade Colour (LBC) at stem elongation to heading stage with classes (i) Light green, (ii) Green, (iii) Dark green; 4. Panicle Axis (PnAK) at milk stage to mature stage with classes (i). Straight, (ii) Droopy; 5. Lemma and Palea Colour (LmPC) at mature stage with classes (i) straw, (ii) Black, (iii) Black with golden furrow (iv) Reddish straw (v) Brown with golden patches; seven agronomical traits were recorded, such as 1. Total tillers per hill (TT/hill), 2. Seed Length in cm, 3. Seed Breadth in cm, 4. Seed Length Breadth ratio (L/B ratio), 5. Kernel Length (L) in cm, 6. Kernel Breadth (B) in cm, 7. Length Breadth ratio (L/B ratio) and 8. Weight of 1000 seeds.

Data analysis and clustering

Multivariate statistical tools have found extensive use in analyzing, summarizing and describing the inherent variation among crop genotypes. Descriptive statistics of different rice landraces was analyzed by calculating mean, minimum, maximum, standard error and coefficient of variation. Correlation was calculated between seed length, seed breadth, kernel length, kernel breadth, and seed weight. Data were analyzed using XLSTAT version 2017 statistical packages. Cluster analysis of 22 landraces of rice was based on similarity in quantitative parameters (seed length, seed breadth, kernel length, kernel breadth, and kernel weight). A similarity matrix was generated based on the simple Euclidean distance across all accessions of different

landraces, and this matrix was used with a hierarchical clustering technique of Ward's Minimum variance method (Ward, 1963; Rao, 1964; Kaufman and Rousseeuw, 1990; Chutia *et al.*, 2012).

Results

The diversified Joha rice landraces distributed heterogeneously throughout Assam was collected from diverse populations of different places and assembled. Table 1 and Fig. 1 depict the areas of collection (collection sites), variability in some qualitative traits and diversity in aromatic rice landraces as revealed by field surveys and distribution of these landraces in different regions.

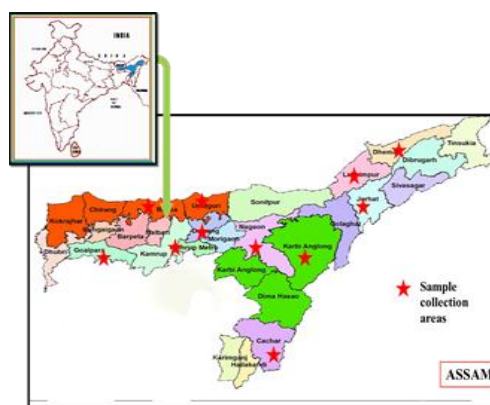


Figure 1. Map showing different sample collection area



Figure 2. Images of different rice varieties

Variation in qualitative traits

Discrepancy for qualitative traits in Joha rice landraces, mainly for seed coat colour, kernel colour, presence or absence of aroma and awns was recorded (Table 1 and 2; Fig. 2). A total of 14 accessions of landraces had Yellowish White 155B, 2 Greenish White 155C, 4 Pinkish white (N155B), 1 Yellowish Grey 156B and 1 White (NN155B) colour. Seed coat colours ranged from Golden (12 landraces), Black (7 landraces), brown (1 landraces), Golden with brown spotted (1 landrace) and Golden with brown in both end (1 landrace). The entire 22 collected landraces aroma was present. 21 accessions of the landraces were awn less and only one accessions viz. Kharika Joha was with awn.

Table 1. Variability studies in 22 traditional aromatic landraces of rice from different districts of Assam, India.

SL.NO.	Collector No.	Landrace name	Districts	Kernal Colour (Code)	Seed Coat Colour	Aroma	Presence of Awn
01	LL/BT/001	Kunkuni Joha	Nagaon	Greenish White 155C	Golden	Present	Awnless
02	LL/BT/002	Boga Kunkuni Joha	Goalpara	Greenish White 155C	Golden	Present	Awnless
03	LL/BT/003	Kola Kunkuni Joha	Kamrup	Yellowish White 155B	Black	Present	Awnless
04	LL/BT/004	Kola Joha	Jorhat	Yellowish White 155B	Black	Present	Awnless
05	LL/BT/005	Kala Jeera	Cachar	Yellowish White 155B	Black	Present	Awnless
06	LL/BT/006	Kola Tulsi Joha	Kamrup	Yellowish White 155B	Black	Present	Awnless
07	LL/BT/007	Tulsi Joha	Baksa	Yellowish White 155B	Black	Present	Awnless
08	LL/BT/008	Jwsa Gufur (Phisa-jhar)	Udalguri	Yellowish White 155B	Golden	Present	Awnless
09	LL/BT/009	Boga Joha	Darrang	Yellowish White 155B	Golden	Present	Awnless
10	LL/BT/010	Badsahbhog Joha	Baksa	Yellowish White 155B	Golden	Present	Awnless
11	LL/BT/011	Betguti Joha	Baksa	Pinkish White N155B	Golden with brown spotted	Present	Awnless
12	LL/BT/012	Kharika Joha	Kamrup	Yellowish Grey 156B	Golden	Present	Awned
13	LL/BT/013	Mukuta Joha	North Lakhimpur	Yellowish White 155B	Golden	Present	Awnless
14	LL/BT/014	Rampal Joha	Kamrup	Pinkish White N155B	Brown	Present	Awnless
15	LL/BT/015	Keteki Joha	North Lakhimpur	Pinkish White N155B	Golden	Present	Awnless
16	LL/BT/016	Gobinda Tulsi Joha	North Lakhimpur	Pinkish White N155B	Golden with brown in both end	Present	Awnless
17	LL/BT/017	Dangor Joha	Dhemaji	Yellowish White 155B	Black	Present	Awnless
18	LL/BT/018	Kon Joha	Jorhat	Yellowish White 155B	Golden	Present	Awnless
19	LL/BT/019	Guti Joha	Jorhat	Yellowish White 155B	Golden	Present	Awnless
20	LL/BT/020	Nepali joha	Baksa	Yellowish White 155B	Golden	Present	Awnless
21	LL/BT/021	Krishna joha	Jorhat	Yellowish White 155B	Black	Present	Awnless
22	LL/BT/022	Joha Bora	Karbi Anglong	White NN155B	Golden	Present	Awnless

Characteristics revealed by different farmers about local landraces were recorded and presented in Table 2. Among the landrace diversity, Kola Kunkuni Joha and Kola Tulsi Joha, were highly aromatic types collected from this region. The production of Keteki Joha was maximum and Kunkuni Joha have minimum among the aromatic rice landraces of this region. The landrace Joha Bora is moderately aromatic, more sticky than other landrace and traditionally useful for the preparation of rice beer.

Morphological characters

Five morphological characters were recorded for 22 collected aromatic rice landraces. The

morphological characters included leaf length (1), leaf width (2), leaf blade colour (3), Panicle Axis (4), lemma and palea colour (5), and the results of morphological characters recorded for 22 accessions are discussed in Table. 3 (Character 1 to 5). The leaf length ranges from 71.5cm (Joha Bora) to 32.2cm (Badsahbhog Joha). Leaf breadth of collected aromatic rice landraces ranges in between 0.8-1.4 cm. Out of 22 collected aromatic rice landraces 9 light green, 5 dark green and 8 were green in leaf blade colour, Except 6 landraces all the others have straight panicle axis. Maximum number of landraces has straw and black color of Lemma and palea.

Table 2. Characteristic features recorded in 22 landraces of rice based on feedback from farmers

SL. NO.	Landrace name	Specific note recorded from farmers	Remarks
01	Kunkuni Joha	Aromatic, low production	Feedback from farmers
02	Boga Kunkuni Joha	Aromatic	Feedback from farmers
03	Kola Kunkuni Joha	Highly Aromatic	Feedback from farmers
04	Kola Joha	Aromatic,	Feedback from farmers
05	Kala Jeera	Aromatic,	Feedback from farmers
06	Kola Tulsi Joha	Highly Aromatic,	Feedback from farmers
07	Tulsi Joha	Aromatic,	Feedback from farmers
08	Jwsa Gufur (Phisa-jhar)	Aromatic,	Feedback from farmers
09	Boga Joha	Aromatic,	Feedback from farmers
10	Badsahbhog Joha	Aromatic,	Feedback from farmers
11	Betguti Joha	Aromatic,	Feedback from farmers
12	Kharika Joha	Aromatic,	Feedback from farmers
13	Mukuta Joha	Aromatic,	Feedback from farmers
14	Rampal Joha	Aromatic,	Feedback from farmers
15	Keteki Joha	Aromatic, Good yield	Feedback from farmers
16	Gobinda Tulsi Joha	Aromatic,	Feedback from farmers
17	Dangor Joha	Aromatic,	Feedback from farmers
18	Kon Joha	Aromatic,	Feedback from farmers
19	Guti Joha	Aromatic,	Feedback from farmers
20	Nepali joha	Aromatic,	Feedback from farmers
21	Krishna joha	Aromatic,	Feedback from farmers
22	Joha Bora	Moderately Aromatic, Sticky, traditionally Use for the production of Rice Beer	Feedback from farmers

Table 3. Various morphological characteristic features recorded in 22 landraces of rice

SL.NO.	Landrace name	No of tiller	Length of leaf (cm) at heading stage	Breadth of leaf (cm) at heading stage	Leaf blade colour at stem elongation to heading stage	Panicle Axis (PnAK) at milk stage to mature	Lemma and Palea Colour (LmPC)
01	Kunkuni Joha	3	58.8	1.1	Light green	Straight	Straw
02	Boga Kunkuni Joha	3	61.2	1.4	Light green	Straight	Straw
03	Kola Kunkuni Joha	3	43.5	0.9	Light green	Straight	Black with golden furrow
04	Kola Joha	2	54.5	1.0	Light green	Straight	Black
05	Kala Jeera	2	57.2	0.8	Light green	Straight	Black with golden patches
06	Kola Tulsi Joha	2	68.4	1.1	Drak green	Straight	Black with golden patches
07	Tulsi Joha	2	46.6	0.9	Green	Straight	Black with golden patches
08	Jwsa Gufur (Phisa-jhar)	3	60.2	1.3	Light green	Straight	Straw
09	Boga Joha	4	66.3	1.1	Green	Straight	Straw
10	Badsahbhog Joha	3	32.8	1.2	Green	Straight	Straw and brown
11	Betguti Joha	4	54.2	1.2	Green	Straight	Straw with brown patches
12	Kharika Joha	5	48.9	1.1	Drak green	Dropy	Straw
13	Mukuta Joha	4	43.5	0.8	Light green	Straight	Straw
14	Rampal Joha	6	43.5	0.9	Green	Dropy	Brown with golden patches
15	Keteki Joha	6	40.1	1.2	Drak green	Dropy	Straw
16	Gobinda Tulsi Joha	4	38.9	1.1	Green	Straight	Straw with brown patches
17	Dangor Joha	4	61.9	0.8	Drak green	Dropy	Black
18	Kon Joha	3	38.9	0.9	Light green	Straight	Reddish straw
19	Guti Joha	3	50.5	1.3	Light green	Straight	Straw
20	Nepali joha	4	59.8	1.0	Green	Straight	Straw
21	Krishna joha	3	56.3	1.2	Green	Dropy	Black
22	Joha Bora	5	71.5	1.4	Drak green	Dropy	Light straw

Agronomical characters

22 landraces of traditional aromatic rice group were evaluated for agronomical traits like total tillers per hill were recorded from five competitive plants of middle row of each entry. The tiller number per hill ranges in between 2 to 6. Keteki Joha have maximum i.e. 6 tiller/hill.

Variation in quantitative traits

The landraces was unusual in the seed morphological characters. Variability was also measured for quantitative traits (Table 4) of rice landraces particularly for kernel length with and without husk, kernel breadth with and without husk, ratio l/b (with husk), ratio l/b (without husk) and 1000 kernel weight (g). Maximum seed length 8.80 mm was recorded for Keteki Joha while minimum seed length was 5.00 mm in Joha Bora. Maximum seed breadth (5.00 mm) was recorded for

Krishna Joha and Joha Bora while minimum seed breadth (1.50 mm) was recorded for Kunkuni Joha. Maximum kernel length 7.00 mm was recorded for Keteki Joha and Rampal Joha while minimum kernel length was 4.00mm. Maximum kernel breadth (4.00mm) was recorded for Krishna Joha and Joha Bora while minimum kernel breadth (1.10mm) was recorded for Kunkuni Joha. Maximum kernel weight (17.29g) was recorded (Table 4) for Kharika Joha while minimum kernel weight (9.77g) was recorded for Betguti Joha. Rice landraces germplasm showed considerable variability for quantitative traits as indicated by coefficients of variation (CV) (Table 4). Among the traits studied the highest variability (CV per cent) was observed for 1000 kernel weight (29.31) followed by ratio of kernel length/breadth without husk (20.21), ratio of kernel length/breadth with husk (17.06) and kernel breadth without husk (16.60).

Table 4. Descriptive statistics of different rice landraces collected from different districts of Assam, India.

Sl. No.	Variables	Minimum	Maximum	Mean	SE Mean	CV
1	Kernel length with husk (mm)	5.00	8.80	6.20	0.034	18.24
2	Kernel length without husk (mm)	4.00	7.00	5.30	0.006	88.33
3	Kernel breadth with husk (mm)	1.50	5.00	2.60	0.02	13
4	Kernel breadth without husk (mm)	1.10	4.00	2.10	0.003	70
5	Ratio l/b (with husk)	1.76	3.33	2.39	0.17	14.06
6	Ratio l/b (without husk)	1.5	3.68	2.68	0.2	13.4
7	1000 Kernel weight (g)	9.77g	17.29g	12.56	0.33	38.06

Table. 5 Pearson correlation coefficients among quantitative characters in aromatic landraces

	Seed Length	Seed breadth	Kernel Length	Kernel breadth	Seed weight
Seed Length	1				
Seed breadth	-0.0394	1			
Kernel Length	-0.0397	0.6734*	1		
Kernel breadth	-0.0264	0.9663**	0.6551*	1	
Seed weight	0.4096	0.5881*	0.3113	0.5433*	1

Correlations among quantitative characters

The correlation matrix showed the relationships among the analyzed quantitative characters values of seed length, seed width, kernel length, kernel width and seed weight the twenty-two traditional aromatic rice landraces (Table 5). Significant positive correlations between Kernel Length and Seed weight with Seed breadth ($r = 0.6734^*$) and (0.5881^*) respectively as well as Seed weight with Kernel breadth ($r = 0.5433^*$) were shown at the 0.05 level, which indicates that rice landraces high in weight may likely to be high Kernel Length, Seed breadth and Kernel breadth. The correlation between Seed breadth and Kernel breadth (0.9663^{**}) showed significantly positive relationship which shown at 0.01 level (Table. 5).

Cluster analysis

Cluster analysis based on quantitative characters and distance matrices is given in Fig. 3. Dendrogram have indicated two major cluster groups. Major cluster - I comprised 10 landraces while cluster - II comprises 12 landraces. Cluster - I further grouped in two sub-clusters based on similarity in quantitative characters seed type and kernel type was of Boga Joha, Badsahbhog Joha, Gobinda Tulsi Joha, Keteki Joha and Boga Kunkuni Joha (5 landraces) in sub-group (A). In sub-group (B) landraces like Guti Joha, Betguti Joha, Mukuta Joha, Jwsa Gufur Phisa Jhar and Kala Jeera (5 landraces) were grouped on the basis on similarity in quantitative characters.

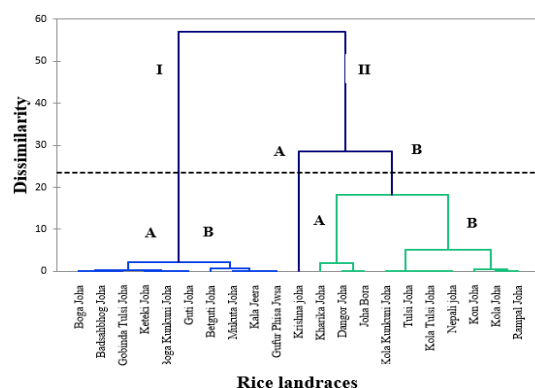


Figure 3. Dendrogram generated by Ward's method based on similarity in quantitative parameters (seed size, kernel size, and kernel weight) for 22 rice landraces collected from Assam, India.

Cluster II also grouped further in two sub-clusters based on similarity in characters like maximum 1000 kernel weight with Krishna Joha (A) which form a

group. The (B) sub-group further divided into two subgroups consisting landraces like Kharika Joha, Dangor Joha and Joha Bora (3 landraces) sub group (a) and Kola Kunkuni Joha, Tulsi Joha, Kola Joha, Kola Tulsi Joha, Nepali Joha, Kon Joha and Rampal Joha sub group (b) depending on the similar quantitative characters and traits like kernel types. The accessions in clusters (I) and (II) are distant, and some traits like seed size, kernel size, seed coat colour, aroma, 1000 kernel weight and presence of awn help to form same cluster which can be help in the crosses successfully for distinct traits that might helpful in plant breeding programme. Different landraces collected from diverse areas of Assam were clustered together.

Discussion

The magnitude of genetic variability and the extent to which the desirable characters are heritable (Ravi *et al.*, 2003) is the basis of successful crop improvement programme. For assessing genetic diversity among morphologically distinguishable rice cultivars in aromatic rice cultivars from Asia morphological traits were useful for preliminary evaluation (Hien *et al.*, 2007). Analysis of genetic variability in landraces of traditional rice landraces can help in identifying diverse parental combinations for further selection and to help introgression desirable genes (Semwal *et al.*, 2014).

Morphological data

The results presented here, for landraces from Assam, reveal high diversity of B landraces. Maximum kernel length (0.7 cm) was recorded for Keteki Joha and Rampal Joha while minimum kernel length was 0.4 cm in Kunkuni Joha. Maximum kernel breadth (0.4 cm) was recorded for Krishna Joha and Joha Bora while minimum kernel breadth (0.11cm) was recorded for Kunkuni Joha. During household survey it was observed that these landraces were cultivated by many farmers for over a long period of time. Similar results were also reported in rice by several workers (Patra, 2000; Deb, 2000; Singh *et al.*, 2005; Chakravorty and Ghosh, 2011; Mishra and Sinha, 2012; Tirkey *et al.*, 2013; Semwal, *et al.*, 2014). Due to unique morphological characters of seeds and adaptability to local environments, the landraces are known for significant variability (Frainkel *et al.*, 1995; Hore, 2005). Rice landraces collected from Assam were studied and significant variability was noted for vegetative and seed characters involving different quantitative traits.

Areas of collection of landraces and data analysis

In the present investigation, 22 aromatic rice landraces of Assam were mainly analysed for variability in seed morphology which showed the diversity for morphological characters. Landrace like Keteki Joha is very popular indigenous aromatic rice grown in a small pocket of Assam for its high yield. Landrace Kola Kunkuni Joha though is relatively smaller sized seed of black colour and low yield but had high marketability due to its high aroma as compared to other landraces. Several studies were carried out using morphological and molecular markers in different cereal species (Glaszmann, 1987; Thanh *et al.*, 1999; Sun *et al.*, 2001; Zhang *et al.*, 1994). Analysis of diversity with traditional landraces like Kalo, Rato, Seto, Marshi, Mehele and Darime based on traits like colour of grain, length of kernel, period of maturity etc., showed low level of morphological and molecular diversity (Chakravorty and Ghosh, 2013). For the latter, adaptation, selection and cultural variation were probably responsible for variability among the different landraces from same region.

Conclusions

In conclusion, the present investigation provides the guidelines for the selection of parents based on agronomic traits with special reference to qualitative characters for rice improvement program. Many of these landraces are poor yielder and grown only in restricted pockets in the area of collection. Special drive is desirable for their collection and conservation. Due to the significant aroma, good taste and their regional importance local farmers are conserving these landraces from generation to generation. The traits recorded during germplasm collection are listed on the basis of feedback from farmers and present data gives preliminary observations and require further validation after characterization/evaluation. In rice improvement program characterization of landraces could help breeders to utilize appropriate characters. The present investigation provides the base material for the rice breeders for exploitation of landraces possessing one or more desirable characters.

Acknowledgements

Funding support received from Department of Biotechnology (DBT), Govt. of India for the research project entitled "Development of high yielding, non-lodging and biotic resistant varieties of black scented rice of Manipur and joha rice of Assam through biotechnological intervention" vide sanction number DBT-NER/AGRI/29.2015, dt. 19/10/2016 is greatly acknowledged.

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Cite this article as:

Lipika Lahkar and Bhaben Tanti. Study of morphological diversity of traditional aromatic rice landraces (*Oryza sativa* L.) collected from Assam, India. *Annals of Plant Sciences* 6.12 (2017) pp. 1855-1861.

doi: <http://dx.doi.org/10.21746/aps.2017.6.12.9>

Source of support: Department of Biotechnology (DBT), Govt. of India

Conflict of interest: Nil