

Inducing Variability in Rice for Enriched Iron and Zinc Content through *In vitro* Culture

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Abstract

De-husked rice of four rice varieties *viz.*, Iratom24, BRR1 dhan 58, BRR1 dhan 62 and Binnatoa was used to observe their regeneration efficacy and produce somaclones. MS supplemented with different combinations of plant growth regulators; MS + 2,4-D + BAP + NAA, MS + BAP + NAA + Kn + IAA and MS + IBA were used for callus induction, shoot regeneration and root induction, respectively. Transcendent frequency of callus was obtained in BRR1 dhan58 (80.95%). MS supplemented with 1.5 mg/l 2, 4-D + 0.5 mg/l BAP + 0.5 mg/l NAA was found as the best for callus induction in BRR1 dhan58. Maximum nutrient of shoot was found in BRR1 dhan62 (80%) followed by BRR1 dhan58 (66.66%). For shoot regeneration, MS supplemented with 1.5 mg/l BAP + 0.5 mg/l NAA + 0.5 mg/l Kn + 0.5 mg/l IAA performed better compared to other growth regulator combinations were tested. The highest numbers of roots were produced in all varieties under MS + 0.5 mg/l IBA media. BRR1 dhan58 showed maximum number of plants was established at field condition after hardening. A total of 11, 17, 5 and 3 tissue derived plants was established in field condition from Iratom24, BRR1 dhan58, BRR1 dhan62 and Binnatoa, respectively. Among the tissue culture variants, the variant number 36 derived from Binnatoa had the highest Fe 26.93 and 21.51 mg/l and Zn 42.67 and 38.84 mg/l contents in brown and polished rice grain, respectively. This high iron coupled with zinc content rice line could be the potential breeding material for developing Fe and Zn enriched rice cultivar.

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Introduction

Rice, *Oryza sativa* L. ($2n = 2x = 24$) is cultivated as an annual plant, belongs to Graminae. *Oryza* consists of two cultivated species and 22 wild species (Jena 2010). Among the two cultivated species, *Oryza sativa* is extensively grown in Asia, therefore, it is called Asian rice. On the other hand, *O. glaberrima* is cultivated merely in western part of African continent. It has been reported that the Fe and Zn contents of rice endosperm is very low ranging from 2 to 20 mg/l Fe and 12 to 32 mg/l Zn in brown rice; but in polished rice the amount is relatively low ranging from 2 - 8 mg/l Fe and 5 - 10 mg/l Zn (Banerjee et al. 2010). It is recommended to take 11 mg Zn/day for an adult, while a pregnant woman needs to take 12 mg Zn/day and children need >8 mg Zn/day (FAO 2013). There is huge deficiency of Fe and Zn in Bangladeshi children (Sabuktagin et al. 2016). Fe deficiency in pregnancy makes risk; like sepsis, maternal mortality and low birth weight for both mother and infant (CDC; Centers for Disease Control and Prevention 2012). Growth and development of the children become impaired due to zinc deficiency. Zn deficiency also may cause diarrhea, hair loss, impotence, loss of appetite, eye and skin lesion, white spot in the nails for both of the adult and children (WHS; World Health Statistics 2012). Iron and Zn are supplemented as medicinal supplement in severe deficiency cases, however, these might cause some hyper-sensitivity symptoms in children and for the poor people the medicinal supplement could be hard to effort (Griffin et al. 2004).

In Indian sub-continent, people uptake rice almost three times as their daily meals, therefore idea is to bio-fortification of the rice through breeding will increase the chance to take iron and Zn with rice based daily meals. Several works have been done for Fe and Zn bio-fortificate in rice by influencing nitrogenous fertilizer (Zhang and Wu 2008), agronomic bio-fortification (Dipender et al. 2018) and marker aided backcross breeding (MABS) (Reddy et al. 2018). Bangladesh Rice Research Institute (BRRI) released zinc enriched two rice varieties; BRRI dhan62 and BRRI dhan64 contained 18 - 20, 22 - 25 mg/l Zn, respectively. Meanwhile, Bangladesh Institute of Nuclear Agriculture (BINA) developed one iron enriched rice variety; Binadhan-20 contained 20 - 31 mg/l Fe (Digital Herbarium 2018) following MABS. But Fe and Zn content tended to decrease upon milling and failed to attain farmer's attraction due to their low amylase content. Therefore, it is hypothesized that somaclonal variation in indigenous rice varieties could be one of the alternative solutions to enrich rice grain with Fe and Zn. Genetic transformation for enriched Fe was implied for biofortification in local rice varieties (Hiroshi et al. 2013) but endosperm specific expression of target gene was unstable. While somaclonal variation induces variability for Fe and Zn content could be used to develop Fe and Zn enriched rice cultivars. Fe and Zn contents can be increased more than 50% and milling as well as cooking loss can be checked to 3 - 5% by selecting from somaclonal variations (Fablo et al. 2018).

Atomic absorption spectrophotometer (AAS) is widely used to estimate Fe and Zn content because absorption of optical radiation through free atoms in the gaseous state is

accurate rather compared to other methods. AAS is widely used to estimate the presence of heavy metals in cereals and pulse crops. Therefore, AAS is a proof tool for selecting somaclones with enriched Fe and Zn in the tissue culture experiment. Present objective is to create somaclonal variants through *in vitro* culture of de-husked rice from four rice cultivars and selection of Fe and Zn enriched somaclones using AAS and based on field performance. Our developed protocol and the material will be potential source to accelerate Fe and Zn enriched rice cultivar development research.

Materials and Methods

Four rice genotypes, namely Iratom-24 (a rice variety for Aus season developed by BINA: Bangladesh Institute of Nuclear Agriculture), BRRI dhan58 (a short duration high yielded variety for Aman season developed by BRRI: Bangladesh Rice Research Institute), BRRI dhan62 (a zinc rich high yielding variety for Aman season developed by BRRI) and Binnatoa (a local primitive cultivar with red aleurone color cultivated at Aus season showed salt tolerance features) of *Oryza sativa* ssp. *indica* were used as source of explants. De-husked seeds were obtained from mature rice and placed on MS supplemented with different combinations and concentration of phyto-hormones for callus induction.

For callus induction, following combination and concentration of phyto-hormones supplemented in MS were used: MS + 1.0 mg/l 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA; MS + 1.5 mg/l 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA; MS + 2.0 mg/l 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA; MS + 2.5 mg/l 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA. For shoot regeneration, the hormonal combination was used as follows: MS + 2.0 mg/l BAP + 0.2 mg/l NAA + 0.2 mg/l Kn + 0.2 mg/l IAA; MS + 1.5 mg/l BAP + 0.5 mg/l NAA + 0.5 mg/l Kn + 0.5 mg/l IAA. Root initiation was investigated upon using the following hormonal combinations: MS + 0.3 mg/l IBA and MS + 0.5 mg/l IBA.

Ten de-husked seeds from each genotype were sterilized using 0.1% Hg₂Cl₂ aqueous solution for 15 min and rinsed 3 times with sterilized distilled H₂O. Surface sterilized seeds were dried out on sterilized filter paper and afterward seven seeds in one Petri dish were placed on MS and incubated in the dark at 25 ± 1°C after properly sealed with parafilm. Three to four days after inoculation, mesocotyl and radicle region of the mature embryo was swelled to form calli. Well developed calli found after 2 weeks of incubation; from which different calli were categorized as embryogenic calli (dry, milky white in color and compact calli with nodular in shape and formed numerous organized globular and translucent structures) and non-embryogenic unorganized calli (soft and watery calli; mostly white but some had brown in color). For shoot induction, proliferated calli were placed on above mentioned shoot inducing medium following 3 replicates for each hormonal combinations and incubated in culture room at 25 ± 1°C with 16/8 hrs light/dark condition. Shoots with 4.0 - 5.0 cm in length were rescued aseptically from the batch of regenerants in a vial and sub-cultured the individual shoot separately for rooting on MS with different concentration of IBA. The sub-cultured vials were incubated in the same condition as mentioned for shoot induction.

Garden soil, sand and cow-dung were mixed well at 1 : 2 : 1 and autoclaved at 121°C at 1.06 kg cm⁻² for 1 hr. Ten cm diameter size plastic pots were filed with the autoclaved soil mixture and individual plant with complete root and shoot system was planted and covered with polyvinyl bag for hardening. Half strength Hoagland was sprayed regularly to maintain higher humidity inside bag and ensured ready food to transplanted baby plants. Poly bag was removed after five days by increasing the number of gradual perforation on bag and thereafter plants were kept in filed condition in a regular size earthen bucket and allowed them to grow until harvest. The somaclonal variants were compared with their parents and evaluated for phenotypic traits and ion contents, like plant height (cm), effective tillers/plant, panicle length (cm), filled grains /panicle, 1000 grain weight (g), yield/plant (g) and Fe and Zn concentration in brown and polished rice.

For estimating Fe and Zn content in rice, grains were de-husked (for brown rice) and polished by polyurathin roller (254 mm width × 254 mm outer diameter) and Millman machine (Model: YF-75 at BRRRI), respectively. Firstly, the de-husked and polished grains were dried in oven at 50 - 60°C for 24 hrs and ground into powder using stainless steel grinder. 0.5 g ground rice grain sample was digested using 10 ml di-acid digestion mixture (HNO₃ : HClO₄ = 2 : 1) and heated at 180°C until the sample became colorless. The digested sample was filtered (Whitman 42 filter paper) in a 50 ml volumetric flask and made the volume up to the mark by adding distilled water. The AAS machine was set and calibrated with seven different iron concentrations viz., 0.0, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l Fe with -0.001, 0.045, 0.085, 0.164, 0.242, 0.300 and 0.361 absorbance, respectively for estimating Fe. Moreover, the AAS machine was set and calibrated with eight zinc concentrations viz., 0.000, 0.025, 0.050, 0.100, 0.150, 0.200, 0.300 and 0.400 mg/l Zn with 0.002, 0.024, 0.039, 0.071, 0.101, 0.131, 0.184, 0.232 mg/l absorbance, respectively for Zn. Fe and Zn estimation was performed using 2 repeats for each sample as 2 technical replicates. Fe and Zn concentration estimated with the formula, amount of iron/zinc content (mg/l) = Atomic Absorption Spectrophotometer concentration reading × dilution factor.

ANOVA among the genotypes for different traits was calculated following augmented design using PLABstat (Utz 2007). Mean comparison was made among 40 genotypes (4 controls and 36 somaclones) for different traits using static software Minitab 17 (State College, PA, USA) following Tukey test. In the augmented design four parents were planted as 4 replicates but each tissue culture variant was planted as single entry. The genotypes were assigned in 4 separate blocks and each block contained 10 genotypes.

Results and Discussion

Among four rice genotypes, BRRRI dhan58 showed the highest callus induction frequency (80.95%) in MS + 1.5 mg/l 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA within the shortest time (14 days) (Table 1). Even the increased level of 2,4-D did not enhance the callusing frequency, but showed reverse effect in most of the genotypes. BRRRI dhan62

showed the lowest frequency in callusing (42.86%) in MS + 2.0 mg/L 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA and the Binnatoa genotype required the highest number of days (>18 days) for callusing in MS + 2.5 mg/l 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA. Iratom 24 produced the largest size (3.89 cm²) callus in MS + 1.5 mg/l 2,4-D + 0.5 mg/l BAP + 0.5 mg/l NAA followed by BRRi dhan62 (3.28 cm²), but calli were brown color with compact texture and brown with loose texture for Iratom 24 and BRRi dhan62, respectively (Table 1) and they were non-embryogenic in nature. While, the calli derived from BRRi dhan58 in different phyto-hormone combinations were white in color with compact texture, which all had embryogenic in nature. This result indicated that in case of rice formation of embryogenic calli, proliferation of calli (size), texture and color varied on number of factors, like genotypes and combination and concentration of phyto-hormones specially concentration of 2,4-D. Embryogenic calli with whitish color could be achieved from optimum concentration of phyto-hormones. Non-embryogenic calli produced from very low and high concentration of 2,4-D, although it was influenced by genotype itself. Calli became fragile and tended to be dried up in increasing concentration of 2,4-D. MS supplemented with 1.5 mg/l 2,4-D was found the most effective in callus induction for all genotypes (Table 1). It produced 71.42, 80.95, 71.42 and 61.92% callus in Iratom-24, BRRi dhan58, BRRi dhan62 and Binnatoa, respectively supported by the results reported previously in *Oryza sativa* (Islam et al. 2005). There was claim that maximum callus induction could be achieved in BRRi released genotypes than traditional landraces (Shahnewaj et al. 2010). BRRi genotypes responded quickly to auxin, 2,4-D for accelerating the cell enlargement and increases cell wall plasticity through formation of cellulose enzyme. Auxin increased the intercellular fluid concentration by accepting mineral and chemical component in cytoplasm resulting unorganized cell mass, namely callus. However, with increased concentration of 2,4-D behind 1.5 mg/l dropped down the callus induction because of inhibiting cellulose enzyme activity in the explants cells (Iman et al. 2017). Induction of callus was found best in Pajam variety by using calcium silicate and 1.0 mg/l 2,4-D (Islam et al. 2005). Modified N6 medium was proved to be effective in callus induction supplemented with 2.0 mg/l 2,4-D and AgNO₃ in rice (Niroula et al. 2005). Effect of genotype and age of explant were reported as important factors for callusing in indica rice (Hoque et al. 2004, Diah et al. 2006).

Transition from callus to green buds/plantlets attained within 14 days in BRRi dhan58 in MS + 1.5 mg/l BAP + 0.5 mg/l NAA + 0.5 mg/l Kn medium (Table 2). Due to decrease of BAP concentration (0.5 mg/l) and increase of NAA and Kn concentration (0.3 mg/l) in medium, shoot induction frequency reduced by 17% in Iratom 24; 17% in BRRi dhan62 and 33% in Binnatoa. Average number of shoot production was the highest in BRRi dhan62 at MS +1.5 mg/l BAP + 0.5 mg/l NAA + 0.5 mg/l Kn followed by BRRi dhan58. BRRi dhan58 showed maximum rooting and the highest number of shoot with root was obtained in MS + 0.5 IBA (Table 3). Shahnewaj et al. (2010) found maximum callus induction and plant regeneration in BRRi dhan29. Per cent of plant establishment was the highest in BRRi dhan58 (73); whereas 50, 62 and 47 plant establishment were

Table 1. Effects of genotypes and different combinations of phyto-hormones supplemented in MS on callusing parameters in rice.

Genotypes	MS + 2,4-D + BAP + NAA (mg/l)	Days to callusing	Callusing frequency (%)	Size of callus (cm ²)	Texture of callus	Color of callus
Iratom 24	1.0 + 0.5 + 0.5	15.66 f-h	66.66 d	2.26 de	1.33 f	3.00 a
	1.5 + 0.5 + 0.5	15.33 g-i	71.42 c	3.89 a	1.00 g	2.33 b
	2.0 + 0.5 + 0.5	17.00 c-e	57.15 f	2.00 fg	1.66 e	1.33 e
	2.5 + 0.5 + 0.5	17.33 b-d	38.10 j	2.24 de	3.00 a	1.66 d
BRR1 dhan58	1.0 + 0.5 + 0.5	14.66 h-j	76.20 b	2.19 e	1.33 f	3.00 a
	1.5 + 0.5 + 0.5	14.00 j	80.95 a	2.37 d	1.66 e	3.00 e
	2.0 + 0.5 + 0.5	14.33 ij	76.20 b	2.35 d	1.00 g	3.06 d
BRR1 dhan62	2.5 + 0.5 + 0.5	16.00 e-g	52.38 g	1.95 g	1.33 f	2.00 c
	1.0 + 0.5 + 0.5	15.33 g-i	47.62 h	2.00 fg	2.00 d	1.00 f
	1.5 + 0.5 + 0.5	15.66 f-h	71.42 c	3.28 b	2.66 b	1.33 e
Binnatoa	2.0 + 0.5 + 0.5	17.33 b-d	42.86 i	2.14 ef	2.00 d	1.66 d
	2.5 + 0.5 + 0.5	18.00 a-c	38.10 j	2.11 ef	2.67 b	2.00 c
	1.0 + 0.5 + 0.5	16.66 d ef	47.62 h	3.38 b	2.33 c	1.00 f
	1.5 + 0.5 + 0.5	17.66 ad	61.91 e	2.35 d	2.00 d	1.33 e
Binnatoa	2.0 + 0.5 + 0.5	8.33 ab	57.15 j	2.19 e	2.66 b	1.66 d
	2.5 + 0.5 + 0.5	18.66 a	52.38 g	3.00 c	3.00 a	1.33 e
CV (%)		4.02	3.56	3.38	4.46	8.17

Callus color and texture were recorded as; 1 = White, 2 = Creamy, 3 = Brown and 1 = Loose texture, 2 = Compact texture, 3 = Fragile texture, respectively. The means with the different and same letter in a column represent significant and insignificant difference respectively at the 0.05 level.

Table 2. Effects of genotypes and different combinations of phyto-hormones supplemented in MS on shooting parameters in rice.

Genotypes	MS + BAP + NAA + Kn + IAA (mg/L)	Days to green bud formation	Per cent shoot induction	Average no. of shoot/callus
Iratom 24	2.0 + 0.2 + 0.2 + 0.2	17.00 c	33.33 e	2.66 f
	1.5 + 0.5 + 0.5 + 0.5	16.33 d	50.00 d	3.33 cd
BRR1 dhan58	2.0 + 0.2 + 0.2 + 0.2	14.66 g	66.66 c	4.50 b
	1.5 + 0.5 + 0.5 + 0.5	14.33 h	100.0 a	4.66 b
BRR1 dhan62	2.0 + 0.2 + 0.2 + 0.2	15.00 f	66.66 c	3.17 de
	1.5 + 0.5 + 0.5 + 0.5	15.33 e	83.33 b	5.00 a
Binnatoa	2.0 + 0.2 + 0.2 + 0.2	18.66 a	50.00 d	3.00 e
	1.5 + 0.5 + 0.5 + 0.5	18.33 b	83.33 b	3.50 c
CV (%)		0.43	2.24	2.96

The means with the different and same letter in a column represent significant and insignificant difference respectively at the 0.05 level

recorded in Iratom 24, BRR1 dhan62 and Binnatoa, respectively using MS + 0.3 IBA. Number of roots/shoot was accelerated due to increase of root inducing hormone IBA. Maximum number of multiple shoots was obtained in rice by using 1.5 mg/l BAP and 0.5 mg/l NAA in MS (Oinam et al. 1995). Cytokinin accelerates the level of reduced nitrogen that trigger somatic embryogenesis from callus, i.e. reduce nitrogen is pre-requisite in embryo induction (Moura et al. 1997). High concentration of BAP stimulates green shoot bud initiation but increased concentration of Kn and NAA has significant influence on somatic embryo maturation and plantlet formation (Adita and Anuradha 2014). The highest percentage of shooting was recorded in MS supplemented with 1.5 mg/l BAP + 0.5 mg/l NAA + 0.5 mg/l Kn (Chu et al. 2003). The highest number of shoot with roots was found in polyploidy breeding by using 0.3 mg/l IBA to 0.5 mg/l IBA (Hoshino et al. 2011). It has been revealed that NAA significantly assists to root proliferation, whereas IAA and IBA solely accelerate root elongation in cereals (Liu et al. 2013). Root inducing hormone specifically IBA promotes activities of enzyme concerned with producing root and gives access to enter food material by improving surface areas. The plantlets were transferred to glasshouse for hardening with 73% survival rate. Hardening reduces mortality rate of plantlets and creates favorable condition to survive in field condition.

Tissue culture variants showed highly significant differences compared to their corresponding parents for different phenotypic, yield and yield contributing traits. It is retrieved total 36 tissue variants (TCV) from 4 parental genotypes and tested them for their performance at field condition. Among the 36 TCVs, 11, 17, 5 and 3 TCVs were regenerated from Iratom 24, BRR1 dhan58, BRR1 dhan62 and Binnatoa, respectively (Table 4). TCV 34 derived from BRR1 dhan62 was the tallest genotype among all TCVs and parents. On the other hand, TCV 22 derived from BRR1 dhan58 was the shortest genotypes even compared with the BRR1 released two varieties, BRR1 dhan58 and BRR1 dhan62 (Table 4). This result indicates that tissue culture could be effective tool for selecting short stature rice genotype from somaclonal variants.

Number of effective tillers/plants was recorded as the highest in TCV 33 (29.43) derived from BRR1 dhan62. Number of effective tiller/plant was the lowest in the TCV variant 23 derived from Binnatoa. The panicle length/plant was observed as the highest in TCV 20 (26.32 cm) derived from parent BRR1 dhan58. Small panicle length was observed in TCV 19 (17.39 cm) derived from BRR1 dhan58. TCV 33 derived from BRR1 dhan62 produced maximum number of filled grains/panicle (157.75), but this trait was found as minimum in TCV 30 (60.75) derived from Binnatoa, which were deviated largely from their parents. Thousand-grain weight was recorded as the highest in TCV 36 (25.25 g) derived from Binnatoa and it was recorded as the lowest in TCV 20 (18.85 g) derived from BRR1 dhan58; whereas, 1000-grain weight varied from 23.38 to 22.25 g in the parents (Table 4). Yield/plant also tremendously changed in TCVs, one third of the total tissue TCVs showed higher amount of yield/plant compared to the 4 parental genotypes. Maximum yield/plant produced by TCV 33 (31.57 g) derived from BRR1 dhan62 and the lowest yield/plant produced by TCV 21 (7.28 g) derived from BRR1

dhan58 (Fig. 1). While, yield/plant varied between 11.54 and 14.50 g within the parents indicates that somaclonal variation could be used for improving yield in rice effectively. Hossain et al. (2010) reported significant variation for yield, number of effective tillers, filled grain/panicle and panicle length in tissue culture variants of rice. Somaclonal variation induced in tissue culture through: (i) hormonal combination, (ii) change in chromosome number and structure, (iii) gene silencing and gene activation, (iv) DNA methylation and (v) activation of transposable element (Nwazomu and Jaja 2013).

Table 3. Effects of genotypes and different combinations of phyto-hormones supplemented in MS on rooting parameters of rice.

Genotypes	MS + IBA (mg/l)	No. of shoot with root	Average no. of root/plant	Plant establishment (%)
Iratom 24	0.3	2.33 f	12.67 d	50.00 g
	0.5	2.66 e	14.33 c	53.00 e
BRR1 dhan58	0.3	3.66 b	15.00 b	67.00 b
	0.5	4.00 a	16.33 a	73.00 a
BRR1 dhan62	0.3	3.33 c	13.00 d	62.00 c
	0.5	3.00 d	8.66 f	51.66 f
Binnatoa	0.3	2.00 g	10.00 e	47.00 h
	0.5	2.50 ef	10.33 e	55.00 d
CV (%)		3.95	2.11	1.20

The means with the different and same letter in a column represent significant and insignificant difference respectively at the 0.05 level.

Not only phenological traits but also biochemical and mineral content in rice grain could be enhanced in the tissue culture variants due to somaclonal variations (Ferdausi et al. 2009). Maximum iron concentration was found in brown rice of TCV 36 (26.93 mg/l) derived from Binnatoa, while it was recorded as the lowest in brown rice of TCV 13 and TCV 14 (6.44 mg/l) derived from BRR1 dhan58, while BRR1 dhan62 and BRR1 dhan58 had iron content 13.42 and 9.49 mg/l, respectively in brown rice grain (Fig. 2). In polished rice, maximum iron concentration was found in TCV 36 (21.51 mg/l) derived from Binnatoa and minimum TCV 14 which was derived from BRR1 dhan58 had the lowest (5.15 mg/l); whereas iron content varied from 9.23 to 5.51 mg/l in polished rice of Binnatoa and BRR1 dhan58 parental genotypes (Fig. 3). These results indicate that iron content in rice grain could be enhanced remarkable both in brown grain and polished grain through creation of somaclonal variation by tissue culture.

It was also attempted to increase the Zn content in both brown and polished rice grains through effective selection of induced somaclones by tissue culture. In brown rice grain, Zn content was found as maximum in TCV 36 (42.67 mg/l), while it was observed at the lowest level (12.33 mg/l) in TCV 14. Whereas, Zn content in the released Zn enriched rich genotypes was found as 36.05 and 18.07 mg/l in Binnatoa and BRR1 dhan58,

Table 4. Mean performance of tissue cultured variant (TCV) compared to parents in yield contributing traits.

Genotypes	Name	Plant height (cm)	Effective tillers/ plant	Panicle length (cm)	Filled grains/ panicle	1000-grain weight (g)
Parents	Iratom-24	81.12 k-n	5.50 no	17.48 m	101.50 e-k	22.97 g
	BRR1 dhan58	90.62 c-f	8.50 m	21.21 h	136.25 bc	19.00 s-v
	BRR1 dhan62	75.12 op	5.75 no	19.22 k	81.75 m-u	22.25 h
	Binnatoa	97.37 ab	14.00 e-g	22.71 fg	89.50 h-r	23.38 f
Iratom 24	TCV 1	88.19 d-i	8.94 lm	20.16 j	111.25 d-f	23.66 d-f
	TCV 2	84.4 g-m	9.19 k-m	22.50 g	106.25 d-h	24.02 b-d
	TCV 3	79.19 m-p	9.94 j-m	23.78 cd	88.25 i-s	23.78 d-f
	TCV 4	86.94 e-j	9.44 k-m	16.53 no	96.75 e-n	23.96 b-d
	TCV 5	81.94 j-n	6.44 n	15.77 op	84.75 k-t	24.24 bc
	TCV 6	79.18 m-p	9.94 j-m	18.27 l	77.25 o-v	24.26 b
	TCV 7	88.43 d-i	10.43 j-l	21.28 h	80.75 n-u	23.86 c-e
	TCV 8	89.43 c-h	9.44 k-m	11.22 q	110.75 d-g	20.79 i
	TCV 9	81.93 j-n	14.18 e-g	15.33 p	141.25 b	20.13 k-m
	TCV 10	86.43 e-k	14.18 e-g	16.01 no	110.25 d-g	19.32 q-t
BRR1 dhan58	TCV 11	85.18 f-l	10.93 i-k	17.22 m	83.25 l-t	18.87 uv
	TCV 12	85.93 e-l	12.43 g-i	18.37 l	91.75 h-q	19.95 l-n
	TCV 13	84.43 g-m	10.43 j-l	16.33 no	86.75 i-s	20.01 l-n
	TCV 14	85.43 f-l	14.43 ef	216.33 no	93.75 g-p	19.09 r-v
	TCV 15	83.43 i-n	6.44 n	23.58 de	72.75 r-v	20.48 i-k
	TCV 16	89.93 c-g	11.43 h-j	23.67de	99.75 e-l	19.39 p-s
	TCV 17	86.93 e-j	12.43 g-i	20.34 ij	81.75 m-u	19.26 q-u
	TCV 18	81.43 j-n	14.18 e-g	22.45 g	94.25 f-u	20.46 i-k
	TCV 19	81.43 j-n	17.18 d	26.47 a	77.25 o-v	19.25 q-v
	TCV 20	93.18 b-d	18.93 c	19.38 k	152.25 ab	18.85 v
	TCV 21	83.93 h-m	4.44 o	25.01 cd	122.75 cd	19.98 l-n
	TCV 22	74.43 p	9.44 k-m	23.78 cd	79.75 n-u	19.61 n-q
	TCV 23	80.43 m-o	8.44 m	19.03 k	68.75 t-v	20.29 j-l
	TCV 24	88.93 c-i	14.18 e-g	25.33 b	113.25 de	19.79 m-o
BRR1 dhan62	TCV 25	88.43 d-i	20.18 c	19.03 k	102.25 e-j	19.43 o-r
	TCV 27	85.68 e-l	12.93 f-h	16.40 n	85.25 j-t	19.88 mn
	TCV 33	96.93 ab	29.43 a	26.02 a	157.75 a	23.68 d-f
	TCV 34	99.93 a	15.43 e	22.52 g	76.75 p-v	24.15 bc
	TCV 35	84.43 g-m	23.18 e	23.15 ef	92.25 h-p	23.54 ef
	TCV 29	97.93 ab	9.44 k-m	26.36 a	102.75 e-i	20.57 ij
	TCV 30	77.93 n-p	6.44 n	22.83 fg	60.75 v	18.96 t-v
Binnatoa	TCV 36	88.18 d-i	23.93 b	26.36 a	98.25 e-m	25.25 a

The means with the different and same letter in a column represent significant and insignificant difference respectively at the 0.05 level.

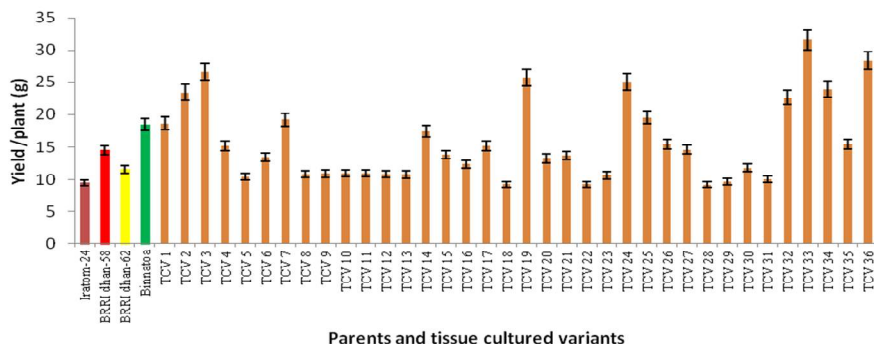


Fig. 1. Mean performance for yield/plant (g) of 36 tissue cultured variant compared to 4 parents.

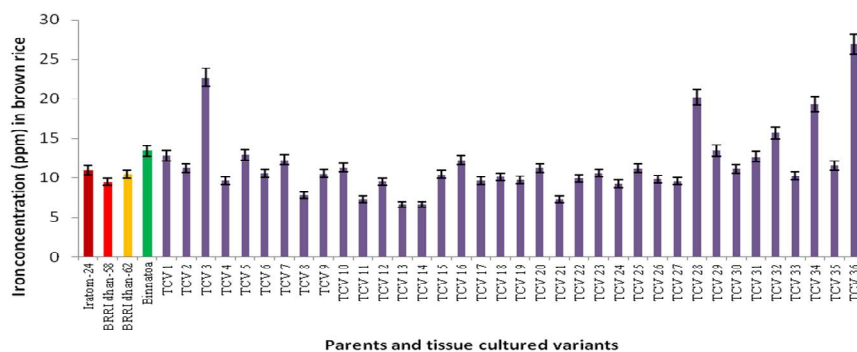


Fig. 2. Mean performance for iron (Fe) content (mg/l) in brown rice of 36 tissue cultured variants compared to 4 parents.

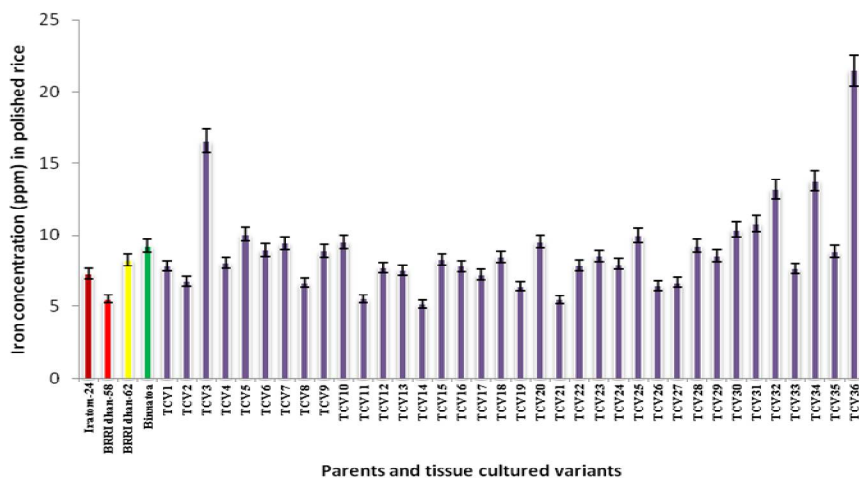


Fig. 3. Mean performance for iron (Fe) content (mg/l) in polished rice of 36 tissue cultured variants compared to 4 parents.

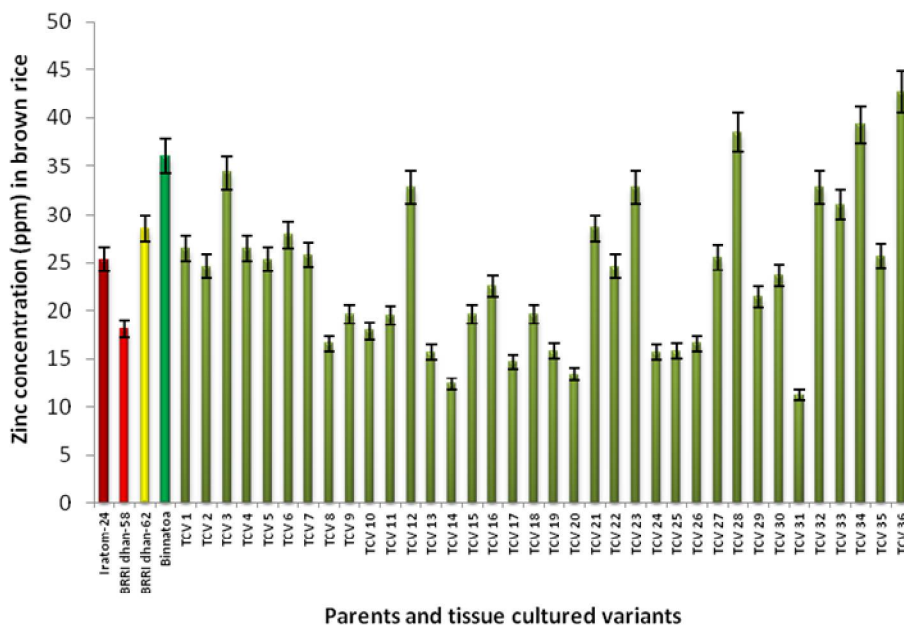


Fig. 4. Mean performance for zinc (Zn) content (mg/l) in brown rice of 36 tissue cultured variants compared to 4 parents.

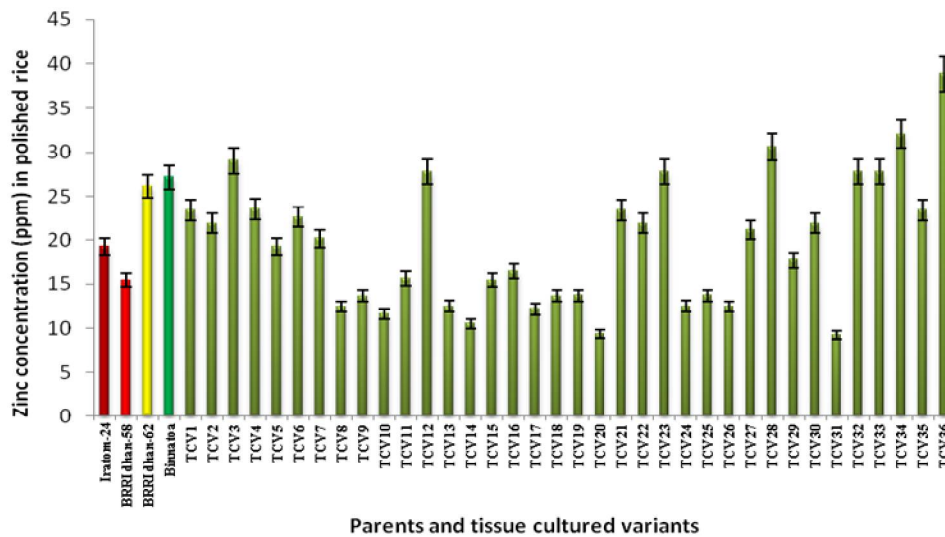


Fig. 5. Mean performance for zinc (Zn) content (mg/l) in polished rice of 36 tissue cultured variants compared to 4 parents.

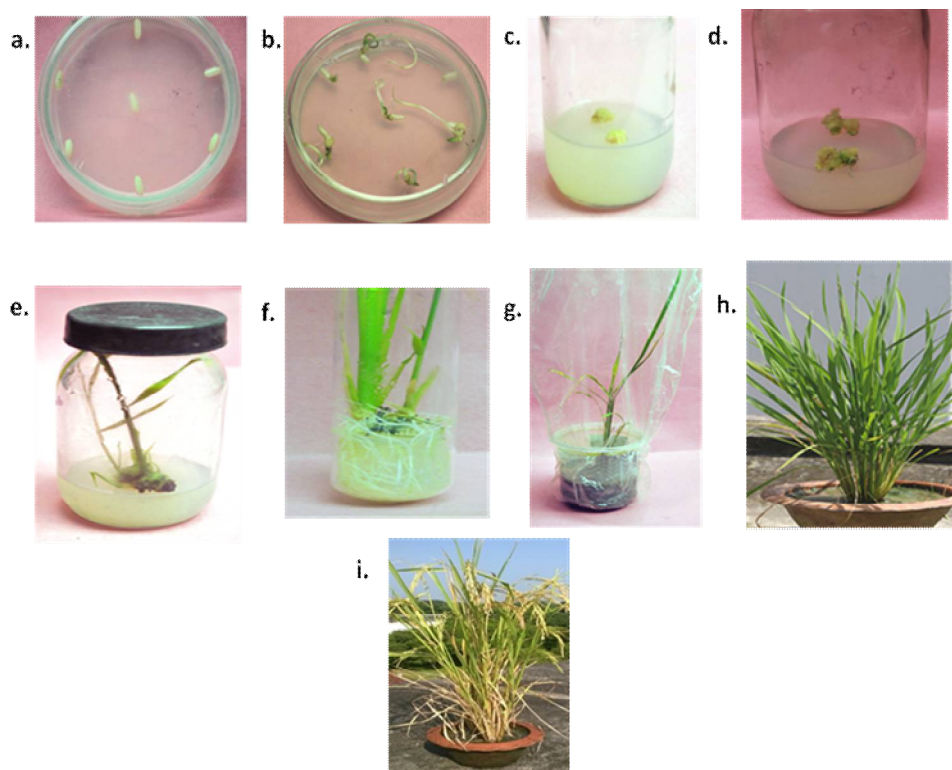


Fig. 6. Whole work flow followed in tissue culture for creation and selection of desired somaclones. (a) Seed placement, (b) initiation of callus, (c) maintenance of calli derived from mature embryo of BRR1 dhan58, (d) green bud initiation, (e) induction of shoot, (g) hardening of regenerated plantlet of BRR1 dhan-58, (h) established plantlet of somaclone and (i) grain maturation in tissue culture derived plants.

respectively (Fig. 4). In polished rice, TCV 36 which was derived from Binnatoa had maximum Zn concentration (38.84 mg/l) and it was recorded as minimum in polished rice of TCV 20 (9.37 mg/l) derived from BRR1 dhan58. In contrast the parental genotypes Binnatoa and BRR1 dhan58 had Zn content 27.17 to 15.37 mg/l, respectively in polished grains (Fig. 5). Fe concentration increased up to 40 and 62% in polished and brown rice of TCV 36, respectively, while Zn increased 16.9 and 7.69% in polished and brown rice, respectively in the TCV 36 and TCV 34. Results showed that somaclonal variants attained 28.33 and 5% higher Fe compared to BRR1 dhan62. Similarly, Zn content in TCV 36 increased up to 25.56 and 33% in brown and polished rice grains, respectively compared to the Zn enriched released cultivar BRR1 dhan62. The overall work flows to select the Fe and Zn enriched somaclones presented in Fig. 6. Fe and Zn concentrations in unpolished rice grains increased 23 and 36%, respectively in the bio-fortified transgenic progenies (Laura et al. 2016). Somaclonal variation might have altered the proportion of Fe and Zn transporter in regenerated roots which may ultimately create differential Fe and Zn accumulation pattern in grains of TCVs.

Fe and Zn enriched somaclonal variants TCV 36 and TCV 34 could be evaluated in multi-locational trial for testing their consistency in Fe and Zn content in the polished and brown. Upon consistent results for Fe and Zn content those two TCVs could be recommended as new rice cultivars for farmers' cultivation.

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