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Nutritional evaluation of different rice (*Oryza sativa*) straw of bio-fortified varieties, bio-fortified breeding lines and breeding lines

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Zinc deficiency is a well-established global health problem, affecting nearly half of the world population especially in developing countries, where high proportion of cereal crops, such as rice and wheat are consumed as staple food (Wei *et al.* 2012). Rice (*Oryza sativa*) is major straw available for feeding livestock contributes to around 24.7% of total dry matter (Gorti *et al.* 2012). Bio-fortification refers to genetic enhancement of key food crops with enhanced nutrients and differs from fortification (using addition of exogenous nutrients). Very fundamental purpose of bio-fortifcation is to enhance the key nutrients like protein, zinc, iron or β-carotene (Neeraja *et al.* 2017). Hence, straws arising out of bio-fortified crops are precious value-added items for feeding livestock.

Keeping in view the enormous quantities of straw, chemical methods were attempted to break the ligno-cellulosic bonds to make them more digestible to beef cattle (Klopfenstein 1978). However under Indian perspective, the chemical methods are more costly and high input oriented. Alternatively, plant breeders are aiming to improve the grain yield and quality traits through conventional approaches or molecular techniques. Several workers have shown a lack of correlation of straw quality and grain yield [Tuah *et al.* 1986 (barley, oat and wheat), Reed *et al.* 1987 (sorghum; Bainton *et al.* 1991 (rice)]. Williams *et al.* (1996) observed cultivar differences in straw have a genetic component which could be enhanced by selection and concluded that plant breeders need to consider the quality of straw as well as the grain to have sustainable holistic approach, sought for farming systems. Further, they observed that variation in nutritional quality of crop residues can be due to many factors like genetic, climatic and cultivation practices such as fertilizer application, water management, and stage of maturity at harvesting and post-harvest storage. The present work was taken up with an objective to evaluate the rice straw varieties of bio-fortified varieties (BFV), bio-fortified breeding lines (BBL) and breeding lines (BL) and find out any correlation between straw quality parameters with any of the plant phenological parameters so as to suggest plant breeders in their varietal development programmes.

A total of 13 varieties of rice straw including eight bio-fortified varieties (BFV), two bio-fortified breeding lines (BBL), two breeding lines (BL) and one check variety of rice (*Oryza sativa*) straw were grown at Indian Rice Research Institute, Rajendranagar, Hyderabad (Table 1). After the harvest of crop, straw samples were subjected to straw quality evaluation at National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru.

The samples were ground in Cyclotec Mill (Foss) using 1 mm sieve. The samples were analyzed for crude Protein (990.03: AOAC 2012), total ash (942.05: AOAC, 2012) and crude fiber (962.09: AOAC 2012). The minerals such as Zn and Fe were estimated using dry ashing procedure and extracted with 5N hydrochloric acid and the mineral extract was injected to inductively coupled plasma-optical emission spectroscopy (ICP-OES) using a Perkin Elmer instrument (Sun *et al.* 2000).

In vitro gas production test was conducted on test samples for finding out *in vitro* organic matter digestibility (IVOMD) (Menke and Steingass 1988). Blanks and 200 mg well ground samples were incubated in triplicates with 30 ml of mixture of strained rumen liquor and buffer. The rumen liquor was obtained from bullock before morning feeding which was well adapted to roughage based diets [green fodder and finger millet (*Eleusine corocana*)]. The incubation was arrested at the end of 24 h and the contents refluxed with double strength Neutral Detergent Solution. The IVOMD was determined after ashing the crucibles.

The individual fatty acids like acetate, propionate, butyrate, iso-butyrate, valerate and iso-valerate in the incubation medium were estimated using the procedure (Cottyn and Boucque 1968) using gas chromatograph (Agilent – 7890A) equipped with flame ionization detector (FID) capillary column (Agilent J&W DB-WAX GC column 40 mm × 0.18 mm × 0.18 μm).

The various straw quality and volatile fatty acids parameters were analyzed for significance between broad

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Table 1. Details of bio-fortified varieties/lines along with check samples grown at IIRR, Hyderabad

Name of variety/breeding line	Particulars
Zinco rice	Released biofortified variety
Surabhi	Released biofortified variety
Protozin	Released biofortified variety
CGZR2	Released biofortified variety
DRR Dhan 45	Released biofortified variety
DRR Dhan 48	Released biofortified variety
DRR Dhan 49	Released biofortified variety
CR Dhan 311	Released biofortified variety
IET 25443/CNBC	Biofortified breeding line
IET 24773/CNRB	Biofortified breeding line
SJ3	Breeding line
MV/2	Breeding line
BPT5204	Check variety

groups of BFV, BBL and BL using analysis of variance. Similarly, correlation co-efficients were calculated among all the parameters using Statistical Package for the Social Sciences software (PASW Statistics Release 18.0.0 (30 July 2009). The significance level of 0.05 was taken for comparison of treatment means (Snedecor and Cochran 1994).

The crude protein of rice straw ranged from 4.2 in Zinco Rice to 6.0 in CGZR2 with average of 5.05 in BFV. In case of BBL and BL, the CP was found to be 5.28 and 4.04, respectively (Table 2). Straw CP was negatively correlated with CF of straw and positively correlated with IBR, ZBR and ZPR (Table 3). Contrary to the similar protein levels across different varieties, improved N level of rice straw was observed by Khan *et al.* (2003) due to application of zinc in various forms (soil application, 10 kg Zn/ha), root dipping (1.0% $ZnSO_4$) and foliar spray (0.2% $ZnSO_{4)}$.

The total ash of rice straw ranged from 16.8 in CGZR2 to 19.1 in Zinco Rice with average of 17.9 in BFV. In case of BBL and BL, the total ash was found to be 17.1 and 18.1, respectively. The straw ash was not correlated with any of the parameter studied.

The CF (%) of rice straw ranged from 38.1 in CGZR2 to 50.8 in DRR Dhan 49 with average of 45.2 in BFV. In case of BBL and BL, the total ash was found to be 45.0 and 45.2, respectively. The straw crude fiber was negatively correlated with many parameters such as SPDW, FLL, IBR, ZBR, ZPR, straw CP and positively correlated with Straw Fe.

Zinc (ppm) of rice straw ranged from 5.5 in Zinco Rice to 9.4 in Protozin in with average of 7.49 in BFV. In case of BBL and BL, the straw Zn was found to be 15.1 and 11.9, respectively. The contents were significantly higher than BBL indicating further scope of improving zinc concentration. The straw zinc was negatively correlated with IPR. Higher zinc values in the rice straw (22.1 to 30.1 ppm) were reported Khan *et al.* (2003) by due to application of zinc in different forms (soil application, 10 kg Zn/ha), root dipping (1.0% $ZnSO_4$) and foliar spray (0.2% $ZnSO_{4)}$ compared to observed values in this study. The Fe (ppm)

Table 2. Straw quality parameters of different bio-fortified varieties of rice

Variety	Straw quality parameters					IVOMD
	CP	Total ash	CF	Zn	Fe	(%)
	(%)			ppm		
Released bio-fortified varieties						
Zinco Rice	4.20	19.10	49.20	5.50	121.00	40.40
Surabhi	5.70	17.50	41.80	7.60	52.90	41.00
Protozin	5.60	19.00	48.50	9.40	223.00	48.30
CGZR2	6.00	16.80	38.10	6.60	89.50	42.00
DRR Dhan 45	4.40	17.00	46.40	7.70	69.80	37.90
DRR Dhan 48	4.60	17.70	45.30	6.70	228.00	43.90
DRR Dhan 49	4.50	18.90	50.80	8.70	227.00	49.60
CR Dhan 311	5.50	17.20	41.50	7.80	65.90	41.00
Average	5.05	17.90	45.20	7.49[b]	135.00	43.00
Bio-fortified breeding lines						
IET 25443/ CNBC	5.90	17.80	43.40	15.90	98.10	45.60
IET 24773/ CNRB	4.70	16.40	46.60	14.30	140.00	39.90
Average	5.28	17.10	45.00	15.10[a]	119.00	42.70
Breeding lines						
SJ/3	4.00	18.80	44.30	14.60	73.00	43.60
MV/2	4.10	17.50	46.00	9.20	98.80	43.80
Average	4.04	18.10	45.20	11.90[a]	85.90	43.70
Sig.	0.18	0.51	1.00	0.01*	0.69	0.97
BPT-5204 (Control)	5.50	18.10	47.10	5.00	117.00	49.20

*P<0.01.

of rice straw ranged from 52.9 in Surabhi to 228 in DRR Dhan 48 in with average of 135 in BFV. In case of BBL and BL, the straw Fe was found to be 119 and 85.9, respectively. The straw Fe was positively correlated Straw CF and straw IVOMD.

The straw IVOMD is very important parameter from animal nutrition point of view. IVOMD ranged from 37.9 in DRR Dhan 45 to 49.6 in DRR Dhan49 with an average of 43.0 in BFV. In case of BBL and BL, the IVOMD was found to be 42.7 and 43.7, respectively. The straw IVOMD was positively correlated to Straw Fe only. Vadiveloo (1995) observed small non-significant (P>0.05) differences in canonical correlations between composition parameters and IVD and its morphology and agronomy of whole straw across 32 varieties of rice straw.

The measure of VFA reflects fermentation potential of straw. Acetate is the end product of cellulose fermentation and concentrations (meq/1000 ml) ranged from 23.7 in DRR Dhan 45 to 34.1 in Zinco Rice with an average of 28.9 in BFV. In case of BBL and BL, the acetate was found to be 24.2 and 29.7, respectively (Table 4). Propionate is the end product of soluble carbohydrate fermentation and ranged from 6.5 in DRR Dhan 45 to 9.1 in Zinco Rice and CGZR2 with an average of 7.8 in BFV. In case of BBL and BL, the propionate was found to be 6.59 and 8.02, respectively. Butyrate is the end product of carbohydrate fermentation

Table 3. Correlation co-efficient between phenological and quality parameters in case of different bio-fortified varieties of rice

Parameter	Straw quality parameters						VFA			
	CP	Ash	Crude fibre	Zn	Fe	IVOMD	C2	C3	C4	TVFA
PH	–0.01	–0.13	–0.47	0.45	–0.54	–0.42	–0.02	0.12	–0.08	0
SPDW	0.38	–0.36	–0.79*	–0.30	–0.71	–0.50	0.32	0.33	0.37	0.33
NT	–0.01	0.10	0.00	0.19	0.17	0.24	0.18	0.22	0.12	0.17
NP	0.12	0.03	–0.10	0.12	0.28	0.30	0.23	0.27	0.17	0.22
FLL	0.45	–0.48	–0.74*	0.19	–0.32	–0.22	–0.01	0.09	–0.07	0.01
PL	0.30	–0.28	–0.09	0.43	–0.24	0.00	–0.37	–0.30	–0.34	–0.35
SPY	–0.10	0.08	–0.10	0.20	–0.48	–0.20	0.18	0.15	0.04	0.16
TW	–0.37	–0.01	0.29	0.44	0.11	–0.29	–0.13	–0.05	–0.25	–0.14
IBR	0.55*	–0.24	–0.69*	–0.08	–0.21	–0.09	0.41	0.44	0.38	0.42
ZBR	0.75*	–0.32	–0.77*	–0.03	–0.24	–0.08	0.14	0.13	0.17	0.15
IPR	0.19	–0.11	–0.21	–0.61*	–0.05	0.13	0.07	–0.02	0.12	0.06
ZPR	0.73*	–0.25	–0.63*	–0.07	–0.08	0.03	0.19	0.16	0.21	0.19
Straw CP	1.00	0.58	–0.56*	–0.07	–0.11	0.21	–0.11	–0.16	0.04	–0.11
Straw Ash		1.00	0.58	–0.07	0.41	0.59	0.49	0.45	0.47	0.49
Straw CF			1.00	–0.05	0.61*	0.21	–0.06	–0.07	–0.06	–0.06
Straw Zn				1.00	–0.10	0.00	–0.36	–0.29	–0.50	–0.36
Straw Fe					1.00	0.59*	0.11	0.09	0.03	0.09
Straw IVOMD						1.00	0.14	0.11	0.13	0.13
Acetate							1.00	0.98*	0.94*	1.00*
Propionate								1.00	0.91*	0.99*
Butyrate									1.00	0.95*
TVFA										1.00

*P<0.05. PH, Plant height; SPDW, Single plant dry weight; NT, Number of tillers; NP, Number of productive tillers; FLL, Flag leaf length; PL, Panicle length; SPY, Single plant grain yield; TW, Total weight (100 grain); IBR, Iron in brown rice; ZBR, Zinc in brown rice; IPR, Iron in polished rice; ZPR, Zinc in polished rice; CP, Crude protein; IVOMD, *In vitro* organic matter digestibility; C2, Acetate; C3, Propionate; C4, Butyrate; TVFA, Total volatile fatty acids.

Table 4. Total volatile fatty concentration (meq/1000 ml) incubation medium in different bio-fortified varieties, lines and breeding lines

Variety	Acetate	Propionate	Isobutyrate	Butyrate	Valerate	Isovalerate	Total
Released bio-fortified varieties (*BFV*)							
Zinco Rice	34.1	9.10	0.60	3.50	0.90	0.40	48.6
Surabhi	25.9	6.70	0.40	2.50	0.60	0.20	36.2
Protozin	29.2	7.60	0.40	2.70	0.60	0.30	40.7
CGZR2	33.4	9.10	0.60	3.20	0.80	0.30	47.5
DRR Dhan 45	23.7	6.50	0.40	2.20	0.50	0.20	33.5
DRR Dhan 48	29.9	7.90	0.40	2.80	0.60	0.30	41.9
DRR Dhan 49	27.8	7.80	0.40	2.60	0.60	0.30	39.6
CR Dhan 311	27.7	7.70	0.50	2.80	0.70	0.30	39.5
Average	28.9	7.80	0.46	2.80	0.68	0.27	40.9
Bio-fortified breeding lines (*BBL*)							
CNBC	24.1	6.50	0.40	2.30	0.60	0.20	34.2
CNRB	24.3	6.70	0.40	2.20	0.50	0.20	34.3
Average	24.2	6.59	0.39	2.26	0.56	0.23	34.3
Breeding lines (*BL*)							
SJ/3	33.1	9.10	0.50	3.00	0.70	0.30	46.8
MV/2	26.3	7.00	0.40	2.30	0.60	0.20	36.9
Average	29.7	8.02	0.47	2.69	0.67	0.28	41.8
BPT5204 (Control)	29.6	7.80	0.50	3.10	0.80	0.30	42.1

important in maintaining rumen and colon epithelial health and ranged from 2.2 in DRR Dhan 45 to 3.5 in Zinco Rice and CGZR2 with an average of 2.8 in BFV. In case of BBL and BL, the butyrate was found to be 2.26 and 2.69, respectively. Valerate was in range of 0.5 to 0.9 in different rice varieties. The isobutyrate and isovalerate concentrations ranged from 0.4–0.6 and 0.2–0.4 in different varieties.

On the whole, total VFA gives a measure of fermentation of complex carbohydrates present in the straw. The concentration ranged from 33.5 in DRR Dhan 45 to 48.6 in Zinco Rice with average 40.9 in BFV. In case of BBL and BL, the TVFA was found to be 34.3 and 41.8, respectively.

Overall results indicated that no significant differences were observed between BFV, BBL and BL with regard to volatile fatty acids. Correlation analysis revealed that individual VFA as well as TVFA did not have positive or negative significant correlation in any of the plant phenological parameter or straw quality parameter. However, positive significant correlations were obtained between acetate and propionate, butyrate and TVFA. Similarly, propionate was positively correlated with butyrate and TVFA. Butyrate was also positively correlated with TVFA.

SUMMARY

A total of 13 varieties of rice straw including eight bio-fortified varieties (BFV), two bio-fortified breeding lines (BBL), two breeding lines (BL) and one check variety of rice straw were subjected to straw quality evaluation. The Straw CP was negatively correlated with CF of straw and positively correlated with Iron and zinc content of brown rice and zinc content of polished rice. CF of rice straw was negatively correlated with important plant breeding parameters such as single plant dry weight, flag leaf length, iron content of brown rice, zinc content of both brown and polished rice. Improved bio-fortified breeding lines had more zinc than bio-fortified varieties indicating further scope of improvement of zinc content in straw. *In vitro* organic matter digestibility (IVOMD) ranged from 37.9 in DRR Dhan 45 to 49.6 in DRR Dhan49 with an average of 43.0 in different bio-fortified varieties. Thus, the straw obtained from different bio-fortified crops is valuable feed item which needs to be utilized in livestock rations.

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