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Evaluation of grain quality and yield attributes traits in biofortified rice (*Oryza sativa* L.) Lines

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ABSTRACT

An experiment was conducted for the ten rice lines developed by crosses between 2 parents Varalu having high iron and zinc content and NDR-359 have good yield in Randomized complete block design at student instructional farm kumarganj, Ayodhya during *Kharif season* 2023. Ten lines of rice were evaluated during 2023 on the basis of grain quality parameters and yield attributing traits with three replications. The maximum protein (mg/g) content (9.54) was observed in varalu and in NDR 2305 (9.09). Total starch content (78.71%) in NDR 359 and in developed line NDR 2302 (77.91%). The maximum iron and zinc content (ppm) was found in varalu (17.29 ppm, 26.76ppm) and in line NDR 2303 (17.04ppm), in NDR 2302 (25.33ppm) respectively. Phytic acid content high in lines NDR 2301(11.17µg/100ml). Maximum panicle length was recorded (24.83cm) in NDR 2305. Highest number of grains per panicle was recorded (128.33) in NDR 2302. Maximum test weight (g) was recorded (24.73) in NDR 2305. Maximum grain yield per plant was recorded (24.42g) in NDR 359 and (22.39g) in line NDR 2305.

Keywords; Quality parameters, Starch content, Test weight etc.

INTRODUCTION

Rice is the second most important food crop in the world. It is the primary staple for half of the world population and for more than 70% of the poor. In the Indian scenario it is estimated that the rice demand will be 140 million tonnes in 2025 (Mishra, 2004). By 2030, the world's population is predicted to rise from 6.1 billion to 8.0 billion, and to satisfy the rising demand, rice production needs to rise by 50%. Only by continuing to raise production steadily over time will this anticipated demand be satisfied. The modern period of the World Trade Organization requires that enough rice be produced for both export and self-sufficiency. It is necessary to create the exportable excess of high-quality rice at a competitive price. The goal of the current study was to find early maturing genotypes with high yields. The genotypes were evaluated based on attributes associated with yield and quality metrics. A rise in rice output is essential to ensuring food security. One beneficial chance for selection is to comprehend genetic heterogeneity in the experimental material (Singh *et al.,* 2020). The variations among members of a population brought about by genetic makeup and the environment in which they grow are known as phenotypic variability (Sumanth *et al.,* 2017). The level of genetic diversity determines how breeding effort aimed at improving quantitative traits is planned and carried out. Thus, the presence of genetic variability with regard to desired qualities and the plant breeder's selection competence are the only factors that can make plant breeding efforts successful (Adhikari *et al.,* 2018).

Essential minerals including iron and zinc support immune system function, cell growth and development, and protein synthesis (Roohani *et al.,* 2013). According to Read *et al.,* (2019), zinc also possesses antiviral qualities against a number of virus species that frequently harm people. The metabolic functions of living things, such as electron transport, deoxyribonucleic acid (DNA) synthesis, and oxygen transport, depend on iron (Abbaspour et al., 2014). Thus, the body requires enough consumption of the minerals zinc and iron. In underdeveloped nations, children's health issues are caused by a lack of iron and zinc. Globally, the prevalence of stunting was 29.1%, wasting was 6.3%, and underweight was 13.7% (Ssentongo *et al.,* 2021). The human body uses iron and zinc primarily for a variety of metabolic processes.

Thus, insufficient consumption of iron and zinc can result in immunological dysfunction mediated by cells, cognitive decline, and, most significantly, growth retardation. Anaemia results from a reduction in the body's circulating haemoglobin and erythrocyte synthesis brought on by an iron shortage. The main and most important foods with significant health benefits are functional food products. The use of supplements seems to be a promising way to avoid and counteract the detrimental effects of deficits in zinc and iron (Abdollahi *et al.,* 2019). However, because these essential elements will chelate with phytic acid in food grains, resulting in low Fe and Zn bioavailability for the human body, staple food crops like rice cannot supply enough of these elements to meet daily dietary demands (Wang *et al.,* 2021). In order to address the issues of malnutrition worldwide, a number of national and international organizations, including the FAO, the International Rice Research Institute (IRRI), the Indonesian Centre of Rice Research (ICRR), the Indian Council of Agricultural Research (ICAR), and others in Asia-Africa, are working with partner nations to develop rice cultivars with high Fe and Zn content. (Swamy *et al*., 2021).

MATERIALS AND METHODS

There were 10 genotypes of rice developed by crossing 2 parents (Varalu and NDR-359). An experiment was conducted in the *Kharif* season 2023. It was conducted at the Student Instructional Farm (SIF) in Acharya Narendra Deva of Agriculture and Technology University Kumarganj, Ayodhya. The RILs lines have been developed by the crosses of varalu have high level of iron and zinc quantity and NDR-359 have a good yield and then lines seeds were sown at SIF and for sowing, a raised bed nursery was utilized, then single seedling were transplanted in irrigated conditions. After 25 days, single seedlings were transplanted in the Randomized Complete Block Design (RCBD) trial field. Twelve rice genotypes were planted with three replication. Every entry was moved onto a plot measuring 1m in length and 1m in width, with 20 cm separating rows, 15 cm separating plants, and 60 cm separating plots. Within replication, the Parents were randomized. For regular crop growth, typical agronomic methods were implemented. Different Observations were recorded, which were Plant Height (cm), Number of tillers, Days to 100 percent flowering, Days to maturity, panicle length (cm), number of grains per panicle, 1000 grain weight (g), grain yield per plant (g) and after harvesting of seeds then quality parameters had been analysed.

Estimation of quality parameters

A mortar and pestle were used to grind a random sample of seeds from each rice genotype. 0.2M phosphate buffer (pH 7.2) solution was well mixed with 0.50 g of powdered powder samples. The samples were then centrifuged for 10 minutes at 6000 rpm, and the supernatants were gathered in a new tube. filled a tube with 0.2 ml of supernatant and added distilled water to reach a final amount of 1.0 ml. Five millilitres of the alkaline copper sulphate solution were added to each test tube, properly mixed, and incubated for ten minutes. Each tube was filled with 0.5 ml of diluted Folin's reagent, properly mixed, and allowed to sit at room temperature for 30 minutes. The absorbance was measured against a blank at 660 nm. Using the standard curve technique, the protein content of the rice sample was determined.

The amylose content in the grains of rice genotypes was estimated by the modified iodometric method on the basis of amylose -iodine binding mechanism (Juliano *et al.,* 1978). In this, rice grain sample of each genotype crushed separately and homogenized with sodium hydroxide and absolute ethanol. Mixture was kept in water bath as per protocol and incubated it 15 min for reaction. The sample was kept off from water bath and 50 ml water was added in gelatinized sample. The diluted samples were put in amber tubes and iodine solution was added with acetic acid. The samples were further diluted with distilled water and kept it at room temperature for 20 min for appearance of blue colour complex. The optical density (OD) of each sample were recorded at 620 nm against blank with the help of benchtop double beam spectrophotometer (Zonotech, ZT-2201). The amylose content in OD was calculated by preparation of standard curve with rice amylose and result showed as g/100 g sample basis.

Estimation of Fe and Zn

Standard procedure was followed for estimation of Fe and Zn contents (George *et al.*, 2013).

One gram of sample weighed separately for each genotype, transferred quantitatively into 100 mL pyrex digestion tubes. Ten ml of di-acid mixture (HNO_3+$HClO_4$, 2:1) was added to each tube and allowed to stand overnight until the vigorous reaction phase was over. After preliminary digestion, digestion tubes were placed in a cold-block digester and temperature was raised to 150 °C for 60 minutes until all traces of HNO_3 disappeared. Then, temperature was slowly raised to 235 °C. When white dense fumes of $HClO_4$ appeared, digestion was continued for 30 more minutes, until white precipitates settled down at the bottom of digestion tubes. dH_2O was added in small increments for dissolving the crystals and washing of the tube walls. The contents were filtered through Whatman No. 41/42 filter paper and 50 mL dilutions were made with dH_2O. Finally, extract was fed to atomic absorption spectrophotometer (200 Series AA, Agilent Technologies, USA. Each digestion batch contained 12 samples and one blank (no plant material). The analysis was repeated thrice for each genotype and mean value is presented in results. Fe and Zn concentrations were expressed in parts per million (ppm).

Phytic acid extraction

The estimation of phytic acid in seeds, the colorimetric assay was used. It is given by Lorenz *et al.* (2007). Ground white rice seed samples of each genotypes weighing 100 mg were put in 2 ml eppendorf tube and 1.5 ml of 0.65 M HCl was added to each tube. The sample tubes were shaken overnight in a shaker at 120 rpm and were centrifuged at 10000 rpm for 10 minutes. 1.25 ml of Wade reagent (1.25% sulpho-salicylic acid and 0.03g ferric Chloride ($FeCl_{3}$).) was added to each tube and was left at room temperature for 15 to 20 minutes to develop brown color and subsequently their optical densities were measured at 420 nm using spectrophotometer (Uv-VIS, Double beam Zonotech, ZT-220).

RESULTS AND DISCUSSION

The mean performances of 12 rice genotypes for quality parameters and yield attributing traits were presented (Table 1& 2) significant variation within the genotypes for the 14 different characters studied over one year which provided enough scope for improvement on the traits through selection.

Quality parameters

The protein content in grains was ranged from 7.17 mg/g to 9.54 mg/g. The maximum protein content was found in varalu (9.54 mg/g) followed by NDR 2305 (9.09 mg/g) and NDR 2309 (9.06 mg/g) respectively while minimum in NDR 2304 and NDR 2308 (7.17 mg/g). The traditional cultivars had higher protein content as compared to the enhanced ones shows their nutritional value. Coefficient of variation was observed for protein 6.08 at 5% significance level and critical difference was 0.85. Total starch content in the grains of rice lines varies 74.35% to 78.71%. The maximum starch content was recorded in NDR 359 (78.71%) followed by NDR 2302 (77.91%) and NDR 2305 (77.47%). The minimum starch content was recorded in rice genotypes NDR 2301 (74.1%). Average starch content percentage was recorded in the remaining genotypes of rice. Similar experimental finding has been reported by Kuril *et al.,* (2024).

Table no.1 Biochemical parameters on the basis of mean performance of 12 rice genotypes/lines in *Kharif season* 2023

Sr.no.	Genotypes	Protein(mg/g)	Starch (%)	Amylose (%)	Iron(ppm)	Zinc(ppm)	PA(µg/100ml)
P1	Varalu	9.54	75.08	22.27	17.29	26.76	8.86
P2	NDR-359	7.83	78.71	20.03	10.06	21.37	9.14
1	NDR 2301	7.59	74.10	22.91	16.40	23.21	11.17
2	NDR 2302	8.88	77.91	21.40	15.48	25.33	8.91
3	NDR 2303	8.54	74.35	18.79	17.04	23.51	9.26
4	NDR 2304	7.17	76.07	18.63	13.95	22.69	9.83
5	NDR 2305	9.09	77.47	21.65	16.03	24.58	8.91
6	NDR 2306	8.86	75.82	16.84	13.85	21.64	10.95
7	NDR 2307	7.71	75.40	23.02	14.59	20.98	8.82
8	NDR 2308	7.17	75.92	20.90	12.81	21.11	9.97
9	NDR 2309	9.06	77.36	21.50	15.89	23.58	8.81
10	NDR 2310	7.38	75.84	18.03	11.05	21.31	10.43
	Grand mean	8.23	76.17	20.50	14.54	23.01	9.59
	SE(m)	0.29	0.27	0.33	0.26	0.26	10.64
	CV at 5%	6.08	0.61	2.74	3.06	1.92	1.92
	CD at 5%	0.85	0.79	0.96	0.76	0.76	3.14

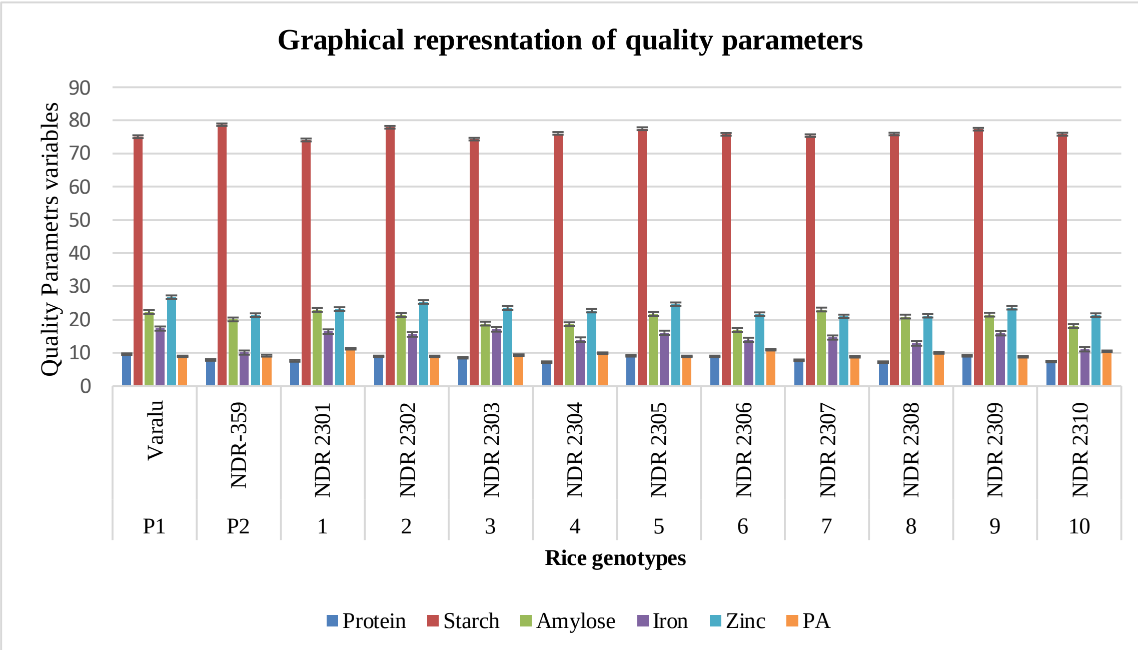

Fig.1. Graphical representation of quality parameters of 12 rice genotypes; Protein, Starch, Amylose, Iron, Zinc and Phytic acid (PA)

The Maximum Amylose content was estimated in NDR 2307 (23.02%) followed by NDR 2301(22.91%) and Varalu (22.27%). Minimum starch content had been recorded NDR 2306 (16.84%) preceded by NDR 2310 (18.03%). The maximum iron content (ppm) was estimated in varalu (17.29) followed by NDR 2303 (17.04) and NDR 2301 (16.40). The minimum iron content was reported in NDR 2310 (11.05). The maximum zinc content (ppm) was recorded in varalu (26.76) followed by NDR 2302 (25.33) and NDR 2305 (24.58). Minimum zinc content was observed NDR 2307 (20.98) and NDR 2308 (21.11). The Maximum phytic acid (µg/100ml) content was recorded in NDR 2301 (11.17) and (10.95) in NDR 2306. It was followed by NDR 2310 (10.43), minimum in NDR 2309 (8.81). Phytic acid contains multiple negative charges due to the presence of phosphate groups that bind to divalent cations iron and zinc and makes them unavailable for absorptions in the human digestive tract (Graham *et al.,* 2001).

Yield and yield attributes traits

The rice genotypes had significant variability in growth, yield contributing traits, and yield under normal environmental condition (table 2). The plant height (cm) at physiological maturity ranges from 83.83 to 134.5 cm. According to the current study's findings, under typical climatic circumstances, genotype NDR 2301 had the highest plant height at maturity (134 cm) whereas genotype NDR had the lowest (83.83cm) succeeded by NDR 2302 (86.33cm). Rice genotypes character like plant height, tillers number, days to flowering, panicle length, grains per panicle, test weight and yield are controlled by genetics and environmental factors. significant diversity in agro-morphological traits was reported in rice genotypes by Bardoloi et al., (2024). The number of tillers plant^{-1} was recorded at flowering stage and varied from 10.33 to 15. The maximum tiller number (count) was noted in NDR 2302 and Varalu (15) and followed by NDR 2305 (14) while minimum tiller number was observed in NDR 2306 (10) succeeded by NDR 2308 (10.33) and NDR 2309(10.33). The days to 100 % flowering ranged from 84–112.67in rice genotypes. The maximum days to 100 % flowering (112.67) was noted in NDR 2310 while minimum (84) in NDR 2306. Days to physiological maturity of rice genotypes varied from 118 to 133.33 days. Highest days to maturity was NDR 2302 (133.33) followed by 2301 (132), NDR 2304 (131.33) and while minimum in NDR 2305 (118 days) succeeded by NDR 2306 (119 days). Days to 100% flowering is associated with the days to maturity and it is genotypic traits which is varied among rice genotypes. Similar results were also reported by Rathod *et al.,* (2017).

The panicle length of rice genotypes ranging from 20.83 to 24.83 cm. The maximum panicle length was noted in line NDR 2305 (24.83 cm) followed by NDR 359 (24.33), NDR 2302 (24.1) while the minimum panicle length was recorded in NDR2307 (19.67) succeeded by NDR 2309 (20.50 cm), NDR 2306 (20.83 cm). The number of grains panicle^{-1} of rice genotypes was varied from 96 to 149.33. The highest grain panicle^{-1} was recorded in NDR 359 (149.33) followed by NDR 2302 (128.33), NDR 2305 (125.67) while minimum in NDR 2306 (95.33) succeeded by NDR 2303 (96), NDR 2301 (97). The range of Test weight in rice genotypes was observed from 16.9 g to 24.73 g (table 2). The highest test weight was noted in NDR 2305 (24.73) followed by NDR 359 (24.17) NDR 2302 (23.06), and NDR 2307 had the lowest

(16.9 g) succeeded by NDR 2303 (18.58). Panicle length, number of filled grain panicle^{-1} and test weight were varied among rice genotypes and major contributor for grain yield of rice genotypes. Similar findings were reported by Ahamad *et al.,* (2014) and Islam *et al.,* (2019).

Grain yield per plant was varied from 13.78 g to 24.42 g. Maximum grain yield per plant was observed in genotypes NDR 359 (24.42 g) followed by NDR 2305 (22.39g), NDR 2309 (22.35 g) a and minimum grain yield per plant in NDR 2308 (13.78g) succeeded by NDR 2310 (16.19), NDR 2306 (16.32 g). The grain yield plant^{-1} is associated with yield contributing traits of rice genotypes. Effective tillers, filled grains panicle^{-1} and test weight are major yield contributing traits and determinant yield of rice genotypes under existing environmental condition. Similar results has been found by Kumar *et al*., (2020).

Table no.2. Yield and yield attribute traits on the basis of mean performance of rice genotypes in *Kharif season* 2023 where PH-Plant height, NOT- Number of tillers, FP-Flowering percentage, DTM-Days to maturity, PL-panicle length, NOGPP-Number of grains per panicle, TW-Test weight, GYPP-Grain yield per plant

Sr. no.	**Genotypes**	**PH**	**NOT**	**FP**	**DTM**	**PL**	**NOGPP**	**TW**	**GYPP**
P1	**Varalu**	91.50	15.00	103.33	127.33	22.77	123.67	21.88	20.08
P2	**NDR 359**	101.67	11.00	108.33	130.00	24.33	149.33	24.17	24.42
1	**NDR 2301**	134.50	12.00	106.33	132.00	21.50	97.00	20.74	20.79
2	**NDR 2302**	86.33	15.00	109.67	133.33	24.17	128.33	23.06	21.53
3	**NDR 2303**	87.83	12.67	106.67	125.00	21.67	96.00	18.58	16.88
4	**NDR 2304**	87.83	12.00	109.00	131.33	23.83	118.00	20.96	22.10
5	**NDR 2305**	109.67	14.00	95.00	118.00	24.83	125.67	24.73	22.39
6	**NDR 2306**	95.33	10.00	84.00	119.00	20.83	95.33	20.58	16.32
7	**NDR 2307**	83.83	12.67	95.00	124.00	19.67	101.67	16.90	17.86
8	**NDR 2308**	88.00	10.33	107.33	130.00	23.27	102.67	21.19	13.78
9	**NDR 2309**	109.50	10.33	104.33	119.00	20.50	124.67	22.77	22.35
10	**NDR 2310**	88.83	12.33	112.67	128.67	22.17	102.67	19.72	16.19
	Grand mean	97.07	12.28	103.47	126.47	22.46	113.75	21.27	19.56
	SE(m)	1.29	0.97	2.09	1.51	0.56	2.89	0.42	0.38
	CV at 5%	2.31	13.77	3.49	2.07	4.33	4.41	3.44	3.35
	CD at 5%	3.81	2.80	6.15	4.45	1.65	8.55	1.25	1.12

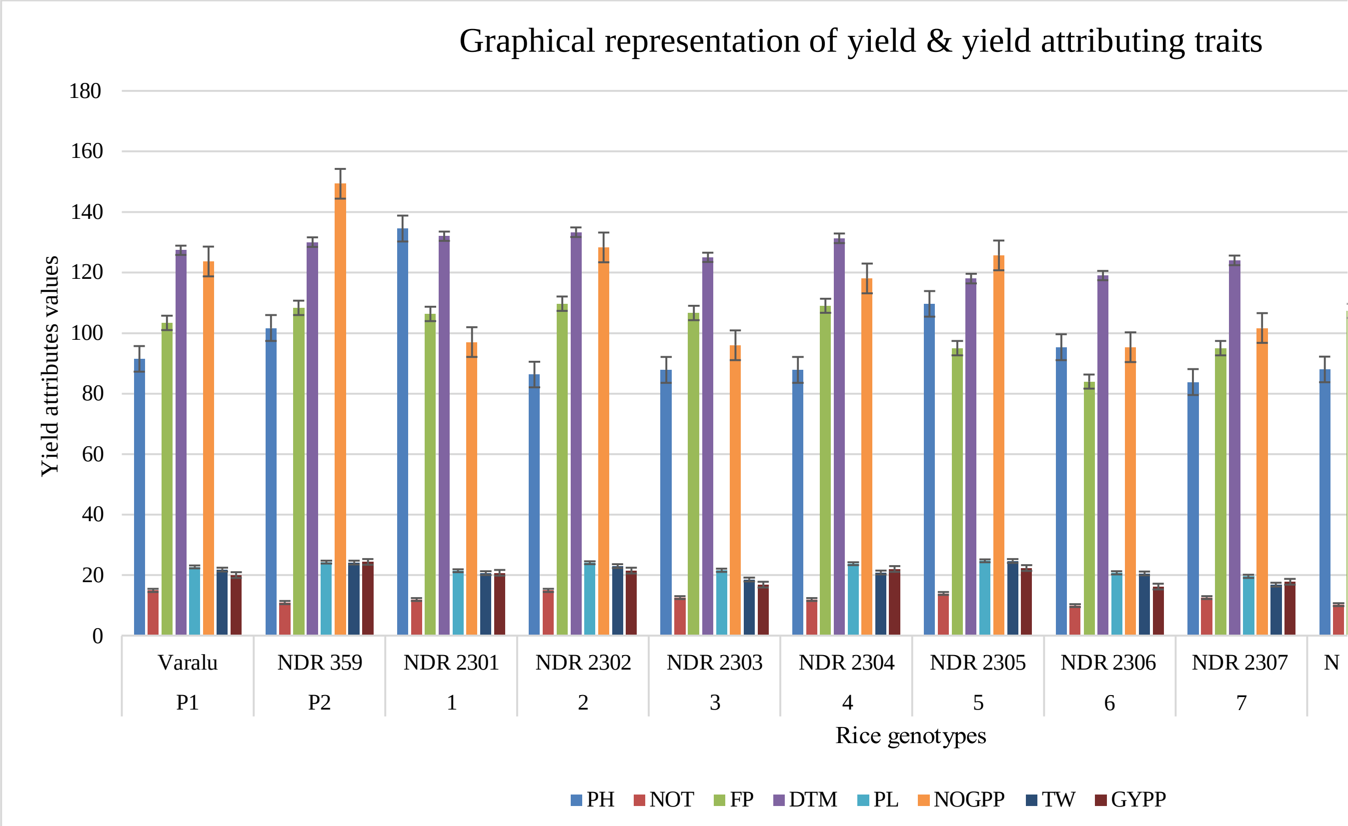

Fig.2 Graphical representation of yield and yield attributing Traits; Where PH-Plant height, NOT-Number of tillers, FP-Flowering percentage, DTM -Days to maturity, PL-panicle length, NOGPP-Number of grains per panicle, TW-Test weight, GYPP-Grain yield per plant.

CONCLUSION

From this study, it can be concluded that sufficient variability exists in most of the parameters. There were three lines NDR 2302, NDR 2305 and NDR 2309 out of 10 lines has been found with better qualities and good yield. These lines are better for the future prospects to overcome the problems such as malnutrition and anaemia.

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Conflict of interest

All the authors declare that there is no conflict of interest.

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