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Seed storage protein evaluation of few rice varieties used by tribal people of Chhattisgarh

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Abstract

Seed storage protein profiling of seven local rice varieties namely Jeeraphul, Karheni, Sighar, Ratua, Lusari, Chhindmauri, Kalinga collected from Pratappur, Chhattisgarh were analyzed in the present study. Albumin was observed to be notably lowest in all rice varieties (1.2 mg/g to 14.4 mg/g of seed flour) where as glutelin fraction was found to be the highest (19.6 mg/g to 67.6 mg/g of seed flour) in all varieties. The percentage yield of prolamin is high in Lusari and Ratua i.e. 58.5 mg/ g of seed flour where as Karheni and Sighar varieties were found to have least prolamin i.e. less than 2 mg/g of seed flour. Seed storage protein profile analysis revealed polymorphic prolamin banding pattern in Kalinga and Karheni rice varieties where as similar globulin and glutelin profiles observed among all the studied varieties.

Key words: Rice, Seed storage protein, Prolamin

Introduction

Chhattisgarh is responsible for more than 70% of the Country's rice production and popularly known as Rice bowl of India (Rahman et al., 2006). Many rice varieties have been documented from this region that are consequence of centuries of rice farming by native communities through adaptation and selection to a variety of micro-ecosystem conditions. Cereals are the major source of energy, protein, vitamins and minerals for the world's largest population (McKevith 2004). In addition, rice protein is hypoallergenic and rich in lysine (Wang et al., 2014). Therefore, rice protein is commonly used in baby foods of limited formula for children with food sensitivity. Rice seed storage protein is a significant source of energy and nutrition, the second most copious ingredient of rice after starch (Chen et al., 2018). The major classes of seed storage protein in rice have been classified according to their relative solubility into four fractions: albumin, globulin, prolamin and glutelin. Many reviews and research papers have been published in the recent years on rice seed storage proteins and they have confirmed that storage globulin constitute major endosperm storage protein in rice. The rice proteins are usually not soluble in dilute salt solutions and categorised as glutelins, but they actually belong to the 11–12S globulin family (Shewry and Halford 2002).

Rice protein is considered to be of high-quality as it contains eight out of ten essential amino acids. Rice has elevated level of lysine in comparison to wheat and maize, which provides high digestibility and dietary quality (Santos et al., 2013). The identification of protein rich rice genotypes not only revolutionized plant breeder's but also increase the nutritional quality of the diet in poor tribal communities where rice act as the staple food. The present study is an attempt to determine the contents of four seed storage protein fractions (albumin, globulin, prolamin and glutelin) among few rice varieties of Chhattisgarh.

Materials and Methods

Plant materials: Seven local rice varieties such as Jeeraphul, Karheni, Sighar, Ratua, Lusari, Chhindmauri, Kalinga were collected from Pratappur, Surajpur district of Chhattisgarh (Figure 1). Whole seeds were crushed to fine powder that is used as the raw material in this study.

Extraction, Quantification and electrophoresis of various seed storage proteins: The protein extraction was performed at room temperature. Rice flour (200 mg) of different varieties successively extracted with (i) 500 ul of deionized water, (ii) 1 M NaCl (iii) 80% alcohol and (iv) 0.01 M NaOH for the extraction of four major seed storage

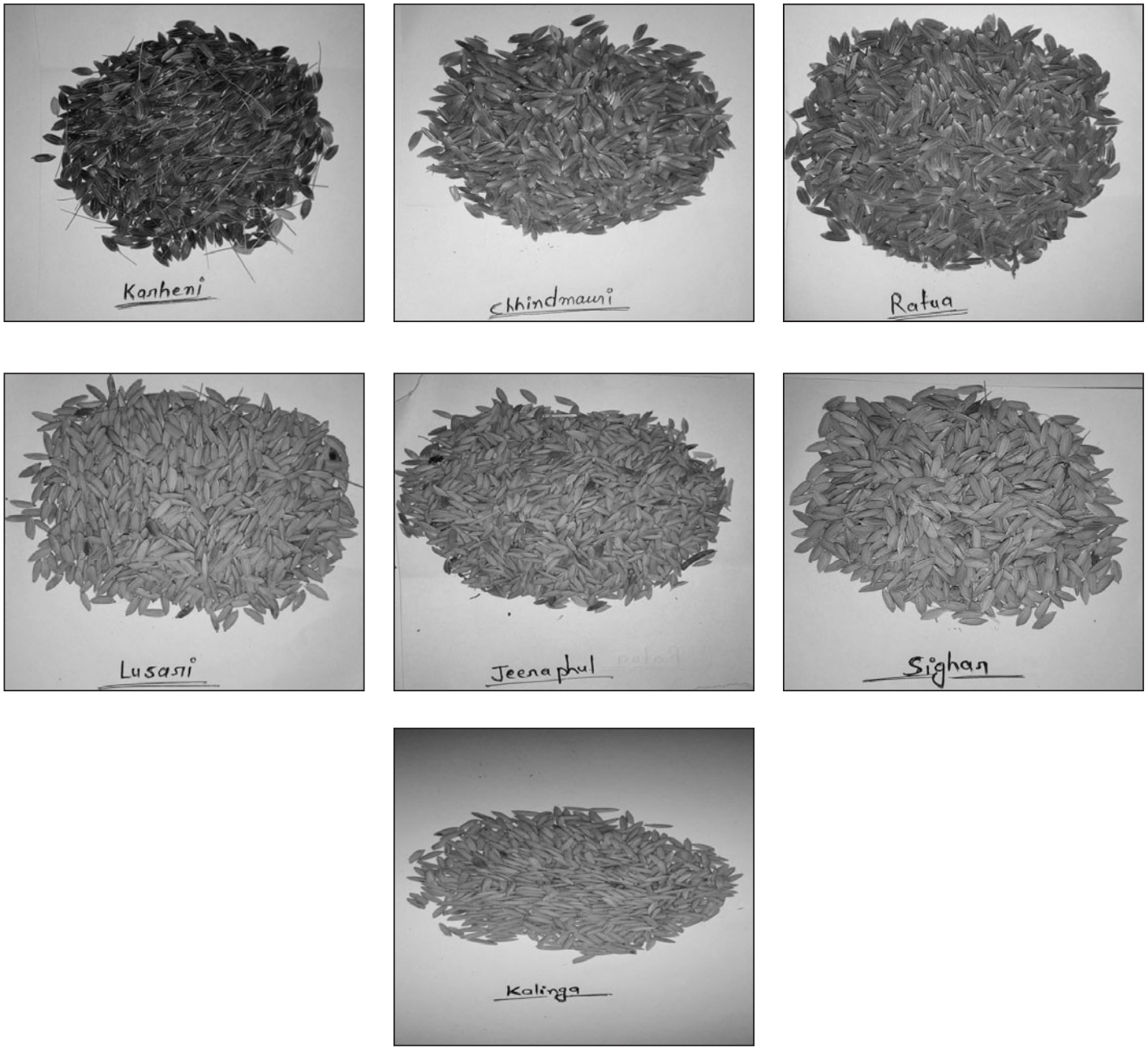

Figure 1: Photographs of collected rice varieties

proteins i.e. albumin, globulin, prolamin and glutelin respectively (Figure 2). Protein concentrations were estimated by Lowry method using BSA as standard (Lowry et al., 1951). The percentage yields were calculated as per the formula: (Protein fraction/ Total seed storage protein X 100) (Khanzada et al., 2016). Seed storage protein profile was done by Laemmli's discontinuous buffer system (Laemmli 1970) on a vertical gel containing 4% stacking gel and 12 % resolving gel of 29.2 % acrylamide / 0.8 % N,N'-methylene- bis-acrylamide (BIS). 5 µl each of protein were mixed separately with 5 µl of sample buffer (1.25 ml 1M Tris-HCl, 2.5 ml glycerol, 2 ml 10% SDS,0.2 ml 0.5% BPB final volume make up to 10 ml). These mixtures were boiled at 100°C for 2 minutes and then loaded on to the wells of a polyacrylamide gel. Protein gels were stained with 50% methanol, 10% acetic acid, and 0.25% Coomassie brilliant blue. The molecular weight of protein bands determined manually by calculating the Rm (relative mobility) and considering BSA as the standard.

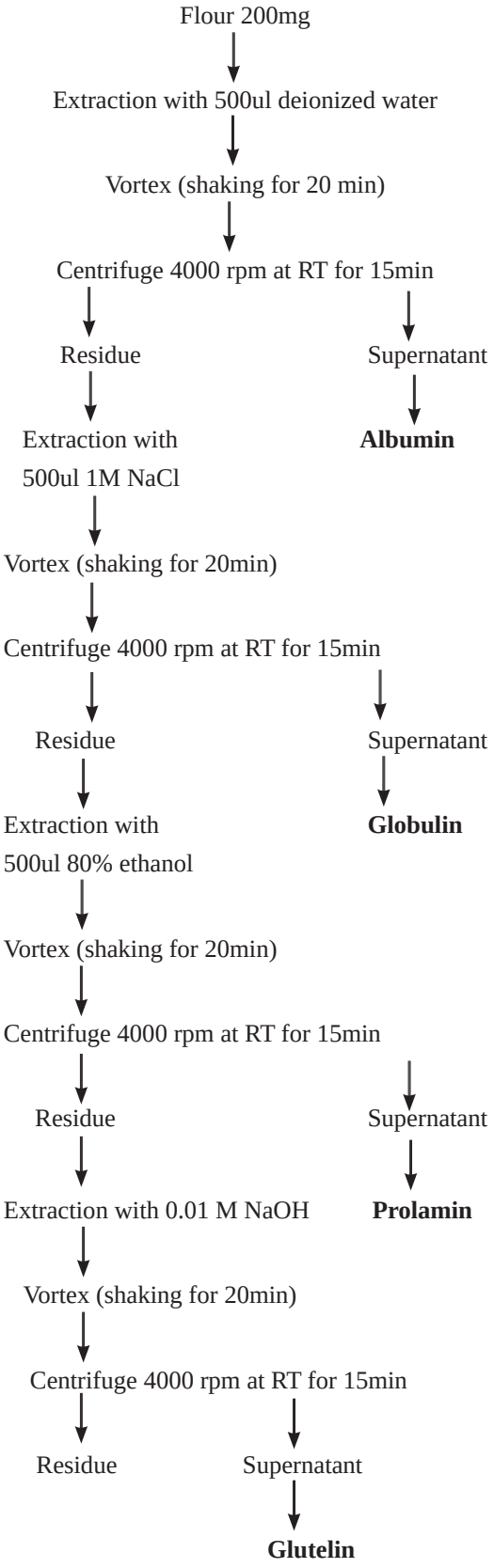

Figure 2: Flow chart for rice seed storage protein extraction

Results and Discussion

The collected local rice varieties varied in their protein contents. Variation in kernel color, grain size and protein content of all rice varieties are summarized in Table 1. The color of seeds in different rice varieties are red, light red and white.

Table 1: Grain colour, size and total seed storage protein of the collected rice varieties

S. No	Rice varieties (Local name)	Seed color	Grain size mm	Total seed storage protein (mg/g of seed flour) (Albumin+ Globulin + Prolamin + Glutelin)
1	Lusari	Light red	5.0	89.65
2	Karheni	White	7.0	1.85
3	Sighar	White	6.2	24.73
4	Chhindmauri	White	6.0	72.75
5	Kalinga	White	4.8	59.25
6	Ratua	White	6.8	122.1
7	Jeeraphul	White	6.3	88.6

Fractionation of seed storage protein based on their solubility: The storage proteins are generally categorized in four types due to their solubility: albumin- H_2O soluble, globulin- NaCl soluble, prolamin- alcohol soluble and glutelin- NaOH soluble protein. Albumin was found to be significantly low in all rice varieties except Ratua (14.4 mg/ g of seed flour). Globulin yield is low in Karheni and Jeeraphul (0.8 mg/ g of seed flour) where as Chhindmauri , Lusari and Kalinga contain 3.2, 5.95 and 6.75 mgof globulin/g of seed flour. . The percentage yield of prolamin is high in Lusari and Ratua i.e. 49.24 and 47.91 % respectively, where as Karheni and Sighar varieties were found to have least prolamin i.e. less than 2 mg/g of seed flour (Table 2). Glutelin fraction was found to be the highest in all varieties except Sighar i.e. 19.6 mg/ g of seed flour (Table 2). Ratua variety identified with the highest levels of albumin, prolamin and glutelin protein fractions (Table 2).

Seed storage protein is a distinctive quantitative trait usually affected by environment (Shewry, 2007). The combination of conventional breeding and marker assisted selection will provide a more proficient move towards improving the storage protein content of the rice grain than traditional breeding (Zhang et al., 2008).There are reports that prolamin and glutelin constitute 80-85% of rice total seed protein and are the good pointer of high protein content (Vithyashini and Wickramasinghe 2015). Glutelin

protein is repoted to be rich in essential amino acids and recognized as easily digestible protein (Resurreccion *et al*., 1993). The present study revealed that out of the seven studied local rice varieties six are glutelin rich.

Polymorphism of storage proteins in different rice varieties

There were differences in protein banding patterns between the local rice varieties. As shown in Figure 5, similar prolamin band was observed for Jeeraphul, Sighar and Lusari respectively. However, marked difference observed in the prolamin banding pattern of Kalinga and Karheni rice varieties. The prolamin band with aproximate size of 14 kDa was found in all studied varieties except Karheni. All the rice varieties showed monomorphic globulin (approximate size of 26kDa) and glutelin (approximate size of 22kDa) profile (Figure 3 and 4). Jin et al. (2006) have reported that 80% of rice varieties had similar seed storage patterns, suggesting that storage protein polymorphism in rice cannot be used to distinguish different ecotypes. In contrast the present study showed different patterns of prolamin seed storage proteins.

Table 2: Comparison of the concentrations and total percent yield of the major seed storage proteins

S.No.	Rice varieties	Albumin		Globulin		Prolamin		Glutelin	
		mg/g	% Yield	mg/g	% yield	mg/g	% yield	mg/g	%yield
1	Lusari	1.2	1.33	5.95	4.40	**58.5**	42.94	**24**	26.77
2	Karheni	ND	--	0.8	43.24	1.05	46.75	ND	--
3	Sighar	3.2	12.93	ND	--	1.93	7.80	19.6	79.25
4	Chhindmauri	1.8	2.47	3.2	4.39	**29.25**	40.20	**38.5**	52.92
5	Kalinga	1.6	2.70	6.75	11.79	6.8	11.47	**44.1**	74.43
6	Ratua	**14.4**	11.79	ND	--	**58.5**	47.91	**49.2**	40.29
7	Jeeraphul	1.4	1.58	0.8	0.90	18.8	21.21	**67.6**	76.29

Bold font indicates high values of seed storage proteins observed in samples, ND – Not Detectable, -- Not Measurable

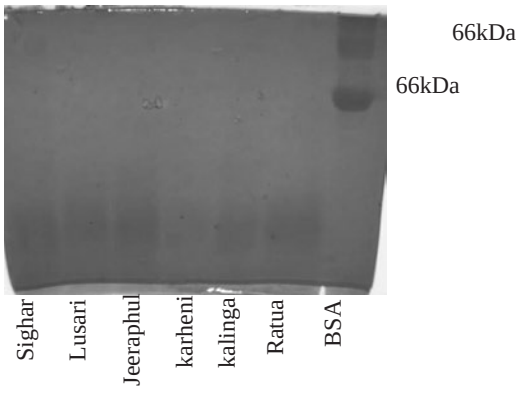

Figure 3: SDS-PAGE analysis Globulin protein bands

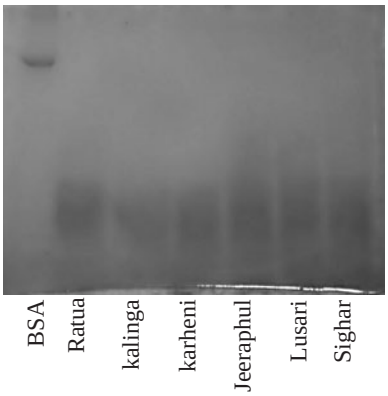

Figure 4: SDS-PAGE analysis of Glutelin proteins

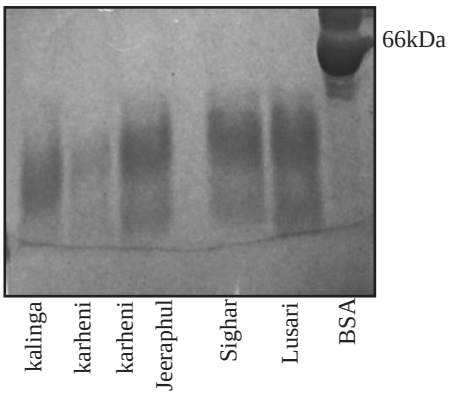

Figure 5: SDS-PAGE analysis of Prolamin proteins (Black arrow shows polymorphic fragments)

Conclusions

The present work provides information on seed storage protein profiling of seven local rice varieties. In conclusion, this study revealed that out of the seven local rice varieties collected, six varieties are glutelin rich. Seed storage protein profile analysis revealed polymorphic prolamin banding pattern in Kalinga and Karheni rice varieties. These polymorphic fragments may be exploited as markers for rice breeding.

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