NBU Journal of Plant Sciences
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Vol. 9, No. 1 March 2015 p. 78-86
ISSN No. 0974692?

Morphological characterization of rice cultivars their root colonization with arbuscular mycorrhizal fungi and screening for field resistance caused by brown spot disease

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Abstract

Variability in seed morphology was studied in 15 rice cultivars using qualitative and quantitative characters. Germplasm of these indigenous rice landraces were collected from Bijanbari, Kalimpong, Sikkim, Malda, Siliguri and UBKV (Uttar Banga Krishi Viswavidyalaya). Data were recorded for traits such as Kernel colour, Seed coat colour, Aroma, Presence of Awn and Length of the seed. A total of 9 landraces had white kernel colour while 4 had brown and 2 had greyed-orange .The seed coat colour variation in different landraces ranged from Golden yellow, Yellow, Red and Black. 6 landraces were having aroma whereas 9 had no aroma and lastly 11 landraces were found to have awn and 4 were awnless.UBKV-4 was longest in length with 1.1 cm and Sano masuri being the smallest of 0.4 cm. Establishment of disease in naturally infected rice cultivars were observed and disease index was calculated. Arbuscular Mycorrhizal Fungi (AMF) were screened from rhizosphere of fifteen rice cultivars grown on experimental field using wet sieving and decanting method. Microscopical observation revealed the presence of different genus of AM fungi present in the roots as hyphae, spores and sporocarp. Among the different AM fungi species of *Glomus* sp.were found to be high in all the fifteen cultivars of rice plants followed by *Gigaspora*, *Scutellospora* and *Acaulospora*. Histopathological study of roots showed the presence of vesicles and arbuscules. AMF infection and total number of spores per 100 grams of soil were recorded. Present study evaluates the study of different AMF population and their histopathology harbouring in the rhizosphere of rice.

Keywords: Rice cultivars, Morphological traits, AM Fungi.

Introduction

Rice (*Oryza sativa* L., family Poaceae) is the leading staple food crop of India, 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). West Bengal is called as 'bowl of rice' with over 450 rice landraces (Deb D., 2005; Chatterjee *et al.*, 2008). Diversity studies in rice using morphological characters were done on improved and ancestral rice varieties of Philippines (Caldo *et al.*, 1997; Juliano *et al.*, 1998) 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 M.M., 1972). Variation due to adaptation to specific ecosystems selection and socio-economic condition resulted in differentiation in different named landraces of a region (Bajracharya *et al.*, 2006). Variability study for rice landraces from West Bengal was undertaken by (Chakravorty *et al.*, 2013). Keeping in view the under representation of rice landrace diversity from West Bengal and Sikkim 15 different rice cultivars were selected and studied for seed morphology, associated knowledge on local use of collected landraces was recorded to help in characterization of rice germplasm from this region.

Arbuscular Mycorrhizal Fungi (AMF) are vital components of the microbial soil community forming the most commonly occurring underground symbiosis between members of phylum *Glomeromycota* and roots of 80% of all terrestrial plant species (Wang *et al.*, 2008; Schüßler *et al.*, 2001). AMF are the key species groups that inter-connect plants into a functional web (Hegalson *et al.*, 1998), extending plant root systems and thereby, facilitates plants uptake of soil nutrients of poor mobility, especially phosphorus (Smith and Read, 2008).

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Besides, AMF improve plant fitness by improving seedling establishment, plant fecundity, tolerance to some root pathogens, water relations and formation and stability of soil aggregates (Read, 1999; Newsham *et al.*, 1995a). Efforts are being undertaken to develop a bio formulation which can reduce the disease occurrence.

Considering the importance of AMF for disease resistance in rice plants present investigation was made to assess the AMF population from fifteen different rice varieties collected from different areas of hilly and plain regions and were grown on experimental field of Immuno Phytopathology Lab, Department of Botany, NBU.

Materials and methods

Collection of Rice Seeds

Rice seeds were collected from different regions of West Bengal and Sikkim. Brimful, Champasari and Black Nuniya from Bijanbari. Kaberi 9090, Loknath 505 and Gouraknath 509 from Siliguri. Sano masuri and Adde from Sikkim. Attheu and Maiti from Kalimpong .Swarnamasuri and Tulai panji from Malda and finally UBKV-1, UBKV-4 and UBKV-5 from Uttar Bangha Krishi Viswavidyalaya (UBKV) respectively.

Morphological study and measurement of seeds

Seed morphology was recorded paying attention to Kernel colour, seed coat colour, aroma, presence of awn and finally the length of the seed was noted.

Disease Assessment

Establishment of naturally occurring brown spot disease was observed and disease severity was assessed in terms of lesion number per leaf and infection index calculated as described by Adlakha et al. (1984). For percent disease index (PDI) calculation, the following formula was used-[(class rating x class frequency)/(total no. of leaves x maximum rating)] x 100.

Isolation of AMF spores from soil

Arbuscular mycorrhizal fungal spores were screened from soil samples of fifteen rice varieties rhizosphere by the wet sieving and decanting method (Gerdeman & Nicholson, 1963). Soil samples (100gm each of the root zone) were collected, suspended in water (1 lt) in order to obtain a uniform suspension. Soil clusters are carefully dispersed in the water and is kept for 10 minutes to settle down the heavy particles. Aqueous suspension was passed through a set of sieves of different pore size (200, 170, 150, 80, 50 µm) arranged one below the other. The spores were picked by the help of bristles / brushes and transferred to grooved slides or vials and observed under dissecting microscope. Few spores were stained with Melzar's reagent and studied under sterio-microscope. Healthy spores are separated by fine brush and are stored in autoclaved glass vials either in sterile distilled water or Ringer's Solution (8.6gm NaCl, 0.3gm KCl, 0.33gm $CaCl_2$ in one litre of boiled distilled water) at 4°C for further study and observation. It is evident from various studies that each plant has multiple AM fungi population.

Identification of AMF spores

Spore samples were separated according to their morphology size, colour, shape, wall thickness, wall layers, and other accessory structures like hyphal attachment etc. for the purpose of identification. The spores were identified with the help of standard keys (Walker, 1981; Schneck and Perez, 1987). Spores were critically examined with special reference to variation in vesicles (size, shape, wall thickness, wall layer, position and abundance), hyphal branching patterns, the diameter, structure (specially near entry points) and the staining intensity of hyphae.

Spore count

Rhizosphere soil (100gm) was taken and suspended in 250ml water. Wet sieving and decanting method was used for isolation of spores. Total number of spores was then counted and spore percentage of different genera was obtained.

Histo-pathological analysis

The root specimen were taken from field and washed with tap water. The roots were cut into pieces, after washing treated with 10% KOH added, kept in water bath for 1hr, then 1% HCl was added to neutralize the alkalinity. The root pieces were then washed with water (after 30 min) and staining was done by shimmering the roots in cotton blue: lactophenol (1:4) for 3-4 min with mild heating. Degree of contrast between fungal tissues and back ground plant cell was obtained according to the duration of storage of tissues. 1% HCl was added to acidify the tissues, as most histological stains are acidic. A little amendment in this process is noteworthy because it has been noticed that extraradical spore bearing hyphae and other extraradical fungal tissue with root segments are destroyed or dissolved when it is boiled in hot water bath at 90°C twice with 2% KOH followed by 0.05 cotton blue and lacto glycerol for staining the internal structures of AMF inside the root segment i.e. arbuscules, vesicles, auxilliary cells etc. The total staining process can be done without heating but keeping the root fragments in 1-2% KOH for 24-48 hrs in a Petri dish and another 2-18 hrs in cotton blue and lactoglycerol with minimum movement of the samples yields remarkable results. In this method the spore bearing hyphal structures, auxiliary cells etc. are clearly visible and percent colonization can be determined with better accuracy. After preparing the roots the hyphal structures were viewed under dissecting stereo-microscope under 20X and 40X magnification.

Root colonization

Percent root colonization was estimated by using slide method by (Giovannetti and Mosse, 1980). All the infected and uninfected segments of root tissue and the percentage of infection was calculated as follows

AMF infection (%)=[infected root segments/total fragments of root taken] X 100.

Results and Discussion

Fifteen different rice cultivars were collected from different regions of West Bengal and Sikkim the cultivar name, type, origin and its GPS Location is given in (Table 1).

Table 1. Rice cultivars and its localization.

Sl.No.	Rice Cultivars	Cultivar type	Origin	GPS Location
1.	Brimful	Ethnic	Bijanbari	27°02'N 88°07'E/ 27.04°N 88
2.	Champasari	Ethnic	Bijanbari	27°02'N 88°07'E/ 27.04°N 88
3.	Black Nuniya	Local	Bijanbari	27°02'N 88°07'E/ 27.04°N 88
4.	Kaberi 9090	Commercial	Siliguri	26.7100°N,88.4300°E
5.	Loknath 505	Commercial	Siliguri	26.7100°N,88.4300°E
6.	Gouraknath 507	Commercial	Siliguri	26.7100°N,88.4300°E
7.	Sano Musuri	Ethnic	Sikkim	27.3300°N,88.6200°E
8.	Adde	Ethnic	Sikkim	27.3300°N,88.6200°E
9.	Attheu	Ethnic	Kalimpong	27°04'N 88°28'E/27.06,88.47°
10.	Maiti	Ethnic	Kalimpong	27°04'N 88°28'E/27.06,88.47°
11.	Swarnamasuri	Local	Malda	25.0000°N,88.1500°E
12.	Tulaipanji	Local	Malda	25.0000°N,88.1500°E
13.	UBKV-1	Research	UBKV	26°24'15" N, 89°23'5"E
14.	UBKV-4	Research	UBKV	26°24'15" N, 89°23'5"E
15.	UBKV-5	Research	UBKV	26°24'15" N, 89°23'5"E

Fig. 1.Fifteen different rice cultivars.

Table 2: Morphological diversity of rice cultivars.

Sl. No.	Rice Cultivar	Area of collection	Kernel colour	Seed coat colour	Aroma	Presense of Awn	Length of the seed (cm)
1.	Brimful	Bijanbari	Brown	Red	Present	Absent	0.9
2.	Champasari	Bijanbari	White	Red	Absent	Present	0.8
3.	Black Nuniya	Bijanbari	Brown	Black	Present	Absent	0.7
4.	Attheu	Kalimpong	White	Yellow	Present	Absent	0.9
5.	Sano Masuri	Sikkim	White	Yellow	Absent	Absent	0.4
6.	Loknath 505	Siliguri	White	Golden Yellow	Absent	Absent	0.8
7.	Gouraknath 509	Siliguri	White	Golden Yellow	Present	Absent	0.7
8.	Kaberi 9090	Siliguri	White	Golden Yellow	Absent	Absent	0.9
9.	Adde	Sikkim	Brown	Yellow	Present	Absent	0.5
10.	Maiti	Kalimpong	Brown	Yellow	Absent	Absent	0.6
11.	Swarnamasuri	Malda	Greyed orange	Red	Absent	Absent	0.7
12.	Tulaipanji	Malda	Greyed orange	Golden Yellow	Present	Present	0.7
13.	UBKV-1	UBKV	White	Yellow	Absent	Present	0.9
14.	UBKV-4	UBKV	White	Red	Absent	Present	1.1
15.	UBKV-5	UBKV	White	Yellow	Absent	Absent	1.0

Table 3: Population count of AM Fungi in rhizosphere of fifteen different rice cultivars and percentage colonization in root

Sl. No.	Rice Cultivars	Percentage of VAM spore in soil (%)					Root colonization (%)
		Glomus	*Gigaspora*	*Scutellospora*	*Acaulospora*	*Entrophospora*	
1	Loknath 505	78.04	19.59	1.68	0.33	0.33	99 %
2	Gouraknath 509	83.85	15.09	-	1.04	-	91 %
3	Kaberi 9090	67.17	27.30	0.61	4.90	-	93%
4	Champasari	80.0	20	-	-	-	90%
5	Brimful	85.52	13.15	1.3	-	-	99%
6	Black Nuniya	83.33	13.88	-	2.77	-	94%
7	Adde	66.66	31.81	-	1.51	-	95%
8	Sano Masuri	65.94	33.74	-	.30	-	93%
9	Maiti	83.30	13.88	-	2.77	-	97%
10	Attheu	78.02	17.48	1.1	3.36	-	96%
11	Swarnamasuri	69.7	23.25	5.81	1.16	-	93%
12	Tulai Panji	65.19	33.77	1.04	-	-	95%
13	UBKV-1	90.39	5.64	1.12	2.82	-	98%
14	UBKV-4	60.30	36.43	0.75	2.51	-	100%
15	UBKV-5	50.07	41.07	2.52	6.31	-	98%

Seed Morphological Diversity of all the cultivar was observed (Fig. 1) and was seen that a total of 9 landraces had white kernel colour while 4 had brown and 2 had greyed-orange .The seed coat colour variation in different landraces ranged from Golden yellow, Yellow, Red and Black. 6 landraces were having aroma whereas 9 had no aroma and lastly 11 landraces were found to have awn and 4 were awnless. UBKV-4 was longest in length with 1.1 cm and Sano masuri being the smallest of 0.4 cm as shown in (Table 2). Table 3 shows the percentage of different AM fungi in the each soil samples and the maximum population was found to be of *Glomus* sp. followed by *Gigapora* sp., *Acaulospora* sp., *Scutellospora* sp. and

Fig. 2 AMF population collected from rhizospheric soils of rice

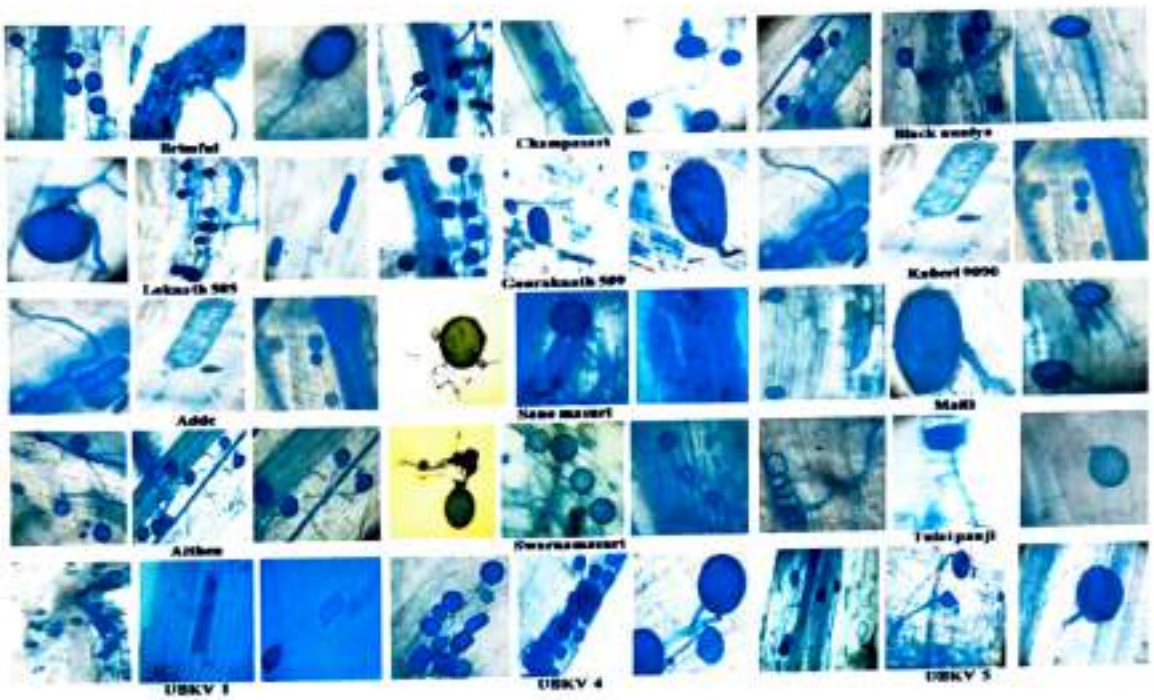

Fig. 3: Observation of rice root colonisation by AMF.

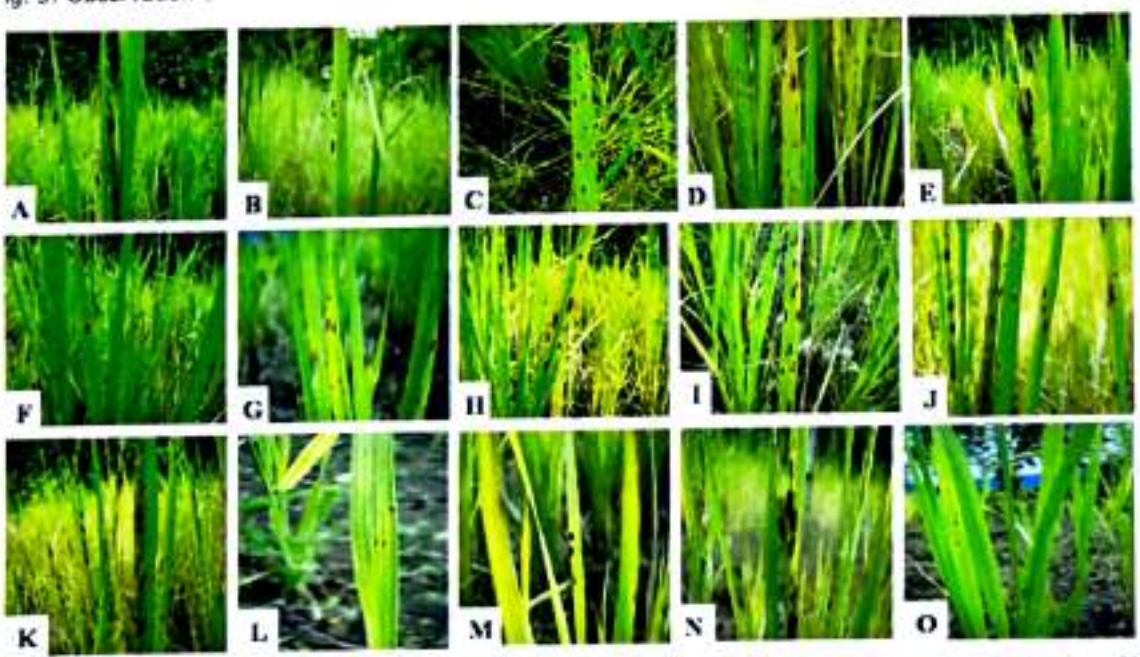

Fig. 4: Rice cultivars grown in experimental plots for study of AMF population and root colonisation. (A) Brimful,(B) Champasari, (C) Black nuniya, (D) Loknath 505, (E) Gouraknath 509,(F) Kaberi 9090 (G) Adde, (H) Sano masuri, (I) Maiti, (J) Attheu, (K) Swarnamasuri, (L) Tulai panji, (M) UBKV-1,(N) UBKV-4,(O)UBKV-5.

Enthropospora sp. was found in the rhizosphere of one of the cultivars.

Morphological and topographical characteristics of plant organs such as the shape and size of seeds and the structure of incidental

features have been useful weapons in identifying and classifying the plant and weed species (Noda *et al.*, 1985). Awn less seed is an improved trait and high diversity in seed shapes and pericarp color may be important for developing quality rice to meet diverse consumer demand.

Table 4: Disease index showing the establishment of natural disease.

Sl.No	Rice cultivars	Disease index (PDI %)
1.	Brimful	62.28
2.	Champasari	51.76
3.	Black Nuniya	52.72
4.	Kaberi 9090	58.47
5.	Loknath 505	41.66
6.	Gouraknath 507	50.05
7.	Sano Musuri	48.36
8.	Adde	59.82
9.	Attheu	49.44
10.	Maiti	47.89
11.	Swarnamasuri	53.33
12.	Tulaipanji	51.85
13.	UBKV-1	41.62
14.	UBKV-4	43.78
15.	UBKV-5	43.66

The role of below-ground soil organisms interacting with plant roots has gained increased attention in recent years (e.g. Reynolds et al., 2003; van der Putten, 2003; Callaway et al., 2004), and the interactions between beneficial and pathogenic organisms have been identified as being particularly relevant due to their important implications for plant fitness (e.g. Schippers et al., 1987; Fitter and Garbaye, 1994; Bever, 2003). Arbuscular Mycorrhizal Fungi were collected and screened from the rhizospheric soil of fifteen rice cultivars grown on experimental plots. The different types of spores which were observed in the rhizosphere of rice soil have been identified. On observation it was found that species of *Glomus* sp. and *Gigaspora* sp. dominated the AM population in all the soil sample (Fig. 2).

Histopathological study revealed the presence of vesicles and arbuscules in the root segments determining the fact that the rice roots has been infected by AMF spores (Fig. 3).

Organisms of AMF have a bimodal pattern of differentiation (Morton 1990). The vegetative thallus consists of arbuscules intraradical vesicles (shared only by species in the suborder Glomineae), extra radical auxiliary cells (shared only by species in the suborder Gigasporineae), and intraradical and extra radical hyphae (Smith and Read, 1997; Morton and Benny, 1990). Arbuscules are finely branched structures in close contact with the cell plasma membrane, functioning in exchange of nutrients between host and fungal cells (Smith and Read, 1997). Hyphae are important in nutrient acquisition and as propagules to initiate new root colonization (Graham *et al.*, 1982; Friese and Allen, 1991). Vesicles are globose structures arising from swelling of the hyphae and filled with glycogen granules and lipids are considered to be storage structures (Bonfante-Fasolo, 1984; Brundrett, 1991).

Under the natural condition the establishment of the brown spot disease was observed after four month growth of the rice plants grown on experimental plots (Fig.4) and Disease index (PDI%) was calculated. DI of rice cultivar Brimful was found to be the maximum with 62.28 and that of UBKV-1 to be minimum with 41.62 PDI% (Table 4).

Conclusion

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. Characterization of landraces could help breeders to utilize appropriate characters in rice improvement programme. The present investigation provides the base material for the rice breeders for exploitation of landraces possessing one or more desirable characters. The overall results of the present study have shown some of the important facts of the indigenous AM Mycorrhizal fungi present in experimental soils capable of infecting rice roots. Among the different types of AM fungi collected and observed *Glomus* sp. was found to be widely distributed in rhizosphere of rice plants in experimental plots. The present results also suggested that the rice plant may be considered as an initial stock plant which may be

used for inoculum production in departmental climatic condition. In future, the most modern and advanced technology should be considered for large-scale inoculum production of AM fungus under field condition.

Acknowledgement

Financial help received from Indian Council of Agricultural Research (ICAR) Govt. of India under AMAAS network project is gratefully acknowledged.

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