Study of Variability in Seed Morphology, Screening of Seed Borne Fungi and Uses of Rarely Cultivated Indigenous Rice Landraces (*Oryza sativa* L.) of Kerala, India

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10.18805/BKAP406

ABSTRACT

Background: Conservation is the key to sustainability. Kerala is known to be a host to number of traditional rice varieties, which are dying at a rapid pace. The first and foremost step in the conservation of traditional germplasm sources is to assess the various qualitative and quantitative features. Various scientists across the globe, have been studying the genetic diversity, morphological characteristics and fungal activities on rice since appreciable amount of time. This has proved to be helpful in sustenance and refinement of the rice varieties.

Methods: Variability was accessed for quantitative and qualitative characters for 30 landraces. Notable variability was observed in the quantitative characters of the selected landraces. Highest variability was seen in ratio of length/breadth (l/b) without husk (25.55), followed by 50 kernel weight (25.51), kernel breadth without husk (21.21), ratio l/b with husk (19.19), kernel breadth with husk (17.90), kernel length without husk (16.58) and kernel length with husk (13.91). A dendrogram was generated by Ward's method from the data acquired. Ten selected rice varieties were screened for seed borne fungi by standard blotter paper method. The percentage of disease incidence and percentage of frequency were calculated.

Result: The rice varieties *Choman* and *Ponnaryan* showed highest percentage of disease incidence (100). Lowest percentage of disease incidence was seen in *Kumkumashala* and *Gandhakashala*. Highest frequency of occurrence was shown by *Aspergillus sps.* Other fungal species reported were *Chaetomium sps., Curvularia sps., Helminthosporium sps*., Unidentified *sps.* from *Dematiaceae*, *Phoma sps.* and *Trichococcus sps.*

Key words: Landraces, Rice, Seed morphology, Variability.

Abbreviations: CV- Coefficient of variation, SE- Standard error.

INTRODUCTION

Genetic heterogeneity is the key to achieve 'productivity in perpetuity'(Swaminathan, 2010). Before the advent of commercial agriculture, farmers across the globe have been conserving and cultivating a large number of landraces (Gopi and Manjula, 2018). Since the beginning of cultivation of rice, farmers were known to protect varieties of rice that condoned various biotic and abiotic stresses like drought, salinity, floods, pests and diseases. Many varieties of rice were also closely associated with religious and cultural practices across the world, especially in India. However, severe erosion in plant genetic diversity has been observed since the early 1900s (Gopi and Manjula, 2018).

The three major staples, rice, wheat and maize contribute to 60% of the energy and protein source from sourced from plants by humans. Rice is an important crop in India, where centuries of cultivation, adaptations to diverse environments and culinary preferences have given rice to a rich genetic diversity in rice varieties (Gopi and Manjula, 2018). With various systems of medicine like Ayurveda originating in India, rice accomplished itself as a major constituent in traditional medicine. An estimated 140,000 landraces are recorded in the world and India alone has about 86,330 accessions.

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How to cite this article: Rachana, K. and Sheikh, S. (2022). Study of Variability in Seed Morphology, Screening of Seed Borne Fungi and Uses of Rarely Cultivated Indigenous Rice Landraces (*Oryza sativa* L.) of Kerala, India. Bhartiya Krishi Anusandhan Patrika. 37(3): 245-252. DOI: 10.18805/BKAP406.

Submitted: 13-12-2021 **Accepted:** 23-08-2022 **Online:** 02-09-2022

The state of Kerala has an estimated 2000 traditional rice varieties (Leena 2012). Given their remarkable adaptations and ability to perform under harsh climatic conditions, conservation of these varieties is imminent for promoting climate resilient agriculture (Gopi and Manjula, 2018). Improvement in our lifestyle, better opportunities and greed has led to people overlooking agriculture as a profession. With every passing year, the area under cultivation of paddy is showing an alarming rate of decline. Also, the trend of replacing traditional varieties with genetically improved varieties is a major threat to the indigenous landraces. During 1973-74, the area under paddy

was 8.73 lakh ha, which came down to just 1.98 lakh ha during 2014-15. Similarly, the coverage of high yielding varieties in gross rice area was 18.17% in 1970-71, which increased to 92.98% in 2010-11. To conserve the traditional genetic resources, the appraisal of the genetic and morphological variations is considered as an initial step for management (Toro and Cabellero 2005).

Studying the variation in seed morphology and uses of traditional varieties of rice of Kerala, will help give an insight on various adaptations of these varieties against changing climate of Kerala. Since traditional varieties have been grown for generations, they have a better hand at surviving than the genetically improved varieties, which on the other hand requires ideal growth conditions.

Grain morphological characteristics is an important component of grain yield and quality which have been used as a criterion of selection since cereals were first domesticated (Wang *et al.,* 2012). The quantitative characters like kernel length, kernel breadth, kernel weight is some of the very important characters that determine the commercial demand for rice varieties. Cooking quality, palatability, grain color, aroma, calorie content, satiety are the main attributes the choice of a traditional cultivar for consumption in Kerala (Kumar *et al.,* 2010). The preferences for grain size and shape differ from one group of consumers to the other (Xiongsiyee and Thai 2016). It is seen that long grains are preferred in Indian subcontinent, but medium to medium-long rice are preferred in Southeast Asia. Most of the traditional varieties satisfying all the requirements are vanishing due to the ignorance and the scientific world must invest its time and resources in saving these varieties than developing new varieties.

Another challenge that has affected the traditional varieties of rice is the infection of pathogenic and non-pathogenic fungi. The tropical climate, humidity and heavy rainfall and floods, especially near harvest makes the rice varieties in India more susceptible to invasion by filamentous fungi and bacteria (Makun *et al.,* 2007). Improper storage methods adopted by farmers also can be a reason for the infestation by fungi.

Rice disease such as blast, brown leaf spot, seedling blight dwarf disease have, from time to caused heavy damages to rice in India (Alam *et al.,* 2014). In India, rice seeds infected with *Helminthosporium oryzae*, *Curvularia lunata, Cochliobolus lunatus, Alternaria tenuis* and *Epicoccus sp*. were reported (Mazumdar and Chattopadhyay ,1976) . The disastrous epiphytotic of rice blast caused losses up to 2/3 of rice yield in India (Kulkarni 1959). The quality of the rice has direct impact on public health. Hence, it is important to isolate, screen and study various fungi that infest different rice varieties.

MATERIALS AND METHODS

Variation in rice seed morpholog

Collection of indigenous rice germplasm

Thirty rarely cultivated rice varieties were collected from farmers of 'Pulari', Aravath on August, 2018. The organization is mainly comprised of farmers who are involved in the conservation of traditional rice varieties and spread awareness among people to protect the diverse rice germplasm. During the course of collection, a brief interaction with farmers were done regarding issues they face in the present scenario and steps that can be taken to improve the condition of farmers and agriculture in Kerala. Traditional knowledge about the various rice varieties were recorded along with the information from published records.

Evaluation of qualitative and quantitative characters

Samples were collected by random sampling method. The qualitative characters such as husk color, kernel color, presence of aroma and presence of awn were recorded for each sample. 50 seeds weight was measured in grams and kernel length (with and without husk), kernel breadth (with and without husk) were measured with the help of a centimeter scale. Length/breadth ratio (with and without husk) and length of awn if present, was calculated.

Data analysis and clustering

Descriptive statistics based on mean, standard deviation, standard error mean and coefficient of variation were analyzed from the quantitative characters. Data were analyzed using SPSS statistical software. Cluster analysis of 30 rice varieties was done based on their similarity in quantitative parameters (grain length, grain breadth, kernel length, kernel breadth and kernel weight). A dendrogram was generated by a hierarchical technique called Ward's method using Euclidean distances across all varieties of rice.

Screening of seed borne fungi

A. Selection of rice varieties for fungal screening

Following rice varieties having different degree of resistance against fungal infestations were selected:

a) *Thonnuran*
b) *Jeerakashala*
c) *Chennellu*
d) *Gandhakashala*
e) *Ponnaryan*
f) *Choman*
g) *Kumkumashala*
h) *Mullan kayama*
i) *Koduveliyan*
j) *Karindadichal*

B. Fungal isolation and identification

Fungal isolation was carried out in department of Botany, St Aloysius College (Autonomous), Mangaluru by Standard blotter paper method (Fig 2). Healthy sterilized seeds selected from each variety, were placed on three sets of moist blotter paper (10 seeds per petri plate). Distilled water was sprayed on the petri plates every third day in order to keep the blotter paper sufficiently moist. The seeds were examined regularly for fungal growth. At the end of 7 days, the conidia and hyphae of the fungi was carefully picked from each seed using forceps and mounted on a slide using

cotton phenol blue indicator. Fungal colonies were carefully examined under a compound microscope and identified. The percentage of disease incidence, percentage of frequency were calculated using following formula:

$$\text{Disease incidence (DI)} = \frac{\text{Total number of diseased seeds}}{\text{Total number of seeds plated}}$$

$$\text{Frequency of occurrence (\%)} = \frac{\text{Number of seeds on which a fungal species occur}}{\text{Total number of seeds}}$$

RESULTS AND DISCUSSON

Variation in morphology and uses of landraces

A. Variability in qualitative characters

Variation in the qualitative traits were recorded for all the 30 landraces collected, mainly for husk color, kernel color, presence of awn and presence of aroma (Table 1 and 2, Fig 1).

Significant variation in both husk color and kernel color were observed. Kernel color of varieties evaluated showed various shades of red, brown, yellow and white. 8 varieties showed orangish brown color, 5 accessions showed reddish brown color, 3 varieties showed dark brown color, 2 land races showed light yellow color, 2 varieties showed orangish red, 2 landraces showed blackish red, 2 varieties showed light brown, 2 varieties showed whitish yellow, 2 varieties showed white color, while another 2 varieties showed brown and orange color. Husk color of the accessions recorded were greyed yellow, light yellow, brownish yellow, golden yellow, blackish yellow and brown.

Out of the landraces collected, 11 were Awned rice varieties, while 19 of them were Awnless. 9 of the landraces were aromatic and 21 of them lacked aroma. Specific notes given by the farmers regarding each variety, along with the information from the published records were recorded in Table 2. 4 varieties were identified as pest resistant and 9 varieties were identified as tolerant to abiotic stresses.

B. Variability in quantitative characters

The landraces recorded significant variation in quantitative characters and the variation were recorded for kernel length with and without husk, kernel breadth with and without husk, ratio l/b (with and without husk) and 50 kernel weight (Fig 3).

The maximum kernel length with husk (9.5 mm) was recorded for *Machan* and the minimum kernel length with husk (0.5 mm) was recorded for *Kunjinellu*. *Veliyan* showed

Fig 1: a). Visiting a farmer's field during the study; b.) Varieties of landraces collected.

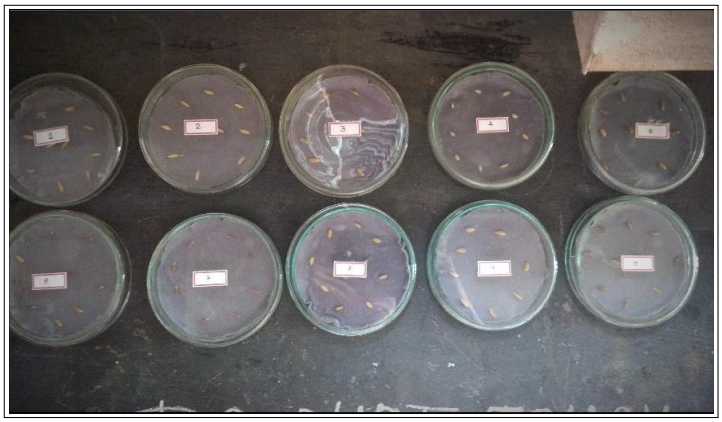

Fig 2: Setup for fungal screening of rice varieties by standard blotter paper method.

maximum kernel breadth with husk (0.4 mm), while *Choman* showed minimum kernel breadth with husk (0.15 mm). Maximum l/b ratio with husk (3.52) was observed for *Jeerakashala* and minimum l/b ratio with husk (2.06) was observed for *Chennell thondi.*

Among the Awned varieties, *Machan* showed highest awn length (2.5 cm) and Mullankayama showed minimum awn length (0.9 cm). Maximum 50 kernel weight (1.65 g) was observed in *Mannuveliyan* and minimum 50 kernel weight (0.7 g) was observed in Choman.

Table 1: Variability studies in qualitative characters among the 30 landraces collected.

Name of the landrace	Kernel colour	Husk colour	Aroma	Presence of awn
Rakthashali	Reddish brown	Brown	Absent	Awnless
Mannuveliyan	Reddish brown	Light yellow	Absent	Awnless
Kothamapalari kayama	Light yellow	Golden yellow	Present	Awnless
Undakayama	Orangish red	Greyish yellow	Present	Awned
Machan	Blackish red	Blackish yellow	Absent	Awned
Adukkan	Reddish brown	Light yellow	Absent	Awnless
Vellari	Reddish brown	Greyish yellow	Absent	Awned
Chennell thondi	Orangish red	Blackish yellow	Absent	Awnless
Mundodan	Orangish brown	Greyish yellow	Absent	Awnless
Thonnuran thondi	Orangish brown	Greyish yellow	Absent	Awnless
Thonnuran	Orangish brown	Greyish yellow	Absent	Awnless
Chennellu	Orangish brown	Blackish yellow	Absent	Awned
Choman	Light brown	Brown	Absent	Awnless
Jaadhisukhi	Dark brown	Brownish yellow	Absent	Awnless
Veliyan	Light brown	Light yellow	Absent	Awned
Jeerakashala	Light yellow	Golden yellow	Present	Awned
Ponnaryan	Blackish red	Brownish yellow	Absent	Awnless
Mullankayama	Whitish yellow	Golden yellow	Present	Awned
Gandhakashala	Whitish yellow	Golden yellow	Present	Awnless
Karindadichal	Orange	Blackish yellow	Absent	Awnless
Kayama	Reddish brown	Greyish yellow	Present	Awnless
Vella thouvan	Orangish brown	Greyish yellow	Absent	Awned
Koduveliyan	Dark brown	Light yellow	Absent	Awnless
Kuttusan	Dark brown	Greyish yellow	Absent	Awnless
Naadan punjankayama	Orangish brown	Greyish yellow	Present	Awnless
Kumkumashala	Brown	Brown	Absent	Awnless
Kunjinellu	White	Light yellow	Present	Awned
Rajakayama	White	Light yellow	Present	Awnless
Chenthadi	Orangish brown	Blackish yellow	Absent	Awned
Karuvaalicha	Orangish brown	Blackish yellow	Absent	Awned

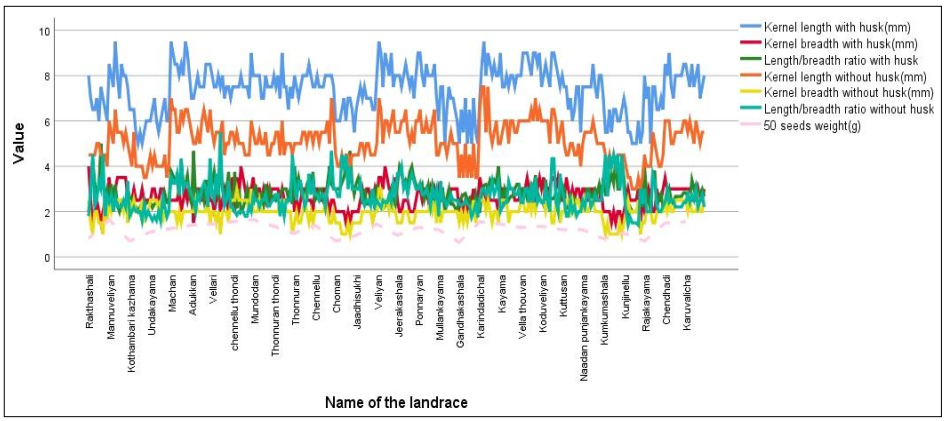

Fig 3: Graph showing variability in quantitative characters among the 30 landraces.

Table 2: Characteristic features recorded in 30 landraces based on feedback from farmers and published records.

Landrace	Notes recorded from farmers	Remarks (References cited)
Rakthashali	Non-aromatic variety	Diuretic (Leena, 2004)
Mannuveliyan	Grown in Wayanad	Drought tolerant (Rekha *et al.,* 2011)
Kothamapalari kayama	Resembles coriander seeds	High yielding variety (Gopi and Manjula, 2018)
Undakayama	Highly resistant variety	Suitable for dry conditions (Sebastian *et al.,* 2014)
Machan	Grows really well	Feedback from farmers
Adukkan	Pest resistant variety	Flood resistant (Gopi and Manjula, 2018)
Vellari	Large sized grains	Long duration cultivar (Joseph and Abdul, 1998)
Chennell thondi	Grown in Wayanad	Pest resistant (Sebastian *et al.,* 2014)
Mundodan	Grown in distinct season	Short duration variety (Joseph and Abdul, 1998)
Thonnuran thondi	Matures in 90 days	Grown in upland ecosystem (Joseph and Abdul, 1998)
Thonnuran	Low pest resistance	Direct seeding in Wayanad [22]
Chennellu	Grown in flood plains	Anti-diarrheic (Joseph and Abdul 1998)
Choman	Grown in upland ecosystem	Suitable for dry land (Sebastian *et al.,* 2014)
Jaadhisukhi	Red rice	Anti- dysenteric (Gopi and Manjula, 2018)
Veliyan	Aromatic	Biotic stress tolerant (Gopi and Manjula, 2018)
Jeerakashala	Gives high energy	Fine grains (Joseph and Abdul, 1998)
Ponnaryan	Red rice	Feedback from farmers
Mullankayama	Aromatic	Distinguishable panicle
Gandhakashala	Culturally important	*Kharif* crop
Karindadichal	Characteristic blackish husk	Feedback from farmers
Kayama	High yielding variety	Weakly photosensitive (Leena and Shylaraj, 2015)
Vella thouvan	Grown in upland ecosystem	Drought tolerant (Singh, 2014)
Koduveliyan	-	Cold tolerant (Latha *et al.,* 2013)
Kuttusan	Tall variety	Salinity tolerant (Chandramohanan and Mohanan, 2012)
Naadan punjankayama	III crop	Seasonal specific (Latha *et al.,* 2013)
Kumkumashala	Characteristic brown husk	Feedback from farmers
Kunjinellu	Salinity tolerant	Low glycemic index (Leena 2007)
Rajakayama	Royal aromatic rice	Grown in High lands (Leena 2016)
Chenthadi	Grown in Wayanad	Flood resistant (Gopi and Manjula, 2018)
Karuvaalicha	I crop	Blast disease tolerant (Manjunath, 2016)

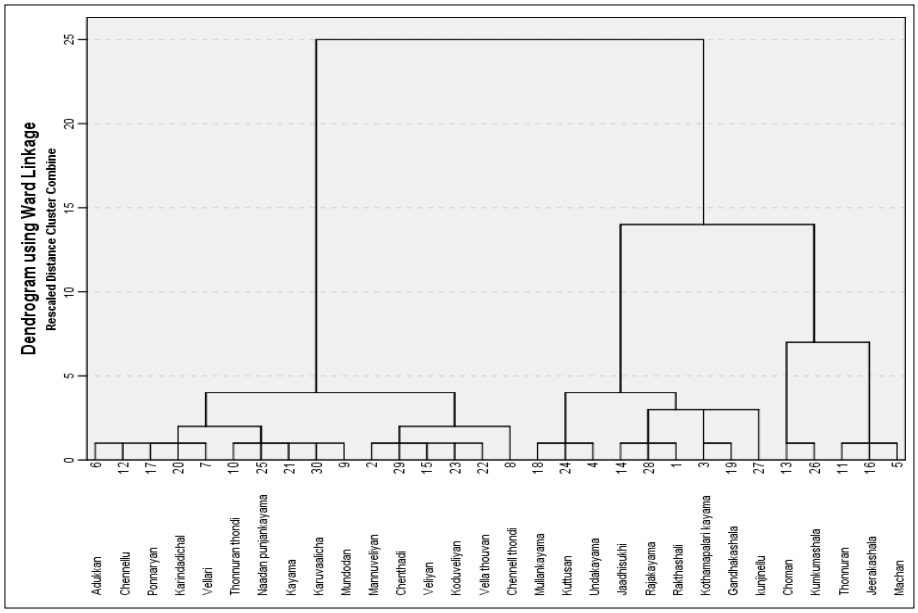

Fig 4. Dendrogram generated by Ward's method based on similarity in quantitative characters among 30 landraces collected.

Coefficient of variation measured indicated significant amount of variation among the traditional varieties collected (Table 3). Highest variability was seen in ratio of length/ breadth without husk (25.55), followed by 50 kernel weight (25.51), kernel breadth without husk (21.21), ratio l/b with husk (19.19), kernel breadth with husk (17.90), kernel length without husk (16.58) and kernel length with husk (13.91).

A. Cluster analysis

Hierarchical clustering gives an insight of similarity between various rice varieties and clusters them into various groups based on their similarities. A dendrogram was generated on the basis of quantitative characters of 30 traditional landraces collected (Fig 4). Dendrogram generated indicated two main cluster groups.

Cluster I comprised of 16 landraces and Cluster II comprised of 14 landraces. Cluster I was again sub-clustered into two groups based on their similarity in quantitative characters. Sub-group I (a) consisted of *Adukkan, Chennellu, Ponnaryan, Karindadichal, Vellari, Thonnuran thondi, Naadan punjankayama, Kayama, Karuvaalicha* and *Mundodan.* Sub-group II (b) consisted of *Mannuveliyan, Chenthadi, Veliyan, Koduveliyan, Vella thouvan* and *Chenthadi.*

Cluster II was sub-clustered into two sub groups. Sub-group II (a) consisted *Mullankayama, Kuttusan, Undakayama, Jaadhisukhi, Rajakayama, Rakthashali, Kothamapalari kayama, Gandhakashala* and *Kunjinellu.* Sub-group II (b) consisted of *Choman, Kumkumashala* and *Thonnuran.*

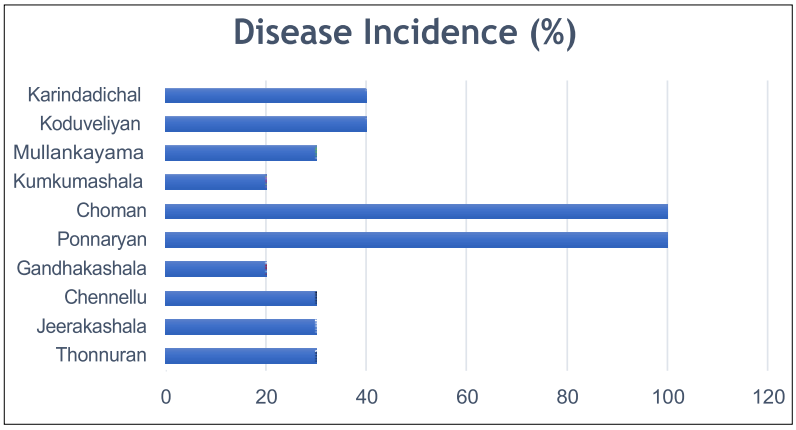

Fig 5: Graph showing disease incidence of selected 10 traditional rice varieties.

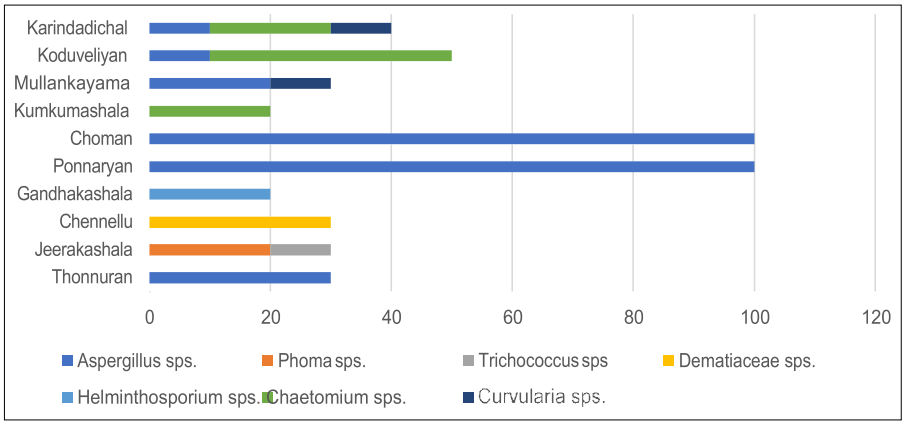

Fig 6: Graph showing fungal frequency of different rice varieties.

Table 3: Descriptive status of 30 landraces collected based on quantitative characters.

Variables	Maximum	Minimum	Mean	SE mean	CV
Kernel length with husk (mm)	9.50	5.00	7.38	0.059	13.91
Kernel length without husk (mm)	7.50	3.00	5.13	0.049	16.58
Kernel breadth with husk (mm)	4.00	1.50	2.66	0.027	17.90
Kernel breadth without husk (mm)	3.00	1.00	2.00	0.024	21.21
Ratio l/b with husk	5.00	1.66	2.84	0.031	19.19
Ratio l/b without husk	5.50	1.40	2.67	0.039	25.55
50 seeds weight (g)	1.65	0.65	1.20	0.056	25.51

The rice varieties from different groups can be selectively interbred to develop better hybrid varieties.

Screening of seed borne fungi

10 selected rice varieties were screened for fungal infestation by standard blotter paper method. The rice varieties showed varied resistance against the fungal pathogens. The rice varieties *Choman* and *Ponnaryan* showed highest percentage of disease incidence (100), followed by *Koduveliyan* and *Karindadichal* (40), *Chennellu, Jeerakashala*, *Mullankayama* and *Thonnuran* (30). Lowest percentage of disease incidence was seen in *Kumkumashala* and *Gandhakashala* (Fig 5).

Highest frequency of occurrence was shown by *Aspergillus sps*. Occurring in 6 varieties, followed by *Chaetomium sps.* Occurring in 3 varieties, *Curvularia sps.* Infecting 2 varieties. *Helminthosporium sps*., Unidentified sps. from *Dematiaceae*, *Phoma sps.* and *Trichococcus sps.* occurring in one variety each (Fig 6).

The study done on the variability in the seed morphology indicated that the rice varieties showed considerable amount of variation in their quantitative and qualitative characters. The rice varieties also showed variable resistance against the fungal pathogens. These traits can be exploited by the breeders to develop new varieties according to consumer preferences, from the parental generation having different characters and sustainability. The interaction with farmers revealed that the traditional varieties held an important role in the cultural and ritual practices. The medicinal values of these rice varieties are being ignored by the people and the scientific world.

Similar works were done by Semwal *et al.,* (2014) and Sinha *et al.,* (2015) on the morphological diversity of traditional rice varieties of West Bengal. They also came to a conclusion that the varying geographical and climatic conditions of West Bengal, had a huge role to play in the diversity of rice varieties. Due to unique morphological characters of seeds and adaptability to local environments, the landraces are known for significant variability (Frankel, 1995).

The study has revealed a wide range of fungi associated with the rice seeds. Similar studies by Alam *et al.,* (2014) and Gautam *et al.,* (2012) in stored grains of Uttar Pradesh and Himachal Pradesh respectively, reported various fungal pathogens associated with rice grains. The seeds highly susceptible to fungal attack could be avoided by the farmers or stored in conditions which prevent fungal growth. A minimal amount of fungicides may be used or the grains could be safely sterilized in the rice varieties that are known to be highly prone to the fungal attack. Fungal infested rice seeds bring huge loss the farmers and also may prove fatal to the consumers.

However, the vegetative characters also need to be studied to understand the sustainability of a specific variety in a given geographical area. Famers cannot select a particular variety, solely based on the seed morphology studies. In the present study, the visual characteristics like color of the husk and kernel may differ in another sample collected, due to reasons like age and storage conditions of the seeds. The age of the stored grains may also have affected their resistance against the fungal pathogens which could have shown some errors in the results obtained.

CONCLUSION

The main concern that is to be addressed through this study is the loss of traditional varieties of rice at an alarming rate in Kerala. During the field exploration, it was found that most of the farmers preferred hybrid varieties than traditional landraces. The condition of the farmers in India, with poverty, debt, lack of government schemes being the highlight, has led them shift to more profitable hybrid rice varieties than the less profitable rice varieties. Government should provide more funds to the farmers and encourage them to grow traditional varieties, considering their significant role in the conservation of these varieties. It is a matter of appreciation that farmers themselves have formed an organization to conserve and grow these rice varieties and help students and researchers study these varieties. They have proved that it is possible to conserve them by giving awareness to the public, especially students.

Once a rare rice variety is lost, its unique medicinal values and properties are also lost. Nature in its store has cure for all the diseases and problems we face today, losing a part of it may prove costly to the mankind.

ACKNOWLEDGEMENT

The authors would like to express great appreciation to K Manikandan, Sr Technical Officer, KVK, CPCRI, Kasaragod, for helping to connect with the farmers. Grateful thanks are extended to Sri. Balakrishanan K.V. and Sri. Koran A, Pulari, Aravath, for providing the rice varieties for conducting the study. The authors acknowledge the financial support by Funding agency, Star college scheme, Department of Biotechnology, India.

Compliance with ethical standards

Conflict of interest

The authors declare that no conflict of interest has been reported.

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