Plant Science Research

ISSN 0972-8546

Genotypic variations of protein banding pattern in indigenous rice genotypes from Koraput, India in relation to drought stress

Debabrata Panda[Ψ], Swati S. Mishra and Prafulla K. Behera

Department of Biodiversity and Conservation of Natural Resources, Central University of Odisha, Koraput-763 004, Odisha, India

ARTICLE INFO

Article history:
Received : 25 September 2023
Revised : 23 October 2023
Accepted : 20 November 2023

Keywords:

Drought stress
Indigenous rice
Protein banding
SDS-PAGE

ABSTRACT

Indigenous rice landraces are an invaluable resource for restoring genetic diversity. Precise biochemical profiling based on protein banding provides information about the extent of genetic diversity, which helps for effective breeding programs. The present study evaluated SDS-PAGE protein profiling of selected six indigenous rice genotypes from Koraput along with tolerant (N22) and susceptible (IR64) check varieties under control and simulated drought stress. A total of 50 polypeptide bands ranging from 47.7 kDa to 200 kDa were harvested from studied rice genotypes under drought stress, whereas 47 polypeptides bands ranging from 47.13 to 200 kDa was observed in control plants. A total of 17 unique bands were noticed under drought-treated plants, which were absent in control plants. The number of protein bands were higher in some indigenous rice landraces than that of drought-tolerant and susceptible check variety under drought stress. Based on the genetic similarity analysis, some indigenous rice landraces such as Machhakanta, Haladichudi and Kalajeera showed highest genetic similarity with drought tolerant check (N22) variety and formed one cluster. The presence of some unique protein bands in these genotypes indicates their importance for drought tolerance breeding and can be further used for 'omic' studies to better understand stress responses.

1. Introduction

Traditional/indigenous rice genotypes exhibit huge genetic diversity and reservoirs for many potential genes that remain untapped (Samal *et al*., 2018). Different indigenous rice genotypes differ in adaptation to soil types, seeding time, maturity, height, nutritive value, use and other stress-tolerant properties (Arunachalam *et al.*, 2006). These varieties were grown for specific traits like maturity duration, plant stature, panicle features, yield potential, tolerance to biotic and abiotic stresses and other elusive traits like aroma, cooking quality and grain quality (Patra and Dhua, 2003; Roy *et al.*, 2016). The introspection of such diversity is vital for reaching a consensus and making decisions on the conservation and proper utility in breeding programs. Plants respond to drought by regulating gene expression both at the transcriptional and translation levels for the synthesis of stress protein. During water deficit conditions, rice tissue synthesizes more soluble proteins and therefore contributes towards the stress tolerance phenomena (Farooq *et al.*, 2009). Proteins responsible for biosynthesis of osmolytes, ROS scavenging and protection of cellular structure have been identified (Borah *et al.*, 2017). The changes in protein concentration are an indication of stress response towards drought (Qureshi *et al.*, 2007; Choudhary *et al.*, 2009). Majority of the research carried out on this aspect was on high yielding irrigated variety, which is highly susceptible to drought stress. However, detail analysis of protein banding pattern in indigenous rice landraces is lacking.

Koraput district of Odisha, India, is a hotspot of folk rice diversity and secondary centre of origin of Asian cultivated rice (Mishra *et al.*, 2018). Varied agroclimatic

[Ψ] *Corresponding author; Email:* dpanda80@gmail.com

ecosystems of these region, scarce rainfall, low soil moisture during post-monsoon season and varied topography with an altitude varied from 500 to 1600 mean sea level favour for rice diversity (Mishra *et al.*, 2019). Recently the importance of the region and genetic potentiality of rice diversity in relation to different agronomic traits highlighted (Mishra *et al.*, 2018; Mishra *et al.*, 2019; Panda *et al.*, 2020). However, there is a dearth of biochemical profiling reports and genetic variability studies of protein banding pattern in indigenous rice landraces with respect to drought tolerance. Therefore, the present study aims to characterise the protein banding pattern in selected drought-tolerant folk rice genotypes in relation to drought stress and genotypic relationship will be established between tolerant and susceptible check varieties, which will be helpful for future breeding programs.

Six indigenous rice genotypes namely, Pandakagura, Machhakanta, Haladichudi, Mugudi, Kalajeera and Dangarbayag undar of Koraput, India along with drought-tolerant and susceptible check varieties such as N22 and IR64, respectively were selected for the study. These rice genotypes were popular in the region and were recently identified as most drought-tolerant genotypes of the region (Mishra *et al.*, 2018; Mishra *et al.*, 2019). The rice plants are grown in the hydroponic system using Yoshida nutrient solution and details of growth conditions were recently described in our laboratory by Mishra *et al*. (2018). Briefly, after 30 days of normal growth in a hydroponic system, the plants were treated with drought by application of (36.0%) of polyethylene glycol (PEG)-6000 for 10 days. A control set was also run along with the treatment without the application of PEG. The fresh leaf samples (500 mg) of control and drought-treated plantswere homogenized with extraction buffer (50 mM sodium phosphate buffer (pH 7.0) containing 0.1% polyvinylpyrrolidone (PVP), 0.1% triton X-100 and 10 mM β-Mercaptoehanol). The samples were mixed for 5 minutes in vortex mixer and kept for one hour at room temperature. After incubation, the samples were kept in a boiling water bath for 3 minutes and allowed to cool, and centrifuged at 12000 rpm for 15 min at 4 °C. The crude extract was used for the estimation of protein according to the method of Lowry *et al*. (1951). Protein was denatured by addition of equal volume of SDS-sample buffer and heated at 95 °C for 3 minutes. The Electrophoresis was carried out in vertical electrophoresis unit (Bangalore GeNei maxi.). The 10% Gel cast (20×20 cm and 1mm thickness) was used for the separation of proteins. The protein extract (100 ìg) of different samples was loaded into the wells. Extracted soluble proteins were fractionated by one-dimensional SDS-PAGE gel electrophoresis according to the method of Laemmli (1970). The gel was photographed using gel documentation system (Bio-Rad Gel Doc, California. USA). The number of bands and band density was calculated by the densitometer equipped with the instruments. The presence/absence of bands were transformed into a binary character matrix (1 for presence and 0 for absence of a band at a particular position). The similarity index among different genotypes were constructed by dendogram using protein bands and were measured through Jaccard's similarity coefficient and Nei and Lee Dice coefficient using *PAST-3* (Palaeontological Statistics) software.

2. Results and Discussion

Protein banding pattern in rice genotypes under control and drought stress was shown in Fig. 1. A total of 50 poly peptide bands ranging from 47.7 kDa to 200 kDa were harvested from studied rice genotypes under drought stress, whereas 47 polypeptides bands ranging from 47.13 to 200 kDa was observed in control plants (Table 1). A total of 17 unique bands (66.0 kDa, 66.5 kDa, 72.2 kDa, 75.9 kDa, 80.1 kDa, 85.3 kDa, 92.3 kDa, 93.2 kDa, 93.6 kDa, 98.6 kDa, 99.1 kDa, 110.4 kDa, 116.0 kDa, 129.3 kDa, 130.5 kDa, 136.4 kDa and 154.5 kDa) were noticed under drought treated plants, which were absent in control plants. Similarly, 13 unique bands (67.8 kDa, 73.7 kDa, 77.7 kDa, 78.1 kDa, 78.9 kDa, 109.0 kDa, 109.4 kDa, 119.3 kDa, 123.7 kDa, 124.8 kDa, 135.2 kDa, 162.9 kDa and 170.4 kDa) were present only in control samples. A common protein band of 200 kDa was found in all the tested rice varieties both under control and drought conditions. Under control conditions the protein bands were more or less similar among the genotypes but numbers of protein bands were increased during drought. In this study, the number of protein bands was higher in indigenous rice landraces than that of drought-tolerant and susceptible check variety under drought stress. In particular the drought-tolerant variety (N22) exhibited a greater number of protein bands than that of susceptible (IR64) variety under drought treatment. Some drought responsive polypeptides might be over expressed in the indigenous landraces to withstand the adverse effect of drought as compared to the susceptible variety. It has been additionally observed that different chemical signals transduced under drought stress initiate a variety of genes, prompting the synthesis of proteins and metabolites, providing drought resistance (Mishra *et al.*, 2006). These drought responsive proteins play a vital role for the tolerance mechanism of the studied rice genotypes.

The pair-wise genetic similarity is the measure to categorise the underlying genetic relationship among the genotypes. The genetic similarity was calculated by Jaccard's similarity coefficient and it ranged from 0.012 to 0.667 among the studied rice genotypes under drought stress (Table 2). Based on the genetic similarity analysis, some indigenous rice landraces such as Machhakanta, Haladichudi and

Table 1

List of protein bands with their molecular weights in different rice genotypes under control and drought treatments.

Genotypes	Control			Drought		
	Band No.	Molecular weight (kDa)		Band No.	Molecular weight (kDa)	
Kalajeera	10	1	200.00	9	1	200.00
		2	138.88		2	138.88
		3	114.23		3	114.06
		4	112.57		4	112.08
		5	109.01		5	101.03
		6	100.29		6	99.12
		7	86.62		7	85.36
		8	73.72		8	72.29
		9	63.35		9	63.35
		10	47.14			
Pandakagura	7	1	200.00	11	1	200.00
		2	114.90		2	154.53
		3	113.23		3	115.57
		4	109.49		4	113.73
		5	100.58		5	110.45
		6	79.32		6	102.21
		7	63.35		7	100.58
					8	86.62
					9	75.91
					10	64.92
					11	47.72
Dangarbayagundar	7	1	200.00	8	1	200.00
		2	114.90		2	115.74
		3	102.21		3	111.42
		4	91.40		4	102.66
		5	78.17		5	93.21
		6	64.60		6	79.71
		7	48.42		7	64.60
					8	48.54
Mugudi	10	1	200.00	9	1	200.00
		2	163.00		2	127.06
		3	119.39		3	115.40
		4	114.90		4	103.27
		5	111.10		5	101.77
		6	102.21		6	93.66
		7	89.63		7	80.10
		8	77.79		8	65.88
		9	64.44		9	48.54

		10	48.19			
Macchakanta	12	1	200.00	9	1	200.00
		2	170.41		2	129.34
		3	127.06		3	116.08
		4	115.23		4	103.27
		5	111.75		5	91.40
		6	103.27		6	79.71
		7	101.92		7	66.52
		8	89.63		8	55.93
		9	79.32		9	48.66
		10	65.88			
		11	55.93			
		12	48.78			
Haladichudi	6	1	200.00	7	1	200.00
		2	123.72		2	130.50
		3	103.27		3	103.72
		4	101.77		4	102.07
		5	78.94		5	92.30
		6	67.84		6	80.10
					7	65.72
N22	4	1	200.00	5	1	200.00
		2	124.82		2	136.43
		3	104.02		3	104.94
		4	65.24		4	80.10
					5	66.04
IR64	5	1	200.00	3	1	200.00
		2	135.23		2	98.69
		3	104.79		3	86.20
		4	79.71			
		5	65.56			

Table 2.

Jaccard's similarity coefficient (below diagonal) and Nei and Lee Dice coefficient (above diagonal) among different rice genotypes based on protein banding patterns.

Variety	Kalajera	Pandakagura	Dangarbayagundar	Mugudi	Macchakanta	Haladichudi	N 22	IR 64
Kalajera	1	0.333	0.010	0.011	0.400	0.556	0.500	0.091
Pandakagura	0.500	1	0.125	0.125	0.091	0.200	0.182	0.429
Dangarbayagundar	0.012	0.222	1	0.333	0.100	0.0120	0.010	0.286
Mugudi	0.012	0.222	0.500	1	0.010	0.100	0.010	0.286
Macchakanta	0.571	0.167	0.182	0.012	1	0.400	0.500	0.091
Haladichudi	0.714	0.333	0.012	0.182	0.571	1	0.500	0.010
N 22	0.667	0.308	0.012	0.012	0.667	0.667	1	0.083
IR 64	0.167	0.600	0.444	0.444	0.167	0.013	0.154	1

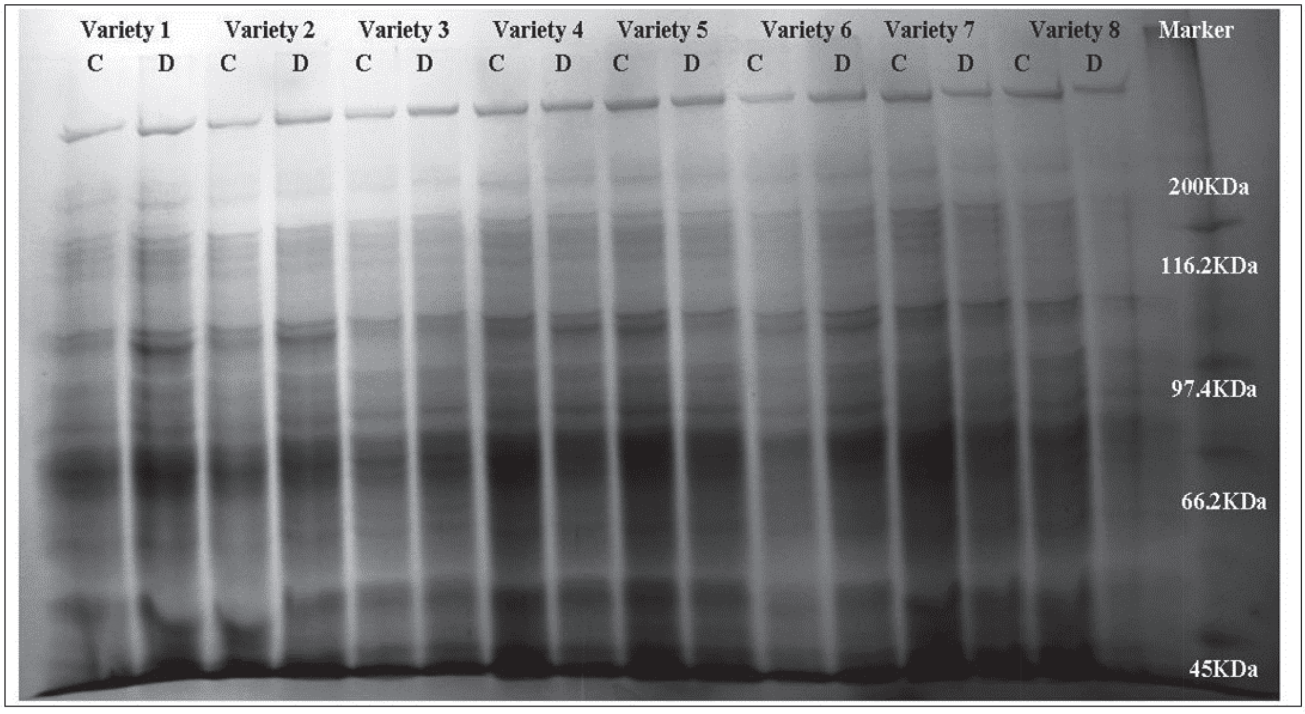

Figure 1: Changes of protein banding pattern in leaf tissue in different folk rice genotypes under control and drought stress. Genotypes 1: Kalajeera; 2: Pandakagura; 3: Dandarbayagundar; 4: Mugudi; 5: Machhakanta; 6: Haladichudi; 7: N22; 8: IR 64.

Kalajeera showed highest genetic similarity with drought tolerant check (N22) variety. Similarly, genetic distance varied from 0.010 to 0.556 among the studied genotypes (Table 2). Based on the results, Machhakanta, Haladichudi, and Kalajeera showed higher Nei and Lee Dice coefficient and showed highly genetically distant from other genotypes.

As genetic diversity is an important requirement for a successful breeding programme and biochemical characteristics such as proteins are powerful tools for the analysis of genetic diversity in rice (Das *et al.*, 2010). Cluster analysis based on the Bray-Curtis paired linkage revealed the percent of similarity in protein banding pattern among rice genotypes presented in Fig. 2. Based on the protein banding pattern under drought stress studied rice genotypes wereforming three major clusters. Indigenous rice landraces such as Machhakanta, Haladichudi, and Kalajeera forming one cluster with drought tolerant check (N22) cultivar showed 66% similarity where as, *Dangarbayagundar* and *mugudi* formed separate cluster with 45% similarity (Fig.2). The genotypes Pandkagura formed separate cluster with drought susceptible check (IR64) cultivar with 55% similarity.

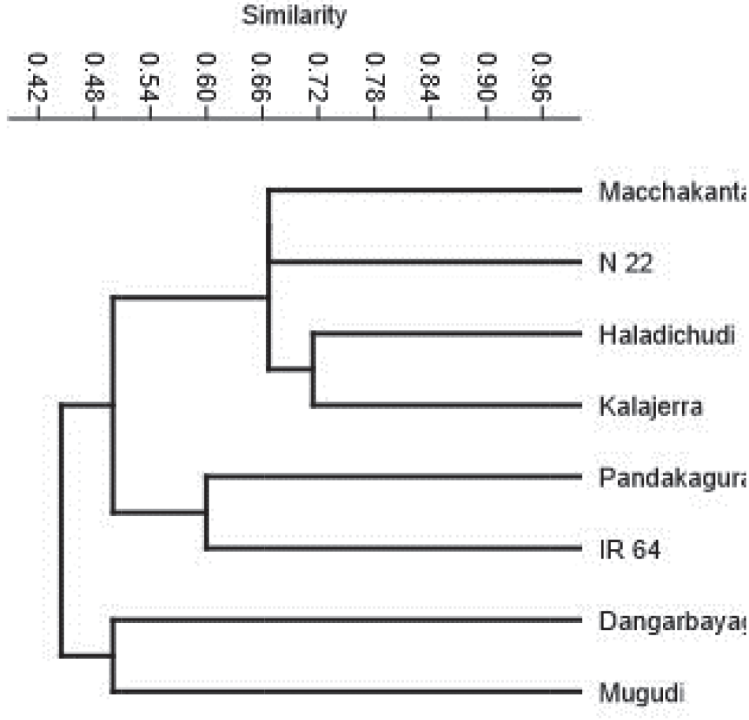

Figure 2: Dendrogram showing the Bray-Curtis similarity index among folk rice genotypes constructed using different protein banding pattern.

3. Conclusion

In conclusion, three genotypes such as Kalajeera, Haldichudi and Machhakanta showed highest genetic similarity with drought-tolerant check variety (N22). Presence of some unique protein bands in these genotypes indicating their importance for varietal identification in drought tolerance breeding. These three indigenous rice genotypes from Koraput are proved to have drought tolerance ability and can later be used for 'omic' studies to better understand stress responses. Further, gene expression studies are aimed to know their molecular mechanism for drought tolerance.

Acknowledgements

The authors are grateful to Science and Technology Department, Govt. of Odisha [Ref. No. 3340 (Sanc.)/ST / 22.06.17] for financial support and University Grants Commission, New Delhi, Govt. of India for providing Non-NET Fellowship. The Regional Director, MS Swaminathan Research Foundation (MSSRF), Jeypore, Odisha and the Director, National Rice Research Institute, Cuttack, Odisha are highly acknowledged for providing the rice seeds for the experiment.

References

Arunachalam, V., Chaudhury, S.S., Sarangi, S.K., Ray, T., Mohanty, B.P., Nambi, V.A. and Mishra, S. (2006). Rising on rice: The story of Jeypore. Chennai: MS Swaminathan Research Foundation. 1: 39.

Borah, P., Sharma, E., Kaur, A., Chandel, G., Mohapatra, T., Kapoor, S. and Khurana, J.P. (2017). Analysis of drought-responsive signalling network in two contrasting rice cultivars using transcriptome-based approach. Sci. Rep. 7: 42131.

Choudhary, M.K., Basu, D., Datta, A., Chakraborty, N. and Chakraborty, S. (2009). Dehydration responsive nuclear proteome of rice (*Oryza sativa* L.) illustrates protein network, novel regulators of cellular adaptation, and evolutionary perspective. Mol. Cell. Proteom. 8(7): 1579-1598.

Das, A., Kesari, T.V. and Rangan, L. (2010). Aromatic joha rice of Assam-A review. Agric. Rev. 31:1–10.

Farooq, M., Kobayashi, N., Wahid, A., Ito, O. and Basra, S.M. (2009). Strategies for producing more rice with less water. Adv. Agron.101: 351-387.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227(5259): 680-685.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. J. Biol. Chem. 193(1): 265-275.

Mishra, G., Zhang, W., Deng, F., Zhao, J. and Wang, X. (2006). A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in *Arabidopsis*. Science. 312(5771): 264-266.

Mishra, S.S., Behera, P.K. and Panda, D. (2019). Genotypic variability for drought tolerance-related morphophysiological traits among indigenous rice landraces of Jeypore tract of Odisha, India. J. Crop Improv. 33: 254-278.

Mishra, S.S., Behera, P.K., Kumar, V., Lenka, S.K. and Panda, D. (2018). Physiological characterization and allelic diversity of selected drought tolerant traditional rice (*Oryza sativa* L.) landraces of Koraput, India. Physiol. Mol. Biol. Plants, 24(6): 1035-1046.

Panda, D., Mohanty, B., Behera, P.K., Barik, J. and Mishra, S.S. (2020). Harnessing leaf photosynthetic traits and antioxidant defence for multiple stress tolerance in three premium indigenous rice landraces of Jeypore tract of Odisha, India. Funct. Plant Biol. 47: 99-111.

Pandey, V. and Shukla, A. (2015). Acclimation and tolerance strategies of rice under drought stress. Rice Sci. 22(4): 147-161.

Patra, B.C. and Dhua, S.R. (2003). Agro-morphological diversity scenario in upland rice germplasm of Jeypore tract. Genet. Resour. Crop Evol. 50(8): 825-828.

Qureshi, M.I., Qadir, S. and Zolla, L. (2007). Proteomics-based dissection of stress responsive pathways in plants. J. Plant Physiol. 164(10): 1239-1260.

Roy, P.S., Patnaik, A., Rao, G.J.N., Patnaik, S.S.C., Chaudhury, S.S. and Sharma, S.G. (2016). Participatory and molecular marker assisted pure line selection for refinement of three premium rice landraces of Koraput, India. Agroecol. Sustain. Food Syst. 41(2): 167-185.

Samal, R., Roy, P.S., Sahoo, A., Kar, M.K., Patra, B.C., Marndi, B.C. and Gundimeda, J.N.R. (2018). Morphological and molecular dissection of wild rices from eastern India suggests distinct speciation between *O. rufipogon* and *O. nivara* populations. Sci. Rep. 8(1): 2773.