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Research Article

Development of Iron and Zinc Biofortified Rice Variety for Nutrition Improvement in Bangladesh

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Abstract: Depleting micronutrients such as iron and zinc poses a serious risk to a population's health in regions where nutrient-rich meals are unavailable, such as Bangladesh. We can engross the essential minerals using biofortified rice varieties enriched with essential minerals. We experimented to develop and evaluate a new high-yielding rice variety, Advanced Chemical Industries (ACI) dhan1, specifically for the rainy season in Bangladesh. We used high-yielding rice parent lines with high iron and zinc in the hybridization process, then grew and selected the resulting breeding lines during the rainy season of Bangladesh to identify the most suitable rice lines for the rainy season. The plant height and days to maturity traits were the same as shown in both cultivars, ACI dhan1 and Bangladesh Rice Research Institute (BRRI) dhan62 (standard check), which are 104 cm and 103 days, respectively. On the other hand, ACI dhan1 produced 6.05 tons per hectare, and BRRI dhan62 yielded 4.33 tons per hectare. A quality analysis of the grain quantified that ACI dhan1 contained 8.21% protein and 25.12% apparent amylose. Similarly, ACI dhan1 had very high levels of Zn (24.8 ppm) and Fe (9 ppm). We observed a more than 70% improvement in milling capabilities and head rice recovery. In proposed variety trials, the Seed Certification Agency of Bangladesh identified an average yield advantage of more than 10% for ACI dhan1 in 10 locations. National Seed Board, Bangladesh, released it as a candidate for high-iron and zinc-enriched rice variety and approved its extensive cultivation in Bangladesh. Due to its high micronutrient content, excellent agronomic performance, and non-sticky cooking quality, ACI dhan1 presents itself as a variety of choice in Bangladesh's pursuit of food and nutrition security. Thus, the present study demonstrates the large achievement of biofortification by ACI dhan1 and hence promises to be a strong weapon in the battle against malnutrition.

Keywords: iron, zinc, biofortification, nutrition, high yielding rice

1. Introduction

Resolving micronutrient deficiencies, specifically iron and zinc, is a critical issue in the area of public nutrition. Lack of these minerals is associated with serious health hazards, particularly in developing countries where individuals have less access to a variety of nutrient-dense foods and their diets are less varied [1]. These deficiencies affect many people, potentially leading to health problems such as weakened immune systems, delayed cognitive development, and increased susceptibility to infectious illnesses [2]. Developing and disseminating rice cultivars with high iron and zinc content is critical to tackling this worldwide health concern. It provides a long-term and economical strategy to lessen

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malnutrition and its effects [3].

Zinc deficiency affects about 40% of the world's population; in Bangladesh, the problem is especially serious since a large amount of low-zinc grains are consumed. In Bangladesh, anemia brought on by an iron deficiency is quite common in women and children [4]. 41% of children under five suffer from stunting, which is closely associated with zinc deficiency [5]. Utilizing rice varieties that are high in iron and zinc may improve public health and promote sustainable development in regions affected by malnutrition [6]. Biofortified rice may effectively alleviate iron and zinc shortages and enhance the welfare of disadvantaged individuals [7]. The development and distribution of iron and zinc-rich rice varieties will increase the nutritional content of basic food crops [8]. This research has the potential to profoundly impact food systems, improve public health outcomes, and promote nutrition goals via research, collaboration, and policy support [9].

Since more than 50% of the world's population relies on rice as their major food source, improving the nutritional quality of rice is essential for ensuring global food security and nutrition. Nevertheless, people who eat rice may be at risk of deficiencies because regular rice cultivars lack sufficient critical micronutrients [10]. This nutritional quality-related information will be of great help to breeders who might use modern breeding techniques to develop biofortified rice varieties that could make a big impact in the fight against hunger and malnutrition [11]. Rice lines with higher concentrations of micronutrients are selected for hybridization to create new rice varieties with improved nutritional qualities, such as higher levels of iron and zinc, as well as important agricultural traits like yield, disease resistance, and grain quality [12].

The Advanced Seed Research and Biotech Centre (ASRBC), a subsidiary of ACI Limited, and the International Rice Research Institute (IRRI) are collaborating to improve the nutritional value of rice. The objective of ASRBC is to methodically breed rice varieties that are suited to the dietary requirements of various populations and are high in grain quality [13]. These rice varieties have undergone biofortification to enhance iron and zinc content and maintain the best agronomic characteristics for cultivating all over the country. Initially, Bangladesh did not widely adopt biofortified rice varieties due to consumer dissatisfaction and the challenge of boosting farm productivity [14]. If a significant number of rice producers accept it, it may resolve any uncertainties regarding its feasibility [15, 16]. This research aimed to develop a new rice line through hybridization techniques and release it as a variety by the National Seed Board, Bangladesh, to enhance the bioavailability of iron and zinc.

2. Materials and methods

2.1 *Progeny selection*

Hybridization was completed at the International Rice Research Institute (IRRI), Philippines, using high-yielding, high iron and zinc content parent lines. The process of selecting 100 progenies began considering the traits of the parent lines and morphology of the progenies from the IRRI, Philippines. These populations served as the basis for later breeding initiatives designed to improve the nutritional content of rice lines.

2.2 *Pedigree selection*

As the parent lines are iron and zinc rich, we used a rigorous pedigree selection procedure at the Advanced Seed Research and Biotech Centre (ASRBC) that included plant morphology and field performance of the offspring derived from IRRI, Philippines. Once breeding lines became homogenous, we assessed the gross morphology of rice grain and estimated the micronutrient content. The objective of this stage was to find advanced lines that may have better features, with a particular emphasis on higher iron (Fe^{+}) and zinc (Zn) contents.

2.3 *Morphology and micronutrient calculation*

We carried out a gross morphological assessment at ASRBC and micronutrient estimation at the Bangladesh Rice Research Institute (BRRI). At this phase of the investigation, we conducted a comprehensive assessment of the offspring's physical characteristics and precisely measured the amounts of essential micronutrients. The assessment of micronutrient content, particularly zinc and iron, was done by the ICP-OES-9820 machine in the BRRI Central

Laboratory. For inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis, 0.600-0.625 g of ground whole-grain brown rice was digested with 20 mL of 1% HNO_3. The digested samples were analyzed for mineral content using the method outlined by Molina et al. [17].

2.4 *Multi locations trial (MLT)*

We conducted a multi-location trial (MLT) to evaluate the practicality and appropriateness of the top advanced line. We conducted this MLT in the Gazipur, Rajshahi, Cumilla, and Satkhira regions of Bangladesh to evaluate the candidate line's performance under a variety of environmental conditions. The MLT was carried out using a randomized complete block (RCB) design, and there were three replications in the experiment. The area was 5.4 meters in length and had 10 rows (10.8 square meters). The plants were transplanted at a density of two to three plants per hill, with a distance of 20 cm between each plant. The statistical analyses were performed on the collected data: plant height (cm), days to maturity, and grain yield per plot (metric tons per hectare). The data were subjected to analysis via analysis of variance (ANOVA), and any mean discrepancies were determined using Duncan's multiple range test (DMRT) in R software using the agricolae package.

2.5 *Proposed variety trial (PVT)*

Following multi locations trial (MLT), we chose the most high-performing advanced breeding line to proceed with for further levels of evaluation and authorization. We sent the advanced breeding line to the National Seed Board (NSB) through the Seed Certification Agency (SCA) for evaluation and approval, aiming to name it a recognized variety for cultivation and use. SCA conducted a proposed variety trial (PVT) following the guidelines of NSB at ten locations-BRRI Gazipur, BRRI Rangpur, BRRI Faridpur, BINA Mymensingh, Dinajpur, Bogra, Feni, Cumilla, Jessore, and Barishal to evaluate the candidate line's performance under a variety of environmental conditions. The PVT was carried out using a randomized complete block (RCB) design, and there were three replications in the experiment. The area was 5.4 meters in length and had 10 rows (10.8 square meters). The plants were transplanted at a density of two to three plants per hill, with a distance of 20 cm between each plant.

2.6 *National seed board (NSB) release*

The NSB reviews and approves the chosen advanced breeding line before putting it into cultivation. If the advanced line consistently outperforms the standard check variety, with yield advantages of 10% at six locations (on stations and firms) during the proposed variety trial (PVT), then NSB will release it as a new variety. We focused our efforts on creating a rice variety that satisfied farmer and consumer tastes in response to the shortcomings of previous biofortified varieties. We were improving important attributes such as the Zn and Fe content, yield potential, lodging tolerance, grain appearance, and cooking qualities.

3. Results

Among the breeding population, 506 homozygous advanced breeding lines showed a short growth duration, high yield potential, and lodging tolerance in the F7 generation. The 152 lines were selected considering the plot yield higher than 4 ton/ha and were evaluated under an observational yield trial against the short duration BRRI dhan62 fortified with zinc by BRRI as a check, and the top-10 lines yielded were tested in an advanced yield trial. We then tested 1 exceptional line in a multi-location trial at four different sites throughout the rainy season (Figure 1). From this MLT, the advanced breeding line labeled IR102481-BK-BK-ACI28-3-1 (Parentage: IR79156B/IR93562B) was selected to progress to the proposed variety trial (PVT) stage (Table 1).

In our research, we compared this newly produced rice line, IR102481-BK-BK-ACI28-3-1, with previously existing BRRI dhan62 concerning agronomic characteristics and grain characters (Figure 2). There were some similarities found to BRRI dhan62 for plant height at 104 cm and growth duration of 103 days for IR 102481-BK-BK-ACI28-3-1. But IR102481-BK-BK-ACI28-3-1 produced 6.05 tons per hectare, while BRRI dhan62 produced 4.33 tons per hectare (Table 1). Simultaneously, the assessment of micronutrient content, particularly zinc and iron, was done by the ICPOES-9820 machine in the BRRI Central Laboratory (Table 2). This analytical step aimed to verify the attainment of targeted micronutrient enrichment in selected advanced lines. The grain quality testing findings indicate that the IR102481-BK-BK-ACI28-3-1 variety has a protein percentage of 8.21% and an apparent amylose content of 25.12%. Table 2 presents a concise overview of the results obtained from the micronutrient investigation. The findings indicate that IR102481-BK-BK-ACI28-3-1 had a more favorable nutritional composition, including 9 ppm of iron and 24.8 ppm of zinc. Furthermore, IR102481-BK-BK-ACI28-3-1 demonstrated enhanced milling capabilities as compared to BRRI dhan62. The result of the study was head rice recovery over 70%, with the outrun of the milling process also above 70%.

Table 1. Multi-location trial of the proposed variety ACI dhan1 (IR102481-BK-BK-ACI28-3-1), T. Aman 2022

Designation	Plant height (cm)	Duration (days)	Yield (t/ha)				
			Gaz	Raj	Cum	Sat	Mean
ACI dhan1	104	103	6.2 a	6.5 a	6.0 a	5.5 a	6.05
BRRI dhan62	100	100	4.0 b	4.5 b	4.9 b	3.9 b	4.33
Level of significance	-	-	0.001	0.001	0.001	0.001	-
CV	-	-	23.6	19.7	11.4	18.7	-

* Gaz = Gazipur, Raj = Rajshahi, Cum = Cumilla and Sat = Satkhira

Figure 1. ACI dhan1 (IR102481-BK-BK-ACI28-3-1) field during multi-location trial

Table 2. Grain properties of the candidate variety ACI dhan1 (IR102481-BK-BK-ACI28-3-1)

Designation	Grain length (mm)	Grain width (mm)	Grain shape	1,000 g·wt (g)	Cook rice property	Amylose (%)	Protein (%)	Zinc (PPM)	Iron (PPM)	Milling outrun (%)	Head rice recovery (%)
ACI dhan1	6.5 b	1.9 b	LS	20.8 b	Non-sticky	25.12 a	8.21	24.8 a	9.0 a	70.05	70.1 a
BRRI dhan62	7.0 a	2.3 a	LS	25.6 a	Sticky	19.0 b	9.0	19.0 b	8.0 b	69.0	62.5 b
Level of significance	0.001	0.001	-	0.001	-	0.001	-	0.001	0.001	-	0.001
CV	3.84	9.72	-	10.56	-	14.17	-	13.53	5.99	-	5.88

* LS = Long Slender, 1,000g wt (g) = Thousand grains weight in gram

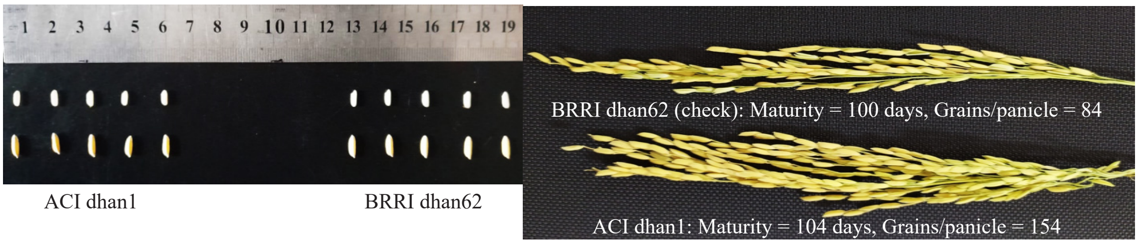

Figure 2. Grain of the ACI dhan1 (IR102481-BK-BK-ACI28-3-1) and BRRI dhan62

The lines demonstrating the highest yield response, shortest duration, non-lodging habit, and high grain micronutrient content were selected for distinctness, uniformity, and stability (DUS) testing and proposed variety trials at 10 different locations by the Seed Certification Agency (SCA). In the DUS tests, the characteristics of the candidate variety IR102481-BK-BK-ACI28-3-1 (proposed variety) were compared with the standard check variety, BRRI dhan62. Table 3 presents the results, highlighting distinct differences between the two varieties in several characteristics. Specifically, the candidate variety exhibited an erect flag leaf attitude, a medium culm diameter, a long culm length, a medium number of effective tillers per plant, a medium weight of 1,000 fully developed grains (at 12%), a medium decorticated grain length after dehulling and before milling, late and slow leaf senescence, a light brown color of decorticated, unpolished grain, and a high endosperm amylose content. In contrast, the check variety, BRRI dhan62, showed a semi-erect flag leaf attitude, a small culm diameter, a medium culm length, a high number of effective tillers per plant, a high weight of 1,000 fully developed grains (at 12%), a long decorticated grain length, intermediate leaf senescence, a white color of decorticated, unpolished grain, and an intermediate endosperm amylose content. At 50% heading date time, only 0.5% off-type was observed for both lines, indicating that the candidate variety IR102481-BK-BK-ACI28-3-1 is uniform according to the UPOV standard. No remarkable variation or segregation was noted in the test plots over two consecutive seasons, which implies the stability of the candidate variety.

Table 3. DUS tests result of ACI dhan1/IR102481-BK-BK-ACI28-3-1 (proposed variety) with check BRRI dhan62 at control farm, seed certification agency, T. Aman 2021-22 & 22-23

S. N.	Characteristics	(Check variety) BRRI dhan62		(Proposed variety) IR102481-BK-BK-ACI28-3-1		Remarks
		Code	State	Code	State	
1	Leaf sheath: anthocyanin color	1	Absent	1	Absent	
2	Leaf color	2	Green	2	Green	
3	Penultimate leaf: pubescence of blade	3	Weak or only in the margin	3	Weak or only in the margin	
4	Penultimate leaf: anthocyanin color of auricles & collar	1	Absent	1	Absent	
5	Penultimate leaf: ligule	9	Present	9	Present	
6	Penultimate leaf: shape of the ligule	3	Split or two-cleft	3	Split or two-cleft	
7	Flag leaf: attitude of blade	3	Semi-erect	1	Erect	Distinct
8	Time of heading (50% of plants with heads)	3	Early	3	Early	
9	Male sterility	-	-	-	-	
10	Lemma & palea: anthocyanin coloration	1	Absent	1	Absent	
11	Lemma & palea: anthocyanin coloration below apex	1	Absent	1	Absent	

Table 3. (cont.)

S. N.	Characteristics	(Check variety) BRRI dhan62		(Proposed variety) IR102481-BK-BK-ACI28-3-1		Remarks
		Code	State	Code	State	
12	Lemma: anthocyanin coloration of apex	1	Absent	1	Absent	
13	Spikelet: color of stigma	1	Absent	1	Absent	
14	Culm diameter	1	Small	3	Medium	Distinct
15	Culm: length	5	Medium	7	Long	Distinct
16	Stem: anthocyanin coloration of nodes	1	Absent	1	Absent	
17	Stem: intensity of anthocyanin coloration of nodes	-	-	-	-	
18	Stem: anthocyanin coloration of internodes	1	Absent	1	Absent	
19	Panicle: length	5	Medium	5	Medium	
20	Panicle: curvature of main axis	5	Medium	5	Medium	
21	Panicle: number of effective tillers in plant	7	Many	5	Medium	Distinct
22	Spikelet: pubescence of lemma & palea	7	Strong	7	Strong	
23	Spikelet: color of tip of lemma	2	Yellowish	2	Yellowish	
24	Panicle: awn in spikelet	1	Absent	1	Absent	
25	Panicle: length longest awn	-	-	-	-	
26a	Panicle: distribution of awns	-	-	-	-	
26b	Panicle: color of awns	-	-	-	-	
27	Panicle: attitude of branches	3	Semi-erect	3	Semi-erect	
28	Panicle: exertion	9	Well exerted	7-9	Well exerted	
29	Time of maturity	3	Early	3	Early	
30	Grain: wt of 1,000 fully developed grains (at 12%)	7	High	5	Medium	Distinct
31	Grain: length (without dehulling)	9	Very long	9	Very long	
32	Spikelet: sterile lemma length	3	Medium	3	Medium	
33	Decorticated grain: length (after dehulling, before milling)	5	Long	3	Medium	Distinct
34	Leaf senescence	5	Intermediate	1	Late & slow	Distinct
35	Decorticated grain: shape (length-breadth ratio)	9	slender	9	slender	
36	Decorticated, unpolished grain: color	1	White	2	Light brown	Distinct
37	Polished grain: size of white core or chalkiness	1	Absent	1	Absent	
38	Endosperm: content of amylose	3	Intermediate	5	High	Distinct
39	Decorticated grain: aroma	1	Absent	1	Absent	
40	If any (zinc)	19.50 mg/kg		24.8 mg/kg		

Yield testing was carried out following RCB design with 3 replications by the Seed Certification Agency of Bangladesh at the four on-station and six on-farm sites. The results indicated that IR102481-BK-BK-ACI28-3-1 was consistently superior over BRRI dhan62, with > 10% higher grain yield in all of the trial locations (Table 4). According to these findings, it seems that the IR102481-BK-BK-ACI28-3-1 rice variety can be an advantageous, nutrient-dense, and high-producing variety for Bangladesh's extensive rice cultivation. Considering the DUS test and PVT results, the line IR102481-BK-BK-ACI28-3-1 was named ACI dhan1 and released by NSB as high Iron and zinc-enriched rice for cultivation throughout Bangladesh.

Table 4. Yield performance of the candidate variety ACI dhan1 (IR102481-BK-BK-ACI28-3-1) at proposed variety trial, T. Aman 2023

Variety	Yield on station (t/ha)				Yield on firm (t/ha)					
	BRRI Gazipur	BRRI Rangpur	BRRI Faridpur	BINA Mymensingh	Dinajpur	Bogra	Feni	Cumilla	Jessore	Barishal
ACI dhan1	4.91	6.68	6.38	7.69	5.93	3.22	5.70	5.22	4.73	4.14
BRRI dhan62	4.23	6.01	4.85	5.87	5.34	2.65	3.87	2.66	4.24	3.76
Yield advantage (%)	16%	11%	32%	31%	11%	22%	47%	96%	11.55%	10.11%

4. Discussion

The successful identification and characterization of homozygous, elite quality converted line IR102481-BK-BK-ACI28-3-1 of the F7 generation was indeed a big moment in the breeding process to ensure a significant breakthrough towards better bio-fortified rice. These included quite a few desired characteristics, including a shorter growth duration, high yield potential, and resistance to lodging, which is a crucial component in providing a successful farmer entry point to the release of this advanced line. This achievement underscores the effectiveness of the breeding strategy employed, which focused on enhancing both agronomic performance and nutritional content simultaneously [18].

The comparative yield trials conducted with the leading BRRI dhan62 variety were highly informative about the performance of this advanced line, IR102481-BK-BK-ACI28-3-1 under natural conditions. IR102481-BK-BK-ACI28-3-1 produced 6.2, 6.5, 6.0, and 5.5 tons per hectare in Gazipur, Rajshahi, Cumilla and Satkhira, respectively, while BRRI dhan62 produced 4.0, 4.5, 4.9, and 3.9 tons per hectare in Gazipur, Rajshahi, Cumilla, and Satkhira, respectively (Table 1). The average yield of IR102481-BK-BK-ACI28-3-1 was 6.05 tons per hectare, while BRRI dhan62 produced 4.33 tons per hectare. Those trials provided a good system for comparing the yield potential and adaptability of the candidate line [19]. Due to the thorough evaluation process, some top performers were pinpointed that will help build towards the latter stages of the selection.

The performance of this line, IR102481-BK-BK-ACI28-3-1 was further validated in subsequent adapted trials (proposed variety trial) during the wet season in diverse locations. The yield of IR102481-BK-BK-ACI28-3-1 was 4.91, 6.68, 6.38, 7.69, 5.93, 3.22, 5.70, 5.22, 4.73, and 4.14 tons per hectare in BRRI Gazipur, BRRI Rangpur, BRRI Faridpur, BINA Mymensingh, Dinajpur, Bogra, Feni, Cumilla, Jessore, and Barishal, respectively. In comparison, the variety BRRI dhan62 yielded 4.23, 6.01, 4.85, 5.87, 5.34, 2.65, 3.87, 2.66, 4.24, and 3.76 tons per hectare in the same locations. This line exhibited consistent and impressive performance across various environmental conditions, demonstrating its adaptability and resilience, which are essential traits for ensuring stable and reliable crop production [11, 20].

Meanwhile, micronutrient profiling, specifically of zinc and iron, served as an important benchmark for the biofortification strategy. A subsequent comprehensive quantitative assessment of these micronutrients indicated that targeted levels of enrichment had indeed been achieved in our selected advanced lines and thus reinforced their potential to alleviate major nutritional challenges faced by target populations [21]. This represents a key validation of the effectiveness and reliability of biofortified crop varieties.

A holistic approach was adopted to make the final selection of the line in terms of yield potential, growth period, lodging tolerance, and privilege enrichment with essential trace elements. The candidate line was further subjected to characteristic evaluation for distinctiveness, uniformity, and stability (DUS) and conducted a proposed variety trial across different (10) locations to ensure top performance across all locations and was therefore eligible for the candidate line for state-wide cultivation [22]. This stringent evaluation confirmed its superiority over other available lines and had to pass the Seed Certification Agency (SCA) assessment process to be released officially as ACI dhan1.

The new rice variety, ACI dhan1, is a major milestone in biofortification efforts worldwide and provides a powerful tool for reducing malnutrition globally and improving public health. ACI dhan1 may play a significant role in food and nutrition security considering its high zinc and iron content, high yield potential, and Aman (wet) season growing, which will help improve the cropping intensity. In addition, its non-sticky cooking properties increase its desirability to consumers, leading to acceptance and preference in target markets [3]. Together, ACI dhan1 demonstrates a successful combination of targeted breeding for enhanced nutrition and biofortification techniques, with rich contents and agronomic performance.

5. Conclusion

The development of ACI dhan1, a wet-season rice variety with high yields, short duration, and lodging tolerance, is a huge step forward in solving Bangladesh's agricultural and nutritional problems. This variety demonstrates the potential of biofortification in addressing micronutrient shortages and satisfies the long-awaited demand for a cultivar that can be grown after winter crops such as oil crops, legumes, wheat, and potatoes on the same site. ACI dhan1 represents a determined step towards bettering the general welfare of the people of Bangladesh and provides an uplifting example for such endeavors across the world.

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Authors contribution

All the authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Md. Ariful Islam and Md. Moniruzzaman Hasan. The first draft of the manuscript was written by Md. Ariful Islam and Md. Moniruzzaman Hasan. All the authors commented on the draft version of the manuscript and read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest regarding this manuscript or research.

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