ISSN 2319-3670

Journal of Rice Research

Volume 8, No.1

June 2015

Society for Advancement of Rice Research

Society For Advancement of Rice Research

(Regd. No.2175/2004 of A.P. Society Regn. Act. 2001)

The Society for Advancement of Rice Research is a registered society for researchers, research managers extension personnel, institutions, development agencies, trade and industry who practice and promote activities for the advancement of rice science and development. The society has been started with overall objectives of providing a platform for exchange information and knowledge and disseminate the latest developments in rice research and to bring together all persons / institutions working for the cause of rice.

Aims and Objectives

- To advance the cause of rice research and development in the country.
- To disseminate knowledge on latest development in rice research through publications, seminars, lectures and training programs.
- To provide a platform for exchange of knowledge and information on rice research and developments through organizing workshops, symposia, conferences, etc.,
- To provide consultancy in rice production and development.
- To facilitate research and Industry collaboration and public private partnership at national level.
- To honour outstanding achievers in rice research and development.
- To cooperate with other organizations having similar aims and objectives
- To promote any other scientific/professional activities conducive for the advancement of science of rice and rice improvement.

Executive committee 2014-15 to 2015-16

President: Dr. V. Ravindra Babu; Vice President: Dr. A.K. Singh; Secretary: Dr. Vijay Pal Bhadana;
Joint Secretary: Dr. R. Robin; Treasurer: Dr. P. Revathi

Membership: Membership of the society is open to all persons interested in furthering the aims and objectives of the society and in the opinion of the executive committee have the necessary qualification and experience.

Category of the Members: a) Patrons b) Honorary members c)Life members d) Annual members e)Student members f)Corporate/ Institutional members.

Admission fee and Annual subscription fee

Class of Membership	Admission fee	Annual Subscription	
	INR	INR	US $
Ordinary Members	100.00	500.00	100
Life Members	100.00	2200 (one time)	600
Student Members	50	300.00	-
Corporate Members	Nil	10,000 (one time)	-
Library	Nil	2000	400

All membership subscriptions will become due on the 1st of January each year. Membership subscriptions should be paid within four months from the due date

Published by :

Dr. V. Ravindra Babu, President, Society for Advancement of Rice Research, Hyderabad.

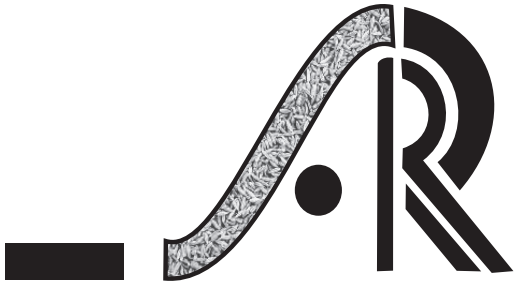
Printed at :

Balaji Scan Pvt. Ltd., Hyderabad - 500 004. Ph: 040-23303424 / 25 E-mail: bsplpress@gmail.com

ISSN 2319-3670

Journal of Rice Research

Volume 8, No. 1 June 2015



Society for Advancement of Rice Research

Journal of Rice Research

Volume 8: Issue No. 1 June 2015

Contents **Page No.**

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Genetic Diversity in Aromatic Rice (*Oryza sativa* L.) Genotypes using Microsatellite Markers

Neha Agrawal*and Rajeev Shrivastava

Department of Genetics and Plant Breeding, IGKVV, Raipur, Chhattisgarh

*Corresponding author-pnehaagrawal@gmail.com

Received: 30th December, 2014; Accepted: 10th May, 2015

Abstract

The genetic diversity and DNA fingerprinting of 40 aromatic rice genotypes constituting landraces collected from different regions of Chhattisgarh were assessed using 21 microsatellite markers distributed over 12 chromosome of rice. The results revealed that all the SSRs were polymorphic with different alleles among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism. A total of 50 alleles were amplified among the 21 SSRs. Hundred per cent similarity was observed between genotypes Jeeradhan and Jawaphool; Bisni-I and Bisni –III. Principal component analysis was done to visualize genetic relationships among the aromatic rice landraces. The information obtained from the DNA fingerprinting studies helps to distinctly identify and characterize 40 aromatic rice landraces using 21 different RM primers. A basic molecular database created for aromatic landraces of rice will be useful for future reference and to protect this unique rice under IPR regime. This information can be used in background selections during backcross breeding programs.

Key words: Genetic diversity, microsatellite marker, dendogram.

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops that provide food for more than half of the world population. India has a long history of rice cultivation and stands first in rice area and second in rice production, after China. Chhattisgarh is very rich for biodiversity but the resources are not yet properly utilized. This geographical region has vast diversity of rice. Some of the local rice or land races having unique identity and taste are very much in demand by traders and consumers. The research efforts have been focused to develop high yielding dwarf rice varieties having resistance to biotic stresses but less emphasis has been given to improve the local aromatic rice of Chhattisgarh. Though the land races are being maintained by the farmers traditionally, they are not released as varieties and are not notified and not in seed production chain.

Rice is also a model crop for the study of genetics and genome organization due to its diploid genetics, relatively small genome size (430 Mb) (Causse *et al*., 1994; Kurata *et al.,* 1994) and significant level of genetic polymorphism (McCouch *et al.,* 1998; Tanksley, 1983, Wang *et al.,* 1992). Scope of crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programmes and also with the use of molecular markers. A large amount of well conserved genetically diverse material (approximately 23250 accessions of rice germplasm) is available at Indira Gandhi Krishi Vishwavidyalay, Raipur, Chhattisgarh.

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains. Diversity based on physiological and morphological characters usually varies with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. The rapid development of biotechnology allows easy analysis of a large number of loci distributed throughout the genome of plants.

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Several molecular markers *viz.,* RFLP (Becker *et al.,* 1995; Paran and Michelmore, 1993;), RAPD (Tingey and Deltufo, 1993; Williams *et al.,* 1990), SSRs (Levinson and Gutman, 1987), ISSRs (Albani and Wilkinson, 1998; Blair *et al.,* 1999), AFLP (Mackill *et al.,* 1996; Thomas *et al.,* 1995; Vos *et al.,* 1995; Zhu *et al*., 1998) and SNPs (Vieux *et al*., 2002) are presently available to assess the variability and diversity at molecular level (Joshi *et al.,* 2000). Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique

germplasm that compliments existing cultivars. In the present investigation, assessment of genetic variability and diversity at molecular level among 40 aromatic rice genotypes was assessed using 21 SSR markers spanning 12 chromosomes.

Materials and Methods

Forty aromatic rice landraces from different parts of Chhattisgarh were collected *viz*., Jeeradhan, Jawaphool (Raigarh), Dubraj-II (Dhamtari), Mai dubraj, Chinnor, Karigilas, Dubraj-I (Nagri Sihwa), Dubraj-III, Dujai (Pendra), Dubraj-IV (Bemetra), Kasturi, Anterved, Lallu-14, Kapoorsar, Badhsaahbhog (Jagdalpur), Kalikamod, Gangabaru (Bastar), Jeeraphool (Ambikapur), Aatmasheetal (Bastar), Chhatri, Shyamjeera (Surajpur), Kubrimohar (Bemetra), Gopalbhog, Shuklaphool, Tilkasturi, Samudrafan, Bisni-II, Bisni-I, Bisni-III, Lohndi (Garola), Jaigundi, Dubraj-V (Kharora), Keragul, Jaophool, Katarnibhog, Srikamal, Tulsiprasad, Tulsimanjari, Elayachi and Vishnubhog. These genotypes are very popular among consumers due to their unique aroma and taste.

Plant materials and genomic DNA isolation

Fourty aromatic landraces of rice were planted in nursery bed, after 2 weeks of sowing about one gram leaf of seedlings were collected and DNA was isolated from these leaves by using mini prep method of DNA extraction (Dellaporta *et al*., 1983). The DNA samples were quantified on Nano Drop Spectrophotoscopy (NANODROP 2000). After quantification, the DNA was diluted with TE such that the final concentration of DNA was approximately 40-50 ηg/µl for PCR amplification.

PCR amplification and electrophoresis

A set of 21 microsatellite markers distributed over 12 chromosomes of rice were used. 2 ml of diluted template DNA of each genotype was dispensed at the bottom of PCR plate (OXYGEN). Separately cocktail was prepared in an Eppendorf tube as described in Table 1. About 18ml of cocktail was added to each sample and the PCR (Life Technologies, Applied Bio system Ltd) was set up as the profile depicted in Table 2. Five per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified products, since polyacrylamide gel (PAGE) have better resolution for amplified products.

Results and Discussion

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Selection increases the frequency of alleles or allelic combinations with favorable effects at the expense of others, eventually eliminating many of them (Cao *et al*., 1998). In the present investigation microsatellites (Rice microsatellites) or SSR markers (Simple Sequence Repeats) were used to characterize and assess genetic diversity among 40 aromatic rice land races of Chhattisgarh. A total of 21 RM primers were utilized to provide genetic diversity among 40 aromatic rice landraces. Eighteen RM primers showed polymorphism in these cultivars.

Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (Mc Couch *et. al*., 2001). Rice similarity ratio revealed that high degree of similarity to the extent of 100 per cent exists between Jeeradhan and Jawaphool, and Bisni-I and Bisni-III, lowest level of similarity of 54 per cent exists between Elaychi and Visnubhog. It is important to note here that Bisni-I and Bisni-III are the sister lines selections and though Jeeradhan and Jawaphool were collected from fields of different farmers but from the same village. Highest similarity co-efficient among them indicated similar genetic background. Whereas, Elaychi land race showed different plant characters with all these lines and Vishnubhog also showed least similarity with rest of the entries. Similar studies were made by different authors using SSR markers (Panaud *et al*., 1996; Chakravarthi and Naravaneni, 2006.).

The UPGMA cluster analysis was performed by using Jaccard's similarity coefficient matrix prepared by binary score generated by using 21 microsatellite markers situated on different chromosomes of rice. The similarity coefficient ranged from 43-100 per cent. Two major clusters were formed which exhibited 46 per cent genetic similarity. First cluster consisted of 13 genotypes, whereas second cluster consisted of 27 genotypes of aromatic rice.

Second cluster is further divided into two groups, among which genotype Elaychi and Vishnubhog included and these genotypes exhibited 54 per cent genotypic similarity. The other genotype of this cluster exhibited 56 per cent genetic similarities further divided into two groups of which one group consisted of 24 genotypes whereas Tulsimanjari alone formed a separate group.

In first cluster, two major groups were formed at 46 per cent similarity. 11 genotypes clustered in one in group and exhibited 62 per cent genetic similarity, whereas other group had two genotypes Anterved and lallu-14 with 66 per cent genetic similarity.

For the loci studied, genotypes Jeeradhan and Jawaphool exhibited 100 per cent similarity. In the accessions three of

them from Bisni (aromatic rice collected from Ambikapur and Bagicha Districts of Sarguja). Bisni-I and Bisni-III also exhibited 100 per cent genetic similarity, but with Bisni-II these two showed 90 per cent genetic similarity these all 3 lies in 2nd cluster. Similarly five different accessions of Dubraj were also studied for SSR markers. Out of which Dubraj-II and Maidubraj exhibited 95 per cent genetic similarity. Dubraj-I and Dubraj-III has 88 per cent genetic similarity. Between Dubraj-I and III and Dubraj-IV 67 per cent genetic similarity was recorded. An accession of Dubraj-V recently collected from farmers laid on 2nd cluster exhibited that between Dubraj-5 and rest of the Dubraj accessions *i.e.,* Dubraj-I, II, III, IV, and Mai Dubraj only 43 per cent genetic similarity exists.

In second major cluster, two major groups were formed. Two entries of the second group *i.e.,* Elaychi and Vishnubhog have 53 per cent genetic similarity and with rest of the entries of the group these two showed 46 per cent genetic similarity. There results indicated that all the genotypes have high amount of genetic variability. It can be further utilized in breeding program for developing new varieties. Similar observations were made by Akagi *et al*. (1997).

Cluster analysis was used to group the varieties and to construct a dendrogram. This dendrogram revealed that the genotypes are derivatives of genetically similar type clustered more together. In this study, the larger range of similarity values for cultivars revealed by micro satellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs. Principle component analysis was also done to visualize genetic relationships among the elite breeding lines.

This fingerprinting makes identification and characterization of genotype very easy and further it will be of greater help in background selections during back cross breeding programmes.

References

Akagi, H., Yokozeki, Y., Inagaki, A. and Fujimura, T. 1997. Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. *Theoretical and Applied Genetics* 94:61-67.

Albani, M.C. and Wilkinson, M.J. 1998. Inter simple sequence repeat polymerase chain reaction for the detection of somaclonal variation. *Plant Breeding* 117:573-575.

Becker, J., Vos, P., Kuiper, M., Salamini, F. and Heun, M. 1995. Combined mapping of AFLP and RFLP markers in barley. *Mol. Gen. Genet*., 249: 65-73.

Blair, M.W., Panaud, O. and McCouch, S. R. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 98: 780-792.

Cao, T., Duprez, E., Borden, K.L.B., Freemont, P.S. and Etkin, L.D. 1998. Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. *J. Cell Science* 111:1319-1329.

Causse, M.A., Fulton, T.M., Cho, Y.G., Ahn, S.N., Chunwongse, J., Wu, K., Xiao, J., Yu, Z., Ronald, P.C., Harrington, S.E., Second, G., McCouch, S.R. and Tanksley, S.D. 1994. Saturated molecular map of the rice genome based on a inter specific backcross population. *Genetics*138:1251-1274.

Chakravarthi B.K. and Naravaneni Rambabu. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *African Journal of Biotechnology* 5 (9), pp. 684-688.

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA mini preparation: version II. *Plant Molecular Biology*1:19-21.

Joshi, S.P., Gupta, V.S., Aggarwal, R.K., Ranjekar, P.K. and Brar, D.S. 2000. Genetic diversity and phylogenetic relationship as revealed by Inter simple sequence repeat polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics* 100:1311-1320.

Kurata, N., Nagamura, Y., Yamamot, K., Harushima, Y., Sue, N., Wu, J., Antonio, B.A., Shomura, A., Shimizu, T. and Lin, S.Y. 1994. A 300 kilo base interval genetic map of rice including 883 expressed sequences. *Nature Genetics* 8:365-372.

Levinson, G. and Gutman, G.A. 1987. Slipped-strand mis-pairing: A major mechanism of DNA sequence evolution. *MolecularBiology Evolution* 4:203-221.

Mackill, D. J., Zhang, Z., Redona, E. D. and Colowit, P. M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome* 39: 969-977.

McCouch, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y. and Khush, G.S. 1988. Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics*76: 815-829.

McCouch, S.R., Temnykh, S., Lukashova, A., Coburn, J., Declerck, G., Cartinhour, S., Harrington, S., Thomson, M., Septiningsi, E., Semon, M., Moncada, P. and Jiming, L. 2001. Microsatellite markers in rice: Abundance, diversity and applications. *In: Rice Genetics IV*. IRRI. Manila, Philippines. pp: 117-135.

Panaud, O., Chen, X. and McCouch 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSR) in rice (*Oryza sativa* L.). *Mol. Gen. Gene*t., 252: 597–607.

Paran, I. and Michelmore, R.W. 1993. Development of reliable PCR based markers to downy mildew resistance genes in Lettuce. *Theoretical and Applied Genetics* 85:985-993.

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Molecular Biology Reports 1:3-8.

Thomas, C.M., Vos, P., Zabeau, M., Jones, D. A. and Norcottet, K.A. 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato Cf-9 gene for resistance to Cladosporium fluvum. *Plant Journal* 8:785-794.

Tingey, S.V. and Deltufo, J.P. 1993. Genetic analysis with Random Amplified Polymorphic DNA. *Plant Physiology* 101:349-352.

Vieux, E.F., Kwok, P.Y. and Miller, R.D. 2002. Primer design for PCR and sequencing In:High-throughput analysis of SNPs. *Biotechniques* 32: 28-30.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee T, Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407- 4414.

Wang, Z.Y., Second, G. and Tanksley, S.D. 1992. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. *Theoretical and Applied Genetics* 83: 565-581.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18:6531-6535.

Zhu, J., Gale, M.D., Quarrie, S., Jackson M. T. and Bryan, G.J. 1998. AFLP markers for the study of rice biodiversity. *Theoretical and Applied Genetics* 96:602-611.

Table 1. PCR mix for one reaction

Reagent	Stock concentration	Volume (ml)
Sterile and nanopure H_2O	-	13.5
PCR buffer with 15 mM $MgCl_2$	10X	2.0
dNTPs (Mix)	1mM	1.0
Primer (forward+ reverse)	5ρmol	1.0
Taq polymerase	1 u/ml	0.5
DNA template	40 ηg/ml	2.0
	Total	20

Table 2. Temperature profile used for PCR amplification using micro-satellite markers

Steps	Temperature (°C)	Duration (min.)	Cycles	Activity
1	95	5	1	Denaturation
2	94	1		Denaturation
3	55	1	34	Annealing
4	72	1		Extension
5	72	7	1	Final Extension
6	4	∞	1	Storage

Table 3. SSR primers used to amplify the *O. sativa* in study

Chro. Numbers	SSR Primers	PRIMER SEQUENCES	
		FORWARD 5' → 3'	REVERSE 5' → 3'
1	RM 5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
1	RM 431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
2	RM 13541	CTCCTCGCTTCGTCCACTTCC	CCATGTGTCACCGACTCAACG
2	RM 7485	GCCAGTTTCTCCAAAAGACG	AACTAGCCTCGACAGCGAAC
3	RM 55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG
3	RM 514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
4	RM 307	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
5	RM 507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
5	RM 161	TGCAGATGAGAAGCGCCGCCTC	TGTGTCATCAGACGGCGCTCCG
6	RM 162	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG
6	RM 454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
7	RM 455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
8	RM 44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
8	RM 408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
9	RM 105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
9	RM 316	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
10	RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
11	RM 144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG
11	RM 536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
12	RM 1261	GTCCATGCCCAAGACAAC	GTTACATGGGTGACCCC
12	RM 277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG

1. Jeeradhan
2. Jawaphool
3. Anterved
4. Kapoorsar
5. Badhsaahbhog
6. Dubraj-III
7. Aatmasheetal
8. Karigilas
9. Chhatri
10. Elayachi
11. Gopalbhog
12. Tilkasturi
13. Tulsimanjari
14. Maiubraj
15. Shuklaphool
16. Dubraj-I
17. Kalikamod
18. Dujai
19. Lallu-14
20. Gangabaru
21. Dubraj-IV
22. Dubraj-II
23. Jeeraphool
24. Kubrimohar
25. Jaophool
26. Katarnibhog
27. Shrikamal
28. Vishnubhog
29. Shyamjeera
30. Dubraj-V
31. Kasturi
32. Lohndi
33. Keragul
34. Chinnor
35. Samudrafen
36. Jaigundi
37. Tulsiprasad
38. Bisni-III
39. Bisni-II
40. Bisni-I

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Genetic Diversity in Grain Quality Traits of Rice Genotypes

G. Nirmala Devi*, V. Ravindra Babu, G. Padmavathi and T. Sunitha

Department of Genetics & Plant Breeding, Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana
* Corresponding author: nirmalalaxman2002@gmail.com

Received: 25th November, 2014; Accepted: 18th June, 2015

Abstract

Genetic diversity in grain quality among 92 rice cultivars was evaluated using Mahalanobis D2 statistic. Highly significant ($p < 0.01$) differences were observed among varieties for 14 quality characters namely hulling (%), milling (%), head rice recovery (%), kernel length and breadth (mm), length/breadth ratio, 1000 grain weight (g), kernel length after cooking (mm), kernel breadth after cooking (mm), kernel elongation ratio, volume expansion ratio and water uptake, amylose content (g%), gel consistency (mm) and alkali spreading value. Based on cluster analysis, the genotypes were grouped into 10 clusters of which clusters VII and IX are the largest clusters consisting of 16 genotypes each while cluster V was the smallest with only a single genotype. The maximum intra cluster distance (D = 14066.5) was found in cluster VI consisting of two traditional varieties AS 100 and Chittimutyalu. The most divergent clusters found were Clusters V and VI. Minimum inter cluster distance (D = 5144.43) was found between Clusters VIII and IX. Kernel breadth (0.01%), volume expansion ratio (0.39 %) and elongation ratio (0.30%) failed to contribute significantly towards genetic diversity. Amylose content (25.2%), gel consistency (20.4 %), 1000 grain weight (12.6 %) and head rice recovery (10.1%) were found to be the most contributing traits towards genetic diversity. Hence these traits could be focused for selection while improving grain quality.

Key words: Rice, Genetic diversity, Grain quality, cluster analysis, Mahalanobis D2

Introduction

Rice (*Oryza sativa*. L) is the most important cereal food crop in the world. Globally around three billion people depend on rice which provides 50 to 80 per cent of daily calories. Next to China, India is the second largest producer and consumer of rice. For meeting the dietary requirements of increasing population, genetic improvement of rice with higher yield, good grain quality, resistance to biotic and abiotic stresses is the most logical and promising approach. Genetic improvement mainly depends upon inclusion of genetically diverse parents having wider variability for different yield and quality characters in hybridization program. The more diverse the parents, the higher are the chances of obtaining more amounts of heterotic expression in F_1s and superior recombinants in the segregating generations. The present investigation aimed to assess the nature and magnitude of genetic divergence present in the 92 rice genotypes and to select suitable diverse genotypes as parents for further utilization in grain quality improvement programs.

Material and Methods

The experiment was conducted at DRR farm, ICRISAT campus, Patancheru during *kharif*, 2013. Ninety two rice varieties were transplanted randomly 20 cm apart between rows and 15 cm within row to each unit plot of 5 m^2 following randomized complete block design (RCBD) with three replications. Recommended crop management practices and need based plant protection measures were taken up. Observations were recorded on 14 grain quality traits: physical quality traits *viz.,* hulling (%), milling (%), head rice recovery (%), kernel length and breadth (mm), length/breadth ratio, 1000 grain weight (g) and cooking quality traits *viz.,* kernel length after cooking (mm), kernel breadth after cooking (mm), kernel elongation ratio, volume expansion ratio and water uptake; chemical quality attributes *viz.*, amylose content (%), gel consistency (mm), alkali spreading value. Genetic diversity analysis was done following D^2 statistics proposed by Mahalanobis (1936). The varieties were classified into a number of clusters by Toucher's method as described by Rao (1952).

Results and Discussion

The analysis of variance showed significant differences among genotypes for each character indicating the existence of variability among the genotypes (Table 1). The genotypes were grouped into 10 clusters based on D^2 value (Table 2, Fig1a &1b). Clusters VII and IX were the largest consisting of 16 genotypes followed by cluster VIII (15 genotypes), cluster I (13 genotypes), cluster X

(12 genotypes), cluster II & IV (6 genotypes), cluster III (5 genotypes) and cluster VI (2 genotypes). The smallest cluster (V) contained a single genotype. The genotypes of the biggest clusters VII and IX originated from different States like Andhra Pradesh, Telangana, Uttar Pradesh, Punjab, Chhattisgarh, Karnataka, West Bengal, Gujarat, Odisha, Assam, Punjab, Maharashtra, Tripura, Madhya Pradesh, Bihar and Haryana. It indicates that there was no relationship between clustering pattern and geographical distribution of genotypes.

Cluster II consisted of medium slender grain genotypes. There are six genotypes in this cluster and majority (66%) of them namely GR-103, Swarna, White Ponni and DL-184 possessed medium slender grains. In cluster IX, 10 of 16 genotypes possessed long slender grains. They are Manoharsali, NLR-33359 (Shravani), SGT-1, PR-116, Khitish, Kavya, Karjat-2, Amulya and Sahyadri. The clustering pattern of the hybrids appeared to be very distinct. All of them grouped into different clusters namely cluster 1, cluster 9 and cluster 10.

The genotypic distribution also indicated that the genotypes originated from similar geographic regions were distributed in different clusters. Therefore the kind of genetic diversity found among the genotypes belonging to same geographic origin might be due to differences in adaptation, selection criteria, selection pressure and environmental conditions. The genotypes having bold grain type were clustered together in cluster III. Among the five genotypes of cluster III, three genotypes Jalpriya, Jyothi and Madhukar possessed long bold grain type. The remaining two genotypes, Varsha and Kranti possessed short bold grains. Similarly cluster VI possessed genotypes, AS 100 and Chitti muthyalu with short bold grains. As expected all the aromatic varieties were found in same cluster grouped as cluster IV. They included Basmati-386, Taroari Basmati, Ranbir Basmati, Yamini, Type-3 and PR-115. However PR 115, a non aromatic variety is an exception in this cluster. Cluster IV had only one genotype Pusa Basmati 1 which is a most popular aromatic rice variety and being used as a quality check in basmati improvement programs. It was diverse and recorded high genetic distances from other clusters.

In general, the clustering pattern across locations showed that genotypes collected from the same geographic origin were found to be distributed in different clusters. Similar findings of non correspondence of genetic divergence with geographic diversity were reported earlier in rice by Murthy and Arunachalam, (1966), Sinha *et al*., (1991), Vivekananda and Subramanian, (1993), Manan *et al.,* (1993), Rahman *et al.,* (1997), Chaudhury *et al.,* (1999), Shanmugasundaram *et al.,* (2000), Masud *et al.,* (2003), Patil *et al.,*(2005), Raju *et al.,* (2004), Chandra *et al.,* (2007), Arun Sharma *et al.,* (2008) and Rajesh *et al.,* (2010). Murthy and Arunachalam, (1966) stated that genetic drift and selection in environment could cause greater diversity than geographic distances. Considering this, parents should be selected on the basis of genetic diversity rather than geographic diversity which are supported by the findings of Hasan *et al.,* (2000). Grouping of materials of similar origin into different clusters was an indication of broad genetic base of the genotypes belonging to that origin. So genotypes originating from same place may have different genetic architecture or *vice versa* (Shanmugam and Rangaswamy, 1982).

The intra cluster D^2 values ranged from zero (cluster V) to 14066.5 (cluster VI) followed by cluster II (6442.21); cluster IV (5662.09); cluster III (5422.41); cluster X (4764.55); cluster VII (4747.5); cluster I (4458.51); cluster IX (4245.36) and cluster VIII (3062). The highest intra cluster distance (14066.5) in cluster VI indicates wide genetic variation among the genotypes belonging to these clusters. The V^{th} cluster consisted of only one genotype (Pusa Basmati1) hence; it lacked intra-cluster distance (0.00).

The inter cluster D^2 was maximum between clusters V and VI (53342.32) indicating that genotypes in V were far diverse from those of VI. Choosing of genotypes belonging to distant clusters was expected to execute maximum heterosis in crossing and to be used in hybridization program for obtaining a wide spectrum of variation among the segregants. This was in conformity with Hossain *et al*., (2003). The least distance was observed between cluster VIII and IX (5144.43) which indicated genotypes included in them were closely related (Table 3).

The cluster wise mean values for fourteen quality characters were presented in Table 4. These are helpful to assess the superiority of clusters during the improvement of characters through hybridization programme. The cluster mean values showed a wide range of variation for majority of the characters undertaken in the present study. The diversity was also supported by the appreciable amount of variation among the cluster means for different characters. Cluster III exhibited highest kernel breadth (2.29 mm), amylose content (26.92%) and 1000 grain weight (28.69 g) while cluster IV contained genotypes with highest KLAC (12.63 mm) and elongation ratio (1.98). Cluster V recorded highest value of kernel length (6.82 mm), L/B ratio (4.15) and ASV (6.66). Cluster VII had highest value for hulling (79.73 %), milling (71.24 %), HRR (67.99 %), volume expansion ratio (5.95), water uptake (289 ml), gel

consistency (62.56 mm) and 1000 grain weight (28.69 g). Thus, these genotypes hold great promise as parents for obtaining promising elite lines through hybridization and to create further variability for these characters (Mishra and Pravin, 2004).

It is indicated from the study that hybridization between the genotypes of the clusters VI and VII (high HRR, medium and desirable AC, ASV, GC) with cluster V (high L/B ratio, high kernel length, high kernel length after cooking and low kernel breadth) forms a good cross combination producing superior recombinants. Likewise cluster V (high L/B ratio, low kernel breadth and medium and desirable AC) is suitable for hybridization with cluster VII (high VER) and cluster IV (high ER).

The genotypes from cluster VI *i.e.,* AS 100 and Chittimuthyalu having desired quality characters like high HRR, desirable medium ASV and AC, low kernel breadth; the genotypes from another cluster VII such as Kalanamak, NLR 33654 and WGL 14 possessing desirable quality characters namely high HRR, high L/B ratio indicating long slender grains, desirable medium AC and ASV; one genotype namely Pusa Basmati 1 from cluster V having preferred grain qualities like long slender grains with high l/b, desirable KLAC and medium desirable AC can be selected as ultimate parents for hybridization program. Ravindra Babu *et al*., (2006) and Subudhi *et al.,* (2009) also proposed to choose diverse parents for quality traits such as HRR, KLAC, GC, ER and AC from the most divergent clusters so that they produce larger variability and desirable segregants that would be productive in rice breeding program

Contribution of different quality characters to total divergence is presented in Table 4. At Andhra Pradesh the amylose content was having maximum contribution *ie.*, (25.2%) followed by gel consistency (20.4 %), 1000 grain weight (12.6%) and HRR (10.1 %). The lowest value is observed in kernel breadth (0.01 %). Hence amylose content and gel consistency were found to be potential contributors to genetic divergence in the genotypes. Subudhi *et al*., (2009) evaluated physico-chemical and cooking characters in rice to study the diversity pattern among the genotypes and reported that the characters amylose content, alkali spreading value, kernel length after cooking and kernel breadth together accounted for 83.78 per cent to the total divergence.

In our study 1000 grain weight was one of the main contributors to the total genetic diversity. Senapati and Sarkar, (2005), Ramesh Chandra *et al*., (2007) and Iftekharuddaula *et al.,* (2010) also found that 1000 grain weight to be the chief contributors towards genetic divergence and they suggested that this character must be given importance while selecting parents in crossing programme as well as selection of segregants in succeeding generations reported. Chand *et al*., (2005) conducted experiments to study genetic divergence in 57 genotypes of rice germplasm lines based on 14 agro morphological traits and found that 1000 grain weight character prominently contributed to total genetic divergence.

Ravindra Babu *et al*., (2006) reported genetic divergence using quality characters found that the characters gel consistency, water uptake, and head rice recovery per cent contributed maximum towards genetic divergence. However, Garg *et al*., (2011) observed the gel consistency contributed maximum towards genetic divergence. These observations corroborate well with those of earlier researchers (Sandhya Kishore *et al.,* 2007, Patil *et al.,* 2005). These traits should be given importance during hybridization and selection of segregating populations.

In the present experiment hybridization among the varieties AS 100 and Chittimutyalu from cluster VI for high HRR, soft gel consistency and intermediate amylose and ASV; Pusa Basmati 1 from cluster V for high L/B ratio, more kernel length and more KLAC would result in obtaining wide spectrum of variation and desirable heterotic recombinants in the segregating generations amenable for selection in improving grain quality.

References

Arun Sharma., Gupta, K.R. and Rakesh Kumar. 2008. Genetic divergence in basmati rice (*Oryza sativa*) under irrigated ecosystem. *Crop Improvement* 35 (1):8-10.

Chand, S.P., Roy, S.K. and Senapati, B.K. 2005. Genetic divergence in Aman rice under semi-deep rain fed condition. *Crop Research* 30 (1): 46-49.

Chandra, B.S., Reddy, T.D. and Ansari, N.A. 2007. Genetic divergence in rice (*Oryza sativa* L.). *Research on Crops* 8:600-603.

Chaudhury, D.A., Hamid A., Bhowmik A., Mian, G.U. and Islam, M.S. 1999. Genetic divergence in indigenous rice (*Oryza sativa* L.) varieties of Bangladesh. *Bangladesh Journal of Plant Breeding and Genetics*12 (1&2):33-36.

Garg, P., Pandey, D.P. and Kaushik, R.P. 2011. Genetic Divergence for Yield and Quality Traits in Rice (*Oryza sativa* L.) *Journal of Rice Research* 4 (1 & 2):1-5.

Hasan, M.J., Rasul, M.G., Mian, M.A.K., Hasanuzzaman, M. and Chowdhury, M.M.H. 2000**.** Genetic divergence of Yam. *Bangladesh Journal of Plant Breeding and Genetics* 13 (1):07-11.

Hossain, M.A., Hamid, A., Hossain, S.M.A. and Bhuiyan, N.I. 2003. Genetic divergence and path analysis in groundnut. *Bangladesh Journal of Plant Breeding and Genetics* 16 (1): 9-15.

Iftekharuddaula, K.M., Khaleda Akter Hassan, M.S., Kaniz Fatema. and Adil Badshah. 2010. Genetic divergence, character association and selection criteria in irrigated rice. *Online Journal of Biological Sciences* 2 (4): 243-246.

Mahalanobis, P.C. 1936. A statistical study at Chinese head measurement. *Journal of Asiatic Society Bengal* 25:301:77.

Manan, M.A., Ahmed, M.S., Rashid, M.M., Bhuiyan, M.K.R. and Gomes, R. 1993. Genetic diversity of *Colocasia esculenta* L. *Indian Journal of Root Crops* 19 2:95-99.

Masud, M.A.T., Hossain, M.A., Saha, S.R., Muhammad, A. and Hossain, S.M.M. 2003. Genetic divergence in ridge gourd (*Luffa acutangula* L.). *Bangladesh Journal of Plant Breeding and Genetics* 16(2):17-22.

Mishra, L.K. and Pravin, J. 2004. Variability and genetic diversity in rice (*Oryza sativa* L.). *Mysore Journal of Agricultural Science* 38: 367-375.

Murthy, B.R. and Arunachalam, V. 1966. The nature of genetic divergence in relation to breeding system in some crop plants. *Indian Jounal of Genetics* 26:188-198.

Patil, S.G., Mairan, N.R. and Sahu, V.N. 2005. Genetic divergence of traditional rice germ plasm accessions. *Journal of Soils and Crops* 15:308-314.

Rahaman, M., Acharya, B., Shukla, S.N. and Pande, K. 1997. Genetic divergence in low land rice germ plasm. *Oryza* 34:209-212.

Rajesh, T., Paramasivam, K. and Thirumani, S. 2010. Genetic divergence in land races of rice. *Electronic Journal of Plant Breeding* 1 (2):199-204.

Raju, C.H.S., Rao, M.V.B. and Sundarshanam, A. 2004. Genetic analysis and character association in F2 generation of rice. *The Madras Agricultural Journal* 91(1-3): 66-69.

Ramesh Chandra, Pradhan, S.K., Sanjay Singh, Bose, L.K. and Singh, O.N. 2007. Multivariate Analysis in Upland Rice Genotypes. *World Journal of Agricultural Sciences* 3 (3): 295-300.

Rao, C.R. 1952. Advanced statistical methods in biometrical research. John Willey and Sons, Inc., New York

Ravindra Babu, V., Sandhya Kishore, Shobha Rani, N. and Ravichandran. 2006. Genetic divergence analysis using quality traits in rice genotypes (*Oryza sativa* L.). *Oryza* 43 (4): 260-263.

Sandhyakishore, N., Ravindrababu, V., Ansari, N.A. and Ravichandran. 2007. Genetic divergence analysis using yield and quality traits in rice (*Oryza sativa* L.). *Crop Improvement* 34 (1): 12-15.

Shanmugam, A.S. and Rangasamy, S.R.S. 1982. Genetic diversity for quantitative characters in green gram (*Vigna radiata* L.). *The Madras Agricultural Journal* 69 (10): 631-636.

Shanmugasundaram, P., Souframanien, J. and Sadasivan, S. 2000. Genetic divergence among rice varieties released from paddy breeding station, Coimbatore, India. *Oryza* 37(3): 225-228.

Senapathi, B.K. and Sarkar, G. 2005. Genetic divergence in tall *indica* rice (*Oryza sativa* L.) under rain fed saline soil of Sundarban. *Oryza* 42 (1): 70-72.

Sinha, P.K., Chauhan V.S., Prasad K. and Chauhan J.S. 1991. Genetic divergence in indigenous up land rice varieties. *Indian Journal of Genetics* 51(1): 47-5.

Subudhi, H.N., Meher, J., Bose, L.K. and Sanjukta Das. 2009. Genetic diversity studies of promising rice varieties of eastern India based on quality characters. *Oryza* 46 (3): 224- 227.

Vivekananda, P. and Subramanian, S. 1993. Genetic divergence in rainfed rice. *Oryza* 30:60-62.

Table1. Analysis of variance of 14 characters for 92 genotypes

Characters	Source of variation (mean sum of squares)		
	Replication	Treatments	Error
Hulling (%)	0.235	12.535**	0.007
Milling (%)	0.137	40.935**	0.006
Head Rice recovery (%)	15.249	641.809**	10.038
Kernel Length (mm)	0.0002	1.298**	0.0006
Kernel Breadth (mm)	0.0006	0.133**	0.0003
L/B ratio	0.001	0.686**	0.0005
Kernel Length After Cooking	0.059	7.248**	0.007
Water Uptake (ml)	129.632	11856.66**	36.733
Volume Expansion Ratio	0.006	0.547**	0.005
Elongation Ratio	0.001	0.146**	0.0002
Alkali Spreading Value	1.981	3.354**	0.172
Gel Consistency (mm)	2.851	723.141**	0.785
Amylose Content (%)	0.008	17.279**	0.016
1000 grain weight (g)	0.204	50.607**	0.098

*Significant at 5% level, **Significant at 1% level

Table 2. Clustering pattern of 92 rice genotypes based on D2 analysis

Cluster No.	Number of genotypes	Name of genotypes
I	13	Aishwaraya, Kanchana, Harsha, Matta Triveni, KHP-2, BR-2655, Jalamagna, VRS-3, Shakthi, Nalini, Mandhya Vijaya, Pratap, Gouri
II	6	BPT-11711, GR-103, Swarna, White Ponni, DL-184, RAU 3043
III	5	Jalpriya, Jyothi, Madhukar, Kranti, Varsha
IV	6	Basmati-386, Taroari Basmati, Ranbir Basmati, Yamini, Type-3, PR-115
V	1	Pusa Basmati 1
VI	2	AS-100, Chittimutyalu
VII	16	Dharithri, WGL-14 (Warangal Sannalu), Pooja, Kalanamak, Nagari Dubraj, KMP-101, CN-1233-33-9-117, PantDhan-16, VRM-3, Dandi, VRM-31, VRS-25, High iron rice, Indravati, NLR-33654 (Apurva), Ranjeet
VIII	15	Bhuban, Jaya, MTU-3636(Prabhat), Sunandana, Birupa, Bhudeb, Suraksha, MTU-1001(Vijeta), MTU 1010 (Cottondora Sannalu), Gajapathi, Giri, Sashi, PR-113, PSD-1, Mahamaya
IX	16	CN-1039-9, MSS-5, NLR-145 (Swarnamukhi), Manoharsali, NLR-33359 (Shravani), SGT-1, Jagabndu, Prachi, MSE-9, PR-118, PR-116, Khitish, Kavya, Karjat-2, Amulya, Sahyadri
X	12	Barah Avarodhi, Sabita, PR-111, PR-114, Vasumati, Jalnidhi, Lalat, Konark, VRS-19, Sahyadri-2, Vikas, IR-64

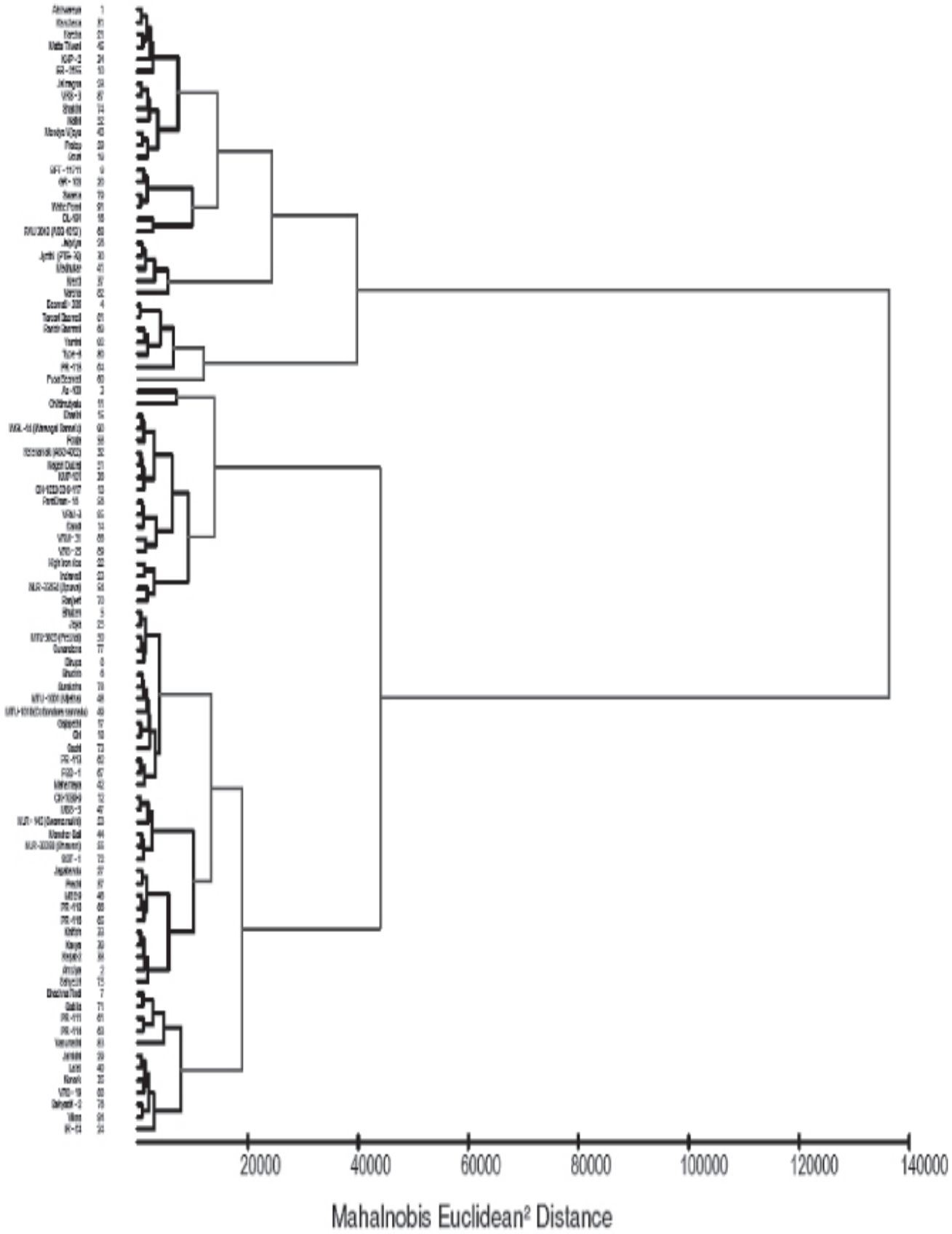


Fig. 1a. Dendrogram showing Clustering of rice genotypes by Tocher's method based on quality traits

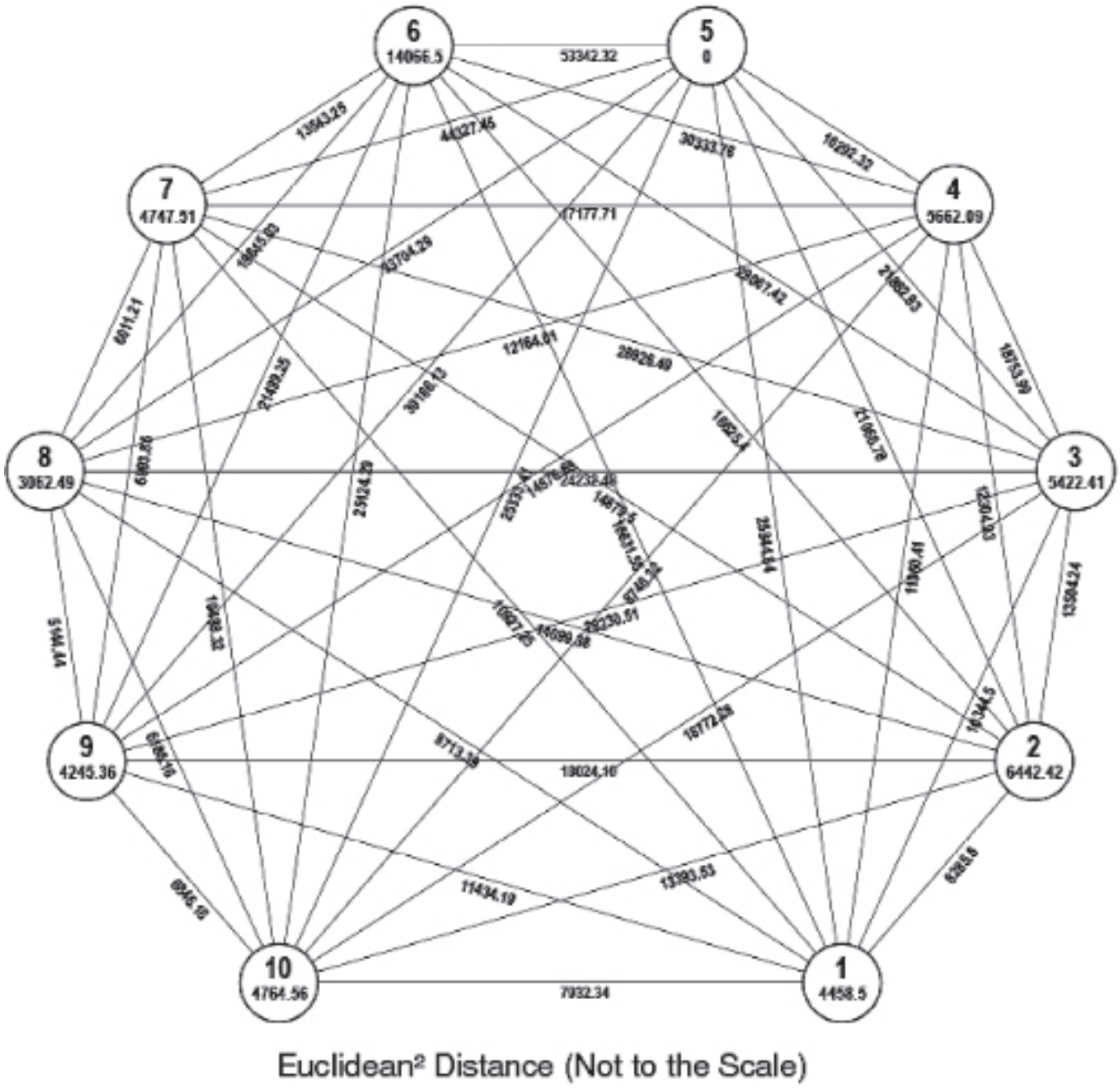


Fig. 1b. Ward Means

Table 3. Average intra (diagonal) and inter cluster distances for quality traits

Cluster	I cluster	II cluster	III cluster	IV cluster	V cluster	VI cluster	VII cluster	VIII cluster	IX cluster	X cluster
I cluster	4458.5	8285.60	10344.5	11960.41	25844.84	16631.55	10927.25	8713.39	11434.19	7932.339
II cluster		6442.41	13504.24	12304.93	21066.78	16625.4	14879.50	14099.38	18024.19	13393.53
III cluster			5422.41	18753.99	21862.93	29067.42	28926.49	24232.48	28230.51	18772.28
IV cluster				5662.09	16292.32	30333.76	17177.71	12164.01	14576.69	8748.324
V cluster					0	53342.32	44327.45	33704.29	39166.13	25333.41
VI cluster						14066.5	13543.25	19645.03	21499.25	25124.29
VII cluster							4747.51	6011.20	6993.85	10488.32
VIII cluster								3062.49	5144.43	6186.15
IX cluster									4245.36	6846.17
X cluster										4764.55

Table 4. Cluster means of quality traits and their contribution to total divergence

Clusters	Hulling (%)	Milling(%)	HRR (%)	KL mm)	KB (mm)	L/B	1000 grain wt (g)	VER	WU (ml)	KLAC (mm)	ER	ASV	AC (%)	GC(mm)
I	78.41	65.33	37.66	5.59	2.19	2.55	23.43	5.00	180.10	9.80	1.76	5.30	25.51	45.84
II	74.78	64.66	51.07	5.062	1.89	2.68	20.09	4.89	127.16	9.41	1.86	5.55	22.12	30.88
III	77.20	59.68	35.23	5.642	2.29	2.46	28.69	4.96	169.93	9.89	1.75	5.60	26.92	54.06
IV	77.01	64.88	40.87	6.374	1.76	3.63	22.96	4.90	156.88	12.63	1.98	5.38	19.53	37.00
V	69.50	58.93	40.76	6.82	1.64	4.15	21.79	4.73	286.00	12.13	1.77	6.66	23.77	27.33
VI	76.88	69.53	67.98	3.86	2.17	1.83	20.02	5.13	133.50	7.60	1.97	4.50	21.78	49.00
VII	79.73	71.24	67.99	5.34	2.13	2.52	28.69	5.95	289.69	9.63	1.81	4.89	22.39	62.56
VIII	79.30	69.86	49.07	6.18	2.16	2.88	24.74	5.05	208.17	10.29	1.66	5.11	24.26	28.62
IX	79.62	70.69	57.89	6.312	2.07	3.05	25.34	4.87	175.58	10.38	1.64	4.66	24.14	60.08
X	78.90	67.30	41.00	6.457	2.06	3.15	24.58	4.76	172.63	11.94	1.85	5.30	24.76	51.08
Mean	78.50	67.93	49.21	5.878	2.09	2.84	23.81	4.93	167.03	10.37	1.77	5.12	23.79	51.08
Contribution (%)	5.10	8.20	10.10	1.59	0.01	3.41	12.60	0.39	2.50	2.10	0.30	8.10	25.20	20.40

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Combining Ability Analysis for Yield and Components Traits in Fine Grain Rice of Mid Hills of Uttarakhand

J. P. Aditya* and Anuradha Bhartiya

Crop Improvement Division, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand
*Corresponding author E mail: jayprakashaditya@gmail.com

Received: 2nd April, 2015; Accepted: 24th June, 2015

Abstract

Nine fine grain rice genotypes *viz.,* VL 30925, VL 30926, VL 30928, VL 30929, VL 30938, VL 31486, VL 31632, VL 31634, VL 31638 were crossed with four basmati varieties Pusa Basmati 1, Pusa Sugandh 2, Pusa Sugandh 3 and Pusa Sugandh 5 in a line x tester mating design. The preponderance of dominant gene action was observed for plant height, days to 50% flowering, days to maturity, grains per panicle, fertile grains per panicle, 1000 grain weight, kernel length, kernel width, L/B ratio and grain yield per plant. Five genotypes *viz.,* VL 30928, VL 30929, VL 31632, VL 30925 and VL 31634 were found to be good general combiners and could be utilized to generate desirable segregants for future breeding programmes. High sca effects were observed in the crosses VL 30926 x P. Sug 5, VL 30929 x P. Sug 3, VL 30938 x P. Sug 5, VL 31486 x P. Sug 2, VL 31632 x P. Sug 5, VL 31634 x P. Bas 1 and VL 31638 x P. Bas 1 for grain yield and components traits.

Key words: Combining ability, gca, sca, grain yield, fine grain rice.

Introduction

Rice is the staple food for more than half of the world's population and the second most widely grown cereal crop of the world. It is the most extensively and the largest grown crop of India having an area of about 44 m ha and is grown in almost all parts of the country. In Uttarakhand, rice is the major cereal crop of *kharif* season accounting an area of about 280 thousand hectares with a production of 594 thousand tonnes and productivity of 2120 kg/ha during 2011-12. Irrigated rice in hills is grown in valleys where water is available in sufficient quantity to meet the growth stage conditions of rice crop. Success of any breeding programme depends primarily on the choice of appropriate parents in the hybridization and combining ability studies helps in selecting the parents for hybridization, provide information on additive and dominance variance (Thakare *et al*., 2010) as well as breeding procedure to be followed to select desirable segregants (Salgotra *et al*., 2009). Line x tester analysis provides information about general combining ability (gca) and specific combining ability (sca) effects of parents and is helpful in estimating various types of gene actions (Muhammad *et al.*, 2007). The present investigation was undertaken to get an idea of the combining ability for yield and other related traits in fine grain rice with a view to identify good combiners for effective breeding.

Materials and Methods

The experimental materials comprised of thirty six hybrids derived from crossing of nine lines with four basmati male parents in line x tester fashion. The parents used as lines were VL 30925, VL 30926, VL 30928, VL 30929, VL 30938, VL 31486, VL 31632, VL 31634 and VL 31638 while Pusa Basmati 1, Pusa Sugandh 2, Pusa Sugandh 3, Pusa Sugandh 5 were used as testers. All the parents and hybrids were grown in randomized block design (RBD) with two replications at experimental farm of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora. The standard agronomic practices were followed for raising good crop. The seedlings were planted at spacing of 20 x 15 cm with two meter length row. Observations were recording on five randomly selected competitive plants of the middle row of each plot for quantitative traits *viz.*, plant height, tillers per plant, flag leaf length, flag leaf width, panicles per plant, panicle length, grains per panicle, fertile grain per panicle, thousand grain weight, kernel length, kernel width, L/B ratio, grain yield per plant whereas days to 50 per cent flowering and days to maturity were recorded on plot basis. The mean value was used as the replicated data and was subjected to statistical analysis using INDOSTAT software package. The combining ability analysis was carried out following the method as suggested by Kempthorne (1957).

Results and Discussion

The analysis of variance revealed significant genotypic effect for all the characters under study except tillers/ plant, flag leaf width, panicles/plant, panicle length. This provides evidence for the presence of sufficient genetic variability among lines, testers and test crosses indicating wide diversity among treatment themselves (Table 1). The mean sum of squares due to parents versus crosses also differed significantly for yield and major yield components *viz*., plant height, days to 50% flowering, days to maturity, grains per panicle, fertile grains per panicle, 1000 grain weight, kernel length, kernel width, L/B ratio and grain yield per plant. Significance of mean squares of lines and testers indicated prevalence of additive variance whereas, non additive variance by line x tester. Preponderance of non-additive variance in the expression of different traits in rice has also reported by Ram *et al.*, (1991) and Khirsagar, (2002). These results emphasized the importance of combining ability studies and indicated good prospects of selection of suitable parents and the crosses for the development of appropriate varieties and hybrids.

The general combining ability (GCA) identifies superior parental genotypes while specific combining ability (SCA) helps in identification of good hybrid combinations which may ultimately lead to the development of hybrids (Shiva Prasad *et al.*, 2013). Line x Tester analysis is one of the most powerful tools for estimating the GCA of parents and selection of desirable parents and crosses with high SCA for the exploitation of heterosis (Tiwari *et al*., 2011). The estimates of general combining ability effects of line and tester (Table 2) revealed that the lines VL 30928, VL 30929 and tester Pusa Basmati 1 were good combiners for grain yield and component traits. However, good general combiners may not necessarily produce good specific combinations for different traits. Similar results were reported by Ramlingam *et al.,* (1997). The desirable gca effects of line VL 31634 and tester Pusa Sugandh 2 were the better general combiner for earliness. Promising parental lines identified for short plant stature were VL 30925, VL 30929, VL 31634 and Pusa Sugandh 3. It is worthwhile to mention here that the selection of parents for days to maturity and plant height depends on the target environment of the breeder. VL 30929 exhibited high gca effects for panicles per plant while good general combiners for panicle length was VL 30928. For grains per panicle, good general combiners were VL 30928, VL 30929, VL 31632, VL 31634 and Pusa Sugandh 3. The good general combiner for important traits like fertile grains per panicle were VL 30926 VL 30928, VL 30929, VL 30938,VL 31632, Pusa Sugandh 3 and Pusa Sugandh 5. The parental lines VL 30925, VL 30928, VL 30929, VL 30938, VL 31486, VL 31638, Pusa Basmati 1 and Pusa Sugandh 3 showed high gca effects for 1000 grain weight and kernel length while VL 30926, VL 30929, VL 30938, VL 31632, VL 31638, Pusa Sugandh 3 and Pusa Sugandh 5 were found to be good for kernel width. Parents with significant negative general combining ability (gca) estimates for days to 50% flowering, plant height and days to maturity and with significant positive gca effects for the remaining characters are considered as good general combiners. The parents with significant positive gca effects for days to 50% flowering, plant height and days to maturity are considered as poor general combiners. The parents with non significant gca estimates for all the characters were considered as average general combiners.

VL 30929 was identified as good general combiner for grain yield per plant, plant height, panicles per plant, grains per panicle, fertile grains per panicle, kernel length, kernel width and L/B ratio whereas, VL 30928 was found to be good general combiner for days to 50 % flowering, panicle length, grains per panicle, fertile grains per panicle, 1000 grain weight, kernel length, L/B ratio and grain yield per plant. It could be mentioned that the parents with significant and positive GCA values might be contributed positive alleles in their hybrid due to its additive nature of gene action for the respective traits. The crosses involving these parents might produce good progenies for the respective traits.

Specific combining ability (SCA) of a cross is the estimation and the understanding of the effect of non additive gene action for a trait which is an indicator for the selection of a hybrid combination (Akter *et al*., 2010). Therefore, a highly significant SCA effect is desirable for a successful hybrid breeding program. Specific combining ability (sca) effects were estimated for all the 36 hybrids and sixteen characters (Table 3). The estimates of hybrid revealed that none of the hybrids was consistently superior for all the traits. In the present study, positive significant sca effects for grain yield per plant was exhibited by seven crosses *viz*., VL 30926 x P. Sug 5, VL 30929 x P. Sug 3, VL 30938 x P. Sug 5, VL 31486 x P. Sug 2, VL 31632 x P. Sug 5, VL 31634 x P. Bas 1 and VL 31638 x P. Bas 1. The high sca effects may be associated with high hybrid vigour (Saidaiah *et al*., 2010). For plant height, negative estimates of sca are desirable and the promising specific combiners were VL 30926 x P. Sug 2, VL 31634 x P. Bas 1, VL 31634 x P. Sug 3, VL 31638 x P. Sug 2. The results confirm the findings of Muhammad *et al*., (2007). Out of 36 crosses, as many as 20 cross combinations showed desirable sca effects for days to maturity. The cross combinations *viz.,*

VL 30925 x P. Bas 1, VL 30926 x P. Bas 1, and VL 31632 x P. Sug 2 showed higher sca effects for flag leaf length. Cross VL 31638 x P. Bas 1 was good specific combiner for tillers per plant whereas cross VL 31638 x P. Sug 2 for flag leaf width. The cross VL 31634 x P. Bas 1, the best specific combination for grain yield per plant was also good with high sca effects for plant height, 1000 grain weight, kernel length, kernel width and L/B ratio. The cross VL 31486 x P. Sug 2 combined positive and significant sca effects for quality traits like kernel length, kernel width, L/B ratio and 1000 grain weight. The cross VL 31632 x P. Sug 5 and VL 30929 x P. Sug 3 expressed desirable significant sca effects for yield component traits *viz.,* days to 50 % flowering, days to maturity, grains per panicle, fertile grains per panicle. The cross VL 31638 x P. Bas 1 showed significantly positive sca effects for grain yield per plant might be due to good specific combination for grains per panicle, fertile grain per panicle, panicle length, tiller per plant and days to maturity.

The proportional contribution of the total variance by lines, testers and interaction revealed that the lines and line x tester interaction have contributed more than testers in respect of all the characters (Table 4). From this study, it is concluded that no cross was good for all the characters but some crosses showed good sca effects for a number of characters. The best general combiners were VL 30928, VL 30929, VL 31632, VL 30925 and VL 31634 could be utilized in future breeding programmes. The crosses *viz*., VL 30926 x P. Sug 5, VL 30929 x P. Sug 3, VL 30938 x P. Sug 5, VL 31486 x P. Sug 2, VL 31632 x P. Sug 5, VL 31634 x P. Bas 1 and VL 31638 x P. Bas 1 could be used for exploitation of heterosis for yield.

References

Akter, A., Hasan, M.J., Begum, H., Kulsum, M.U. and Hossain, M.K. 2010. Combining ability analysis in rice (*Oryza Sativa* L.). *Bangladesh Journal of Plant Breeding and Genetics* 23(2): 07-13.

Kempthorne, O. 1957. An Introduction to Genetic Statistics. John Wiley and Son, Inc., New York.

Khirsagar, R.M. 2002. Genetic analysis of yield and its components in rice. M.Sc. (Ag) thesis (unpublished) submitted to Gujarat Agricultural University, Navsari.

Muhammad, R., Cheema, A.A. and Muhammad, A. 2007. Line x Tester analysis in basmati rice *Pakistan Journal of Botany* 39(6): 2035-2042.

Ram, T., Singh, J. and Singh R.M. 1991. Genetic analysis of yield and components in rice. *Oryza* 28: 447-450.

Ramlingam, J., Nadarajan, N., Vanniarajan, C. and Rangasamy, P. 1997. Combining ability studies involving CMS lines in rice. *Oryza* 34:4-7.

Saidaiah, P., Ramesha, M.S. and Kumar, S.S. 2010. Line x Tester analysis in rice (*Oryza sativa* L.). *Crop Improvement* 37 (1): 32-35.

Salgotra, R.K., Gupta, B.B. and Singh, Praveen. 2009. Combining ability studies for yield and yield components in Basmati rice. *Oryza* 46(1): 12-16.

Shiva Prasad, G., Subba Rao, L.V., Sujatha, M. and Chaitanya, U. 2013. Heterosis and combining ability analysis for few cold tolerant rice germplasm lines at their seedling stage. *International Journal of Development Research* 3(8): 010-012.

Thakare, I.S., Mehta, A.M., Patel, J.S. and Takle, S.R. 2010. Combining ability analysis for yield and grain quality traits in rice hybrids. *Journal of Rice Research* 3(1):1-5.

Tiwari, D.K., Pandey P., Giri S.P. and Dwivedi J.L. 2011. Heterosis studies for yield and its components in rice hybrids using CMS system. *Asian Journal of Plant Sciences* 10: 29-42

Table 1. Analysis of variance for various yield contributing characters

Source of variance	d.f	Plant height	Days to 50% flowering	Days to maturity	Tillers per plant	Flag leaf length	Flag leaf width	Panicles per plant	Panicle length	Grains per panicle	Fertile grains per panicle	Spikelet Sterility %	1000grain weight	Kernel length	Kernel width	L/B ratio	Grain yield per plant
Replications	1	69.28	15.52	18.86	50.00	585.06	1.28	330.61	31.03	1396.93	961.72	11.79	12.42	0.00	0.00	0.00	0.01
Treatments	48	250.19**	78.51**	921.70**	8.46	7.64**	0.02	113.15	11.48	2993.96**	2434.47**	357.66**	40.17**	3.16**	0.21**	1.05**	41.50**
Parents	12	175.94**	38.23**	38.63**	7.29	5.48	0.02	10.08	7.44	2177.53**	816.13**	105.83**	16.19**	1.07**	0.08**	0.86**	18.09**
Lines	8	184.88**	44.51**	44.93**	6.93	4.71	0.02	11.93	10.36	2948.12**	1166.18**	108.12**	21.48**	1.23**	0.06**	0.69**	20.86**
Testers	3	23.59	26.12**	25.83**	10.33	4.63	0.03	8.45	2.08	771.45*	122.45	28.83	3.84	0.33**	0.12**	0.86**	11.86
Lines vs Testers	1	561.56**	24.36**	26.67**	1.09	14.22*	0.02	0.18	0.21	231.00	96.79	318.50**	10.92*	2.05**	0.13**	2.24**	14.65
Parents vs Crosses	1	1241.60**	815.57**	1993.95**	38.22	6.95	0.00	150.35	130.11	18191.68**	12227.23**	109.01	19.69**	1.02**	0.01*	0.76**	66.87**
Crosses	35	247.32**	71.26**	1193.84**	8.01	8.39**	0.02	147.43	9.48	2839.67**	2709.54**	451.11**	48.98**	3.94**	0.26**	1.12**	48.80**
Line Effect	8	490.43*	95.51	844.63	8.18	9.26	0.01	182.59	11.78	3691.07	4463.62*	866.07*	120.16**	9.16**	0.21	2.75**	79.60
Tester Effect	3	53.87	189.74*	1730.93	7.51	5.36	0.03	193.64	5.78	2113.16	7229.97*	760.25	25.49	1.45	0.23	0.46	54.95
Line vs Tester Effect	24	190.46**	48.36**	1243.10**	8.01	8.49**	0.03	129.93	9.17	2646.68**	1559.79**	274.15**	28.19**	2.51	0.27**	0.66**	37.77**
Error	48	18.09	0.27	0.59	5.58	3.47	0.02	109.82	2.93	199.00	115.07	32.58	2.58	0.04	0.00	0.02	4.88
Total	97	133.47	39.14	456.59	7.46	11.53	0.03	113.74	7.45	1594.42	1271.55	193.23	21.28	1.58	0.10	0.53	22.95

** Significant at 1% level of probability.

Table 2. Estimates of general combining ability (GCA) effects of Lines and Testers for sixteen characters in rice

Parents	Plant height	Days to 50% flowering	Days to maturity	Tillers per plant	Flag leaf length	Flag leaf width	Panicles per plant	Panicle length	Grains per panicle	Fertile grains per panicle	Spikelet Sterility %	1000 grain weight	Kernel length	Kernel width	L/B ratio	Grain yield per plant
Lines																
VL 30925	-7.59**	-1.51**	2.36**	1.02	0.34	-0.00	-0.43	-0.56	2.36	5.18	-0.98	1.33*	0.36**	-0.00	0.26**	0.25
VL 30926	6.55**	-3.6**	0.48	-0.97	0.38	0.06	-2.18	-0.09	-1.51	9.18*	-5.61**	-0.70	0.08	0.06**	0.01	0.05
VL 30928	0.65	-3.76**	0.23	-1.09	-1.95**	0.01	-2.43	1.62*	16.73**	24.80**	-6.23**	2.16**	0.17*	0.00	0.20**	2.45**
VL 30929	-4.13**	0.61**	4.48**	1.65	1.27	-0.01	12.56**	0.12	19.86**	15.68**	0.13	0.26	0.23**	0.04*	0.11*	4.99**
VL 30938	10.29*	-2.26**	2.11**	-0.84	-0.53	0.00	-2.18	1.10	-1.63	11.18**	-7.23**	1.05	0.24**	0.10**	0.02	1.43
VL 31486	2.20	-1.88**	1.86**	-0.47	1.03	-0.01	-1.93	0.95	-11.76*	-9.31*	1.13	2.87**	1.07**	-0.05**	0.75**	0.07
VL 31632	4.67**	2.36**	5.98**	1.02	0.63	0.00	-0.43	0.36	15.61**	19.18**	-3.36	-2.51**	-0.09	0.14**	-0.19**	-0.96
VL 31634	-15.25**	4.73**	-26.38**	-0.47	-1.32	0.01	-2.05	-2.21**	10.48*	-41.94**	26.63**	-8.85**	-2.70**	-0.40**	-1.40**	-6.50**
VL 31638	2.60	5.36**	8.86**	0.15	0.14	-0.06	-0.93	-1.28*	-50.13**	-33.94**	-4.48*	4.37**	0.62**	0.10**	0.22**	-1.80*
SEm(±)	1.50	0.18	0.27	0.83	0.65	0.05	3.70	0.60	4.98	3.79	2.01	0.56	0.07	0.02	0.05	0.78
CD(P=0.05)	3.05	0.37	0.55	1.69	1.33	0.10	7.52	1.22	10.12	7.69	4.09	1.15	0.14	0.04	0.10	1.58
Testers																
Pusa Basmati 1	0.37	-2.55**	1.48**	0.94	0.15	-0.02	4.91	0.72	-2.29	-2.51	1.11	0.20	0.18**	0.00	0.17**	1.69**
Pusa Sugandh 2	0.84	-3.05**	-14.29**	-0.44	-0.51	-0.04	-1.69	-0.48	-13.73**	-27.40**	8.55**	-1.72**	-0.41**	-0.16**	-0.21**	-2.31**
Pusa Sugandh 3	-2.53**	2.88**	6.54**	-0.11	-0.34	0.05	-1.47	-0.41	11.87**	15.31**	-3.16*	0.93*	0.17**	0.09**	0.02	0.92
Pusa Sugandh 5	1.31	2.72**	6.26**	-0.38	0.69	0.01	-1.75	0.17	4.15	14.59**	-6.50**	0.58	0.06	0.06**	0.01	-0.31
SEm(±)	1.00	0.12	0.18	0.55	0.43	0.03	2.47	0.40	3.32	2.52	1.34	0.37	0.04	0.01	0.03	0.52
CD(P=0.05)	2.03	0.24	0.36	1.13	0.89	0.06	5.01	0.81	6.75	5.13	2.73	0.76	0.09	0.02	0.06	1.05

Table 3. Estimates of specific combining ability (SCA) effects of hybrid for sixteen characters in rice

Cross combination	Plant height	Days to 50% flowering	Days to maturity	Tillers per plant	Flag leaf length	Flag leaf width	Panicles per plant	Panicle length	Grains per panicle	Fertile grains per panicle	Spikelet Sterility %	1000grain weight	Kernel length	Kernel width	L/B ratio	Grain yield per plant
VL 30925 x P. Bas 1	-2.68	-8.31**	-11.36**	0.80	4.06**	-0.08	-3.29	-2.24	-25.58*	-18.23*	-1.23	-1.59	-0.83**	0.00	-0.51**	-1.38
VL 30925 x P. Sug 2	-5.00	7.18**	18.91**	0.19	-1.29	-0.11	1.31	1.85	5.86	4.15	2.31	3.24**	0.77**	0.11**	0.48**	2.43
VL 30925 x P. Sug 3	3.06	-0.76*	-4.91**	-0.63	-2.07	0.08	0.59	1.67	3.75	6.93	-3.45	-0.27	-0.03	-0.08*	0.03	1.38
VL 30925 x P. Sug 5	4.62	1.90**	-2.63**	-0.36	-0.69	0.11	1.37	-1.28	15.97	7.15	2.37	-1.37	0.09	-0.03	0.00	-2.44
VL 30926 x P. Bas 1	-0.08	2.80**	-2.63*	-1.19	2.87*	0.08	-5.54	-1.56	-29.70**	-18.23*	-4.61	1.24	-0.33*	-0.04	-0.17	-4.57**
VL 30926 x P. Sug 2	-7.65*	-5.69**	6.79**	-0.80	-0.10	-0.14	0.56	2.30	-29.26**	-13.34	-2.05	0.57	0.80**	0.19**	0.35**	-1.67
VL 30926 x P. Sug 3	5.06	-0.63	-5.04**	1.86	0.27	0.06	3.34	-0.27	57.12**	35.93**	3.66	0.81	-0.03	-0.05	-0.02	2.27
VL 30926 x P. Sug 5	2.67	3.52**	-0.26	0.13	-3.03*	-0.00	1.62	-0.46	1.84	-4.34	3.00	-2.63*	-0.43**	-0.08*	-0.15	3.97*
VL 30928 x P. Bas 1	-0.88	3.43**	-0.73	-0.56	-0.67	0.03	-4.79	-0.08	-26.95*	-31.86**	6.51	-2.01	-0.12	-0.20**	0.22**	2.49
VL 30928 x P. Sug 2	2.59	-6.56**	6.54**	0.31	-2.61	-0.09	1.81	-2.02	-42.01**	-17.47*	-8.93*	3.16**	-0.29*	0.47**	-0.63**	1.42
VL 30928 x P. Sug 3	-1.08	3.48**	-1.29*	0.98	2.46	-0.04	2.09	1.64	56.37**	43.80**	0.29	-2.20	0.22	-0.16**	0.26*	-0.99
VL 30928 x P. Sug 5	-0.62	-0.34	-4.51**	-0.73	0.83	0.09	0.87	0.46	12.59	5.52	2.12	1.05	0.20	-0.10*	0.14	-2.93
VL 30929 x P. Bas 1	-2.65	-0.94*	-4.48**	-2.31	-0.72	0.07	31.20**	-1.78	-1.58	2.76	-2.86	0.68	-0.00	-0.05	0.00	-0.34
VL 30929 x P. Sug 2	6.38*	5.05**	15.79**	0.06	-1.29	0.04	-11.18	-0.32	13.36	12.15	0.19	2.01	1.00**	0.20**	0.42**	-0.95
VL 30929 x P. Sug 3	0.10	-1.38**	-5.04**	2.73	1.73	-0.00	-7.90	1.54	22.25*	14.43	0.91	-2.70*	-0.76**	-0.04	-0.39**	6.64**
VL 30929 x P. Sug 5	-3.83	-2.72**	-6.26**	-0.48	0.28	-0.11	-12.12	0.56	-34.02**	-29.34**	1.75	0.00	-0.24	-0.10*	-0.02	-5.34**
VL 30938 x P. Bas 1	-3.57	-7.56**	-10.11**	0.68	-2.25	0.05	-3.04	0.78	-3.58	4.26	-5.48	-0.15	-0.23	-0.05	-0.12	-1.86
VL 30938 x P. Sug 2	-3.39	1.93**	12.66**	0.06	-0.28	-0.12	1.56	-2.34	26.86*	37.65**	-8.43*	0.57	0.29*	0.15**	0.17	-0.94
VL 30938 x P. Sug 3	12.43**	2.48**	-1.66**	-0.26	2.19	0.12	0.84	1.57	-17.25	-28.56**	9.29*	0.76	0.02	-0.03	-0.03	-1.48
VL 30938 x P. Sug 5	-5.46	3.15**	-0.88	-0.48	0.35	-0.04	0.62	-0.01	-6.02	-13.34	4.62	-1.18	-0.07	-0.07	-0.00	4.29**

VL 31486 x P. Bas 1	4.11	1.05**	-2.86**	-2.69	-0.58	0.02	-7.29	-0.41	32.04**	19.76	1.63	-1.08	-0.24	-0.01	-0.20	-6.11**
VL 31486 x P. Sug 2	-4.15	0.55	11.91**	-1.30	1.73	-0.00	0.31	0.65	2.48	6.65	1.19	3.60**	0.54**	0.18**	0.22*	5.19**
VL 31486 x P. Sug 3	1.61	-5.38**	-8.41**	1.86	-0.53	-0.00	3.59	0.92	-17.62	-3.06	-9.08*	-0.36	-0.05	-0.10*	0.06	1.72
VL 31486 x P. Sug 5	-1.57	3.77**	-0.63	2.13	-0.62	-0.01	3.37	-1.16	-16.90	-23.34**	6.25	-2.15	-0.24	-0.07	-0.08	-0.80
VL 31632 x P. Bas 1	-0.61	3.80**	-0.48	0.30	-1.13	-0.15	-4.29	0.47	7.66	10.26	-3.86	0.70	-0.12	0.06	-0.26*	-2.70
VL 31632 x P. Sug 2	-3.42	-1.69**	10.29**	2.19	2.93*	0.17	3.31	-0.86	-25.88*	-2.84	-8.80*	-2.36*	0.71**	0.13**	0.39**	1.14
VL 31632 x P. Sug 3	-2.30	-0.63	-4.54**	-4.13*	-2.38	-0.17	-2.40	0.31	-35.50**	-45.06**	10.41*	1.27	-0.26	-0.18**	0.07	-3.55*
VL 31632 x P. Sug 5	6.34*	-1.47**	-5.26**	1.63	0.57	0.15	3.37	0.07	53.72**	37.65**	2.25	0.37	-0.32*	-0.01	-0.21*	5.10**
VL 31634 x P. Bas 1	-6.72*	3.43**	33.38**	0.80	-1.62	-0.06	-3.16	2.00	-12.20	12.88	-8.36*	4.24**	1.80**	0.42**	0.91**	9.38**
VL 31634 x P. Sug 2	30.05**	-5.56**	-98.33**	1.19	1.60	0.01	2.94	2.76*	59.73**	-42.72**	37.19**	-13.37**	-4.25**	-1.43**	-1.98**	-3.99*
VL 31634 x P. Sug 3	-19.41**	6.48**	37.83**	-0.13	-1.72	0.06	0.72	-6.01**	-43.87**	-10.94	-6.08	4.26**	1.36**	0.58**	0.49**	-3.00
VL 31634 x P. Sug 5	-3.91	-4.34**	27.11**	-1.86	1.73	-0.00	-0.50	1.24	-3.65	40.77**	-22.75**	4.86**	1.09**	0.42**	0.57**	-2.38
VL 31638 x P. Bas 1	13.11**	2.30**	-1.86**	4.18*	0.05	0.02	0.20	2.82*	59.91**	18.38*	18.26**	-2.03	0.10	-0.11**	0.15	5.10**
VL 31638 x P. Sug 2	-15.40**	4.80**	15.41**	-1.93	-0.67	0.24*	-0.68	-2.01	-11.13	15.77*	-12.68**	2.55*	0.40**	-0.03	0.57**	-2.62
VL 31638 x P. Sug 3	0.51	-3.63**	-6.91**	-2.26	0.05	-0.10	-0.90	-1.38	-25.25*	-13.44	-5.95	-1.56	-0.45**	0.08*	-0.47**	-3.01
VL 31638 x P. Sug 5	1.77	-3.47**	-6.63**	0.01	0.56	-0.16	1.37	0.57	-23.52*	-20.72**	0.37	1.04	-0.06	0.06	-0.24*	0.53
SEm(±)	3.00	0.36	0.54	1.67	1.31	0.10	7.41	1.21	9.97	7.58	4.03	1.13	0.14	0.04	0.10	1.56
CD (P=0.05)	6.10	0.74	1.10	3.39	2.67	0.20	15.04	2.45	20.25	15.39	8.19	2.30	0.29	0.08	0.20	3.17

Table 4. Contribution of lines, testers and their interaction

Contribution by	Plant height	Days to 50% flowering	Days to maturity	Tillers per plant	Flag leaf length	Flag leaf width	Panicles per plant	Panicle length	Grains per panicle	Fertile grains per panicle	Spikelet Sterility %	1000grain weight	Kernel length	Kernel width	L/B ratio	Grain yield per plant
Lines (%)	45.32	30.63	16.17	23.33	25.19	9.41	28.30	28.41	29.71	37.65	43.88	56.07	53.13	18.81	55.85	37.27
Testers (%)	1.86	22.82	12.42	8.04	5.47	11.61	11.25	5.22	6.37	22.87	14.44	4.46	3.16	7.66	3.57	9.65
Lines x Testers (%)	52.80	46.54	71.40	68.62	69.32	78.97	60.43	66.35	63.91	39.47	41.67	39.46	43.69	73.52	40.57	53.07

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Proximate Nutritional Evaluation of Rice (*Oryza Sativa* L.)

G. Nirmala Devi [*1], G.Padmavathi[1], V. Ravindra Babu[1] and KavitaWaghray[2]

[1]Department of Genetics & Plant breeding, Indian Institute of Rice Research, Rajendranagar, Hyderabad, Telangana

[2]Department of Food Technology, Osmania University, Hyderabad, Telangana

*Corresponding author: nirmalalaxman2002@gmail.com

Received: 26th February, 2015 Accepted: 25th June, 2015

Abstract

In the present study, ninety two rice varieties were evaluated for estimating proximate composition which is useful in the generation of nutritionally rich rice varieties. Analysis of variance showed significant differences in the proximate compositions of the rice varieties studied. The results indicated high percentage of carbohydrate in all the genotypes (73.6 to 83.7%) and the varieties Bhuban and Prachi had the highest carbohydrate content (83.7%). Sahyadri hybrid showed the highest crude protein (11.0 %), while Swarna had the least crude protein (5. 9 %). Pusa basmati 1 and Swarnamukhi (NLR 145) varieties showed the highest moisture content percentage (11.6%) while Bhuban variety (7.13%) contained the lowest percentage moisture content. MTU 1001 and MSS 5 contained highest fat content (3.7%) while Barah Avarodhi possessed the lowest fat content (0.9%). Crude fibre content was low in majority of the samples. Only two varieties *i.e.,* MTU 3626 and MTU 1010 contained highest crude fibre (0.99%) while Pusa Basmati 1 possessed highest ash content (2.3 %). Carbohydrate was significantly and positively correlated with energy and negatively correlated with moisture %. The association of moisture % with carbohydrate and energy is significant but negative. Thus, the results generated in this study provide first hand information in identifying superior quality rice varieties based on their proximate composition.

Key words: Rice, proximate composition, polishing, Genotypes, correlation

Introduction

Rice is one of the most important staple cereals in human nutrition, consumed by about 75% of the global population. India is the second largest producer of rice. The proximate composition of rice includes moisture, carbohydrates, proteins, dietary fibres, fatty acids, ash and dietary minerals. Rice is an excellent source of carbohydrates containing approximately 87 % in grain. It contains 7 to 8 % of protein which has higher digestibility, biological value and more nutritious; possesses 10% moisture, lower crude fibre and lower fat (1 to 2%) and ash (1 to 2%). Nearly twenty percent of the world's dietary energy is provided by rice alone which is higher than either wheat or maize (Anon, 2004). The crude fibre reduces the risk of bowel disorders. The high proportion of unsaturated fatty acids such as oleic and linoleic acid present in rice bran lowers blood cholesterol. Whole grains are good source of iron, thiamine, niacin and riboflavin. In fact, bran is rich in micronutrients like oryzanols, tocopherols, tocotrienols, phytosterols and dietary fibres like betaglucan, pectin and gum which have hypolipidemic, anti-tumor, anti-oxidant, ergogenic and laxative properties. But rice consumers often prefer to have polished white rice despite the valuable food content of brown rice which is lost when bran is removed while polishing.

Knowledge about the nutritional status of rice is becoming increasingly important among consumers in view of nutritional deficiency disorders. Health conscious consumers are interested in having rice with good cooking quality, eating quality and also nutritional quality. There is limited information about the nutritional composition of the different rice varieties available in India. Therefore, the objective of this study is to investigate the proximate composition of selected rice varieties in terms of nutrition.

Materials and Methods

The experimental material consisted of 92 rice genotypes including land races, improved varieties, aromatic varieties and red rices collected from different Institutes of Indian Council of Agricultural Research and State Agricultural Universities (Table 1). The experiment was conducted during *kharif* 2013 in Randomized Block Design with three replications under rain fed conditions on experimental

farm of Indian Institute of Rice Research, ICRISAT campus, Patancheru, Hyderabad, Telangana State, India. Seedlings of thirty days old were transplanted in 20 cm apart between rows and 15 cm within the row. All the recommended package of practices and necessary plant protection measures were adopted. The seeds were sun dried, dehulled and divided into two sets. One set was kept as unpolished (Brown rice) and the other set was polished up to 5% and 10%. A portion of the brown and polished rice samples was then ground to obtain rice flour suitable for proximate analysis. The rice flour was analyzed for percentage proximate content of rice grain determined using the AOAC (2000) methods, mentioned hereunder.

Determination of Moisture Content

The moisture content in each sample was determined by drying 4g sample in an air forced draft oven maintained at a temperature of 105 ±5 ^{0}C according to the procedure described in AOAC (2000) method No. 44-15 A.

Crude Protein Content

The nitrogen content in rice flour samples was estimated by following the Kjeldahl's method according to the procedure described in AOAC (2000**)** method No. 46-10. The protein percentage was calculated by multiplying nitrogen per cent with a factor 5.95.

Crude Fat

The crude fat content was determined in each rice flour sample by using petroleum ether as a solvent in a Soxhlet apparatus according to the procedure given in AOAC (2000) method No. 30-10.35

Ash Content

The ash content in each rice flour sample was estimated by putting samples in a muffle furnace at a temperature 550 ± 5^{0}C till white grey residue is obtained by following the method described in AOAC (2000) method No. 08-01.

Crude Fibre

For the determination of fibre content, the rice samples were digested with 1.25 % H_2SO_4 followed by 1.25 % Na OH solution and crude fibre content was determined according to AOAC (2000) method No. 32-10 .

Carbohydrates

The total percentage carbohydrate content in the rice sample was determined by the difference method as reported (Onyeike *et al*., 1995). This method involved adding the total values of crude protein, lipid, crude fibre, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate constituent of the sample. Thus, % carbohydrate = 100 – (% moisture + % crude fibre +% protein+ % lipid + % ash).

Statistical Analysis

The data were analyzed using SAS software version 9.1 (SAS, 1998). Differences were declared statistically significant at $P < 0.05$. Where significant differences were detected, the means were separated by the least significant difference (LSD) at 5 % probability level. Inter-relationships among traits values were estimated using the Pearson's correlation coefficient.

Results and Discussion

The moisture, crude fat, crude protein, crude fibre, ash content, iron content and zinc content showed highly significant differences among different rice varieties and white and brown rice milling fractions (Table 2).

Moisture

Moisture content, which plays a significant role in determining the shelf-life (Webb, 1985) varied from 7.1 to 11.6 % with a mean value of 9.70 ± 0.13 in brown rice; 7.1 to 11. 2 % with mean value of 9.43 ± 0.13 at 5 % polishing and 7.01 to 11.2 % with a mean value of 9.31 ± 0.10. at 10 % polishing level. Although moisture content varied between brown and milled rice, it did not differ among the rice samples milled to different degrees (5 to 10%). Moisture is dependent on genetic makeup of varieties and agronomic as well as climatic conditions (Butt *et al*., 1997). The results of the present well supported by the findings of several researchers (Awan, 1996) who found the moisture content variation from 7 to 11%. Pusa basmati 1 and Swarnamukhi (NLR 145) varieties showed the highest percentage moisture content (11.6 %) while Bhuban variety (7.13%) contained the lowest percentage moisture content at 0 % polishing; Pooja and Bhuban contained highest moisture % of 11.3 and 7.1 respectively at 5% polishing; KMP 101 and Bhuban displayed highest and lowest moisture % (11.3 and 7.0 respectively) at 10 % polishing. The high percentage moisture content affects the milling characteristics and the taste of cooked rice (Xheng and Lan, 2006). Anjum *et al*., 2007 found moisture content ranging from 8.61 to 11.08% in different milling fractions of rice and the highest moisture content was found in brown rice of different varieties. Ebuehi and Oyewole (2007) reported that the moisture content of rice also affects

its storage. It follows that Bhuban rice variety may have a longer shelf life compared to the other rice varieties due to the lower moisture content at all polishing levels. The variation observed in moisture content among rice varieties may be attributed to differences in the genetic make up as well as climatic conditions. The results suggested that the moisture content found in the present study is with in safe limits as in all samples it was below 11%.

Crude protein

The nutritional quality of rice depends on the protein **c**ontent which is the second major component of grain next to starch. The protein is a key factor influencing the eating quality of rice. Rice contributes 24.1% of dietary protein out of 207.9 grams of rice consumed per day per person (FAOSTAT, 2001). The rice protein is superior because of its unique composition of essential amino acids (Eggum, 1979). The present results showed appreciably high protein content (> 6 %) in brown rice. Studies on protein content in different Pakistani varieties reported a range of 7.38 to 8.13% protein content (Awan, 1996) which is lower than those obtained in the current study. These levels of proteins in rice are very essential as proteins form the basic building blocks for cells and tissue repairs in the body. Protein content for all the rice varieties evaluated ranged between 5.9 (Swarna) to 11.0 % (Sahyadri) in brown rice with a mean value of 7.4 ± 0.1 in brown rice; 5.3 (Swarna) to 10.8 (Swarna) with a mean value of 7.3 ± 0.13 (at 5% polishing) and 5.2 (Swarna) to 10.3% (Sahyadri) with a mean value of 7.1 ± 0.12 (at 10% polishing). Overall high protein content was recorded in Sahyadri (11.0, 10.9 and 10.3 at 0 %, 5 % and 10 % polishing levels respectively). There was not much variation observed in protein content among different polishing levels. However it varied between brown rice and polished rice (5%). Usually, the average value of total crude protein content is taken as 7.00 % in rice seed. Analyzing the protein in test genotypes will help the nutritionist to assess the protein intake and deficiency of protein among the people of rice consuming countries. In another study, similar range of 6.7 to 11% protein in brown rice as found in present study was observed in 74 varieties from India (Guha and Mitra, 1963). Some varieties from Himachal Pradesh, India were reported to have 6.61 to 7.28% total crude protein (Sing *et al*., 1998). Indigenous cultivars of the north eastern hill states of India possess high protein content with a range of 6.14 to 12.07% (Devi *et al.,* 2008a; Premila Devi *et al.,* 2010). Rice with high proteins provides better growth and development as shown by feeding trials on growing rats (Blackwell *et al.,* 1966; Bressani *et al.,* 1971; Eggum and Juliano 1973, 1975; Murata *et al.,* 1978; Hegsted and Juliano 1974; Pereira *et al.,* 1981). Govindaswami *et al.* (1996) reported 6 to 12.6 % crude protein content in three hundred improved rice varieties in India. Even a wide range of 6.56 to 12.86 % protein content was reported in 40 rice varieties grown in Kashmir. Ahmed *et al.* (1998) reported that the crude protein content of nine aromatic rice cultivars ranged from 9.17 to 11.77 %. Swarna showed lowest protein (5.89 %, 5.74% and 5.89%) content at all polishing levels among the samples. The traditional cultivars are known to possess higher protein in crops. The protein in *Chahou angouba*, a local rice cultivar from Manipur was reported as high as 12.07% (Devi *et al.,* 2008a**)**. In the present study genotypes, CN-1233-33-9-117, Karjat-2, Sahyadri, Sahyadri-2, Yamini, Aishwarya, Amulya, AS-100, Birupa, Bhudeb, Dandi, Jaya, Kranti, Madhukar,Dharitri, DL-184, and Jalapriya recorded more than 10 % crude protein. These cultivars are classified as high protein cultivars of rice with 10 % or more total crude protein following the classification of Resurrection *et al.*, (1979). However, low protein content reported in both brown and milled rice from rice collections of Assam and Himachal Pradesh respectively (Singh *et al.,* 1998). Crude protein values of 1.17 to 7.94 % were noticed in a set of rice varieties (Oko and Ugwu, 2011) whereas Oko *et al.,* (2012) reported 1.6 to 7.9 % which appear to be bit lower than many of the published reports. The variation in protein content observed between brown and white rice is because of bran portion, which is higher in protein and significantly increase the protein content of brown rice as reported earlier (Pederson and Eggum, 1983) and (Anjum *et al*. 2007).

Carbohydrates

Rice is the starchy staple food and a major source of **c**arbohydrates. Carbohydrate content was found to be high in all varieties (> 70%) in the present experiment. The results obtained in this study are in line with those given by Oko and Ugwu (2011) who have reported similar kind of increased carbohydrates in widely cultivated Nigerian rice varieties. The mean carbohydrates in the 92 samples studied ranged from 73.6 to 83.7 % with a mean of 79.6 ± 1.1 at 0 % polishing, 76.7 to 84.0% with a mean of 81.3 ± 1.1 at 5% level and 78.2 to 85.2% with a mean of 82.3 ± 0 .15 at 10 % level of polishing. It is observed that carbohydrate content increased as the level of polishing increases.

Although these values are higher than the values obtained by Eggum, (1982), they are a bit lower than the values (75.37 to 76.37%) reported by Edeogu *et al*., (2007) who analysed the proximate compositions of staple food crops in Ebonyi State. Sipi variety had the lowest carbohydrate content. This low carbohydrate content may be attributed to its high moisture content which also affects the milling

quality and other environmental factors (USA Rice Federation, 2002). The high percent carbohydrate contents of the rice varieties show that rice is a good source of energy. Aishwarya, Bhuban, BPT11711, BR-2655, CN-1039-9,GR-103, Dandi, High iron rice, Prachi, PSD-1, IR-64, NLR-33359,Swarna, Taroari Basmati and VRS-3 contained highest carbohydrates of 82.17% at all polishing levels. The cultivar Sahyadri and Dharitri had lowest carbohydrates of 73%. But most of the samples recorded more than 80 % of the carbohydrates on an average. Such high amount of carbohydrate signifies high level of starch. In the present study most of the cultivars with high carbohydrates had intermediate desirable amylose content resulting in soft textured rice upon cooking. Sahyadri possessed lowest carbohydrates (73.63%) manifested high amylose content (30.1%) which may result dry, fluffy and hard cooked rice.

Fat

Fat in rice is a good source of linoleic acid and other essential fatty acids and rice does not contain cholesterol (Eggum *et al.,* 1982). The mean total fat content was 1.58 %, 0.93 % and 0.54 % at 0 %, 5 % and 10 % level of polishing respectively with the range of 0.50 to 3.77 % at 0 % polishing, 0.30 to 2.42 % at 5 % polishing and 0.10 to 1.46 % at 10 % polishing. The fat content (0.5 to 3.77 %) of brown rice recorded in this study is in agreement with earlier results reported by Oko and Ugwu (2011) and Oko *et al*., (2012)**.** The fat content in brown and polished rice was found to be significantly highest in rice variety MTU 1001 followed by MSS 5, Nalini and Sahyadri. Excess intake of saturated fats is the most important dietary factor causing increased cholesterol and obesity. In this regard, PR 115, SGT 1 and Barah Aavarodhi could be better preferred owing to their lowest fat content in brown rice as well as polished rice.

Juliano (1985) reported that fat content ranged from 0.9 to 1.97 % in different milling fractions. However, this range is lower than the range obtained by Edeogu *et al.,* (2007). This difference may be attributed to the degree of milling which removes the outer layer of the grain where most of the fats are concentrated (Frei and Becker, 2003). The fat content of milled rice has been reported to be about 0.2 to 2.0% **(**Tahira and Chang, 1986). In contrary, a study on 14 varieties of Manipur and Nagaland reported a total fat content ranging from 1.2 to 4.2% with the mean of 2.49% for the local cultivars with low and intermediate amylose contents (Devi *et al*., 2008b). In the present study the genotypes having total fat above the mean (1.04%), had low amylose content. However Singh *et al.,* (1998) observed narrow range of 0.31 and 1.06% of total fat in their samples studied. Further, they found that *indica* type of rice (high amylose) had low lipid content than *japonica* type (low amylose).

Crude fibre

The presence of fibre in diet increases the bulk of feaces, which has a laxative effect in the gut. The standard content of fibre in rice is 0.5 – 1.0% for well milled rice (Oko and Onyekwere, 2010). The crude fibre (g%) ranged from 0.22 to 0.95 with a mean value of 0.5 ± 0.02 in brown rice, 0.08 to 0.78 with a mean value of 0.31 ± 0.02 at 5 % polishing and 0.01 to 0.58 with a mean value of 0.1 ± 0.01 at 10 % polishing. Although this range is a bit lower than the range (1.93 to 4.3 g %) obtained by Edeogu *et al.,* (2007), it is similar to the mean value obtained in the studies of Sotelo *et al.,* (1990). Milling of rice generally decreases the fibre content of rice. The highest fibre content was found in MTU 3626 and MTU 1010 (0.95 %). The lowest fibre content was observed in DL 184 (0.22 %) followed by Dharitri (0.23 %) and Basmati 386 (0.24 %). It has been recommended by the experts to consume at least 25g of fibre every day to decrease the risk of chronic diseases. Fibre-rich foods help to promote proper bowel function and reduce risk of developing intestinal disorders. In the present study, the brown rice contained significantly higher crude fibre content (0.22 to 0.95 %) as compared to white rice which can help reduce chronic diseases. In another study by Sotelo *et al.,* (1990) dietary fibre content was found to be 1.9 ± 0.6% in brown rice fraction whereas Awan, (1996) and Tufail (1997) recorded the fibre content of different Pakistani white rice in the range of 0.20 to 0.35%. The present study showed that preference should be given to brown rice to improve the intake of fibre in the daily diet. The higher fibre content in brown rice fraction may be due to bran portion which is higher in fibre content.

Total ash

The ash content of a food sample gives an idea of the mineral elements present in the food sample. The total ash content varied significantly among different rice varieties and polishing fractions. Ash content was low in majority of the samples. Pusa Basmati-1 showed 2.34 % ash, highest in the lot and 0.17 % was reported in High iron rice.

The ash content ranged significantly from 0.43 to 2.34 % with a mean of 1.19 ± 0.03 in brown rice, 0.28 to 1.22 % in polished rice (5% polishing) with a mean of 0.74 ± 0.01, 0.12 to 1.01 % with a mean value of 0.5 ± 0.02 in polished rice (10 % polishing). The highest ash content (1.69 %) was observed in PusaBasmati1 (2.34%) followed by Sabita (1.72%), Taroari basmati and MTU 1010 (1. 67%) and Vasumati (1.66%) and while the lowest ash content (0.43%) was found in Shakti and Sashi. In earlier

studies ash content varied from 1.46 to 1.61% for brown rice and 0.48 to 0.67% for white rice, respectively (Sotelo *et al.,* (1990), Tufail (1997) and Adu-Kwarteng *et al.*, (2003). Anjum *et al.,* (2007) also found the similar trend for ash content 1.42 % and 0.66 % for brown and white rice respectively. The difference in ash content among rice varieties may be due to difference in the genetic architecture of rice varieties as reported by Butt *et al.*, 1997.

In the present findings, the ash content was higher in brown rice than white rice. The variation among milling fractions suggests that the brown rice contains bran portion which increased the ash content as reported by Anjum *et al.,* (2007) as compared to white rice.

The correlation coefficients among the proximate values for carbohydrate, moisture, fat, protein, fibre, ash and energy are presented in Table 3. As expected, the carbohydrate and energy value were quite high and positively correlated ($r = 0.948$, $p < 0.0001$). It suggests correlated response for high energy value when rice cultivars are selected on the basis of high carbohydrate content. The association between carbohydrate with moisture and fat content were significantly negative which indicates that rice cultivars high in moisture and fat may likely to be low in carbohydrate value. Similarly between moisture and carbohydrate, moisture and energy are also strongly but negatively correlated, implying that carbohydrate and energy are highly correlated traits. Fibre content was also significantly correlated with moisture ($r= 0.246$, $p = 0.071$) and protein ($r = -0.366$, $p = 0.006$), though the observed values were low (Table 3). The negative correlation value between protein content and fibre suggest that rice cultivars high in fibre content may likely be low in protein. Positive correlation between carbohydrate and energy but negative correlation between carbohydrate and moisture, moisture and energy, fibre with protein and carbohydrate with fibre was also reported (Oko *et al.,* 2012).

The correlation between energy and fat was negative in direction, as also was the correlation between percentage fibre and protein among the varieties studied. This suggests a negative relationship occurring between energy and fat as well as fibre and protein content. The percentage carbohydrate and energy value was strongly correlated and positive in direction, suggesting correlated response for high energy value when rice cultivars are selected on the basis of high carbohydrate content.

The present research work was carried out to compare different rice varieties and their polishing fractions for proximate composition. The moisture, fat, crude protein, crude fibre and ash content showed highly significant differences among different rice varieties in brown and polished rice. This work has revealed that the genotypes showed considerable amount of nutrients such as carbohydrate, a good source of energy, protein for body maintenance and the repair and replacement of worn out or damaged tissues; crude fibre for effective digestibility of food as well as fat which contribute to the recommended dietary allowance. Brown rice has more nutritional quality than white rice due to more availability of proximate nutrients.

The varieties Madhukar, Aishwarya Amulya, Bhuban, Jaya, Dandi, Sahyadri, Sahyadri-2, Karjat, Yamini and CN-1233-33-9-117 possessed significantly highest content of protein (10-12 g%). The varieties BPT11711, BR-2655, CN-1039-9, GR-103, Dandi, High iron rice, Prachi, PSD-1, IR-64, NLR-33359, Swarna, Taroari Basmati, Aishwarya and Bhuban contained highest carbohydrates(>80 g%), the sources of energy whereas MTU1001 showed the highest high fat content (2.3 g%). The rice variety MTU 3626 and MTU 1010 possessed the highest content of fibre (0.95%). The result of this study can be exploited by rice consumers in their choices regarding proximate compositions.

References

Adu-kwarteng, E., Ellis, W.O., Oduro, I. and Manful, J.T. 2003. Rice grain quality: A comparison of local varieties with new varieties under study in Ghana. *Food Control* 14: 507-514.

Ahmed, S.A., Baraua, I. and Das, D. 1998. Chemical composition of scented rice. *Oryza* 35 (2):167-169.

Anjum, F.M., Pasha, I., Bugti, M.A. and Butt, M.S. 2007. Mineral composition of different rice varieties and their milling fractions. *Pakistan Journal of Agricuture and Science* 44 (2): 332-336

Anon. 2004. International year of rice. Rice and Human Nutrition.

AOAC. 2000. Official Methods of Analysis. *Association of Official. Analytical Chemists.* Washington, D.C. USA.

Awan, I.A. 1996. Physical and biochemical characterization of some of the Pakiatani rice varieties. M.Sc. (Hons.) Thesis. Dept. of Food Tech., Univ. Agric. Fsd.

Blackwell, R.Q., Yang, T.H. and Juliano, B.O. 1966. Effect of protein content upon growth rates of rats fed highrice diets(Abstr.) Proc. 11th Pacific Science Congress, Tokyo, 8, 15.

Bressani, R., Elias, L.G. and Juliano, B.O. 1971. Evaluation of the protein quality of milled rices differing in protein content. *Journal of Agricultural and Food Chemistry* 19: 1028-1034.

Butt, M.S., Anjum, F.M., Rehman, A. and Ali, V. 1997. Physicochemical characteristics of spring wheats. *Journal of Agricultural Research*. 35 (6): 413-422.

Devi, Th. P., Durai, A., Singh, Th. A., Gupta, S., Mitra, J., Pattanayak, A., Sarma, B.K. and Das, A. 2008a. Preliminary studies on physical and nutritional qualities of some indigenous and important rice cultivars of north eastern hill region of India. *Journal of Food Quality* 31: 686-700.

Devi, Th.P. and Pattanayak, A. 2008b. Medicinal rice of Manipur, their ethnopharmacological uses and preliminary biochemical studies. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* 10(1):139-141.

Ebuehi, O.A. and Oyewole, A.C. 2007. Effect of cooking and soaking on Physical characteristics, nutrient composition and sensory evaluation of indigenous and foreign rice varieties in Nigeria. *Nigerian African Journal of Biotechnology* 6 (8):1016-1020.

Edeogu, C.O., Ezeonu, F.C., Okaka, A.N.C., Ekuma, C.E. and EIom, S.O. 2007. Proximate Compositions of Staple Food Crops in Ebonyi State, South Eastern Nigeria. *International Journal of Biotechnology Biochemistry* 1: 1-8.

Eggum, B.O. and Juliano, B.O. 1973. Nitrogen balance in rats fed rices differing in protein content. *Journal of the Science of Food and Agriculture* 24: 921-927.

Eggum, B.O. and Juliano, B.O. 1975. Higher protein content from nitrogen fertilizer application and nutritive value of milled rice protein. *Journal of the Science of Food and Agriculture* 26: 425-427.

Eggum, B.O., Juliano B.O. and Magnificat, C.C. 1982. Protein and energy utilization of rice milling fractions. *Journal of Human Nutrition* 31: 371-376.

Eggum, B.O. 1979. The nutritional value of rice in comparison with other cereals. In Proceedings, Workshop on Chemical Aspects of Rice Grain Quality, Los Baños, Laguna, the Philippines, IRRI. pp. 91-111.

FAOSTAT 2001. FAOSTAT Statistical Database. Food and Agriculture Organization.

Frei, M. and Becker, K. 2003. Studies on the *in vitro* Starch Digestibility and the Glycemic Index of six Different Indigenous Rice Cultivars from the Philippines. *Food Chemistry* 83: 395-402.

Govindaswami, S., Ghosh, A.K. and Nanda, B.B. 1996. Varietal differences in hulling and cooking qualities. Annual Report of Central Rice Research Institute, Cuttack, India, p 64.

Guha, B.C. and Mitra, N.R. 1963. Studies on the consistency of thiamine and protein contents of pure-bred strains of rice. *Annals of Biochemistry and Experimental Medicine* 23:69-72.

Hegsted, D.M. and Juliano, B.O. 1974. Difficulties in assessing the nutritional quality of rice protein. *Journal of Nutrition* 104:772-781.

Juliano, B.O. 1985. Criteria and tests for rice grain qualities. 2nd ed. In E. T. Champagne, Rice Chemistry and Technology American Association of Cereal Chemists, Inc., Minnesota, U. S. A.

Murata, K., Kitagawa, T. and Juliano, B.O. 1978. Protein quality of high protein rice in rats. *Agricultural and Biological Chemistry* 42: 565-570.

Oko, A.O. and Onyekwere, S.C. 2010. Studies on the Proximate Chemical Composition and Mineral Element Contents of Five New Lowland Rice Varieties in Ebonyi State. *International Journal of Biotechnology Biochemistry* 6(6): 949-955.

Oko, A.O. and Ugwu, S.I. 2011. The proximate and mineral compositions of five major rice varieties in Abakaliki, South-Eastern Nigeria. *International Journal of Plant Physiology and Biochemistry* 3(2): 25-27

Oko, A.O., Ubi, B.E., Efisue, A.A. and Dambaba, N. 2012. Comparative Analysis of the Chemical Nutrient Composition of Selected Local and Newly Introduced Rice Varieties Grown in Ebonyi State of Nigeria. *International Journal of Agriculture and Forestry* 2(2):16-23

Onyeike, E.N., Olungwe, T. and Uwakwe, A.A. 1995. Effect of heat treatment and defatting on the proximate composition of some Nigerian local soup thickeners. *Food Chemistry* 53:173-175

Pereira, S.M., Begum, A. and Juliano B.O. 1981. Effect of high protein rice on nitrogen retention and growth of preschool children on a rice-based diet. Qualitas Planatarum. *Plant Foods for Human Nutrition* 31**:**97-108.

Pederson, B. and Eggum, B.O. 1983.The influence of milling on the nutritive value of flour from cereal grains. *Plant foods Human Nutrition* 33: 267-278.

Premila Devi, T., Anna Durai, A., Anand Singh, Th., Bidyapati Devi, T., Gupta, S., Mitra, J., Pattanayak, A., Dhiman, K.R., Bhadana, V.P., Hore, D.K. and

Ngachan, S.V. 2010. Grain and food quality traits of some indigenous medicinal rice cultivars of Manipur. *International Journal of Food Properties* 13(6): 1244-1255.

Resurrection, A.P., Juliano, B.O. and Tanaka, Y. 1979. Nutritional content and distribution in milling fractions of rice grains. *Journal of the Science of Food and Agriculture* 30: 475-481.

SAS 1998. SAS/STAT users guide, version 9.1.SAS institute Cary, North Carolina, USA

Singh, S., Dhaliwal, Y.S., Nagi, H.P.S. and Kalia, M. 1998. Quality characteristics of six rice varieties of Himachal Pradesh. *Journal of Food Science and Technology* 27 (5):345-348.

Sotelo, A., Saisa, V., Montolvo, I., Hernandez, M. and Hernandez, L. 1990. Chemical composition of different fractions of 12 Mexican varieties of rice obtained during milling. *Cereal Chemistry* 67(2): 209-212.

Tahira, H. and Chang, W. 1986. Lipid content and fatty acid composition of *Indica* and *Japonica* of non glutinous brown rice. *Journal of Agricultural and Food Chemistry* 34: 542-545.

Tufail, S. 1997. Cooking and Eating Quality of Some Cultivars of Rice. M.Sc (Hons) Thesis, Dept.of Food Tech. Univ. of Agri. Faisalabad.

USA Rice Federation. 2002. The natural history of rice. Online Food Cult. Encyclopedia, pp. 1-4.

Webb, B.D. 1985. Criteria of rice quality in the U.S. In B.Juliano (ed) Rice: Chemistry and technology. St. Paul, Minnesota, *Association of Cereal Chemistry* Inc., 774.

Xheng, X. and Lan, Y. 2007. Effects of drying temperature and moisture content on rice taste quality. *Agricultural Engineering International* 49: 24-277.

Table 1. List of genotypes studied

S.No	Genotype
1	Aishwarya
2	Amulya
3	As 100
4	Basmati 386
5	Bhuban
6	Bhudeb
7	Barah Avarodhi
8	Birupa
9	BPT 11711
10	BR 2655
11	Chittimutyalu
12	CN 1039-9
13	CN 1233-33-9-117
14	Dandi
15	Dharitri
16	DL 184
17	Gajapathi
18	Giri
19	Gouri
20	GR 103
21	Harsha
22	High iron rice
23	Indravati
24	IR 64
25	Jaya
26	Jalpriya
27	Jagabandu
28	Jalmagna
29	Jalnidhi
30	Jyothi (PTB 39)
31	Kanchana
32	Kalanamak (ASG 4022)
33	Khitish
34	KHP - 2
35	Konark
36	KMP-101
37	Kranti
38	Karjat-2
39	Kavya
40	Lalat
41	Madhukar
42	Mahamaya
43	Mandya Vijaya

S.No	Genotype
44	Manohar Sali
45	Matta Triveni
46	MSE-9
47	MSS - 5
48	MTU -1001 (Vijetha)
49	MTU-1010 (C. Sannalu)
50	MTU-3626 (Prabhat)
51	Nagari Dubraj
52	Nalini
53	NLR 145 (Swarnamukhi)
54	NLR 33654 (Apurva)
55	NLR 33359 (Shravani)
56	Pant Dhan -16
57	Prachi
58	Pooja
59	Pratap
60	Pusa Basmati
61	PR 111
62	PR113
63	PR 114
64	PR 115
65	PR 116
66	PR 118
67	PSD 1

S.No	Genotype
68	RAU 3043 (ASG 4013)
69	Ranbir Basmati
70	Ranjeet
71	Sabita
72	SGT 1
73	Sashi
74	Shakthi
75	Sahyadri
76	Sahyadri 2
77	Sunandana
78	Suraksha
79	Swarna
80	Type 3
81	Taroari Basmati
82	Varsha
83	Vasumathi
84	Vikas
85	VRM 3
86	VRM 31
87	VRS 3
88	VRS 19
89	VRS 25
90	WGL 14 (W. Samba)
91	White Ponni
92	Yamini

Table 2. Mean values of Proximate composition in rice genotypes

Nutrients	Polishing levels		
	0%	5%	10%
1.Moisture			
Mean	9.70 ± 0.13	9.43 ± 1.11	9.34 ± 0.10
CD 5%	0.16	0.15	0.11
Range	7.13 – 11.60	7.11 - 11.29	7.01 - 11.25
2. Protein			
Mean	7.43 ± 0.13	7.32 ± 0.13	7.18 ±0.12
CD 5%	0.02	0.03	0.02
Range	5.89 - 11.01	5.26 - 10.86	5.22 - 10.29
3.Fat			
Mean	1.58 ± 0.06	0.93 ± 0.03	0.54 ± 0.03
CD 5%	0.13	0.14	0.12
Range	0.50 -3.77	0.30 - 2.42	0.10 -1.46
4. Ash			
Mean	1.19 ± 0.03	0.74 ± 0.01	0.50 ± 0.02
CD 5%	0.03	0.05	0.04
Range	0.43 - 2.34	0.28 -1.22	0.10 -1.01
5. Crude Fibre			
Mean	0.51 ± 0.02	0.31 ±0.02	0.15 ± 0.01
CD 5%	0.09	0.06	0.08
Range	0.20 - 0.95	0.08 - 0.78	0.01 - 0.58
6.CHO			
Mean	79.60 ± 1.12	81.28 ± 1.12	82.29 ±0.15
CD 5%	1.12	1.00	1.01
Range	73.63 -83.73	76.71 -84.05	78.42 -85.18
7. Energy(kcal)			
Mean	362 ±0.59	363 ± 0.46	363 ± o.42
CD 5%	0.54	0.51	0.49
Range	347 -376	354 -373	355 -373

Table 3. Correlation coefficients among proximate composition values

	CHO	Moisture	Fat	Protein	Fibre	Ash	Energy
CHO	1	-0.763	-0.293	-0.173	-0.246	-0.018	0.948
		(<0.0001)	(0.045)	(0.249)	(0.071	(0.906)	(<0.0001)
Moisture		1	0.164	0.002	0.236	-0.058	-0.963
			(0.244)	(0.991)	(0.72)	(0.683)	(<0.0001)
Fat			1	-0.129	0.209	0.177	-0.043
				(0.357)	(0.118)	(0.222)	(0.746)
Protein				1	-0.366	0.059	-0.098
					(0.006)	(0.668)	(0.452)
Fibre					1	0.012	-0.189
						(0.934)	(0.134)
Ash						1	0.067
							(0.609)
Energy							1

Values in parenthesis indicate probability levels

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Genetic Divergence Studies for Yield and its Components in Rice

A. Rajkumar[1], Jyothi Badri[2*], VP Bhadana[2], C. Priyanka[2], N. A. Ansari[1], Susmita Dey[1] and D. Srinivasa Chary[3]

[1]Dept. of Genetics and Plant Breeding, College of Agriculture,
Professor Jayashankar Telangana State Agriculture University, Rajendranagar, Hyderabad

[2]Indian Institute of Rice Research, Rajendranagar, Hyderabad

[3]Dept. of Statistics and Mathematics, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad

*Corresponding author: jyothi_rishik@yahoo.com

Received: 10th April, 2015; Accepted: 18th June, 2015

Abstract

The nature and magnitude of genetic divergence were estimated in 118 rice genotypes using Mahalanobis D^2 statistics by considering 11 quantitative characters. ANOVA revealed the presence of significant differences among all the characters under study, indicating the greater diversity among the genotypes. High estimates of genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for grain yield per plot, followed by number of filled grains per panicle and 1000 grain weight. High heritability coupled with high genetic advance was recorded for number of tillers per plant, number of productive tillers per plant, plant height, panicle length, number of filled grains per plant, grain yield per plant, yield per plot and harvest index. Mahalanobis D^2 analysis revealed considerable amount of diversity in the material. The genotypes were grouped into twelve clusters, cluster III was the largest comprising of 42 genotypes and the higher amount of divergence was observed between clusters IV and XII (3298579) followed by clusters I and XII (2735295) suggesting that the genotypes constituted in these clusters may be used as parents for future hybridization programme. The genotypes falling in cluster V (26469.49) had the maximum divergence, which was closely followed by cluster IV (17711.43). Traits like yield per plot, number of filled grains per panicle and plant height were major contributors towards genetic diversity.

Introduction

Rice is the world's largest food crop, providing the daily caloric needs of more than half of the global population. Globally, rice is cultivated on 154 million hectares with annual production of around 600 million tones and average productivity of 3.9 tons/ha. More than 90% of the rice is produced and consumed in Asian countries. The other continents in which rice is grown are Africa (7.78% of the global area), South America (6.4%) and North America (1.4%) (Viraktamath, 2007). Rice plays a pivotal role in Indian economy being the staple food for two third of the population.

Rice crop has vast amount of diversity at different levels with eleven genera, 24 species and a number of genotypes or accessions within each sub-species. Of the 11 genera *viz*., *Chikusiochloa, Hygroryza, Leersia, Luziola, Prosphytochloa, Rhynchorya, Zizania, Zizaniopis, Parteresian, Potamophila* and *Oryzae; Oryza* is the only one with cultivated species (Ram *et al*., 2013). Genus *Oryza* has two cultivated and 22 wild species. Of the two distinct types of domesticated rice, *O. sativa* (2n=24 AA, Asian rice) is grown worldwide with a high concentration in Asia while *O. glaberrima* (2n=24 AA, African rice) is cultivated on a limited scale in West Africa (Vaughan *et al*., 2003). As genetic variability is a prerequisite for any crop improvement programme, the large spectrum genetic variability present in the gene pool comprising of indigenous and exotic germplasm offers better scope for selection. It is the choice of the germplasm, which virtually determines the success and nature of end product and a wrong choice would result in the wastage of resources. Information on the nature and degree of genetic divergence would help the plant breeders in choosing the right parents for breeding programme (Vivekanandan and Subramanian, 1993).

Efficient and economic crop improvement scheme refers to the collection of superior alleles into a single population. The grain yield is the primary trait targeted for improvement of rice productivity in both favourable and unfavourable environments from its present level. Grain yield is a complex character, which depends on its main

components *viz*, number of panicles per plant, panicle length, number of grains per panicle and 1000 grain weight. These components are further dependent for their expression on several morphological and developmental traits, which are interrelated with each other and therefore, the parents selected for the breeding programmes aimed at increased seed yield should possess wide range of genetic variation for the above said morphological and developmental character.

Genetic diversity can be evaluated with morphological traits, seed protein, isozymes and DNA markers. Conventionally, it is estimated by the D^2 analysis, Metroglyph and Principal Component Analysis (PCA) using morphological traits. The D^2 technique (Mahalanobis, 1936) based on multivariate analysis had been found to be a potent tool in quantifying the degree of divergence in germplasm. This analysis provides a measurement of relative contribution of different components on diversity both at intra and inter-cluster level and genotypes drawn from widely divergent clusters are likely to produce heterotic combinations and wide variability in segregating generation. Recognizing the importance of variability in plant breeding experiments, the present research work was taken up with the objective of assessing the genetic diversity in rice germplasm comprising of various groups such as land races from north east, tropical *japonica* and introgression lines from wild species.

Material and Methods

The plant material consisted of 118 rice genotypes comprising of land races from north eastern part of India, tropical *japonica* accessions and introgression lines from wild species as test entries. Varieties such as Jaya, IR 64, MTU 1010, MTU 1081 and NLR 34449 were used as checks. The investigation was undertaken at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Ramachandrapuram farm, ICRISAT Campus, Patancheru, Hyderabad, during *Kharif* 2014. The experimental material was laid out in Augmented Block Design (ABD) wherein test entries were sown only once while checks were replicated. The layout design was generated using on line 'Design Resources Server' of ICAR-Indian Agricultural Statistical Research Institute (IASRI) website (www.iasri.res.in) by filling in the number of test treatments (118), control treatments (checks-5) and number of blocks (5).

Twenty five days old seedlings of each genotype sown in raised dry bed nursery in lines were transplanted in 3 rows of 6 m length by adopting a spacing of 15 cm between plants and 20 cm between rows. Recommended agronomic practices and plant protection measures for raising a healthy crop were taken up from time to time during the crop growth period. Data was collected on eleven yield component characters *viz.,* number of tillers per plant (TN), number of productive tillers per plant (PTN), plant height (PH), days to 50% flowering (DFF), days to maturity (DM), panicle length (PL), number of filled grains per panicle (GN), 1000 grain weight (TW), grain yield per plant (SPY), yield per plot (Y) and harvest index (HI). The mean values were considered for statistical analysis. The data was subjected to statistical analyses following Gomez and Gomez (1984) using windostat version 9.2 statistical programme. The genetic parameters were computed following Singh and Chaudhury (1985) and estimation of genetic divergence and clustering of genotypes was done using Tocher's method.

Results and discussion

Analysis of variance for the experiment for 11 yield component characters revealed that the mean sum of squares were highly significant for all the characters indicating the greater diversity among the rice genotypes (Table 1).

Genetic parameters

A perusal of genetic parameters revealed that phenotypic and genotypic coefficients of variation were high for characters like number of tillers per plant, number of productive tillers per plant, number of filled grains per plant, harvest index, 1000 grain weight and yield per plot. The values of genotypic and phenotypic coefficients of variation were low for days to 50% flowering, panicle length and days to maturity. High heritability coupled with high genetic advance as per cent of mean was observed for characters like plant height, number of tillers per plant, number of productive tillers per plant, number of filled grains per panicle, harvest index, plot yield, 1000-grain weight and grain yield per plant. This indicated that these traits were controlled by additive type of gene action in the inheritance of these characters. These characters can be further improved by following simple selection procedure. The high estimates of heritability coupled with low genetic advance as per cent of mean for days to 50% flowering and days to maturity indicated the presence of non-additive gene effects, in addition to influence of environment to some extent.(Table 2).

Genetic divergence

The genetic divergence was high and 123 genotypes were grouped into 12 clusters (Fig. 1 and 2). Out of 12 clusters, cluster III was the largest comprising of 42 genotypes followed by clusters I with 24 genotypes, cluster II with 21 genotypes, cluster V with 16 genotypes, cluster IV with 13 genotypes and clusters VI, VII, VIII, IX, X, XI and XII

with one genotype each. The clusters VI, VII, VIII, IX, X, XI and XII were represented by single genotype indicating high degree of heterogeneity among the genotypes.

Inter and intra-cluster distances among the clusters generated are presented in Table 3. The higher amount of divergence was observed between clusters IV and XII (3298579) followed by clusters I and XII (2735295), clusters III and XII (2178405.50) and cluster VIII and cluster XII (1754711), while it was low between clusters VIII and IX (5235.73**)** (Table 4). Maximum intra cluster distance was observed in cluster V (26469.492), followed by cluster IV (17711.43), cluster III (12829.94), cluster II (10504.917), and cluster I (8634.53). Solitary clusters (VI, VII, VIII, IX, X, XI and XII) showed zero intra cluster distances. Based on the inter cluster distances, hybridization between the genotypes of clusters IV and XII, clusters I and XII, clusters III and XII, clusters VIII and XII is suggested to generate promising segregants for grain yield would produce encouraging results.

The intra cluster distance varied from 8634.53 (cluster I) to 26469.49 (cluster V). This reveals the presence of more diversity among the genotypes within the clusters. Therefore due emphasis should be given on the constituents of clusters V and IV for selection of parents for hybridization programme. Hence, selection within these clusters may be exercised based on the highest areas for the desirable traits, which would be made use of in improvement through intervarietal hybridization (Joshi *et al*. 2008).

The clusters VI, VIII, X, XI and XII recorded high mean values for the yield components like number of days to fifty per cent flowering, plant height, panicle length, days to maturity and number of tillers per plant, number of productive tillers per plant, number of filled grains per panicle, 1000 grain weight and harvest index and they were also divergent from each other (Table 4). Hence, crosses between genotypes selected from these clusters may be used to generate rice genotypes with good grain yield**.** None of the clusters contained genotypes with all the desirable traits which could be directly selected and utilized. All the minimum and maximum cluster mean values were distributed in relatively distant clusters. Similar results were also reported by Bose and Pradhan (2005).

Contribution towards Genetic divergence

Table 5 shows the number of times each of the ten characters appeared in first rank and its respective per cent contribution towards genetic divergence. The results showed that the contribution of yield per plot was highest towards genetic divergence (89.47%) ranking first by 6713 times followed by number of filled grains per panicle (7.24%) by 543 times, plant height (3.15%) by 236 times, harvest index (0.07 %) by 5 times, days to 50% flowering 0.04% by 3 times, days to maturity (0.03%) by 2 times and 1000 grain weight (0.01%) by 1 time. The results were in conformity with Ramya and Senthil Kumar (2008) and Vennila *et al*. (2011).

The experimental material comprised of genotypes that were classified into land races, tropical *japonica* accessions, introgression lines from wild species and elite cultivars. Interestingly, clustering of these genotypes did not follow any segregation pattern based on origin or geography or specific groups to which they were classified. Genotypes from all groups were present in different clusters except solitary clusters.

References

Bose, L.K. and Pradhan, S.K. 2005. Genetic divergence in deep water rice genotypes. *Journal Central European Agriculture* 6(4):635-640.

Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultural research (2nd Edition). John Wiley & Sons. Inc. (pp.680).

Joshi, M.A., Singh, P., Sarao, N.K., Sharma, R.C. and Bharaj, T.S. 2008. Genetic diversity among the rice varieties cultivated in Punjab. *Oryza* 45(4):277-279.

Mahalanobis, P.C. 1936. On the generalized distance in statistics. *Proceedings National Institute of Science India* (2):49- 55.

Ramya, K. and Senthil Kumar. 2008. Genetic divergence in rice (*Oryza sativa* L.) *Crop Improvement* 35 (2): 119-121.

Singh, R.K. and Chaudhury, B.D. 1985. Biometrical Methods in Quantitative Genetic Analysis. Kalyani Publishers, New Delhi, India (pp. 229-252).

Ram, T., Bhadana, V.P., Laha, G.S., Padma Kumari, A.P., Jyothi Badri., Azam, M.M., Amtul Waris., Sarla, N., Padmavathi, G., Dinesh Chandra., Divya Balakrishnan and Viraktamath, B.C. 2013. Wild Species in Rice Improvement. Technical Bulletin No. 71, Directorate of Rice Research, 50pp.

Vaughan D.A., Morishima, H. and Kadowaki, K. 2003. Diversity in the *Oryza* genus. *Current Opinion in Plant Molecular Biology* 6:139-146.

Vennila, S., Anbuselvam, Y. and Palaniraja, K. 2011. D^2 analysis of rice germplasm for some quantitative and quality traits. *Electronic Journal of Plant Breeding* 2 (3):392- 4.

Viraktamath, B.C. 2007. Rice research in India current and future prospects, proceedings of 2nd Work shop cum Training Programme on Dus Tests in Rice, Directorate of Rice Research, Hyderabad, pp. 1-3.

Vivekanandan, P. and Subramanian S. 1993. Genetic divergence in rainfed rice. *Oryza* 30:60-62.

Table 1. Analysis of variance for eleven quantitative traits among rice genotypes

	Block (Ignoring treatments)	Treatment (eliminating blocks)	Checks	Checks+ Varieties vs. varieties	Error
d.f	4	122	4	118	116
No. of tillers/ Plant	38.36 ***	7.11***	17.63 ***	6.75 ***	1.01
No. of productive tillers/plant	31.50 ***	6.38 ***	12.55 ***	6.17***	1.38
Plant height (cm)	5736.57 ***	731.12 ***	401.98 ***	742.27 ***	43.89
Days to 50% flowering	159.70 ***	37.21 **	13.94	38.00 **	10.36
Days to maturity	199.41 **	49.78	97.54 *	48.16	31.71
Panicle length (cm)	24.14 **	9.32 *	8.40	9.35*	4.36
Filled grains /Panicle	1216.30 **	2018.38 ***	1629.11 ***	2031.58 ***	184.18
Grain yield/plant(g)	83.526***	26.25 **	48.66**	25.49 **	6.58
1000 grain weight (g)	98.25 ** *	38.10 ***	112.09 ***	35.59 ***	4.62
Plot yield(g/m^2)	311718.300***	99129.83 ***	79755.10 *	99786.60***	21363.85
Harvest index (%)	207.65 **	85.13 **	114.52 *	84.13 **	27.54

* Significant at 5 per cent level; ** Significant at 1 per cent level

Table 2. Estimates of variability, heritability and genetic advance among rice genotypes.

Characters	Genotypic variance	Phenotypic variance	GCV%	PCV%	h2 (bs)%	GA as % of Mean (5%)
No. of tillers/ plant	5.77	6.78	20.72	22.47	85.05	39.37
No. of prod. tillers/plant	4.72	6.10	20.64	23.47	77.33	37.39
Plant height (cm)	587.25	631.14	19.21	19.91	93.05	38.17
Days to 50% flowering	26.19	36.56	5.13	6.07	71.65	8.96
Days to maturity	19.79	51.50	3.37	5.44	38.43	4.30
Panicle length (cm)	4.57	8.93	8.52	11.91	51.17	12.56
Filled grains /panicle	1598.17	1782.36	29.07	30.70	89.67	56.71
Grain yield/plant (g)	18.12	24.70	16.03	18.72	73.37	28.29
1000 grain weight (g)	28.92	33.54	24.32	26.19	86.22	46.52
Plot yield (g)	71823.05	93186.91	41.34	47.09	77.07	74.77
Harvest index (%)	50.34	77.89	22.64	28.17	64.63	37.50

Table 3. Intra (diagonal) and inter cluster average distance (D^2) in Rice genotypes (Tocher method)

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	Cluster XII
Cluster I	8634.53	198724.39	43328.93	38818.68	570346.44	905601.06	312887.16	114916.31	120287.81	926786.00	318634.09	2735295.00
Cluster II		10504.92	77977.02	369684.59	114741.64	269688.66	20807.18	19615.20	21387.63	284582.94	33247.18	1487060.50
Cluster III			12829.94	130426.16	336662.66	602067.75	150602.56	30255.82	32321.10	615259.00	150809.36	2178405.50
Cluster IV				17711.44	838445.00	1243093.75	522047.78	249795.27	255928.28	1263673.00	521416.88	3298579.00
Cluster V					26469.49	60339.10	55863.10	187307.63	180776.64	59913.95	56351.46	844903.63
Cluster VI						0.00	159217.02	392153.28	388247.88	9638.51	182835.00	497607.47
Cluster VII							0.00	55574.84	61191.06	177934.59	23278.08	1208647.13
ClusterVIII								0.00	5235.73	403653.56	56937.70	1754710.63
Cluster IX									0.00	389957.63	49418.19	1748580.25
Cluster X										0.00	172690.64	494238.53
Cluster XI											0.00	1236637.50
Cluster XII												0.00

Table 4. Cluster means for 11 quantitative traits (Tocher method)

	TN	PTN	PH (cm)	DFF	DM	PL (cm)	GN	GY (g)	TW (g)	PY (g/m²)	HI (%)
Cluster I	11.73	10.41	116.86	99.33	130.38	24.44	112.85	21.87	20.48	396.95	23.31
Cluster II	12.13	11.17	119.85	102.54	135.34	25.71	120.58	26.39	21.07	832.12	32.80
Cluster III	11.56	10.69	129.69	99.21	130.29	24.88	143.37	26.73	22.29	575.11	31.52
Cluster IV	11.39	10.15	125.27	99.10	131.18	24.86	124.26	27.06	23.56	235.10	31.85
Cluster V	11.73	10.58	124.66	97.22	133.25	24.67	183.41	32.30	23.11	1137.47	39.58
Cluster VI	**15.59**	**13.63**	98.06	93.36	136.04	25.53	123.26	25.94	15.39	1345.94	40.60
Cluster VII	11.99	11.43	137.38	99.56	130.24	25.93	93.39	34.78	29.57	950.73	55.44
Cluster VIII	7.79	6.03	**169.18**	93.56	138.24	24.93	139.72	27.80	21.17	724.29	25.86
Cluster IX	8.03	6.75	123.38	104.76	134.44	27.53	193.72	20.23	23.38	727.82	25.36
Cluster X	10.23	8.95	106.98	**104.76**	**140.44**	29.93	219.39	30.76	19.88	1351.32	35.50
Cluster XI	5.95	5.91	167.22	103.56	136.64	**30.49**	**238.72**	23.87	**28.27**	940.92	25.34
Cluster XII	12.99	12.03	152.78	89.56	131.24	23.33	146.05	**37.18**	27.57	1048.62	**42.96**

Table 5. Relative contribution of different characters towards genetic diversity in rice genotypes

Character	Times ranked first	Contribution (%)
Number of tillers / plant	0.01	0.00
Number of prod. tillers/plant	0.01	0.00
Plant height (cm)	236	3.15
Days to 50% flowering	3	0.04
Days to maturity	2	0.03
Panicle length (cm)	0.01	0.00
Filled grains/ panicle	543	7.24
Grain yield/ plant (g)	0.01	0.00
1000 grain weight (g)	1	0.01
Plot yield (g)	6713	89.47
Harvest index (%)	5	0.07

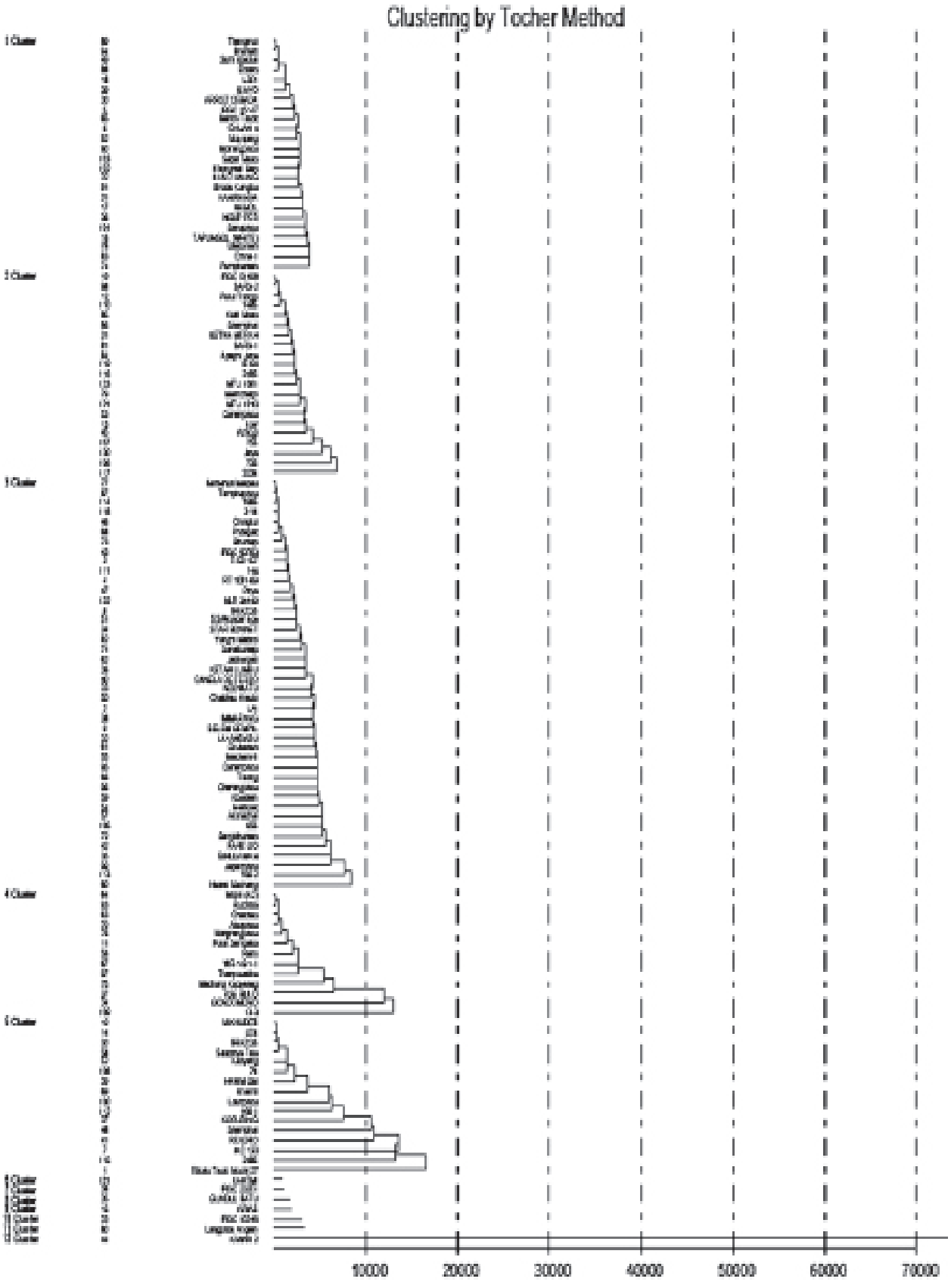


Fig. 1. Clustering of 123 genotypes of rice by Tocher's method

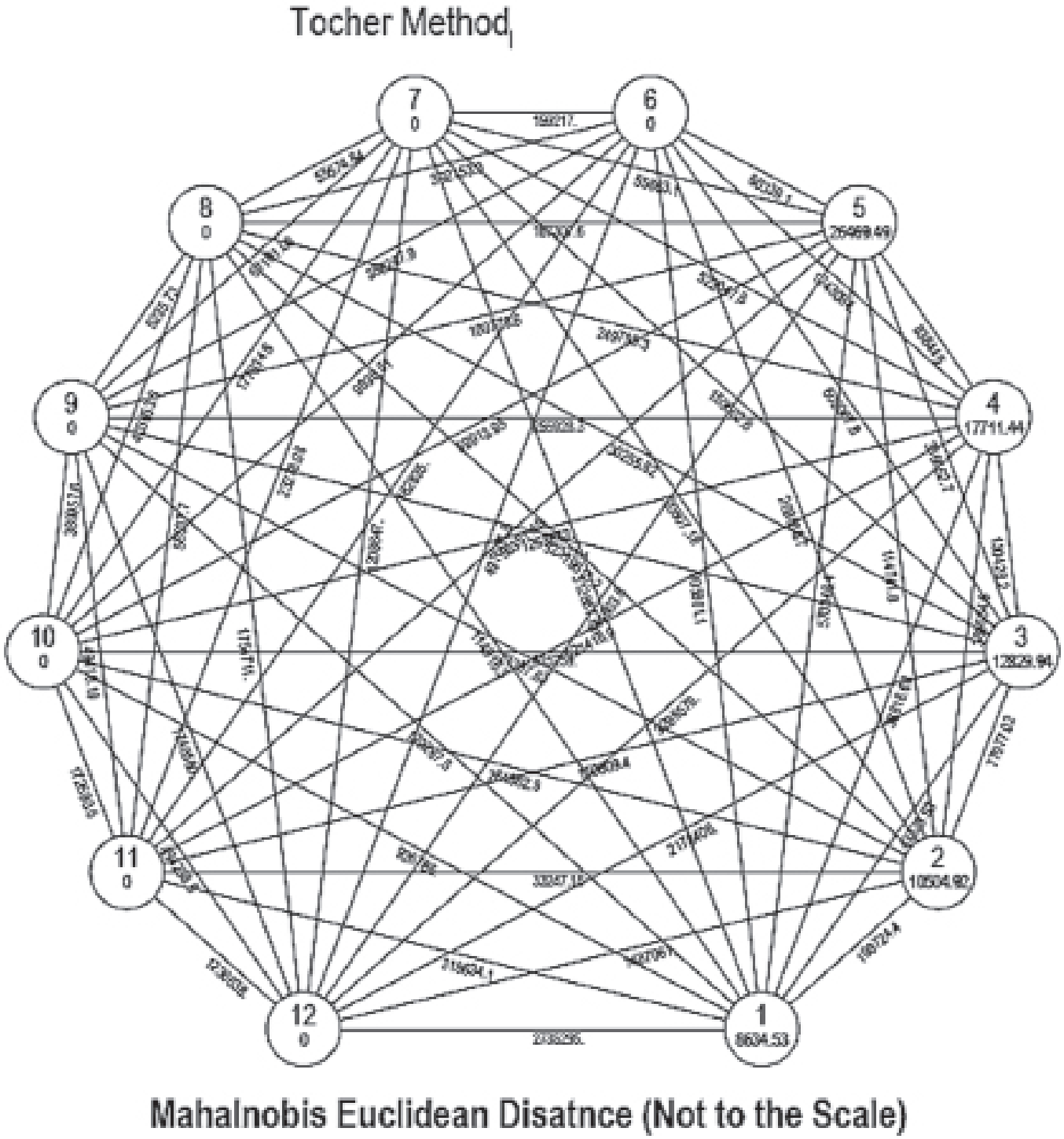


Fig. 2. Statistical distance among 123 genotypes of rice (Not to the scale)

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Evaluation of Crop Establishment Methods for their Productivity, Nutrient Uptake and Use Efficiency under Rice-Rice System

K. Surekha*, R. Mahender Kumar and Ch. Padmavathi

Indian Institute of Rice Research, Rajendranagar, Hyderabad

*Corresponding author: surekhakuchi@gmail.com

Received: 27th November, 2014; Accepted: 8th May, 2015

Abstract

A field experiment was conducted in *kharif* (wet) and *rabi* (dry) seasons of 2010-11 at the Indian Institute of Rice Research (formerly Directorate of Rice Research)- Ramachandrapuram farm on sandy clay loam soil to study the influence of different methods of crop establishment *viz*., System of Rice Intensification (SRI), Eco-SRI and conventional method on rice productivity, nutrients uptake, their use efficiency and soil nutrient status. Three cultivars each in *kharif* and *rabi* were tested. During *kharif*, grain yield was significantly higher in SRI than conventional method and Eco-SRI by 10.3 and 33.4 per cent, respectively. Whereas, SRI and conventional method were on par and superior to Eco-SRI in *rabi*. Among the cultivars, Swarna and DRRH 2 were significantly superior to other varieties in *kharif* and *rabi*, respectively. SRI and conventional method were on par and significantly superior to ECO-SRI with respect to N, P and K uptake in both the seasons. Though the nutrients uptake remained same, the nutrient use efficiency was marginally higher in SRI (by 8, 8 and 12 per cent for N, P and K, respectively during *kharif* and 5 per cent for N during *rabi*) compared to conventional rice. Soil analysis data indicated similar available nutrient status in SRI and conventional methods after two seasons of experimentation.

Key words: Crop establishment methods, conventional method, Eco-SRI, SRI, productivity, nutrient use efficiency

Introduction

Low land rice is being grown under flooded conditions for millennia and such situation may result in several drastic adaptations in the root system of rice such as formation of aerenchyma and subsequent degeneration of root system to the extent of 70 per cent by the time of flowering. Further, the hypoxic condition leads to a reduced soil condition that creates low availability of some nutrient ions and high availability of certain other nutrients. System of Rice Intensification (SRI), originated through participatory on farm experimentation conducted in Madagascar during 1980s by Fr. Henri de Laulanie represents an integrated and ecologically sound approach to irrigated rice cultivation and the productivity is higher in SRI compared to conventional rice farming. A well developed and healthy root system in SRI plays an important role in uptake and translocation of nutrients from the soil than conventional system (Uphoff, 2005) and this ultimately results in healthy plant growth, better tillering, higher biomass and higher yields. Increased yields in SRI compared to conventional method were reported by several authors (Thiyagarajan *et al*., 2005; Uphoff, 2005). Under conditions of modern, high yield rice culture, nutrient removal in double cropping areas is more and continuous cropping under high levels of N and high yield will sooner or later exhaust the phosphate and potash reserves of any soil (Von Uexkull, 1976). Though use of organics alone in SRI has been considered as an important component, non availability of organic manures in large quantities forced the farmers to follow Integrated Nutrient Management (INM). The information on yield, nutrient use efficiency and soil nutrient status under different crop establishment methods is very limited. Keeping this in view, three methods of crop establishment *viz*., SRI-organic (Eco-SRI), SRI-INM (SRI) and conventional method were evaluated for their productivity, nutrient uptake, use efficiency and soil nutrient status during 2006-07 in rice-rice system.

Materials and Methods

The field experiment was conducted in *kharif* (wet) and *rabi* (dry) seasons of 2006-07 at the Indian Institute of Rice Research (formerly Directorate of Rice Research)-Ramachandrapuram farm in ICRISAT campus in a sandy clay loam soil. Initial soil samples were collected from three depths and were analysed for important properties using standard procedures. The soil was alkaline [pH 8.50 - 9.45 in surface (0-15 cm) and sub surface (30-60 cm) depths, respectively]; non-saline (EC- 0.47-0.67 in surface and sub surface depths, respectively); with high organic carbon (0.76-1.27%) content. Available N was

medium (291kg/ha); available P_2O was high (268 kg/ha) and available K_2O was high (527 kg/ha) in surface layer.

The experiment was laid out in a split-plot design with cultivars as main plots (BPT 5204, Swarna & DRRH 2 in *kharif;* MTU 1010, Shanti & DRRH 2 in *rabi*) and methods of crop establishment (ECO-SRI, SRI and Conventional method) as sub-plot treatments in four replications. In SRI and conventional methods, the recommended dose of N @ 100 kg/ha during *kharif* and 120 kg/ha during *rabi* was applied through 50% organics (FYM) + 50% inorganics (urea). P_2O_5 and K_2O @ 60 and 40 kg/ha were given through single super phosphate and muriate of potash, respectively, in both seasons. Whereas, in ECO-SRI method, total nutrients were supplied through organic source, FYM only. Twelve days old seedlings in Eco-SRI and SRI at a spacing of 25x25cm and 30 day old seedlings in conventional method at 20x15cm spacing were transplanted. Water management and other cultural practices were followed as per the principles of SRI in SRI and Eco-SRI and paddy straw was used as mulch in Eco-SRI. Grain and straw yields were recorded at harvest. Further, grain, and straw samples were collected at harvest and were analysed for N, P and K. Plant nutrient uptake was calculated and nutrient use efficiency was computed using grain yield and total nutrient uptake. Soil samples were collected at the end of two seasons and were analyzed for important soil parameters using standard procedures. All the data were analyzed using standard statistical methods (Gomez and Gomez, 1984).

Results and Discussion

Grain and straw yields

Grain yield data presented in Table 1 indicated the superiority of SRI (5.27 t/ha) over conventional method (4.78 t/ha) and Eco-SRI (3.95 t/ha) during *kharif* season by 10.3 and 33.4 per cent, respectively. Whereas, during *rabi*, SRI (3.34 t/ha) and conventional method (3.46 t/ha) were on par and both were significantly superior to Eco-SRI (1.66 t/ha). Among the varieties, grain yield differences were significant where Swarna (5.33 t/ha) during *kharif* and DRRH 2 (4.12 t/ha) during *rabi* were significantly superior to other varieties recording maximum grain yield. The expected higher yields in SRI could not be attained especially, during *rabi* due to sub-soil alkalinity and delayed planting. Plant growth on saline soils is mainly affected by high levels of soluble salts causing ion toxicity, ionic imbalance and impaired water balance and rice is very sensitive during early growth stage (Dobermann and Fairhurst, 2000). Sensitivity of rice to salinity at 1-2 leaf stage and again at flowering stage was also reported by Yoshida (1981). Transplanting at 2 leaf stage and damage caused to the root system due to salt accumulation in the root zone by the upward movement under non-flooded conditions could be the probable reasons for not attaining the potential yield in SRI especially during *rabi* season. The dilution effect due to the advantage of flooding in conventional rice might not have resulted in greater yield reduction. In the arid and semi arid regions, salt accumulation in the root zone of soils with high pH due to upward water movement was reported by Yoshida (1981). Eco-SRI with 100 per cent organics did not perform well because in the initial years of organic farming, yield reduction is expected due to slower release of nutrients and mismatch of nutrient release from organics and crop demand.

In case of straw yields, SRI and conventional method were on par and both systems were significantly superior to Eco-SRI in both seasons. Among the varieties, DRRH 2 recorded maximum straw yield in both seasons.

Nutrients uptake

The major nutrients (NPK) uptake data is presented in Table 2. Total nitrogen uptake ranged from 51.4-109.3 and 29.5-100.1 kg/ha during *kharif* and *rabi* seasons, respectively. In case of methods of cultivation, SRI (103.8 and 73.0 kg/ha in *kharif* and *rabi*) and conventional (100.6 and 82.9 kg/ha in *kharif* and *rabi*) methods were on par and significantly higher than Eco-SRI (72.7 and 42.0 kg/ha in *kharif* and *rabi*) in both the seasons. Among the varieties, Swarna (104.1 kg/ha) in *kharif* and DRRH 2 (82.6 kg/ha) in *rabi* recorded maximum N uptake. Total P uptake ranged from 11.4 – 17.3 and 6.8-18.0 kg/ha during *kharif* and *rabi,* respectively. SRI (15.2 and 12.2 kg/ha in *kharif* and *rabi*) and conventional (14.9 and 12.4 kg/ha in *kharif* and *rabi*) method were on par and superior to Eco-SRI (12.6 and 8.2 kg/ha in *kharif* and *rabi*) in both the seasons. Among the varieties, all varieties were on par during *kharif* (13.3-15.9 kg/ha) and DRRH 2 (15.0 kg/ha) recorded significantly higher P uptake than other varieties during *rabi*. With regard to K uptake, total K uptake ranged from 58.2-101.7 and 36.1-103.6 kg/ha during *kharif* and *rabi*, respectively. SRI and conventional method were on par (81.9-91.9 and 84.1-90.0 kg/ha in *kharif* and *rabi*) and recorded significantly higher K uptake over ECO-SRI (63.9 and 55.9 kg/ha in *kharif* and *rabi*). Varieties did not differ significantly (76.2-84.7 and 70.3-88.1 kg/ha in *kharif* and *rabi,* respectively) in total K uptake.

Nutrients use efficiency

Among the methods of cultivation, in *kharif*, SRI recorded maximum use efficiency in case of N, P and K with 52, 347 and 63 kg grain/kg NPK uptake, respectively (Figure

1) and it was marginally higher than conventional method (48, 320 and 56 kg grain/kg NPK uptake respectively). Whereas, during *rabi,* SRI recorded maximum nutrient use efficiency in case of N alone (44 kg grain/kg N uptake) than conventional method (41 kg grain/kg N uptake) and ECO-SRI (38 kg grain/kg N uptake). P and K use efficiencies were same in SRI and conventional methods but, both were higher than Eco-SRI. Thus, though there was no significant difference in nutrients uptake, the nutrient use efficiency was marginally higher in SRI compared to other systems when grain yield was on par in *rabi* (for N) or significantly higher in *kharif* (for N, P, K) than conventional rice. Similar results were reported by Barison (2002). Among the varieties, Swarna during *kharif* and DRRH2 during *rabi* were superior to other varieties in their nutrient use efficiency of all nutrients (Figure 2).

Soil properties after two crop seasons

Soil properties measured after two seasons of the study indicated no significant treatment differences in pH, EC, organic carbon and available N either due to methods of cultivation (Table 3) or due to different varieties. Available P_2O_5 was same in SRI and conventional method and these two systems were superior to Eco-SRI. Whereas, there was a significant increase in available K_2O in Eco-SRI compared to other two systems which could be attributed to the paddy straw mulching in case of Eco-SRI in both seasons. The increase in soil available K due to paddy straw application was also reported by Ponnamperuma (1984) and Dobermann *et al*. (1998). This indicated that SRI did not exhaust the soil available nutrients after two seasons of experimentation.

Conclusion

From the present study, it can be concluded that SRI resulted in higher yield during *kharif*, non-significant nutrient uptake and marginally higher nutrient use efficiency without depleting the soil available nutrients compared to conventional transplanting, at least up to two seasons. During *rabi*, the expected higher yields could not be achieved due to alkalinity problem. However, long term studies on nutrient uptake and available nutrient status under highly productive SRI in different soils are needed.

References

Barison, J. 2002. Nutrient-use efficiency and nutrient uptake in conventional and intensive (SRI) rice cultivation systems in Madagascar. Master's thesis, Department of Crop and Soil Sciences, Cornell University, Ithaca, New York.

Dobermann, A., Cassman, K.G., Mamaril, C.P. and Sheehy, J.E. 1998. Management of phosphorus, potassium and sulfur in intensive, irrigated lowland rice. *Field Crops Research.*56:113-138.

Dobermann, A. and Fairhurstn, T. 2000. Nutrient disorders and nutrient management. Handbook series. Potash and Phosphate Institute (PPI). .

Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research'. Second Edition. (IRRI John Wiley & Sons Publication: New York).

Ponnamperuma, F.N. 1984. Straw as source of nutrients for wet land rice. In '*Organic Matter and Rice*' pp. 17-136. (International Rice Research Institute, Manila: Philippines)

Thiyagarajan, T.M., Senthilkumar, K., Priyadarshini, R., Sundarsingh, J., Muthusankaranarayan, A., Hengsdijk, H. and Bindraban, P.S. 2005. (*In*): *Transitions in Agriculture for enhancing Water Productivity.* Proceedings of an International Symposium held in Killikulam, Tamil Nadu, India during 23-25, September 2003.

Uphoff, N. 2005. Possible explanations for the productivity gains achieved with the System of Rice Intensification (SRI). (*In)*: *Transitions in Agriculture for enhancing Water Productivity*. Proceedings of an International Symposium held in Killikulam, Tamil Nadu, India during 23-25, September 2003.

Von Uexkull, H.R. 1976. Aspects of Fertiliser use in modern, high yield rice culture. IPI Bulletin No. 3.

Yoshida, S. 1981. Mineral Nutrition of Rice. (*In*): *Fundamentals of Rice Crop Science*. International Rice Research Institute. pp. 175-176.

Table 1. Grain and straw yeilds (t/ha) as influenced by different methods of crop establishment

	Grain yield (t/ha)							
	kharif				*rabi*			
Treatments	BPT 5204	Swarna	DRRH 2	Mean	MTU 1010	Shanti	DRRH 2	Mean
Eco-SRI	3.38	4.83	3.63	3.95	1.30	0.87	2.90	1.69
SRI	5.05	6.00	4.75	5.27	3.32	1.75	4.96	3.34
Conventional	4.52	5.17	4.65	4.78	3.39	2.53	4.45	3.46
Mean	4.32	5.33	4.34		2.67	1.69	4.12	
C.D (0.05)								
Main	0.32				0.58			
Sub	0.15				0.60			
MXS	NS				NS			

	Straw yield (t/ha)							
	kharif				*rabi*			
	BPT 5204	**Swarna**	**DRRH 2**	**Mean**	**MTU 1010**	**Shanti**	**DRRH 2**	**Mean**
Eco-SRI	5.48	4.83	3.68	4.66	2.71	3.81	4.99	3.84
SRI	6.31	6.52	7.47	6.77	6.08	5.36	6.92	6.12
Conventional	5.82	7.07	7.47	6.79	6.45	6.60	6.05	6.37
Mean	5.87	6.14	6.21		5.08	5.26	5.99	
C.D (0.05)								
Main	NS				0.63			
Sub	1.57				1.24			
MXS	NS				NS			

Table 2. Total nutrient uptake (kg/ha) as influenced by different treatments

Kharif (wet season)														
	N uptake					**P uptake**					**K uptake**			
	BPT 5204	**Swarna**	**DRRH 2**	**Mean**		**BPT 5204**	**Swarna**	**DRRH 2**	**Mean**		**BPT 5204**	**Swarna**	**DRRH 2**	**Mean**
Eco-SRI	71.4	95.3	51.4	72.7	Eco-SRI	11.4	14.0	12.4	12.6	Eco-SRI	59.1	74.4	58.2	63.9
SRI	100.2	109.3	101.8	103.8	SRI	14.7	17.3	13.5	15.2	SRI	87.2	101.7	86.8	91.9
Conventional	91.4	107.6	102.9	100.6	Conventional	13.7	16.5	14.6	14.9	Conventional	84.3	77.9	83.5	81.9
Mean	87.7	104.1	85.4		Mean	13.3	15.9	13.5		Mean	76.9	84.7	76.2	
C.D (0.05)					C.D(0.05)					C.D(0.05)				
Main	NS				Main	NS				Main	NS			
Sub	25.67				Sub	3.94				Sub	23.5			
MXS	NS				MXS	NS				MXS	NS			

Rabi (dry season)

	N uptake					P uptake					K uptake			
	MTU 1010	**Shanti**	**DRRH 2**	**Mean**		**MTU 1010**	**Shanti**	**DRRH 2**	**Mean**		**MTU 1010**	**Shanti**	**DRRH 2**	**Mean**
Eco-SRI	29.5	38.4	58.2	42.0	Eco-SRI	7.0	6.8	10.9	8.2	Eco-SRI	36.1	51.9	78.6	55.5
SRI	63.1	55.7	100.1	73.0	SRI	10.8	7.9	18.0	12.2	SRI	83.3	65.2	103.6	84.1
Conventional	77.5	81.8	89.5	82.9	Conventional	11.2	10.1	16.0	12.4	Conventional	91.6	96.5	82.0	90.0
Mean	56.7	58.7	82.6		Mean	9.7	8.3	15.0		Mean	70.3	71.2	88.1	
C.D (0.05)					C.D(0.05)					C.D(0.05)				
Main	NS				Main	4.6				Main	NS			
Sub	12.23				Sub	2.9				Sub	15.7			
MXS	NS				MXS	NS				MXS	NS			

Table 3. Soil properties after 2 seasons as influenced by different crop establishment methods

Treatments	pH	EC (dS/m)	SOC (%)	Available N (kg/ha)	Available P_2O_5 (kg/ha)	Available K_2O (kg/ha)
Eco-SRI	8.51	0.50	1.10	247.0	204	674
SRI	8.43	0.51	1.25	272.0	258	638
Conventional	8.44	0.51	1.18	251.0	256	609
Mean	8.44	0.51	1.18	257	239	641
C.D (0.05)	NS	NS	NS	NS	26	34

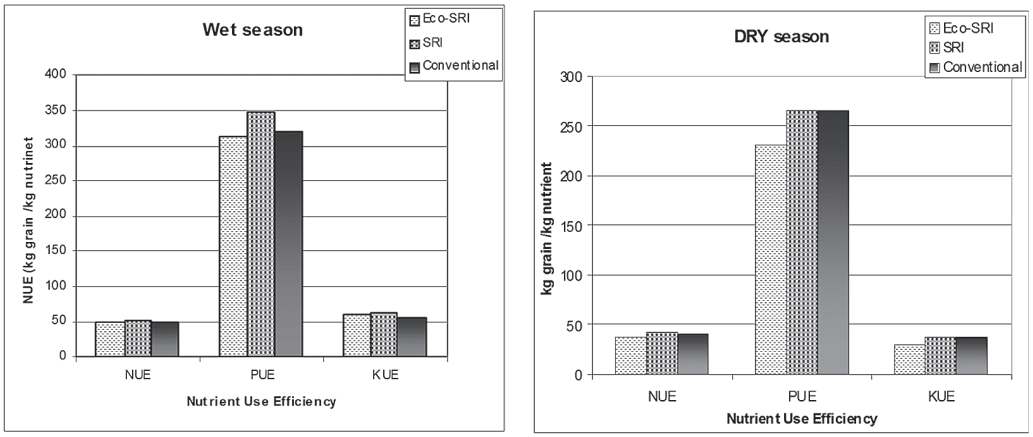


Fig.1. Nutrient use efficiency as influenced by methods of crop establishment

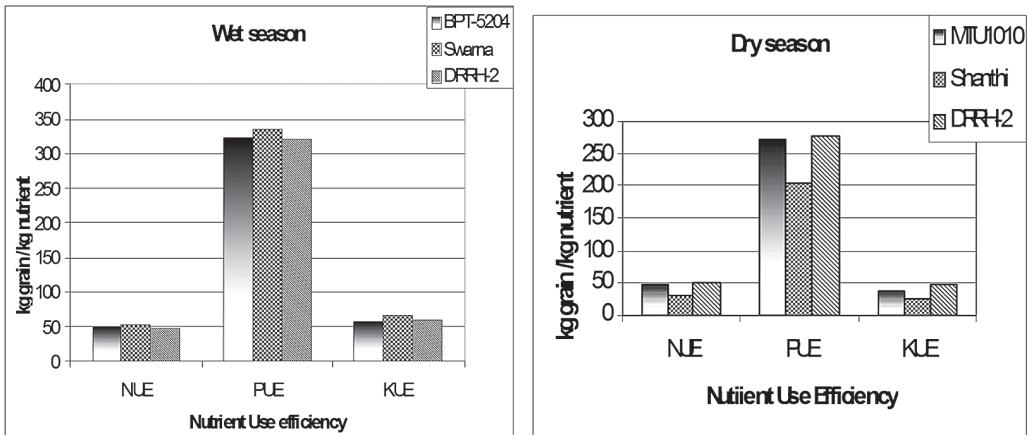


Fig. 2. Nutrient use efficiency as influenced by cultivars

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Influence of Soil Test Based Application of Phosphorus Fertilizers on Yields of Paddy: A Case Study in Khammam District of Andhra Pradesh

A.D.V.S.L.P. Anand Kumar*[1], M. Srinivasa Rao[2] and M. Satyanarayana[3]

[1]District Agricultural Advisory and Transfer of Technology Centre (DAATTC), Kakinada,
[2]DAATTC, Sanga Reddy,

[3]Krishi Vigyan Kendra, Ghantasala, Andhra Pradesh

*Corresponding author: anandkumarento@gmail.com

Received: 27th January, 2015; Accepted: 28th April, 2015

Abstract

In order to create awareness among the farming community on use of phosphatic fertilizers based on soil test values, 17 On farm demonstrations were conducted during *rabi*, 2011-12 on soils having high status of available Phosphorus in Khammam District of Andhra Pradesh. Application of recommended doses of fertilizers based on soil test values recorded similar grain yields in paddy as that of farmers practice and there was a net savings in the cost of P fertilizers applied per hectare to an extent of Rs.1448/-.

Key Words: Soil test, fertilizer, phosphorus, paddy, yield

Introduction

Ensuring food security for escalating population necessitates the production of additional food grains from the same land without losing the production potential of the soil. This, in turn requires balanced nutrition to the food crops for enhancing and sustaining food production as well as soil productivity with minimum environmental degradation. This can be achieved through soil test based fertilizer application.

Fertilizer is one of the costliest inputs in agriculture and the use of right amount of fertilizer is fundamental for farm profitability and environmental protection (Kimetu *et al*., 2004). To enhance farm profitability under different soil-climate conditions, it is necessary to have information on optimum doses for fertilizer use. Traditionally, to determine the optimum fertilizer doses, the most appropriate method is to apply fertilizer on the basis of soil test and crop response studies. During 1956-57 the semi-quantitative soil test calibrations were evolved and advocated for the use. Subsequently in India the quantitative refinements in the fertilizer recommendations based on the soil and plant analysis were made (1967-68) through the All India Coordinated Research Project for Investigation on Soil test crop response correlation (STCRC).

Soil testing is a tool that aids in taking scientifically sound management decisions about fertilizer requirement after assessing the nutrient status in soils. But with continuous and higher application of complex and other phosphatic fertilizers, larger areas of cultivated lands of Andhra Pradesh are being reported to contain higher available P in soils resulting in adverse effects on the availability of other nutrients particularly micronutrients (e.g. Zn) besides increasing the cost of cultivation in different crops. One of the reasons for lower production of rice is imbalanced fertilization of N, P and K nutrients (Reddy and Ahmed, 2000). The most comprehensive approach of fertilizer application by incorporating soil test values, nutrient requirement of the crop, contribution of nutrients from soil, manures, fertilizers and fixing yield-targets is possible only through Soil Test Crop Response (STCR) approach.

Out of 4,00,070 soil samples analyzed during 2010-11 by state soil testing laboratories, 1,22,471 samples constituting 31% were found to register high Phosphorus levels in soils. The research reports of Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad indicate that with applicability of targeted yield equations for soils having high Phosphorus, there is a possibility of saving of Phosphorus fertilizers to an extent of 25 to 75 per cent from currently used phosphorus fertilizer doses in selected crops on such high Phosphorus soils.

In the light of above, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad implemented Bhoochetana project during *rabi,* 2010-11 under RKVY Scheme through its extension institutes like Krishi Vigyan Kendras (KVK) and District Agricultural Advisory and Transfer of Technology Centres (DAATTC) in 10 selected Districts of A.P. Keeping this in view, the present

investigation was carried out to study the effect of soil test based phosphatic fertilizer application on crop yield and cost of production.

Materials and Methods

Seventeen On Farm Demonstrations on soil test based application of Phosphatic fertilizers were conducted during *rabi*, 2011-12 under bhoochetana project in five Mandals of Khammam District with the objective to demonstrate to farming community and to popularize the use of soil test based phosphorus fertilizers in crops for reducing the input cost and sustain the soil health.

The services of the soil testing laboratory, Khammam, Khammam District were utilized for selection of farmers having soils with high levels of available phosphorus (Olsens *et al*., 1954) in the present study (Table1).

Each demonstration consisted of two treatments namely farmer practice (T_1) *i. e* unbalanced use of N, P and K fertilizers and soil test based P recommendation (*i. e* Higher the available phosphorus in soils, 30 per cent reduction in the recommended dose of the nutrient Phosphorus for a particular crop) along with farmers practice with regard to N and K (T_2) and each treatment was imposed in 0.40 ha with same variety (MTU-1010).

The recommended dose of N, P and K per hectare for *rabi* paddy in Central Telangana Zone of A.P. is 120, 60 and 40 kg, respectively. Full dose of P along with 1/3rd N and half dose of K were applied during last puddling in both treatments (T_1 & T_2). The remaining 1/3rd N along with half dose of K were applied at panicle initiation stage in both the treatments. Similar plant protection measures were adopted throughout the crop growth period in both the treatments. Grain yield data per acre was recorded, per hectare yield was computed and subjected to paired t' test.

Results and Discussion

Grain yields were estimated based on crop cutting experiments conducted at the time of harvest and arrived at average figures for grain yield (kg/hectare), cost of fertilizers applied (Rs/hectare) in T_1 and T_2. Economics were also worked out for T_1 and T_2 (Table 2).

Grain yields (kg/ha) obtained in T_1 and T_2 were 6115 and 6150, respectively. According to the data recorded for grain yield, no significant difference (t-calculated < t-tab) was observed in farmers practice and soil test based application of phosphatic fertilizers (Table 3). Cost of P fertilizers applied (Rs. /ha) in T_1 and T_2 were Rs.3435 and 1947/-respectively. This indicates that there is a significant difference in the cost of P fertilizers applied in T_1 and T_2 to the extent of Rs.1488/- per hectare (t-calculated > t-tab). Hence, the present study supports the earlier research reports of Prasada Rao and Bhupal Raj (2001) and Reddy and Ahmed (2000) stating that there is a possibility of saving of phosphatic fertilizers to the extent of 25 to 75 per cent on soils having high status of available P. This suggests that the use of excess P fertilizers does not result in significant marginal increase in the yield besides increasing the cost of cultivation and adverse effects on other nutrients.

Hence present study indicates that application of recommended doses of fertilizers based on soil test values recorded similar grain yields in paddy as that of farmers practice and there was a net savings in the cost of P fertilizers applied per hectare to the extent of Rs.1448/-.

References

Kimetu, M., Mugendi, D.N., Palm, C.A., Mutuo, P.K., Gachengo, C.N., Nandwa, S. and Kungu, B. 2004. African network on soil biology and fertility (http://www.ciat.cgiar.org). pp. 207- 224.

Olsen, S. R., Cole C.V., Watanabe F.S. and Dean L.A., 1954. Estimation of available phosphorous in soils by extraction with sodium bicarbonate. USDA Circular. 1. p. 939.

Prasada Rao, A. and Bhupal Raj. 2001. A text book on Guidelines for soil based technologies to optimize land productivity in Andhra Pradesh.

Reddy, K.C. and Ahmed, S.R. 2000. Soil test based fertilizer recommendation for maize grown in inceptisols of Jagtiyal in Andra Pradesh. *Journal of Indian Society of Soil Science* 48:84-89.

Table 1. Particulars of Farmers along with soil test results of P under Bhoochetana during *rabi* 2011-12

S.No	Village & Mandal	Farmers Name	Crop	Soil test value of P_2O_5 (kg/ ha)	Nutrient recommendation of P (kg/ha)
1	Kothuru (V) Kusumanchi (M)	Lodiga Ramaiah	Paddy	65.9 (H)	42.5
2	Kothuru (V) Kusumanchi (M)	Lodiga Venkata Ramana	Paddy	65.99 (H)	42.5
3	Kothuru (V) Kusumanchi (M)	Banoth Ramu	Paddy	87.12 (H	42.5
4	Paleru (V)Kusumanchi (M)	Nukala Rangareddy	Paddy	63.75 (H)	42.5
5	Paleru (V)Kusumanchi (M)	Bajjuri Venkata Reddy	Paddy	70.12 (H)	42.5
6	Singareddy Palem (V) Nelakondapalli (M)	Pagidikathula Ramu	Paddy	71.22 (H)	42.5
7	ThirumalaPuram (V) Nelakondapalli (M)	Banoth Balaji	Paddy	139.7 (H)	42.5
8	Guvalagudem (V) Nelakondapalli (M)	B. Venkateswarulu	Paddy	76.5(H)	42.5
9	Ammagudem (V) Nelakondapalli (M)	AdapalaVenkata Ramana	Paddy	74.4(H)	42.5
10	Medepalli (V) Mudigonda (M)	S. Pramila	Paddy	51.0 (M)	60.0
11	Kamalapuram (V)Mudigonda (M)	D. Ranga Reddy	Paddy	51.0 (M)	60.0
12	Bhanapuram (V) Mudigonda (M)	Y. Upendar	Paddy	115.0 (H)	42.5
13	Kakarlapalli (V) Sathupalli (M)	B. Rambabu	Paddy	49.0 (M)	60.0
14	Ayyagaripeta (V) Sathupalli (M)	N. Prasada Rao	Paddy	49.0 (M)	60.0
15	Rejarla (V) Sathupalli (M)	K. Himakar Reddy	Paddy	157.25(H)	42.5
16	Yerraboinapalli (V) Kalluru (M)	A. Ramarao	Paddy	79.0 (H)	42.5
17	Yerraboinapalli (V) Kalluru (M)	P. Venkata Krishna Rao	Paddy	91.12 (H)	42.5

Table 2. Economics of treatments (mean)

Particulars (per hectare)	T_1 (Farmers practice)	T_2 (soil test based P recommendation)
Cost of cultivation	35625/-	31500/-
Yield	6115	6150
Gross Returns(Rs.)	67875/-	68265/-
Net Returns(Rs.)	32250/-	33878/-
BC Ratio	1.90:1	2.17:1

Table 3. Particulars on cost of "P"fertililzers applied and Yield obtained under Bhoochetana during *rabi* 2011 - 12

S. No	Farmers Name	Quantity of P applied in T_1 (Farmers practice) (Kg/ha)	Quantity of P applied in T_2 (soil test based P recommendation) (Kg/ha)	Cost of P fertilizers applied in T_1 (Rs/ha)	Cost of P fertilizers applied in T_2 (Rs/ha)	Net savings in the Cost of P fertilizers applied in T_2 (Rs/ha)	Grain Yield in T_1(Farmers practice) (Kg/ha)	Grain Yield in T_2 (soil test based P recommendation) (Kg/ha)
1	Lodiga Ramaiah	55	42.5	3000	2670	330	5873	5971
2	Lodiga Venkata Ramana	55	42.5	3000	2670	330	5888	5995
3	Banoth Ramu	60	42.5	4300	1738	2562	6000	6100
4	Nukala Rangareddy	55	42.5	3000	1165	1835	7280	7405
5	Bajjuri Venkata Reddy	86.25	42.5	3525	1738	1787	6975	7042
6	Pagidikathula Ramu	47.5	42.5	3050	1165	1885	7320	7375
7	Banoth Balaji	60	42.5	2195	1738	457	7560	7500
8	B. Venkateswarulu	57.5	42.5	2350	1738	612	4660	4610
9	Adapala Venkata Ramana	82.5	42.5	4200	1738	2462	5813	5895
10	S. Pramila	82.5	60	4200	2445	1755	6648	6560
11	D. Ranga Reddy	57.5	60	3275	2445	830	6760	6728
12	Y. Upendar	82.5	42.5	4200	1738	2462	4820	4875
13	B. Rambabu	88.75	60	4350	2445	1905	4700	4675
14	N. Prasada Rao	68.75	60	3800	2445	1355	6440	6478
15	K. Himakar Reddy	82.5	42.5	4200	1738	2462	5200	5158
16	A. Ramarao	46.25	42.5	3750	1738	2012	6100	6188
17	P. Venkata Krishna Rao	58.75	42.5	2000	1738	262	5925	6000
			Mean	3435	1947	1488	6115.41	6150.29

Average (D): 1488.41 average (D^2): 2883173
t(test value):2.11 (table value):2.12 (n=17,significant at 5% level)

Average (D): 35.71 average (D^2): 5846.18
t (test value): 7.29 (table value):2 (n=17, non significant at 5% level)

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Yield and Water Productivity of Aerobic Rice (*Oryza sativa* L.) as Influenced by Dates of Sowing and Varieties during *kharif* season

B Balaji Naik*, D Raji Reddy, G Sreenivas and P Leela Rani

Agriculture Research Station, Madhira, Prof. Jayashankar Telangana State Agricultural University, Telangana

*Corresponding author: balajinaikbanoth789@gmail.com

Received: 13th April, 2015; Accepted: 24th June, 2015

Abstract

An experiment was carried out to study the effect of dates of sowing on yield and water productivity of different duration rice varieties with five sowing dates and four varieties under aerobic situation in two consecutive *kharif* seasons during 2012 and 2013 at Agricultural Research Station, Madhira in Telangana State. The higher grain yield and water productivity was realised from the crop sown on 18 June and 7 July. Among the varieties, the long and medium duration cultivars *viz.,* MTU 1061 and JGL 11470 recorded higher yield but the short and extra short duration cultivars *viz*., MTU 1010 and JGL 17004 were proved to be good in terms of higher water productivity due to less water requirement.

Key words: Aerobic rice, dates of sowing, varieties, grain yield, water productivity.

Introduction

Rice is the staple food for nearly half of the world's population. In Asia, more than 90 per cent of rice is produced catering the needs of nearly 560 million hungry people (Mohanty, 2013). Globally, India stands first in rice area and second in production after China. More than 80 per cent of the developed fresh water resources in Asia are used for irrigation purposes and consumes up to 43 per cent of the world's developed irrigation resources (Bouman et al., 2007). About 22 M ha of irrigated dry season rice experience economic water scarcity in South and South-East Asia (Tuong and Bouman, 2002). The availability of water for agriculture is declining steadily due to urbanization and rapid increase in population (Xue et al., 2008). The common method of rice cultivation is transplanting and water requirement of this crop is ranged from 1000 to 1500 mm and besides this, raising nursery is very laborious and time consuming. To address these problems, growing rice under aerobic conditions is evolved. Aerobic rice could successfully be cultivated with 600 to 700 mm of total water in summer and entirely on rainfall in wet season (Hittalmani, 2007 a and b). The exact sowing date for direct seeding of rice also plays a vital role in improving its growth, yield and water productivity. Further, most of our cultivars are developed for puddled transplanted conditions. In the absence of a strong rice improvement programme for dry seeded rice, suitable varieties for aerobic system are generally not available. Therefore, there is a need to identify suitable cultivars among existing varieties developed under transplanted conditions. Considering the above facts, the present investigation was carried out to identify optimum time of sowing and suitable varieties under aerobic condition to realise higher yield and water productivity.

Material and Methods

A field experiment was conducted during *kharif* 2012 and 2013 at Agricultural Research Station, Madhira (at an altitude of 189 m above mean sea level at 16°53' and 80°22' E and 189 m), Telangana State. The location of the site is semi arid tropics. During the crop growth season, the mean maximum and minimum temperatures ranged from 29.5 to 36.9°C with an average of 32.4°C in 2012 and 28.9 to 36.1°C with an average of 32.3°C in 2013, respectively, and the rainfall 1086.3 mm was received in 55 rainy days during first year and 937.8 mm in 47 rainy days during second year, respectively. The experimental soils are clayey with pH 8.2, low in organic carbon (0.52%), available nitrogen (144 kg/ha) and available phosphorus (18 kg/ha) and high in available potassium (398.5 kg/ ha). The experiment was conducted in split plot design with 5 sowing dates as main plots *viz.,*18 Jun. (D_1), 7Jul. (D_2), 20 Jul. (D_3), 4 Aug. (D_4) and 18 Aug. (D_5) and 4 varieties as sub plots *viz.,* JGL 17004 (105 days), MTU 1010 (120 days), JGL 11470 (135 days) and MTU 1061 (160 days), replicated thrice. The net size of each plot was 16 m^2 (4.0 × 4.0 m). Crop was sown at 20 cm apart in solid rows with seed rate of 40 kg ha^{-1}.

One-fourth of the recommended dose of nitrogen (120 kg/ha) and full dose of phosphorus (60 kg/ha) half potash (30 kg/ha) and zinc sulphate (25 kg/ha) were applied at the time of sowing as basal and the remaining nitrogen was top dressed in three equal splits at 15 days after sowing, active tillering (30-35), and at panicle initiation stage, respectively. The remaining half of the potassium was applied at panicle initiation stage. For effective weed control, Pendimethalin (1 kg *a.i.*/ha) was used in moist condition at evening hours in all the treatments just after sowing of rice. Bispyribacsodium @ 25.0 a.i/ha was applied as post emergence spray at 2 to 3 leaf stage of the weeds. One hand weeding was done at 35 to 40 days after sowing to reduce the competition between weeds and crop for nutrients and spaces. Two sprayings of Fe SO_4 was done at weekly interval at 20 to 25 DAS to correct iron deficiency in the crop. Irrigations were given at weekly interval (5 cm) measuring through water meter. Soil samples from every 15 cm depth up to 60 cm were collected prior to each irrigation and after rainfall events and soil moisture % estimated through gravimetric method. The effective rainfall was estimated through soil moisture balance method. The crop water productivity was estimated as ratio of rice yield (kg) to total water used (effective rainfall+ irrigation water applied) to crop in the season.

Results and Discussion

Grain yield

The results obtained from dates of sowing and varieties under aerobic situation was presented in Table 1 and depicted in Fig. 1 and 2. Significantly more grain yield (5422 kg/ha and 4944 kg/ha) was realized from the crop sown on 18 Jun. (D1) and was comparable with grain yield (5254 and 4893 kg/ha) of 7 Jul. (D2) sown crop and thereafter reduction in grain yield was noticed with every successive 15 days delay in sowing from 20 Jul. (D3) to 18 Aug. (D5) during 2012 and 2013, respectively. These results were in conformity with the results of Rai and Kushwaha (2008) who reported that, 15.3 per cent more grain yield of aerobic rice was obtained from 15 Jun. sown crop when compared to late sowing of 15 Jul. which might be due to optimum period available for growth and development resulted in more storage of photosynthates in the grain in early sown crop.

Among the varieties tested, the long duration variety MTU 1061 (V_4) produced more grain yield (5547 kg/ha and 5132 kg/ha) and was on par with the medium duration variety JGL 11470 (V_3), which in turn significantly superior to short (MTU 1010) and extra short (JGL 17004) duration varieties during 2012 and 2013, respectively. These results are in line with the findings of Patra *et al.* (2008) and Gopal (2008) who reported that, the grain yields in short and medium duration varieties were lower than long duration varieties.

Water productivity

The data obtained on effective rainfall, total water use and water productivity of rice varieties sown under different dates during 2012 and 2013 were presented in Table 1 and depicted in Fig.1 and 2. The crop sown on 18 June (D_1) received more effective rainfall of 318mm and 236 mm and it was decreased linearly with every successive 15 days delay in sowing during 2012 and 2013, respectively. Among the varieties the highest amount of effective rainfall (262 mm and 229 mm) was received by the long duration variety during 2012 and 2013, respectively. The medium, short and extra short duration varieties ranked 2nd, 3rd and 4th respectively in terms of quantity of effectively rainfall received by them during both the years under study. The number of irrigations and total water used increased with every successive 15 delay in sowing from 18 June to 18 August in 2012 and in 2013. However, the more amount of total water (759 mm) was used by 4 August sown crop due to prolonged dry spell experienced by the crop in the month of September in 2013. Among the varieties, the total water consumption (712 mm and 849 mm) was high in long duration variety MTU 1061 (V4) and lowest total water (481 mm and 592 mm) was used by the extra short duration variety JGL 17004 (V1) in both the years. This differential response in total water use among the varieties was due to difference in the length of crop growth period.

The water productivity of the aerobic rice was higher (1.04 kg/m^3 and 0.73 kg/m^3) with 18 June sown crop and decreased with every successive 15 days delay in sowing in both the years. Among the varieties, the short duration cultivar MTU 1010 (V_2) recorded the highest water productivity of 0.96 and 0.72 kg/m^3 in 2012 and 2013, respectively, followed by the extra short duration variety JGL 17004 (V_1) which produced 0.84 kg/m^3 in 2012. Whereas, in 2013, the medium duration variety JGL 11470 (V_3) produced 0.66 kg grain yield/m^3 of water. The lowest water productivity (0.78 and 0.61 kg/m3) was recorded by the long duration variety MTU 1061 (V_4) in both the years. James *et al.* (2007) evidently claimed that, the water productivity varied among the varieties depending upon their field duration. The variety PMK 3 with duration of 137 days registered the highest water productivity of 7.06 kg rice per ha mm of water. While Ponni, which matured in 184 days, recorded the lowest water productivity of 1.5kg of rice per ha mm of water.

Conclusions

More grain yield and higher water productivity can be obtained under assured irrigated conditions from the crop sown with onset of monsoon season. Among the varieties, long and medium duration varieties produce more grain yield. However, in terms of higher water productivity, short and extra short duration cultivars would be the better option under aerobic system which has lesser crop growth period.

References

Bouman, B.A.M., Humphereys, E., Tuong, T.P. and Barker, R. 2007. Rice and water. *Advances in Agronomy* 92: 187-237.

Gopal, R. 2008. Zero tillage rice establishment and crop weed dynamics in rice and wheat cropping systems in India and Australia *Project Annual Report* p 27.

Hittalmani, S. 2007a. Aerobic rice cultivation Brochure, MAS lab, Univ. Agric. Sci., GKVK, Bangalore.

Hittalmani, S. 2007b. A new aerobic rice variety for water scarce situation. Aerobic rice cultivation Brochure, MAS lab, Univ. Agric. Sci., GKVK, Bangalore.

James, G., Martin, P.K., Padmanathan and Subramanian, E. 2007. Identification on suitable rice variety adaptability to aerobic irrigation. *Journal of Agricultural and Biological Science* 2:1-3

Mohanty, S. 2013. Trends in global rice consumption. *Rice Today* 44-45.

Patra, P.S., Sonowal, M. and Biswas, S. 2008. Studies on tillering habit and yield of some *kharif* rice cultivars as influenced by transplanting date under terai region of West Bengal. *Journal of Crop and Weed* 4: 7-9.

Rai, H.K. and Kushwaha, H.S. 2008. Effect of planting dates and soil water regimes on growth and yield of upland rice. *Oryza* 45: 129-132.

Tuong, T.P. and Bouman, B.A.M. 2002. Rice production in water scarce environments. *In: proceedings of the water productivity workshop,* 12-14 Nov.2001, International Water Management Institute, Sri Lanka.

Xue, C., Yang, X., Bouman, B.A.M., Deng, W., Zhang, Q., Yan, W., Zhang, T., Rouzi, A. and Wang, H. 2008. Optimizing yield, water requirements, and water productivity of aerobic rice for the North China Plain. *Irrigation Science* 26: 459–474.

Table 1. Grain yield, total water use and water productivity as influenced by dates of sowing and varieties under aerobic culture

Treatment	Effective RF (mm)		Irrigation (mm)		Total water Use (mm)		Grain yield (kg ha^{-1})		Water productivity (kg m^{-3})	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Dates of sowing										
D1- 18 Jun	318	236	213	450	530	686	5422	4944	1.04	0.73
D2- 07 Jul	286	238	263	450	548	688	5254	4893	0.98	0.72
D3- 20 Jul	274	209	338	525	612	734	5005	4754	0.82	0.66
D4- 04 Aug	220	209	425	550	645	759	4769	4377	0.74	0.58
D5- 18 Aug	168	170	488	550	655	720	4573	4257	0.70	0.59
S.Em±							65	48		
CD (p=0.05)							211	156		
Varieties										
V1- JGL 17004	241	192	240	400	481	592	3946	3752	0.84	0.64
V2- MTU 1010	251	204	290	450	541	654	5066	4632	0.96	0.72
V3- JGL 11470	258	223	400	550	658	773	5459	5063	0.84	0.66
V4- MTU 1061	262	229	450	620	712	849	5546	5132	0.78	0.61
S.Em±							63	66		
CD (p=0.05)							183	190		
Interaction							NS	NS		

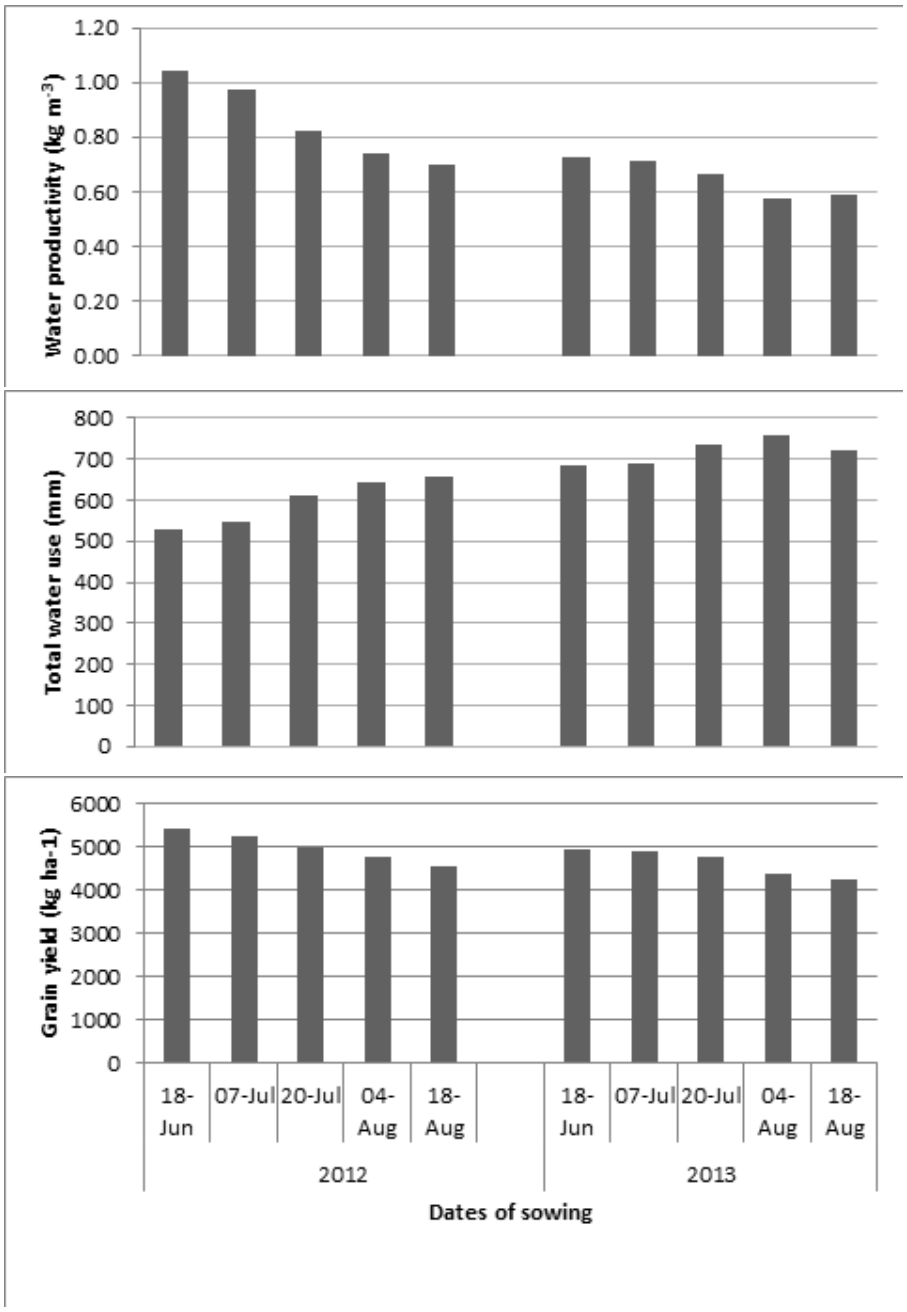


Fig. 1. Grain yield, total water use and water productivity of aerobic rice as influenced by dates of sowing

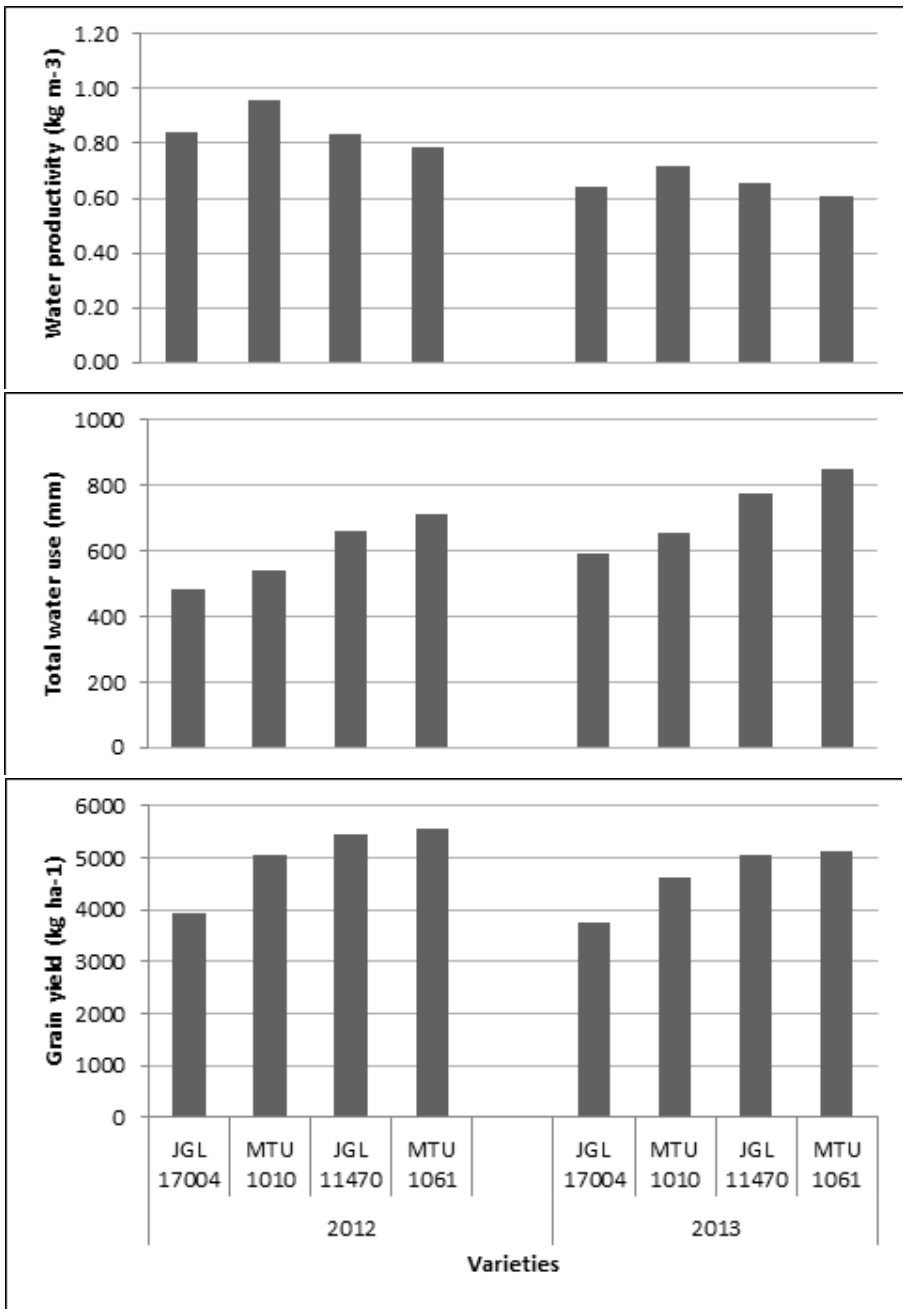


Fig. 2. Grain yield, total water use and water productivity of rice varieties under aerobic culture

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Long Term Fertilization Effect on Soil Organic Carbon and Productivity of Rice Crop under Rice-Rice Cropping System in Godavari Delta, India

Ch.Sreenivas[1]*, D. Srinivas[1], T.V. Sridar[1], K. Surekha[2], and P.V.Satyanarayana[1]

[1]Andhra Pradesh Rice Research Institute & Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Maruteru, W.G. Dt, Andhra Pradesh

[2]Indian Institute of Rice Research, Hyderabad

*Corresponding author: csvasu@yahoo.com

Received: 6th October, 2014 Accepted: 25th May, 2015

Abstract

The effect of long term fertilization with organic and inorganic sources of nutrition on the grain yield and organic carbon content in post harvest soil under rice-rice system in alluvial soils was studied for 22 years during *kharif* and 21 years for *rabi*. Application of 100% NPKZnS + FYM @ 5 t ha^{-1} recorded highest grain yield and soil organic carbon content. 100% NPKZnS produced on par for grain yield production and lower soil organic carbon than 100% NPKZnS + FYM @ 5 t ha^{-1}. Nitrogen substitution with FYM/ GM performed lower than 100% NPKZnS treatment in grain yield production. However nitrogen substitution with FYM/ GM performed superior than 100% NPKZnS in increasing organic carbon content. Between GM and FYM, FYM performed better than GM during *kharif*. Application of FYM@10tha^{-1} alone was found on par with 100%NPKZnS in grain production during *kharif* only but registered higher organic carbon content.

Key words: Rice-rice system, long term effect, organics, inorganics, balanced fertilization, N- substitution, grain yield, organic carbon content

Introduction

Continuous use of high level of chemical fertilizers had lead to soil degradation problems, which also proved detrimental to soil health. A declining trend in the productivity of rice even when grown under adequate application of N, P and K was reported by Nambiar and Abrol (1989). Soil fertility and productivity in Godavari delta are likely to be affected due to intensive rice monoculture with imbalanced fertilization under excessive use of irrigation water. Earlier results of long term fertility management at Maruteru indicated a significant improvement in grain and straw yields due to combined application of organic and inorganic treatments (Anonymous, 2005). Therefore, the present investigation was undertaken to explore the effect of organic and inorganic nutrition on soil fertility and productivity.

Materials and Methods

Experimental site A field experiment on long term soil fertility management in rice- rice system was being assessed over the years (initiated during 1989) on Godavari alluvials (Vertic chromusterts) at Maruteru. The data for *kharif* and *rabi* seasons were pooled (1989 to 2011) and analysed. The soil was clay- loam in texture and with pH of 6.3, EC of 0.79 dSm^{-1}, organic carbon (0.72%), available N (249 kg ha^{-1}), Olsens P_2O_5 (26.9 kg ha^{-1}) and ammonium acetate extractable K_2O (270 Kg ha^{-1}).

Treatmental Details The experiment was laid in randomized block design consisting of 16 treatments *viz*, T1- Control, T2-100% N, T3-100% NP, T4-100% NPKZnS, T5-100% NPK –Zn, T6-100% NPK – S, T7-100% N+50% PK, T8-50 % NPKZnS, T9-50% NPK+ 50% GM-N, T10-50% NPK + 50% FYM-N, T11-50% NPK + 25% GM-N+25% FYM-N, T12-FYM @ 10 t/ ha, T13-100%PK, T14-100% NPKZnS + FYM @ 5t ha^{-1}, T15-STCR fertilizer recommendation, and T16-50%NPK+Azospirillum, in three replications. Treatment No. 1 to 12 being imposed from 1989, treatment No. 13 was started in 1997, treatment 14 from 2000, T-15 from 2008 and T-16 from 2009 *Kharif* seasons on words. Experiment being conducted since 1989 in the same plots regularly except during *rabi*, 1993 and *Kharif*, 1996 where crop was failed due to floods. Thus so far the experiment was conducted for 22 *kharif* and 21 *rabi* seasons were completed. Nitrogen was applied in three equal splits as basal, at tillering and at panicle initiation stage. Entire phosphorus and potassium were applied as basal. Zinc sulphate @ 50 kg ha^{-1} was applied during *rabi* season except in treatments T-5, while in T-6 zinc oxide was applied to exclude sulphur. In T-6 treatment to exclude sulphur, P is supplied through DAP after duly taking care of its nitrogen content and in the rest of the treatments P

was supplied through SSP only. Before each season, the organic manures were analyzed for the nitrogen content to fix the quantity required as per the treatments. Water was maintained at 2 cm depth during vegetative and 5 cm during reproductive stage of the crop until ripening. The surface soil samples before and harvest of the crop were collected and analysed for the physico-chemical properties

Results and Discussion

I. Long term effect of different Fertilizations on Grain Yield

Kharif crop was grown for 22 years in the same plot from 1989 to 2011 excluding *kharif*, 1996. Out of 22 years till the introduction of NPKZnS + FYM @ 5 tha^{-1} (T-14), the treatment 100% NPKZnS (T-4) performed the best in 7 out of 10 years (except T-6 in 1992, T-5 in 1998 & 1999) (Table 1). After the introduction of NPKZnS + FYM @ 5 tha^{-1} (T-14) treatment during *kharif*, 2000 in the rest of the period (2000 to 2011) NPKZnS + FYM @ 5 tha^{-1} (T-14) performed best except for two years (T-11 in 2000, T-4 in 2001).

Rabi Experiment was conducted for 21 years in the same plot from 1990 to 2011 excluding *rabi*, 1993. Out of 21 years till the introduction of NPKZnS + FYM @ 5 tha^{-1} (T-14) treatment, 100% NPKZnS (T-4) performed the best in 7 out of 10 years (except T-6 in 1991, T-9 in 1996 & 1998). After the introduction of NPKZnS + FYM @ 5 tha^{-1} (T-14) treatment from *rabi*,2001 in the rest of the period (2001 to 2011) NPKZnS + FYM @ 5 tha^{-1} (T-14) performed best except for one year (T-11 in 2002).

a) Influence of levels of Fertilization

Increased level of fertilization increased the grain yield. During *kharif*, at the starting year 100% NPKZnS (T-4) treatment increased yield significantly over control (T-1) and 50% NPKZnS (T-8). However, after 22 years of experimentation, it is revealed that graded levels of fertilization increased the yields significantly at both levels over control. During *rabi*, at the starting year and after 21 years of experimentation, it is revealed that graded levels of fertilization increased the yields significantly at both levels over control. This higher response during *rabi* over *kharif* to the added fertilization can be attributed to higher nutrient dose of fertilization to *rabi* crop than *kharif* season.

b) Individual plant nutrient response (Imbalanced fertilization)

During *Kharif* and *rabi*, after 22 years of experimentation, control performed lower than other fertilization treatments which indicates the response to fertilization (Table 2). Balanced fertilization of NPK recorded the highest response than imbalanced fertilization and sole application of FYM@10tha^{-1}(T-12). This indicated the superiority of balanced fertilization of NPK over imbalanced fertilization and organic sources alone (T-12). FYM @ 10tha^{-1} performance is lower during *rabi* than *kharif*, might be due to applied FYM rate ie 10 tha^{-1} is not meeting the requirement of short duration *rabi* crop or slow release of nutrients from FYM.

c) Nitrogen Substitution with organics

Nitrogen substitution with FYM/ GM performed lower than, 100% NPKZnS (T-4) treatment. During *Kharif*, at the starting year (1989) 100% NPKZnS (T-4), T-10 and T-9 treatment increased yield significantly over control (T-1) [Table 3]. Performance of T-10 was on par with 100% NPKZnS (T-4). However, after 22 years of experimentation, it is revealed that N- substitution with GM or FYM performed lower than 100% NPKZnS (T-4).

During *rabi*, at the starting year (1990) and after 21 years of experimentation, it is revealed that N- substitution with GM or FYM performed lower than 100% NPKZnS (T-4). This lower performance of N-substitution with either GM or FYM might be due to

- Lower P and K content in FYM /GM
- Less mineralization of organic sources under prevailing submerged conditions
- Slow release of nutrients from organic sources

d) Integrated Use of Organics and inorganics

During *Kharif*, at the starting year (1989 for all and 2000 for T-14), sole organics (T-12) performance is on par with control (T-1). Inorganics alone (100% NPKZnS, T-4) performed higher than control (T-1), on par with organics (T-12) and lower than conjunctive use over RDF (100% NPKZnS + FYM/PM @ 5 tha^{-1}, T-14). However, after 22 years of experimentation, it is revealed that 100% NPKZnS + FYM/PM @ 5 tha^{-1} (T-14) and 100%NPKZnS (T-4) are on par and 100% NPKZnS (T-4) and T-12 are on par (Table 4).

During *rabi*, at the starting year (1990) and after 21 years of experimentation, NPKZnS + FYM/PM @ 5 tha^{-1} (T-14)) and 100% NPKZnS (T-4) are on par. T-12 is significantly lower to 100%NPKZnS + FYM/PM @ 5 tha^{-1} (T-14), 100% NPKZnS (T-4) and T-10. This higher performance of T-10 over T-12 signifies the conjunctive use over organics alone. Beena and Balachndran (2002) reported that application

of FYM @ 5 tha^{-1} in addition to recommended dose of fertilizer produced significantly higher grain yield in rice. Similar results were also reported by Jayakrishnakumar *et al.* (1994) and Pandey *et al.* (2001).This might be due to

- Conjunctive use of 50% NPKZnS + 50% N-FYM (T-10) is on par with sole organic (T-12) for *Kharif* season only might be FYM@10tha^{-1} meeting the requirement during *Kharif* season only and for *rabi,* rate of application of FYM is not meeting nutrient requirement of the crop.
- Between 100% NPKZnS (T-4) and 100%NPKZnS + FYM/PM @ 5 tha^{-1}(T-14) though the numerical advantage is noticed for both seasons, it will take some more time for making NPKZnS + FYM @ 5 tha^{-1} (T-14) significantly superior over 100% NPKZnS (T-4).

II. Impact on Soil Organic carbon

a) Influence of levels of Fertilization

Increased level of fertilization increased the grain yield and also resulted in higher residues to soil and hence higher organic carbon content recorded (Table 5). During *kharif*, at the starting year, both 100% NPKZnS (T-4), 50% NPKZnS (T-8) treatments increased yield significantly over control (T-1). However, after 22 years of experimentation, only 100% NPKZnS (T-4) treatment increased yield significantly over control (T-1) and (T-8). It is also revealed that graded levels of fertilization increased the yields and hence higher OC content recorded.

During *rabi*, at the starting year and after 21 years of experimentation, it is revealed that graded levels of fertilization increased the yields significantly at both levels over control. However, OC increased significantly with T-4 only after 21 years of experimentation.

b) Individual plant nutrient response

During *kharif* and *rabi* at the end of reporting period, control and nitrogen alone performed lower to the rest (Table 6). During *kharif* application of NPK performed similar to that FYM, but during *rabi*, FYM recorded higher OC content than NPK. Lower performance of N alone and NP combination during *kharif* and N alone during *rabi* can be attributed to deleterious effects of imbalanced fertilization. This resulted in lower yields and hence less crop residue recycling to soil and hence lower OC was recorded.

c) Nitrogen Substitution with organics

Nitrogen substitution with FYM/ GM performed superior than 100% NPKZnS (T-4) and T-1 treatment. Between T-1 and 100% NPKZnS (T-4), 100% NPKZnS (T-4) recorded significantly higher yield than T-1 and hence higher OC content (Table 7). Between GM and FYM, FYM performed better than GM during Kharif, whereas during rabi FYM and GM performed similarly. Yoshida and Padre (1975) also reported that the organic manures reduced N losses and conserved soil N forming organo-mineral complex, maintained supply of N to rice plant and increased the N uptake in grain.

d) Integrated Use of Organics and inorganics

During *Kharif*, at the starting year (1989 for all, 2000 for T-14) FYM performed better in increasing OC content (Table 8). However, after 22 years of experimentation, it is revealed that organics and inorganics performed at par and found significantly superior over control (T-1).

During *rabi*, at the starting year (1990), 100% NPKZnS + FYM/PM @ 5 tha^{-1} (T-14) performed best followed by T-12, T-10 and T-4. After 21 years of experimentation, conjunctive use (T-14) and sole organic (T-10) were significantly superior to sole inorganic, 100% NPKZnS (T-4) treatment (Table 8). However, which were significantly superior to control (T-1). This higher performance of 100%NPKZnS + FYM/PM @ 5 tha^{-1} (T-14), T-10 and T-12 signifies the conjunctive use. Larsen and Clapp (1984) also observed similar effects on grain yield due to combined application of organic and inorganic treatments.

Conclusions

Thus, long term fertilizer experiment results indicated that

- Application of 100%NPKZnS + FYM @ 5 tha^{-1} (T-14) recorded highest grain yield and organic carbon content. This signifies the conjunctive use of organics and inorganics in increasing grain yield and soil productivity also.
- Application of 100%NPKZnS was found on par with of 100%NPKZnS + FYM/PM @ 5 tha^{-1} in grain production only.
- Application of 100%NPKZnS was found superior to imbalanced fertilization
- Application of FYM@10tha^{-1} was found on par with 100%NPKZnS in grain production during *kharif* only but registered higher organic carbon content.
- N substitution with FYM/ GM recorded lower grain yield than100%NPKZnS but resulted in higher organic carbon content in the soil.

Acknowledgement

Financial and technical help to carry out this work by way of sponsoring the 'All India Coordinated Rice Improvement (AICRIP) Programme on Long term soil fertility management in low land rice soils of Godavari delta under rice-rice cropping system at Andhra Pradesh Rice Research Institute & Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Maruteru, W.G. Dt, Andhra Pradesh (India) by Directorate of Rice Research, Hyderabad, Indian Council of Agricultural Research is duly acknowledged.

References

Annonymous, 2005. Annual report of Andhra Pradesh Rice Research Institute, Maruteru, A.P. pp-120-123

Beena, C. and Balachndran, P.V. 2002. Effect of integrated nutrient management on yield in rice-rice cropping system in the oxisols of Kerala. *Crop Research* 23(3):446-449

Jayakrishnakumar, V., Sundresan Nair, S.M., Sahul Hameed, Tajuddin, E. and Ramchandran Nair, V. 1994. Influence of integrated supply of nitrogen through organic and inorganic sources on grain yield of wetland rice. *Oryza* 31:40-42

Larsen, W.E. and Clapp, C.E. 1984. Inorganic matter and rice, International Rice Research Institute, Philippines, 363-386.

Nambiar, K.K.M. and Abrol, I.P. 1989. Long term fertilizer experiment in Indian over view. *Fertilizer News* 34: 11-20 &26.

Pandey, N., Upadhayay, S.K., Jhoshi, B.S. and Tripathi, R.S. 2001. Integrated use of organic manures and inorganic N fertilizers for the cultivation of lowland rice in vertisol. *Indian Journal of Agricultural Research* 35(20):112-114.

Yoshida, T. and Padre, B.C. Jr.1975. Effect of organic matter application and water regimes on the transformation of fertilizer nitrogen in a Philippine soil. *Soil Science and plant Nutrition* 21:281-292.

Table 1. Influence of levels of Fertilization on grain yield (kg ha^{-1}) under rice - rice cropping system in Godavari Western Delta

Treatment	*Kharif*		*Rabi*	
	1989	22 years mean	1990	21 years mean
Control	2422^{a}	3058^{a}	1565^{a}	1905^{a}
50% NPKZnS	2785^{a}	4187^{b}	2849^{b}	4030^{b}
100%NPKZnS	3422^{b}	5100^{c}	4414^{c}	5419^{c}
CD (0.05%)	449	487	384	592

Table 2. Effect of Imbalanced Fertilization on grain yield (kg ha^{-1}) under rice-rice cropping system in Godavari Western Delta

Treatment	*Kharif*	*Rabi*
Control	3058^{a}	1905^{a}
N	4003^{b}	3849^{c}
NP	4384^{bc}	4628^{d}
PK	3889^{b}	2646^{b}
NPK	5100^{d}	5419^{e}
FYM@10tha^{-1}	4454^{c}	3781^{c}
CD (0.05%)	487	592

Table 3. Effect of Nitrogen Substitution with organics on grain yield (kg ha^{-1}) under rice - rice cropping system in Godavari Western Delta

Treatment	*Kharif*		*Rabi*	
	1989	22 years mean	1990	21 years mean
Control	2422^{a}	3058^{a}	1565^{a}	1905^{a}
50% NPKZnS + 50% GM	2931^{b}	4504^{b}	3537^{b}	4753^{b}
50% NPKZnS + 50% FYM	3168^{bc}	4678^{b}	3575^{b}	4737^{b}
100%NPKZnS	3422^{c}	5100^{c}	4414^{c}	5419^{c}
CD (0.05%)	449	487	384	592

Table 4. Effect of Integrated Use of Organics and inorganics on grain yield (kg ha^{-1}) under rice - rice cropping system in Godavari Western Delta

Treatment	*Kharif*		*Rabi*	
	1989	22 years mean	1990	21 years mean
Control	2422^{a}	3055^{a}	1659^{a}	1973^{a}
50% NPKZnS + 50% N- FYM	3168^{b}	4678^{b}	4350^{c}	4793^{c}
FYM@10tha^{-1}	2749^{ab}	4454^{b}	3396^{b}	3781^{b}
100%NPKZnS	3422^{b}	5100^{bc}	5821^{d}	5515^{d}
100%NPKZnS + FYM@5tha^{-1}	3979^{c}	5373^{c}	6002^{d}	6006^{d}
CD (0.05%)	449	487	384	592

Table 5. Influence of levels of Fertilization on soil organic carbon content (%) under rice - rice cropping system in Godavari Western Delta

Treatment	*Kharif*		*Rabi*	
	1989	22 years mean	1990	21 years mean
Control	0.35^{a}	0.97^{a}	0.555^{a}	0.76^{a}
50% NPKZnS	0.60^{b}	1.17^{a}	0.565^{a}	0.79^{a}
100%NPKZnS	0.55^{b}	1.24^{b}	0.575^{a}	1.13^{b}
CD (0.05%)	0.168	0.238	0.073	0.182

Table 6. Effect of Imbalanced Fertilization for 22 years on soil organic carbon content (%) under rice - rice cropping system in Godavari Western Delta

Treatment	*Kharif*	*Rabi*
Control	0.97^{a}	0.97^{a}
N	1.16^{a}	1.12^{a}
NP	1.19^{ab}	1.18^{b}
PK	1.22^{b}	1.23^{b}
NPK	1.24^{b}	1.22^{b}
FYM	1.45^{b}	1.47^{c}
CD (0.05%)	0.23^{8}	0.182

Table 7. Effect of Nitrogen Substitution with organics on soil organic carbon content (%) under rice - rice cropping system in Godavari Western Delta

Treatment	*Kharif*		*Rabi*	
	1989	22 years mean	1990	21 years mean
Control	0.35[a]	0.97[a]	0.555[a]	0.97[a]
50% NPKZnS + 50% N-GM	0.89[c]	1.34[b]	0.920[b]	1.35[bc]
50% NPKZnS + 50% N-FYM	1.12[d]	1.49[c]	0.915[b]	1.42[c]
100%NPKZnS	0.55[b]	1.24[b]	0.575[a]	1.22[b]
CD (0.05%)	0.168	0.238	0.0733	0.182

Table 8. Effect of Integrated Use of Organics and inorganics on soil organic carbon content (%) under rice - rice cropping system in Godavari Western Delta

Treatment	*Kharif*		*Rabi*	
	1989	22 years mean	1990	21 years mean
Control	0.35[a]	0.97[a]	0.555[a]	0.97[a]
50% NPKZnS + 50% N-FYM	1.12[c]	1.49[b]	0.915[b]	1.42[c]
FYM@10tha^{-1}	1.25[c]	1.46[b]	0.985[c]	1.47[c]
100%NPKZnS	0.55[b]	1.24[b]	0.575[a]	1.22[b]
100%NPKZnS + FYM@5tha^{-1}	1.26[c]	1.43[b]	1.20[d]	1.59[c]
CD (0.05%)	0.168	0.238	0.0733	0.182

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Sequence Divergence of Coat Protein Gene among Indian and non-Indian Isolates of *Rice Tungro Bacilliform Virus*

P. Malathi, D. Krishnaveni and Satendra K Mangrauthia*

ICAR-Indian Institute of Rice research (IIRR), Rajendranagar, Hyderabad

*Corresponding author: skmdrr@gmail.com

Received: 8th March, 2015; Accepted: 27th May, 2015

Abstract

Rice tungro disease (RTD), one of the major constraints to rice production in South and Southeast Asia, is caused by combined action of two viruses: *Rice tungro spherical virus* (RTSV) and *Rice tungro bacilliform virus* (RTBV). The present study was undertaken to describe the sequence divergence and evolution of RTBV isolates present in India and other countries. Phylogenetic analysis based on coat protein (CP) sequences of RTBV generated in this study showed distinct divergence of Indian and non-Indian RTBV isolates into two clusters. Further, Indian RTBV isolates formed two groups- one consisted isolates from Andhra Pradesh and Kanyakumari, and other included isolates from Hyderabad, Punjab, and West Bengal. The results obtained from phylogenetic analysis were further supported with the single nucleotide polymorphisms (SNPs), insertion and deletions (INDELs) and evolutionary distance analysis. Signature sequences and amino acid motifs were identified which showed distinct difference between Indian and non-Indian isolates. This study will help in understanding the geographical evolution and adaptation of RTBV in different rice ecosystems.

Keywords: RTBV, CP, Phylogeny, Diversity, *Oryza sativa*

Introduction

Rice tungro disease (RTD) is one of the most important viral diseases of rice in South and Southeast Asia. When plants are infected with RTD in the early seedling stage, yield losses can be as high as 100 percent (Muralidharan *et al*., 2003). The disease is caused by a complex of two viruses; *Rice tungro bacilliform virus* (RTBV), a DNA virus and *Rice tungro spherical virus* (RTSV), an RNA virus (Jones *et al*., 1991). The viral complex is transmitted between plants by the vector Green Leafhopper (GLH), *Nephotettix virescens* (Hibino and Cabauatan, 1987). RTBV is responsible for symptoms development of tungro disease which includes stunting and yellow to orange discoloration of the infected plants (Hibino *et al*., 1978). RTBV is a plant pararetrovirus having ~8.0 Kb circular double-stranded DNA genome with two discontinuities, one on each strand (Hay *et al*., 1991; Qu *et al*., 1991; Hibino *et al*., 1991). It has four open reading frames (ORFs), potentially capable of encoding proteins of 24, 12, 194 and 46 kDa, respectively (Hay *et al*., 1991; Mangrauthia *et al*., 2012a). The longest ORF3 of RTBV encodes a P194 poly protein that contains four functional domains: a putative movement protein (MP), the coat protein (CP), aspartate protease (PR), and reverse transcriptase (RT) with a ribonuclease H activity (Qu *et al*., 1991; Laco *et al*., 1994).

Considering the importance of tungro virus disease from Indian perspective, efforts to understand the RTBV population structure present in India have been very limited. Recently, we studied the molecular diversity of RTBV by utilizing the DNA sequences generated from ORF-I, II and III (Mangrauthia *et al*., 2012a). It is important to extend this study by comparing the sequences of other important viral proteins such as coat protein, movement protein, and replicase. Coat protein (CP) has been most extensively used in understanding the genetic diversity of viruses due to its crucial role in establishing virus infection and multiplication. The objective of this study was to obtain the nucleotide sequence of the coat protein gene of RTBV, and to assess the sequence diversity and phylogenetic relationship among the RTBV isolates. Further to gain better insight, RTBV isolates were analyzed for single nucleotide polymorphism (SNPs), insertion and deletion (INDELs), evolutionary distance, and Ka/Ks ratio.

Materials and Methods

Virus isolates

Rice plants showing symptoms of tungro disease were collected from Hyderabad (ICAR-IIRR, glasshouse). Virus infected plants were maintained through insect (green leaf hopper)-mediated transmission in separate cages in glasshouse to prevent the cross contamination.

Gene amplification

The total DNA from virus-infected rice leaves was isolated by CTAB method (Murray and Thompson, 1980). Sequences of the forward and reverse primers were designed based on the sequence of RTBV genomes available in NCBI database. Forward primer (CCAGAAGTATCCTCAAAAGAT) and Reverse primer (CTCAGGAAGTCTGTCAAATAG) were used to amplify the sequence coding for coat protein sequence of RTBV. The thermal profile used for these primers was 94ºC for 5 min (initial denaturation), 94ºC for 30s (denaturation), 56ºC for 30s (annealing), 72ºC for 1 min (extension), and 72ºC for 10 min (final extension). The amplification reaction was performed in a reaction mixture containing dNTP mix, primers, 10X PCR buffer with 15 mM $MgCl_2$ and recombinant *Taq* DNA polymerase. Resulting PCR products were analyzed on 1 % agarose gel followed by staining with ethidium bromide.

Cloning and nucleotide sequencing

PCR amplified DNA was cloned into pGEM-T (Promega Corporation, Madison, WI) cloning vector using TA cloning strategy. The PCR product was ligated into pGEM-T vector and transformed into competent *E. coli* strain DH5α using heat shock method. Plasmid DNA from potential recombinants (identified through blue-white screening of bacterial colonies and colony PCR) were purified by Wizard plus SV mini preps DNA purification system (Promega) following manufacturer's instructions. Plasmid isolated from these clones was subjected to PCR confirmation with sequence-specific primers and restriction enzyme digestion. Clones containing desired fragment of ~500 bps corresponding to the CP coding region of RTBV were selected for sequencing. Two clones were sequenced in both the directions to eliminate potential sequence heterogeneity introduced by *Taq* DNA polymerase.

Sequence analysis

Nucleotides as well as translated amino acid sequences representing the complete coat protein gene were compared with the already available sequences of RTBV isolates (Table 1). Sequences were aligned using CLUSTAL W programme. Neighbor-joining tree was generated using CLUSTAL X (Thompson *et al*., 1997). The robustness of the lineages in the phylogenetic tree was assessed from the internodes length in the tree by bootstrapping in CLUSTAL X using 1000 resampling. Sequence identity matrix and sequence difference count matrix was calculated using BioEdit sequence alignment editor version 5.09.04 (Hall, 1999). The evolutionary distance was analyzed by using the bioinformatics tool MEGA version 5 (Tamura *et al*., 2011). SNPs and INDELs were calculated by using DnaSP (version 5.10) by comparing the nucleotide sequence with the Hyderabad isolate as reference sequence. Ka/Ks value was also calculated by using the same tool to analyze the synonymous and non-synonymous mutations at the nucleotide level, which really affect the amino acid sequences of the protein. Protein level changes were analyzed by comparing the deduced amino acid sequences using BioEdit–CLUSTAL W tool.

Results and Discussion

Coat protein gene sequence of RTBV from Hyderabad isolate was deciphered. The complete CP gene was found to be 528 bps long coding for 176 amino acid capsid protein in all isolates tested here. Cluster dendrogram based on the nucleotide sequence of the CP gene and the deduced amino acid sequences were similar and only the phylogenetic tree based on the deduced amino acid sequence is shown (Fig. 1). A distinct divergence of CP of RTBV isolates into two clusters was observed, one cluster included all the isolates from India and the other distinct cluster had RTBV isolates from other countries- Japan, Mal (Malaysia), Phil (Philippines) and Thai (Thailand). Cluster containing all Indian isolates was further divided into two groups; one group consisted isolates from Punjab, WB (West Bengal), CWB (Chinsura, West Bengal) and Hyd (Hyderabad) and another group consisted isolates from AP (Andhra Pradesh) and KK (Kanyakumari).

In order to analyze the polymorphism at sequence level, the CP sequences of RTBV were analyzed for the presence of SNPs and INDELs. All the sequences were compared with Hyderabad-CP sequences (reference sequence), as the analysis was done here and polymorphic data was stored for further analysis. At CP sequence level, WB, KK and AP isolates showed very close similarity with the reference sequence (Hyderabad). These sequences had very less deviation from the reference sequence Hyderabad-CP, as it had 0.044, 0.048 and 0.048 evolutionary distances and 22, 24 and 24 SNPs, respectively. The next closest sequences were from Punjab and CWB with evolutionary distance of 0.049 and 0.053, and 24 and 26 SNPs, respectively, when compared with the reference sequence. The farthest sequences were from Japan, Malaysia, Philippines and Thailand with evolutionary distance of 0.168, 0.171, 0.171 and 0.201, respectively. These non-Indian isolates showed 78, 80, 79 and 90 SNPs, respectively, when compared with the Hyderabad CP sequence. INDELs were not observed in any of the sequences from either Indian isolates or non-Indian isolates (Table 2). Ka/Ks value was calculated

to understand the nucleotide change, which affect the amino acid sequence of the protein. The ratios of non-synonymous to synonymous nucleotide substitution rates (Ka/Ks) ranged from 0.0098 to 0.3863. Malaysian isolate had the highest Ka/Ks value of 0.3863, which indicated the mutations in the nucleotide level as well as protein level (Table 2). Indian isolates showed less similarity with non-Indian isolates both at nucleotide (83–87 %) and amino acid level (89–93 %) of CP gene. Within the Indian origin isolates, 94–100 % similarity at nucleotide level and 97–100 % similarity at amino acid level was recorded. Further, the sequence similarity matrix among the Indian isolates revealed 95% (Hyderasbad *Vs*. KK/Punjab/CWB/AP) to ~100% similarity (KK vs. AP) based on nucleotides. Further, 97% (CWB Vs. Hyderabad/ WB) to ~100% similarity (KK Vs. Punjab/AP and Punjab Vs. AP) based on amino acid sequences was observed (Table 3).

CLUSTAL W multiple alignment of amino acid sequences derived from CP gene revealed some of the differential amino acid sequence motifs which were unique to the Indian or non-Indian isolates. For instance, sequence motifs EVS^{4} and II^{44} present in all Indian isolates of CP were replaced by IEA^{4} and LV^{44} in non-Indian isolates. In addition to the sequence motifs, a number of individual amino acids were also recorded as signature sequence which clearly distinguished the Indian isolates from non-Indian isolates (Fig. 2).

In this study, CP gene of RTBV from Hyderabad isolate was sequenced and analyzed. The isolate was supposed to be an Indian member due to its geographical habitat (Hyderabad, India). The sequence analysis clearly indicated strong similarity of Hyderabad isolate with other Indian isolates of RTBV. Out of the 12 RTBV sequences analyzed here, six isolates, *i.e.* Hyd, KK, Punjab, CWB, WB and AP from the Indian origin formed a cluster and the remaining six *i.e.* Japan, Malaysia, Philippines, Philippines-Ic, Philippines-G1 and Thailand isolates from non-Indian clustered separately (Fig.1). This was further strengthened by the observations that greater identity at nucleotide and amino acid level existed within Indian isolates when compared with non-Indian isolates. Fan *et al*., (1996) reported the existence of two strains of RTBV, which was supported by Nath *et al*. (2002) providing molecular evidence. In the present investigation, much emphasis was given to analyze the genetic variation of RTBV isolates present within India. Recently, a similar study was performed to understand the genetic variation of RTBV and RTSV isolates present in tungro endemic states of India (Mangrauthia *et al*., 2012a and 2012b). Our results indicated the existence of habitat correlation in the evolution of nucleotide and amino acid sequences of RTBV. The genetic diversity was less for the amino acid than the nucleotide for all CPs (Table 3) which is supported by Ka/Ks ratio. In general, the natural selection pressure in genome and its functional evolution are reflected by the differences at the nucleotide level and due to the principle of codon degeneracy; these are reduced at the amino acid residue level (Gojobori *et al*., 1990). Analysis of SNPs, INDELs and evolutionary distance also showed similar pattern wherein Hyderabad isolate showed minimum evolutionary distance and divergence with Indian isolates. Evolutionary distance, the number of substitutions per site separating a pair of homologous sequences since they diverged from their common ancestral sequence, is an extremely important measure in molecular evolution and comparative genomics (Rosenberg, 2005). The data obtained from phylogeny, sequence identity matrix, SNPs, INDELs, and evolutionary distance suggest the diversion of Indian RTBV isolates into two major cluster; one includes isolates of AP and KK while other includes isolates of Hyd, CWB, WB, and Punjab. The presence of Zero INDELs among RTBV CP sequences and very low Ka/Ks ratio suggests that though these sequences had the mutations, they may not alter the protein structure and hence its function. One way to evaluate the selection pressures on protein evolution is to compare the rate of synonymous and non-synonymous nucleotide substitutions. Ks is the estimated number of synonymous changes per synonymous site and corresponds to the rate of amino acid-neutral evolution. Ka, on the other hand, is the number of non synonymous substitutions per non-synonymous site (Roth and Liberles, 2006). Interestingly, Malaysian isolate had the highest Ka/Ks value for CP- 0.386 indicating the significant mutation in the nucleotide level as well as protein level when compared with Hyderabad isolate. It was suggested that recombination as well as substitutions and insertions or deletions, have played a significant role in the evolution of RTBV variants (Cabauatan *et al*.,1999). The amino acid sequence motifs unique to CP of Indian isolates EVS^{4} and II^{44} differed from non-Indian isolates (IEA^{4} and LV^{44}). In addition to sequence motifs, a number of individual amino acids were also noticed which were unique to Indian or non-Indian isolates. It would be interesting to define the role of these unique motifs and amino acid residues in diversification and evolution of RTBV in Indian and non-Indian geographical locations.

In conclusion, sequence analysis of CP gene revealed that Indian isolates of RTBV diverged into two lineages: one included AP and KK and other includes isolates of Hyderabad, CWB, WB, and Punjab. Besides deciphering the molecular diversity, the divergence of two major groups

(Indian and non-Indian) of RTBV was revealed. Sequence difference count matrix analysis of the RTBV CP gene revealed that Indian isolates are significantly different in its molecular genetic composition from rest of the world. Further, though mutation and recombination has occurred at nucleotide level that altered genetic composition of these isolates, the protein sequence is not much affected. It would be interesting to ascertain the role of individual amino acids and sequence motifs (identified in Indian and non-Indian RTBV CP sequences) in diversification and adaptation of RTBV in different rice ecologies.

Acknowledgement

Authors are highly thankful to the Project Director (IIRR) for his kind support and cooperation.

References:

Cabauatan, P.Q., Melcher, U., Ishikawa, K., Omura, T., Hibino, H., Koganezawa, H. and Azzam, O. 1999. Sequence changes in six variants of *rice tungro bacilliform virus* and their phylogenetic relationships. *Journal of General Virology* 80:2229-2237.

Fan, Z., Dahal, G., Dasgupta, I., Hay, J. and Hull, R. 1996. Variation in the genome of *rice tungro bacilliform virus*: molecular characterization of six isolates. *Journal of General Virology* 77: 847-854.

Gojobori, T., Moriyama, E.N. and Kimura, M. 1990. Molecular clock of viral evolution, and the neutral theory. *Proceedings of National Academy of Science* USA 87: 10015-10018.

Hall, T.A. 1999. Bio Edit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

Hay, J.M., Jones, M.C., Blakebrough, M.L., Dasgupta, I., Davies, J.W. and Hull, R. 1991. An analysis of the sequence of an infectious clone of *rice tungro bacilliform virus*, a plant pararetrovirus. *Nucleic Acids Research* 19:2615-2621.

Hibino, H. and Cabauatan, P.Q. 1987. Infectivity neutralization of rice tungro-associated viruses acquired by vector leafhoppers. *Phytopathology* 77:473-476.

Hibino, H., Ishikawa, K., Omura, T., Cabauatan, P.Q. and Koganezawa, H. 1991. Characterization of *rice tungro bacilliform* and *rice tungro spherical viruses*. *Phytopathology* 81:1130-1132.

Hibino, H., Roechan, M. and Sudarisman, S. 1978. Association of two types of virus particles with penyakit habang (tungro disease) of rice in Indonesia. *Phytopathology* 68: 1412-1416.

Jones, M.C., Gough, K., Dasgupta, I., Subba Rao, B.L., Cliffe, J., Qu R., Shen, P., Kaniewska, M., Blakebrough, M., Davies, J.W., Beachy, R.N. and Hull, R. 1991. Rice tungro disease is caused by a RNA and a DNA virus. *Journal of General Virology* 72: 757-761.

Laco, G.S. and Beachy, R.N. 1994. *Rice tungro bacilliform virus* encodes reverse transcriptase, DNA polymerase, and ribonuclease H activities. *Proceedings of National Academy of Science* USA 91: 2654-2658.

Mangrauthia, S.K., Malathi, P., Agarwal S., Sailaja, B., Singh, J., Ramkumar, G., Krishnaveni, D. and Balachandran, S.M. 2012a. The Molecular Diversity and Evolution of *Rice tungro bacilliform virus* from Indian perspective. *Virus Genes* 45:126-138.

Mangrauthia, S.K., Malathi, P., Agarwal, S., Ramkukmar, G., Krishnaveni, D., Neeraja, C.N., Madhav, MS., Ladhalakshmi, D., Balachandran, S.M. and Viraktamath, B.C. 2012b. Genetic variation of coat protein gene among the isolates of *Rice tungro spherical virus* from tungro-endemic states of the India. *Virus Genes* 44:482- 487.

Muralidharan, K., Krishnaveni, D., Rajarajeshwari, N.V.L. and Prasad, A.S.R. 2003. Tungro epidemic and yield losses in paddy fields in India. *Current science* 85: 1143–1147.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research 8:4321-4325.

Nath, N., Mathur, S. and Dasgupta, I. 2002. Molecular analysis of two complete *rice tungro bacilliform virus* sequences from India. *Archives Virology* 147(6):1173-1187.

Qu, R.D., Bhattacharyya, M., Laco, G.S., De Kochko, A., Suba Rao, B.L., Kaniewska, M.B., Elmer, J.S., Rochester, D.E., Smith, C.E. and Beachy, R.N. 1991. Characterization of the genome of *rice tungro bacilliform virus*: comparison with *Commelina yellow mottle virus* and caulimo viruses. *Virology*185:354-364.

Rosenberg, M.S. 2005. Evolutionary distance estimation and fidelity of pair wise sequence alignment. *BMC Bioinformatics* 6:102.

Roth, C. and Liberles, D.A. 2006. A systematic search for positive selection in higher plants (Embryophytes). *BMC Plant Biology* 6:12.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology Evolution* 28 (10): 2731-9.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. Nucleic Acids Research 24: 4876-82.

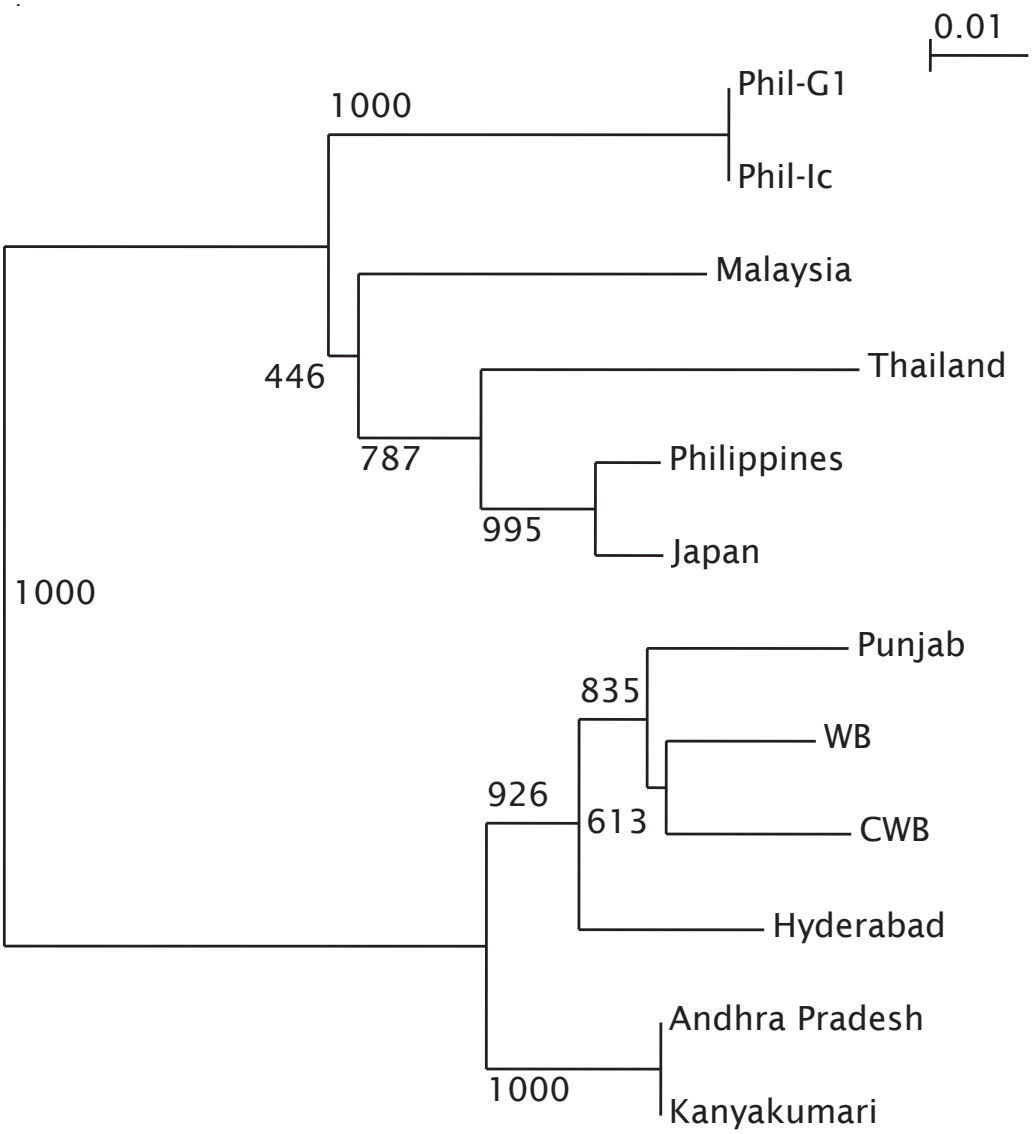


Fig. 1. Neighbor-joining tree based on nucleotide sequences of coat protein of different RTBV isolates. CWB-Chinsura West Bengal, WB-West Bengal

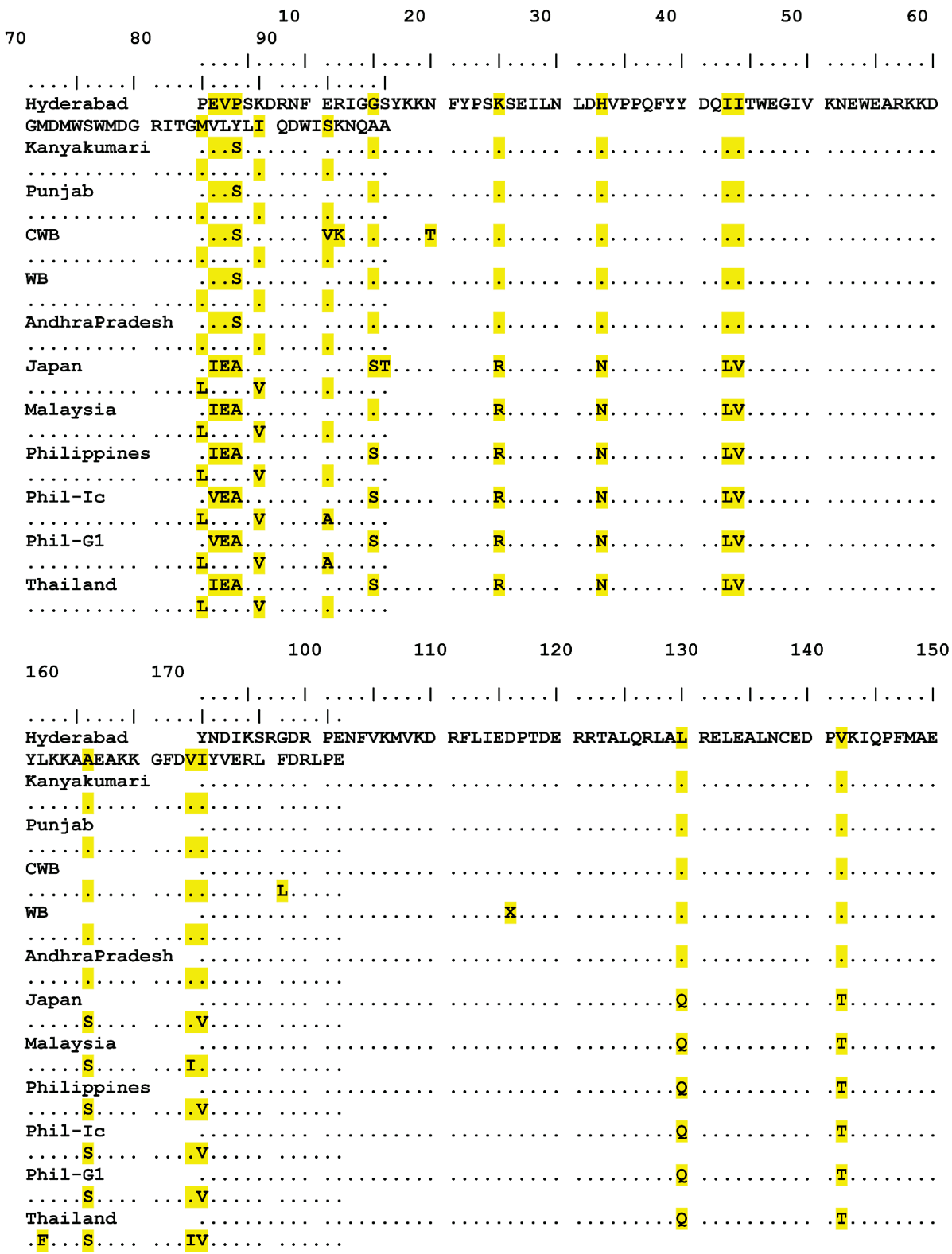


Fig. 2: Amino acid alignment of CP of different RTBV isolates. The CP sequences of Hyderabad, Kanyakumari , Punjab, Chinsura West Bengal (CWB), West Bengal (WB), Andhra Pradesh, Japan, Malaysia, Philippines, Philippines-Ic (Phil-Ic) , Philippines-G1 (Phil-G1) and Thailand isolates were aligned using CLUSTAL W. The motifs and individual amino acids (yellow box) differentiating Indian and non-Indian isolates are highlighted.

Table 1. Sources of coat protein gene sequences of RTBV isolates used in this study for comparison.

S. No.	Origin	Isolates	GenBank accession No.
1	India	Hyderabad	JX644072[a]
2	India	Kanyakumari	HQ385226
3	India	Punjab	JX255736
4	India	Chinsura West Bengal	FN377814
5	India	West Bengal	FN377814
6	India	Andhra Pradesh	AJ292232
7	Japan	Japan	D10774
8	Malaysia	Malaysia	AF076470
9	Philippines	Philippines	NC_001914
10	Philippines	Philippines-Ic	AF113832
11	Philippines	Philippines-G1	AF113830
12	Thailand	Thailand	AF220561

[a] Generated in this study

Table 2. Analysis of evolutionary distance, SNPs, INDELs, and Ka/Ks ratio of coat protein sequence of RTBV isolates

S. No	Name	Evolutionary distance	SE	SNP	INDELs	Ks	Ka	Ka/Ks
1	Hyd	–	–	–	–	–	–	–
2	KK	0.048	0.010	24	0	0.2425	0.0024	0.009896
3	Punjab	0.049	0.010	24	0	0.2412	0.0024	0.009950
4	CWB	0.053	0.010	26	0	0.2173	0.0121	0.055683
5	WB	0.044	0.010	22	0	0.2192	0.0024	0.010948
6	AP	0.048	0.010	24	0	0.2425	0.0024	0.009896
7	Japan	0.168	0.020	78	0	0.9433	0.0469	0.049719
8	Mal	0.171	0.019	80	0	1.0818	0.0418	0.386393
9	Phil	0.171	0.019	79	0	1.0037	0.0444	0.044236
10	Phil-Ic	0.171	0.020	79	0	0.9616	0.0462	0.048044
11	Phil- G1	0.171	0.020	79	0	0.9616	0.0462	0.048044
12	Thai	0.201	0.022	90	0	1.3813	0.0519	0.037573

Hyderabad CP sequence has been taken as reference sequence for this study

Table 3. Nucleotide (Nt) and amino acid (AA) sequence identity matrix showing percent similarity in CP of different RTBV isolates

AA	Nt											
	Hyd	**KK**	**Punjab**	**CWB**	**WB**	**AP**	**Japan**	**Mal**	**Phil**	**Phil- Ic**	**Phil-G1**	**Thai**
Hyd	100	95	95	95	96	95	85	85	85	85	85	83
KK	99	100	94	95	95	100	87	86	87	86	86	84
Punjab	99	100	100	96	96	94	85	84	85	84	84	83
CWB	97	98	98	100	96	95	84	85	85	84	84	83
WB	99	99	99	97	100	95	84	84	84	84	84	83
AP	99	100	100	98	99	100	87	86	87	86	86	84
Japan	91	91	91	89	91	91	100	92	99	94	94	94
Mal	93	93	93	90	92	93	98	100	93	92	92	92
Phil	92	92	92	90	91	92	99	98	100	94	94	95
Phil-Ic	91	91	91	89	91	91	98	97	99	100	100	90
Phil- G1	91	91	91	89	91	91	98	97	99	100	100	90
Thai	91	91	91	89	90	91	98	98	99	98	98	100

Hyd-Hyderabad, KK-Kanyakumari, CWB-Chinsura West Bengal, WB-West Bengal, AP-Andhra Pradesh, Mal-Malaysia, Phil-Philippines, Thai-Thailand

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Compatibility Studies of Insecticide and Fungicide Molecules against Major Pests and Sheath Blight in Rice

K. Karthikeyan

Regional Agricultural Research Station, Kerala Agricultural University, Pattambi.
Corresponding author: entokarthi_65@hotmail.com

Received: 9th February, 2015; Accepted: 28th May, 2015

Abstract

Three insecticide molecules flubendiamide + buprofezin (0.25 ml/lit), triazophos (0.30 ml/lit) and two fungicide molecules hexaconazole (2 ml/lit) and tricylazole (0.60 g/lit) were tested alone as well as in combination with an untreated control against stem borer, whorl maggot, leaffolder and sheath blight during the year 2013-14 at Regional Agricultural Research Station, Pattambi. The pooled results of two crop seasons revealed that flubendiamide + buprofezin followed by combination of triazophos + tricylazole recorded lowest incidence of dead hearts (0.53, 1.07%) and triazophos + tricylazole combination also recorded lowest incidence of white ear (3.90%) and whorl maggot (0.15%). Incidence of whorl maggot was low in all insecticides and in combination treatments. Leaffolder damage was low in flubendiamide + buprofezin and triazophos + tricylazole combination treated plants with 2.08 and 2.75 per cent damaged leaves, respectively. Sheath blight incidence was low in triazophos + tricylazole and tricyclazole treated plots (19.00 and 25.63 %). The grain yield was highest in flubendiamide + buprofezin (3355 kg/ha) followed by flubendiamide + buprofezin + hexaconazole (3268 kg/ha), triazophos + hexaconazole (3143 kg/ha) and triazophos + tricylazole (3116 kg/ha) treated plots.

Key Words: Flubendiamide+Buprofezin, Triazophos, Hexaconazole, Tricyclazole, Stem borer, Whorl maggot, Leaffolder.

Introduction

Rice is an important staple crop of Asia. The losses in rice due to insect pests account at least 20 per cent in India (Pathak *et al.,* 1982). More than 100 insect pests attack rice crop, out of which 20 are major pests (Pathak and Dhaliwal, 1987). Individual effects of insecticides were studied widely but very little information is available for combined effects of pesticides (Singh, 2000). Interactions between different groups of pesticides (fungicides, insecticides and herbicides) can lead to better management of sheath blight of rice (Prakash *et al*., 2013). Keeping this in view, trials were laid out to evaluate the new insecticide and fungicide molecules against major rice pests and sheath blight disease.

Materials and Methods

Two field experiments were conducted at Regional Agricultural Research Station, Pattambi, Kerala Agricultural University during 2013-2014 involving two cropping seasons *viz., kharif* 2013 and *rabi* 2014. Twenty five days old seedlings of variety 'Jyothi' were transplanted in plot size of 7 x 3 m with a spacing of 20 x 15 cm at the rate of two seedlings per hill. The experiment included nine treatments with two insecticides and two fungicide molecules alone as well as in combination with an untreated control. The treatments were replicated four times. The details of treatments and their dosages are given in Table 1. The sprays were made at 30, 50 and 80 DAT with a hand sprayer of 10 litres capacity. The observations were made a day before spraying and a week after spraying on per cent tiller damage (dead heart) at vegetative stage and white ear at reproductive stage for yellow stem borer (*Scirpophaga incertulas* Walker) and per cent damaged leaves in case of whorl maggot (*Hydrellia philipinna* Ferrino), leaffolder (*Cnaphalocrocis medinalis* Guenee) and per cent sheath blight incidence. The grain yield was recorded in kg/ha and the experiments were laid out using completely randomized block design (RBD). The means were compared for significance using CD at 0.05 probability level.

Results and Discussion

Effect on stem borer

The results of the first crop season (*kharif* 13) showed that per cent incidence of yellow stem borer (dead heart) was very low in flubendiamide + buprofezin treated plots with 0.23 per cent followed by triazophos @ 0.3 ml/lit with 0.80 per cent, combination of both insecticides and fungicide *viz*., flubendiamide + buprofezin + hexaconazole, triazophos + hexaconazole, triazophos + tricylazole and flubendiamide + buprofezin + hexaconazole recorded dead

hearts with 1.06, 1.08, 1.54 and 1.83 per cent, respectively, while fungicide treated plots suffered higher dead heart damage (Table 2). For white ear damage, all treatments showed significant reduction over untreated control (Table 2). During the second crop season (*rabi* 2013-14) triazophos + tricylazole suffered low dead heart damage caused by stem borer with 0.60 per cent followed by flubendiamide + buprofezin (0.83%) and flubendiamide + buprofezin + hexaconazole (0.78%) (Table 3). In case of white ear damage, flubendiamide + buprofezin treated plots recorded low incidence of white ear (3.03%) followed by triazophos + tricylazole (4.37%), flubendiamide + buprofezin + tricylazole (4.60%), flubendiamide + buprofezin + hexaconazole (4.71%), respectively, as given in Table 3. The pooled analysis of both the two crop seasons showed that flubendiamide + buprofezin was more effective showing 92.84 per cent reduced dead heart over control followed by triazophos + tricylazole with 85.54 per cent reduced dead heart over control. The incidence of dead hearts was also low in all other combinations of insecticides and fungicides (83.78-81.76%) and white ear incidence was low in insecticides in flubendiamide + buprofezin and triazophos + tricylazole with 49.35 and 49.2 per cent over control and all combinations reduced white ear incidence by 31.56 - 39.35 per cent over control (Table 4). Chlorantraniliprole (0.3 ml / lit) in combination with hexaconazole (2 ml / lit) caused less incidence of stem borer (Bhuvaneswari and Raju, 2013).

Effect on Whorl maggot

The whorl maggot incidence was significantly low in all treatments except control during the first crop *kharif* 2013 (Table 2). Similar results were obtained in second crop season also with low incidence of whorl maggot in all treatments (Table 3). The pooled data of both the crop seasons also showed that all insecticides and their combination with fungicides reduced whorlmaggot incidence from 28. 34 to 34.74 per cent over control as in Table 4.

Effect on Leaf folder

The leaf folder incidence during *kharif* 2013 was low in flubendiamide + buprofezin treated plots (4.15 %) followed by triazophos + tricyclazole and triazophos + hexaconzole treated plots with 5.12 and 5.93 per cent, respectively (Table 2). During second crop season, flubendiamide + buprofezin treated plots showed nil incidence of leaffolder followed by triazophos + tricyclazole (0.37%), flubendiamide + buprofezin + tricylazole (0.63%), triazophos (0.69%) and triazophos + hexaconazole (0.71%), respectively (Table 3). The pooled analysis of both the crop seasons showed that flubendiamide + buprofezin treated plots reduced incidence of leaffolder by 64.32 per cent over control followed by triazophos + tricyclazole and triazophos + hexaconazole (3.32%) flubendiamide + buprofezin + hexaconazole causing 52.83 and 41.17 per cent over control (Table 4). Studies corroborates with Prajapati *et al.,* (2005) who reported that triazophos in compatible with carbendazim and tricylazole was found effective against leaf folder. Raju *et al*., 1988 reported that combined spraying of monocrotophos with fungicides edifenphos, mancozeb, and carbendazim was effective against leaffolder. Combination of edifenphos with quinalphos and carbendazim with phosalone caused high mortality of rice leaf folder (Kalpana, 1992).

Effect on Sheath Blight

Sheathblight was low in triazophos + tricylazole treated plots (10.40%) followed by triazophos + hexaconazole (20.60) and tricylazole (22.50%) treated plots (Table 2). In the second crop season, triazophos + hexaconazole recorded lowest incidence of sheath blight (26.25%) followed by triazophos + tricylazole (27.50%) and tricylazole (28.75%) (Table 3). The pooled analysis of both the crop seasons showed that triazophos + tricylazole recorded lowest incidence of sheath blight with 80.04 per cent reduction over control followed by triazophos+hexaconazole and tricylazole with 75.38and 73.07 per cent over control, respectively (Table 4). Combination of insecticide pymetrozine (0.5g/lit) with hexaconazole recorded less incidence of sheath blight (Bhuvaneswari and Raju, 2013).

Grain Yield

During the first crop season (*kharif* 2013), flubendiamide + buprofezin recorded highest yield with 3636 kg/ha followed by flubendiamide + buprofezin + hexaconazole (3357 kg/ha), triazophos + tricylazole (3232 kg/ha), triazophos + hexaconazole (3048 kg/ha) sprayed plots (Table 2). During the second season, *rabi* 2013-14, triazophos sprayed plots recorded highest yield of 3813 kg/ha followed by triazophos + hexaconazole sprayed plots 3238 kg/ha (Table 3).The pooled analysis of both the seasons also showed that highest grain yield was recorded in flubendiamide + buprofezin with 19.48 per cent increase over control followed by flubendiamide + buprofezin + hexaconazole, triazophos + hexaconazole and triazophos + tricyclazole with 16.38, 11.93 and 10.97 per cent increase over control, respectively (Table 4).

References

Bhuvaneswari, V. and Raju, S.M. 2013. Compatibility of fungicides and insecticides targeting sheath blight and major rice pests. *Journal of Rice Research* 6: 64-71.

Kalpana, T.A. 1992. Compatibility of certain fungicides and insecticides used for the control of major diseases and insect pests infesting the rice crop. MSc (Ag.) thesis. 230pp.

Pathak, M.D. and Dhaliwal, G.S. 1987. Trends and strategies for rice insect problems in Tropical Asia. In IRRI Research Paper Series No.64, IRRI, Los Banos, Philippines.

Pathak, M.D., Khelsa, M.S. and Varma, S.K. 1982. Controlling major pests of rice plant. *Indian Farmers' Digest* 15: 6-9.

Prajapati, K.S., Korat, D.M., Dodia, J.F., Pathak, A.R. and Patel, R.C. 2005. Field evaluation of compatibility of insecticides and fungicides on rice. *Pesticide Research Journal* 17: 30-32.

Prakash, G., Singh, U.D., Sharma, P. and Pandian, R.T.P. 2013. Evaluation of pesticides against rice sheath blight caused by *Rhizoctonia solani*. Indian *Phytopathology* 66: 351-355.

Raju, N., Saroja, R. and Suriachandraselvan, M. 1988. Compatible insecticides and fungicides to control leaffolder (LF) and sheath rot (ShR) in rice. *International Rice Research Newsletter* 13: 27.

Singh, U.D. 2000. Effect of pesticides on sheath blight pathogen of rice. Ph.D., Thesis. Utkal University, Bhubaneswar.

Table 1. Details of treatments with doses

Treatments	Name of the insecticides	Dosage @ ml/gm/litre
T1	Flubendiamide + Buprofezin	0.25
T2	Triazophos	0.30
T3	Hexaconazole	2.00
T4	Tricyclazole	0.60
T5	Flubendiamide+Buprofezin+Hexaconazole	0.25 + 2.00
T6	Flubendiamide+Buprofezin+Tricyclazole	0.25+0.60
T7	Triazophos+Hexaconazole	0.30 + 2.00
T8	Triazophos+Tricyclazole	0.30 + 0.60
T9	Control	

Table 2. Per cent incidence of rice pests during *kharif* 2013 season

Treat-ments	Treatments @ Kg/ml/ ha	Stem borer		Whorl maggot	leaffolder	Sheath blight	Grain yield (Kg/ha)
		(DH%) 50DAT	(WE%) 80 DAT	(DL%) 30 DAT	(DL%) 65DAT	85 DAT(%)	
1	Flubendiamide + Buprofezin	0.23*	4.78	5.88	4.15*	80.25	3636*
		(0.03)	(0.21)	(0.24)	(0.20)	(1.15)	
2	Triazophos	0.80*	4.86	5.53	8.54	88.50	2676
		(0.05)	(0.19)	(0.24)	(0.22)	(1.25)	
3	Hexaconazole	3.39	5.12	4.14	7.13	42.50	2500
		(0.18)	(0.23)	(0.21)	(0.27)	(0.50)	
4	Tricyclazole	2.13	4.60	5.99	9.05	22.50*	1869
		(0.15)	(0.22)	(0.24)	(0.30)	(0.42)	
5	Flubendiamide+Buprofezin+Hexaconazole	1.83	4.63	4.62	5.82	45.50	3357*
		(0.14)	(0.22)	(0.21)	(0.24)	(0.57)	

6	Flubendiamide+Buprofezin+Tricyclazole	1.06	5.92	5.12	9.52	44.20	2387
		(0.10)	(0.23)	(0.23)	(0.31)	(0.54)	
7.	Triazophos + Hexaconazole	1.08*	3.83	3.77	5.93	20.60*	3048
		(0.09)	(0.20)	(0.20)	(0.24)	(0.17)	
8.	Triazophos + Tricyclazole	1.54	3.42	3.25*	5.12	10.40*	3232*
		(0.12)	(0.18)	(0.15)	(0.21)	(0.12)	
9.	Control	6.04	6.64	6.21	9.08	95.60	2687
		(0.24)	(0.26)	(0.25)	(0.31)	(1.30)	
	CD (0.05%)	0.07	0.09	0.08	0.09	0.20	790

Figures in parentheses are arcsine transformed values

*Figures followed by different letters are significantly different at p=0.05

Table 3. Per cent incidence of rice pests in *rabi* 2013-2014 season

Treatments	Treatments @ Kg/ml/ha	Stem borer		Whorl maggot	Leaffolder	Sheath Blight	Grain yield (Kg/ha)
		(DH%)50 DAT	(WE%)80 DAT	(%DL) 30 DAT	(DL%)-65DAT	(%)85DAT	
1	Flubendiamide + Buprofezin	0.83*	3.03*	7.23	0.00*	82.75	3074
		(0.05)	(0.17)	(0.27)	(0.00)	(1.16)	
2	Triazophos	1.60	6.61	6.02	0.69	90.00	3381
		(0.09)	(0.22)	(0.24)	(0.06)	(1.30)	
3	Hexaconazole	5.60	7.23	8.54	1.54	37.50	2798
		(0.15)	(0.28)	(0.30)	(0.09)	(0.36)	
4	Tricyclazole	6.50	8.03	8.10	1.06	28.75*	2786
		(0.20)	(0.29)	(0.29)	(0.11)	(0.56)	
5	Flubendiamide+Buprofezin+Hexaconazole	0.78	4.71	7.42	1.04	30.00	3179
		(0.05)	(0.21)	(0.28)	(0.05)	(0.58)	
6	Flubendiamide+Buprofezin+Tricyclazole	1.65	4.60	9.08	0.63	30.00	3119
		(0.09)	(0.20)	(0.33)	(0.06)	(0.57)	
7.	Triazophos+Hexaconazole	1.62	6.70	10.20	0.71	26.25	3238*
		(0.16)	(0.25)	(0.35)	(0.06)	(0.53)	
8.	Triazophos+Tricyclazole	0.60*	4.37*	7.27	0.37*	27.50*	3000
		(0.03)	(0.19)	(0.27)	(0.03)	(0.54)	
9.	Control	8.75	8.75	12.19	2.57	94.75	2929
		(0.24)	(0.32)	(0.30)	(0.16)	(1.37)	
	CD (0.05%)	0.14	0.12	0.13	0.14	0.10	489

Figures in parentheses are arcsine transformed values

*Figures followed by different letters are significantly different at p=0.05

Table 4. Pooled analysis of both crop seasons

Treatments	Treatments @ Kg/ml/ha	Stem borer		Whorl maggot	Leaffolder	Sheath Blight	Grain yield (Kg/ha)
		(DH%) 50 DAT	(WE%)80 DAT	(%DL) 30 DAT	(DL%)65DAT	(%)85DAT	
1	Flubendiamide + Buprofezin	0.53*	3.91*	6.26	2.08*	81.50	3355*
		(0.04)	(0.19)	(0.27)	(0.10)	(1.16)	
2	Triazophos	1.20	5.74	6.01	4.62	89.25	3029
		(0.10)	(0.21)	(0.26)	(0.15)	(1.28)	
3	Hexaconazole	4.50	6.18	6.34	5.06	40.00	2649
		(0.17)	(0.24)	(0.28)	(0.20)	(0.49)	
4	Tricyclazole	4.52	6.32	7.05	5.35	25.63*	2328
		(0.18)	(0.25)	(0.30)	(0.21)	(0.43)	
5	Flubendiamide+ Buprofezin+Hexaconazole	1.31	4.67	6.02	3.43	37.80	3268
		(0.10)	(0.21)	(0.25)	(0.14)	(0.53)	
6	Flubendiamide+ Buprofezin+Tricyclazole	1.36	5.26	6.60	5.12	37.10	2753
		(0.10)	(0.22)	(0.28)	(0.19)	(0.48)	
7.	Triazophos + Hexaconazole	1.35	5.27	6.00	3.32	23.43	3143*
		(0.10)	(0.23)	(0.26)	(0.14)	(0.36)	
8.	Triazophos + Tricyclazole	1.07*	3.90*	5.26	2.75*	19.00*	3116*
		(0.08)	(0.20)	(0.21)	(0.12)	(0.33)	
9.	Control	7.40	7.70	9.21	5.83	95.18	2808
		(0.24)	(0.28)	(0.36)	(0.22)	(1.34)	
	CD (0.05%)	0.07	0.07	0.07	0.07	0.10	808

Figures in parentheses are arcsine transformed values

*Figures followed by different letters are significantly different at p=0.05

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Impact Assessment of Andhra Pradesh Water Management Project on Socio-economic Conditions of Farmers in Godavari Western Delta Pilot Area

Ch. Sreenivas*, S.Vishnu Vardhan, C. Venkata Reddy and Radha Y

Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India

*Corresponding author: csvasu@yahoo.com

Received: 16th March, 2015; Accepted: 22nd June, 2015

Abstract

The benefits of installing subsurface drainage for salinity control in the Godavari Western Delta region of India are assessed in terms the impact of subsurface drainage on sustainability of agricultural production. The study in the Kalipatnam pilot area revealed substantial farm-level benefits from installing subsurface drainage, as a result of a significant increase in crop yields, the benefit-cost ratio during *kharif* season in the pilot area was increased incrementally from -0.23 during *kharif*, 2004 to 0.50 during *kharif*, 2009. Similarly, during *rabi* season, benefit-cost ratio in the pilot area was increased incrementally from 0.18 during *rabi*, 2004-05 to 0.50 during *rabi*, 2008-09. The land value has been increased from Rs.2.5 lakh/ha in 2004 to Rs. 15.0 lakh ha in 2009. A difference of 3.75 lakh/ha was noticed between pilot area and control area and this difference can be attributed to appreciation of land value due to installation of subsurface drainage system. Among the extension methods used for teaching, trainings followed by demonstrations, kalajathas and campaigns were proved effective in capacity building of farmers.

Key words: Godavari Western Delta, A.P. Water Management Project, Subsurface drainage system, Socio- economic impact.

Introduction

With the prime objectives of land and water productivity management, the Andhra Pradesh Water Management (APWAM) Project has started functioning in A.P. State since November 2003, with Bapatla as the main center and Undi, Garikapadu, Jagtial and Tirupati as net work centers. The APWAM Project is functioning under the aegis of Acharya N. G. Ranga Agricultural University (ANGRAU) with the financial assistance from FAO and technical Assistance from ILRI, Wageningen, Netherlands. Undi network centre is operating in Godavari Western Delta. Subsurface system was installed in 18 ha area in farmers' fields at Kalipatnam pilot area in Godavari Western Delta canal command in India in the year 2005 to combat the problems of water logging, salinity and sodicity. The project has completed the first phase of five years by October 2008 and has been extended for a two-year period, till October 2010. With this background, the present study on 'Impact assessment of Andhra Pradesh Water Management Project on socio-economic conditions of farmers in pilot area and on capacity building of stakeholders in Godavari Western Delta' was conducted.

Materials and Methods

1. Selection of Pilot Areas APWAM Project, Undi center has selected pilot areas, Kalipatnam in West Godavari District for improving the land productivity of problematic soils under saline and water logged conditions.

2. Selection of Sample Farmers All farmers in the pilot area (28) and control area (32) were taken into consideration for collecting the data.

3. Data Collection

Secondary data The secondary data on total cultivated area and number of farmers in the selected pilot area were collected from official records of the pilot areas. The data pertaining to the year 2004 were collected from the Bench Mark Survey Report of pilot areas of the Project.

Primary data The information on socio economic conditions and capacity building on water and land productivity enhancement were collected through pre-tested questionnaires and personnel interviews from sample farmers. The data that were not available in the Bench Mark Survey Report were collected from sample farmers on memory recall basis to the maximum possible extent.

4. Data Analysis The data for the year 2004 was taken as 'Before the Project' situation and the average of four years from 2005 to 2009 was taken as 'After the Project' situation. The data were collected for the year 2009 from the sample farmers of non-pilot areas so as to analyze the impact of the project by comparing 'With Project' and 'Without Project' situations. Simple tabular analysis

was used to prepare text tables. Garrett scoring technique (Garrett and Woodworth, 1969) was used to analyze the importance of various extension methods in capacity building of farmers.

Results and Discussion

The data collected from sample farmers were analyzed as per the objectives and the results are presented hereunder.

1. Social Conditions

Age composition The number of farmers in the age group of more than forty years was more in pilot area and control areas, inferring that more number of young farmers are involved in agriculture and is an indication to show good response to the drainage technology showcased by APWAM Project (Table 1).

When the impact of APWAM Project has been analyzed by taking the number of farmers in different age groups before and after the situations, it was found that there was not any change in > 60 year age group, while the number of farmers in 41-60 group was increased and that in < 40 year age group decreased due to passing of time in 2009.

Educational Status When the educational status was analyzed before and after the situations, it was found that there was decrease in the per cent of illiterates during 2009 over 2004, which is a good indication of better standard of living. However, not much change was observed between pilot and control areas (Table 2).

Community-wise Distribution of Land Holdings

The analysis of community wise distribution of land holdings (Table 3) reveals that the area under cultivation increased with the OC category of farmers in the pilot area. There was increase in per capita land availability in the OC category but over all per capita land availability remained same in the pilot area during the study period.

2. Economic Parameters

Land Holding Particulars It could be revealed from Table 4 that most of the pilot area farmers are cultivating on their own lands and entire area is under irrigated cultivation. The land value has been increased from Rs. 2.5 lakh/ha in 2004 to Rs. 15.0 lakh/ha in 2009. A difference of 3.5 lakh/ha was noticed between pilot area and control area and this difference can be attributed to appreciation of land value due to installation of subsurface drainage system.

Profitability of Agribusiness

The benefit-cost ratio in the pilot area increased incrementally from -0.23 during *kharif*, 2004 to 0.50 during *kharif*, 2009. Similarly, benefit-cost ratio in the pilot area increased incrementally from 0.18 during *rabi*, 2004-05 to 0.50 during *rabi*, 2008-9 (Table 5).

3. Capacity Building of Stakeholders

Impact of Different Extension Methods on Capacity Building of Farmers of Pilot Areas

The impact of various extension methods on the perception of farmers' knowledge on different technological aspects was studied by ranking the extension methods (Table 6). The Garrett's scoring analysis revealed that among the different extensions methods, farmers gave highest preference to trainings followed by demonstrations, kalajathas and campaigns.

Conclusions

1. Social Implications

- Literacy rate increased in the study area from 48% during 2004 to 53% in 2009 due to awareness in adult education programme imparted by APWAM project, Undi centre.
- The area under cultivation increased with the OC category of farmers in the pilot area. The per capita land availability also increased in the OC category but over all per capita land availability remained same in the pilot area during the study period.

2. Economic Implications

- The land value has been increased from Rs.2.5 lakh/ha in 2004 to Rs. 15.0 lakh ha in 2009. A difference of 3.5 lakh/ha was noticed between pilot area and control area and this difference can be attributed to appreciation of land value due to installation of subsurface drainage system.
- The benefit-cost ratio in the pilot area increased incrementally from -0.23 during *kharif*, 2004 to 0.50 during *kharif*, 2009. Similarly, benefit-cost ratio in the pilot area increased incrementally from 0.18 during *rabi*, 2004-05 to 0.50 during *rabi*, 2008-9.
- The net income which was negative before the APWAM Project increased gradually, bringing surplus income to the farmers in Kalipatnam area.

3. Capacity Building of Stakeholders

- Farmers have gained knowledge on improved water management practices that resulted in managing salinity in efficient way and hence significant increase in the yield was noticed.
- Farmer's knowledge on IPM and INM technologies has resulted in increased yields.
- Among the extension methods used for teaching, trainings followed by demonstrations, kalajathas and campaigns were proved effective in capacity building of farmers

References:

Garrett, H.E. and Woodworth, R.S. 1969. Statistics in Psychology and Education, Vakils, Feffer and Simons Pvt. Ltd. Bombay. p. 329.

Table 1. Age composition of sample farmers in Kalipatnam pilot area

Age group	Kalipatnam		
	Pilot Area (2009)	Control (2009)	Before APWAM Project (2004)
<40	18 (64.3)	20 (62.5)	39 (65.0)
41-60	7 (25.0)	8 (25.0)	14 (23.3)
>60	3 (10.7)	4 (12.5)	7 (11.7)
Total	28 (100.0)	32 (100.0)	60 (100.00)

Figures in parentheses indicate per cent to respective total

Table 2. Educational status of sample farmers in pilot areas

Level of Education	Kalipatnam		
	Pilot Area (2009)	Control (2009)	Before APWAM Project (2004)
Illiterate	13 (46.5)	15 (46.9)	69 (52.0)
Primary	10 (35.7)	11 (34.4)	41 (31.0)
Matriculation	4 (14.3)	5 (15.6)	18 (14.0)
Degree	1 (3.5)	1 (3.1)	4 (3.0)
Total	28 (100.0)	32 (100.0)	132 (100.0)

Figures in parentheses indicate per cent to respective total

Table 3. Community-wise distribution of land holdings among sample farmers in Kalipatnam pilot area

Community	Pilot						Control(2009)		
	Number		Area (ha)		Per Capita Land (ha)		Number	Area (ha)	Per Capita Land (ha)
	2004	2009	2004	2009	2004	2009			
OC	12	12	7.06	7.46	0.59	0.62	26	14.52	0.56
	(42.9)	(42.9)	(39.2)	(41.4)			(81.2)	(80.7)	
BC	16	16	10.94	10.54	0.68	0.66	2	2.00	1.00
	(57.1)	(57.1)	(60.8)	(58.6)			(6.3)	(11.1)	
SC & ST	0	0	0	0	-	-	4	1.48	0.37
	(0.0)	(0.0)	(0.0)	(0.0)			(12.5)	(8.2)	
Total	28	28	18.00	18.00	0.64	0.64	32	18.0	0.56
	(100.0)	(100.0)	(100.0)	(100.0)			(100.0)	(100.0)	

Figures in parentheses indicate per cent to respective total

Table 4. Land holding particulars of sample farmers in Kalipatnam pilot area (2009)

Item	Pilot			Control		
	I	ID	T	I	ID	T
Owned (ha)	14.4 (25)	--	14.4 (25)	13.6 (27)	--	13.6 (27)
Leased in (ha)	3.6 (3)	--	3.6 (3)	4.4 (5)	--	4.4 (5)
Leased out (ha)	--	--	--	--	--	--
Present value of land (Lakh. Rs/ ha)	15.0	--	--	11.25	--	--
Rental value of land (Rs/ha/year)	42000	--	--	42000	--	--
Land revenue or tax (Rs/ha)	625	--	--	625	--	--

I: Irrigated, ID: Irrigated Dry, T: Total (Figures in parenthesis indicate number of farmers)

Table 5. Cost of cultivation and income measures of rice in Kalipatnam pilot area

Particulars	2004-05		2005-06		2006-07		2007-08		2008-09	
	kharif	*rabi*	*kharif*	*rabi*	*kharif*	*rabi*	*kharif*	*rabi*	*kharif*	*rabi*
Yield (t/ha)	3.5	6.9	3.77	7.78	5.8	7.70	5.1	7.9	6.5	7.5
Price (Rs/t)	6130	5730	6130	5770	7460	7460	7632	7832	9333	9333
Total cost of cultivation (Rs/ha)	28065	34160	23163	44896	45226	52810	32925	47231	42290	48627
Gross income (Rs/ha)	21455	39537	13119	26186	44828	58970	40923	54108	36611	73314
Net income (Rs/ ha)	-6610	5377	-4370	12353	1398	6160	7998	16133	21321	24687
B:C ratio	-0.23	0.18	-0.15	0.37	-0.01	0.12	0.24	0.34	0.5	0.50
Cost of production (Rs/t)	8019	4951	7303	4185	7798	6858	6455	6757	6506	6484

Table 6. Impact of extension methods on capacity building of farmers of pilot areas

S. No.	Particulars/ Activity	Demonstrations			Trainings			Campaigns			Kalajathas		
		Rank	% Position	Score	Rank	% Posi-tion	Score	Rank	% Posi-tion	Score	Rank	% Posi-tion	Score
1	Crop management aspects	3	62.5	44	1	12.5	74	4	87.5	28	2	37.5	57
2.	Fertilizer application	1	12.5	74	2	37.5	57	4	87.5	28	3	62.5	44
3.	Identification of pests & diseases	2	37.5	57	1	12.5	74	4	87.5	28	3	62.5	44
4.	Use of PP Chemicals	1	12.5	74	2	37.5	57	4	87.5	28	3	62.5	44
5.	Preparation of botanical extracts	1	12.5	74	2	37.5	57	3	62.5	44	4	87.5	28
6.	Use of bio-control agents and bio-pesticides	2	37.5	57	1	12.5	74	4	87.5	28	3	62.5	44
7.	Conservation of natural enemies	3	87.5	28	1	12.5	74	4	87.5	28	2	37.5	57
8.	Water management	2	37.5	57	1	12.5	74	4	87.5	28	3	62.5	44
9.	Mechanised farming	1	12.5	74	2	37.5	57	3	62.5	44	4	87.5	28
Average of scores				60.0			66.4			31.2			43.3
Overall Ranking				II			I			IV			III

SHORT COMMUNICATION OPEN ACCESS

Rice Riche - Rice Bran Oil based Pain Relieving Gel

M. Mohibbe Azam*

Indian Institute of Rice Research, Rajendranagar, Hyderabad

*Corresponding author: mohibbe.azam@gmail.com

Received: 4th April, 2015; Accepted: 29th May, 2015

Rice bran, a by-product of the rice processing industry is a potential source of edible and healthful products and is a rich source of various micronutrients, protein and high quality oil. Oil (RBO) contains various antioxidants including vitamin E, oryzanol. These antioxidants fight with free radicals and slow down the effect of aging. The oil which is popularly known as rice bran oil (RBO) is very light and is easily absorbed in the skin. With this in view, the present product for relieving pain was developed.

The aim of the present invention is to provide a topical composition for fast relief of aches and pains of muscles and joints associated with simple strains, bruises and sprains.

Another object of the present invention is to provide compositions containing analgesic/anti-inflammatory agents dissolved in fast absorbing rice bran oil for quick penetration of the active components.

This product can be prepared in one hour duration and production cost at laboratory level is around Rs. 18.50 (for 33 g) which does not include labor charges and energy cost.

Feature and benefit of the invention

The product of the present invention is a herbal formulation containing rice bran oil. The composition is either in gel form or in liquid form. Both the forms are stable at room temperature for an extended period of time without spoilage. Oryzanol and other anti-oxidants present in the oil improve shelf life of the product. As the rice bran oil has quick absorbing property, dissolve active ingredients in the composition get absorbed fast and provide quick relief. The composition is new, very safe, eco-friendly and does not produce any harmful effects (Fig. 1).

Comparison with related products available in the market

Topical pain relieving compositions are commercially available. These topical compositions are often used to treat sore muscles, pain associated with the joints of a body, arthritis and other similar conditions. Many of these topical compositions are thick and have a heavy texture. Such products typically take a considerable amount of time to reach the desired area of treatment. Additionally, these compositions often leave an oil residue on the surface of the skin leaving the skin feeling greasy, slippery, and wet until the material is either ultimately absorbed into the skin or is sufficiently rubbed off the surface of the skin. This oily residue remaining on the skin can provide an uncomfortable feeling for the person as well as get on clothing worn by the person. Product of the present invention takes care of these problems.

Feedback of the users

Fifty persons (both gender) suffering from different kind of pain were provided with 50 g sample of pain relief composition, along with a questionnaire for continuous use of the product for at least one week. The identified persons were monitored periodically for follow-up action on actual use of the product. The analysis of data revealed that 100 % users reported that they are satisfied or extremely satisfied with the performance of the product. They got immediate relief from muscle pain/sprain/joint pain.

The product absorbs quickly, it was felt by majority of the users (57%). 57% respondents felt that the smell was very-pleasant while remaining (43 % users) also reported that the smell is pleasant. None of the users found the smell unpleasant.

Vey high percentage of the respondents (71.5%) felt that this product was better / much better than the available products in the market. Remaining users (28.5%) also found that the product was at par with the available products. None of the users found the product inferior to market products.

Acknowledgement

The author is thankful to Dr. B.C. Viraktamth former Project Director, IIRR and Dr. V. Ravindra Babu, Project Director (A) for constant encouragement and needed help. The author is also thankful to all volunteers who came forward for using the product and giving feedback.

Fig. 1. Gel made of rice bran oil - *Rice Riche pain relieving gel*

SHORT COMMUNICATION OPEN ACCESS

Variability in Aggressiveness of Rice Blast (*P. oryzae*) Isolates originating from resistant and susceptible cultivars

J. Aruna, S. Vijay Kumar, R. Rambabu, S. Ramesh, K. R. Madhavi, V. Abhilash, G.S. Laha, D. Krishnaveni, D. Ladha lakshmi, V. Prakasam, V. Ravindrababu and M. S. Prasad*

Indian Institute of Rice Research, Rajendranagar, Hyderabad

*Corresponding author: data.msprasad@gmail.com

Received: 28th March, 2015; Accepted: 17th May, 2015

Rice Blast disease caused by fungus, *Pyricularia oryzae* is the most devastating and destructive diseases of rice throughout the world in all the rice growing ecosystems. The fungus attacks the aerial parts of the rice plant *i.e.* leaves, nodes and panicle. The lesions on the leaves are spindle shaped with grey centre. The lesions on the leaves grow and coalesce each other and dries the plant in favourable conditions. It can cause severe loss in yield to the extent of 70-80% in various rice ecosystems (Srinivas Prasad *et al*., 2011). There are several reports on the variability of the pathogen. In the present study, an isolate collected from a resistant cultivar IR-64 was compared with the isolate collected from susceptible cultivar HR-12. The fungus was isolated and pure cultures were maintained by following standard procedures. Ten differential hosts of the blast fungus were grown in small size plastic cups and they were inoculated with these two isolates at four leaf stage. After inoculation, the plants were covered with polythene sheet and sprayed with water regularly to maintain humidity. The disease reaction was noted after one week by following 0-9 SES scale. (IRRI, 1996). The results are presented in the Table-1.

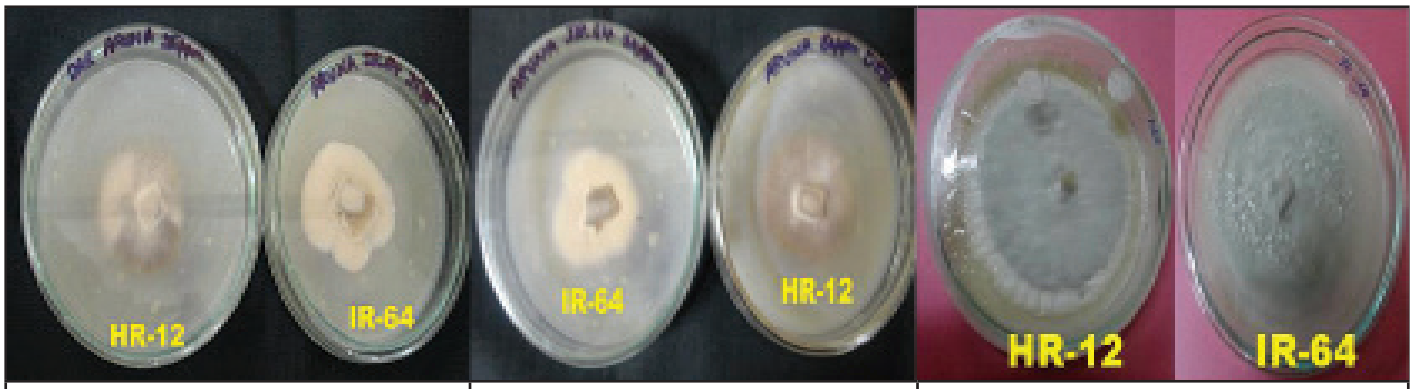
Table 1. Reaction of host differentials to blast isolates collected on HR-12 and IR-64 hosts.

S. No.	Differential host	HR-12 Isolate (Score)	Reaction	IR-64 Isolate (Score)	Reaction
1	*O. minuta*	2	R	5	S
2	Raminad str-3	3	R	5	S
3	Zenith	3	R	5	S
4	NP-125	2	R	5	S
5	Dular	3	R	5	S
6	Kanto-51	3	R	6	S
7	Calaro	2	R	5	S
8	Tadukan	1	R	4	MR
9	IR-64	3	R	6	S
10	Tetep	1	R	4	MR

R-Resistant, S-susceptible, MR-Moderately Resistant

Ten international blast differential lines which are known to contain single or multiple blast resistance genes were tested against these two blast isolates. The IR-64 isolate could infect the differential lines like *O. minuta*, Raminad str-3, Zenith, NP-125, Dular, Kanto-51, Calaro, Tadukan including IR-64 and Tetep while HR-12 isolate showed resistant reaction (score 4) to all these lines.

The efficacy of fungicide Tricyclazole @ 25 ppm & 50 ppm was used by adding it at the time of pouring sterilized Oat meal agar medium (OMA) in petriplate. The medium without fungicide served as control. Seven days old fungus agar block was aseptically transferred to each petriplate and incubated at 28°C. The radial growth of the mycelium was measured periodically at 10 and 15 days. Under *in-vitro* conditions the fungicide at all the concentrations inhibited mycelial growth of *P. oryzae* when compared to control (Fig.1). The growth of the HR-12 isolate was checked more compared IR-64 isolate (Table-2, Fig.1).



Tricyclazole @ 25 ppm	*Tricyclazole* @ 50 ppm	without fungicide

Fig. 1. Radial growth of *P.oryzae* at different concentrations of fungicide

Table 2. Radial growth of *P.oryzae* at different concentrations of fungicide

Isolate	25 ppm concentration*		50ppm concentration*	
	10th day	15th day	10th day	15th day
HR-12 Isolate	3.4	5.4	2.8	4.2
IR-64 Isolate	4.5	6.8	3.0	4.0
Check without fungicide	6.2	8.2	6.8	8.8

*Indicates radial growth of the mycelium in mm

Conclusion

Therefore, the blast isolate collected from IR-64 was more aggressive than isolate from HR-12 and the isolate was different in giving reaction to differential hosts and also *in-vitro* evaluation against fungicide.

References

Srinivas Prasad, M., Sheshu Madhav, M., Laha, G.S., Ladha Lakshmi, D., Krishnaveni, D., Mangrauthia, S.K., Balachandran, S.M., Sundaram, R.M., Aruna Kanthi, B., Madhan Mohan, K., Ratna Madhavi, K., Kumar, V and Viraktamath, B.C. 2011. Rice blast disease and its management. Technical Bulletin No57/2011, Directorate of Rice Research (ICAR), Rajendranagar, Hyderabad-500 030, Andhra Pradesh, India: 52.

IRRI, 1996. Standard Evaluation System for rice. In INGER 4th (ed.) Genetic Resource Centre.

Journal of Rice Research - Authors Guidelines

Scope: Journal of Rice Research is a channel for publication of full length papers covering results of original research, invited critical reviews or interpretative articles related to all areas of rice science, rice based crop systems and rice crop management. The journal also publishes occasional short communications, book reviews and letters to the editor.

Articles reporting experimentation or research in any field involving rice or rice based cropping systems will be accepted as original articles while critical reviews are generally invited. Short articles concerned with experimental techniques or observation of unique nature will be accepted as short communication. Letters to the editor concerning previous articles are welcome and are published subject to review and approval by the editorial board. The original authors will be invited to reply to the points raised in these letter for their response which are also published together.

General Requirement:

Submission to the journal must be reports of original research of at least two crop seasons and must not be previously published or simultaneously submitted to any other scientific or technical journal. At least one of the authors (in case of joint authorship) should be member of the Society for Advancement of Rice Research and not in arrears of subscription. Authors of invited articles are exempted from this.

Submission of manuscript:

Manuscripts should be sent online to the Journal office at sarr_drr@yahoo.com; surekhakuchi@gmail.com, jyothi_rishik@yahoo.com, ento_sam@yahoo.co.in as an attachment. All the enclosed figures (as ppt files), graphs (as MS Excel worksheet with original data) and photographs (as jpg or ppt files with high resolution) may be submitted as separate files. Avoid using more than one font. The manuscript should be typed in double spaced with margins of at least 2.5 cm. On the first page give the title, a byline with the names of authors, their affiliation and corresponding author's e-mail ID. Abstract should be followed by a list of key words, and abbreviations used in the paper. The usual order of sections to be included after title and abstract pages are: Introduction which includes literature review; materials and methods; results and discussion; conclusion (optional), acknowledgements and references followed by figures and tables.

Title and byline should give a clear idea what the articles is about. It should be brief and informative (12-15 words).

References: References are quoted in author-year notation system only. Arrange all the references alphabetically by author. All single author entries precede multiple author entries for the same first authors. Use chronological order within entries with identical authorship and add a low case letter a, b, c, etc., to year for same year entries of the same author. References should be typed as follows:

Research papers

1. Mukherjee JN. 1953. The need for delineating the basic oil and climatic regions of importance to the plant industry. *Journal of Indian Society of Soil Science* 1:1-6.
2. Shin YS, Kim ES, Watson JE and Stokstad EL. 1975. Studies on folic acid compounds in nature. IV. Folic acid compounds in soybeans and cow milk. *Canadian Journal of Biochemistry* 53:338-343.
3. Paul M and Keegstra K. 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. *Plant Journal* 54:559-568.

Thesis

Bhuiyan MDAR. 2010. Phenotypic and genotypic evaluation of selected transgressive variants derived from *Oryza rufipogon* Griff. x *Oryza sativa* L. cv. MR219. Ph D. Thesis. University Kebaangsaan Malaysia, Malaysia, 150 p.

Book chapter

Scott JM. 1984. Catabolism of folates. P. 307-327. In R.L. Blackley and S.J. Benkovic (ed.) Folates and Pterims Vol.1. John Wiley & Sons, New York.

Book

Subba Rao LV, Shobha Rani N, Chiranjeevi M, Chaitanya U, Sudharshan I, Suneetha K, Jyothi Badri and Choudhary DR. 2013. DUS Characterization of Rice Varieties. Directorate of Rice Research, Rajendranagar, Hyderabad-500 030, AP, India. 524 pp.

Figures: Photographs and drawings for graphs and charts should be prepared with good contract of dark and light. Figure caption should be brief specifying the crop or soil, major variables presented, place and year. Give careful attention to the width of lines and size, and clarity of type and symbols.

Tables: Tables are used for reporting extensive numerical data in an organized manner and statistically analyzed. They should be self explanatory. Prepare tables with the word-processing tables feature and tabs or graphics boxes should not be used. Table head should be brief but complete and self contained. Define all variables and spellout all the abbreviations. An exponenetial expression (eg. x 103) in the units line is often needed to keep length of the data reasonably short, and referenced with an explanatory note. Unless otherwise required, two decimal place values are suggested. Follow the articles published in recent journal for table format.

Society for Advancement of Rice Research

ICAR-Indian Institute of Rice Research (ICAR-IIRR) Rajendranagar

Hyderabad – 500 030 (India), Phone No: 091 40 24591221, 24591216
Fax: +9140 24591217, email: sarr_drr@yahoo.com