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RESEARCH ARTICLE

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# Identification of genomic regions associated with grain zinc concentration in RILs derived from popular cultivar MTU1010 and BR2655 of rice

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## Abstract

In the present study seven quantitative traits and grain zinc content were evaluated using 190 recombinant inbred lines (RILs) derived from MTU 1010 and BR 2655 during wet season (WS) 2016 and dry season (DS) 2016-17. A total of 948 SSR markers used for parental polymorphism, of which 119 were found polymorphic. The linkage map of 1874.09 cM with an average marker interval of 331.23 cM was constructed using 60 polymorphic SSR markers with clear resolution. Nine QTLs on chromosome 1, 2, 4, 5, 9, 10 and 12 were detected for eight traits in WS 2016 and DS 2016-17. Among the nine QTLs, number of productive tillers per plant (*qNPT-5.1*) accounted for highest phenotypic variance (7.54%) over both the seasons. A stable QTL for grain zinc (*qZn-2.1*) wasdetected on chromosome 2 flanked between RM13347 and RM262 with phenotypic variance of 7.74% (WS) and 10.2% (DS). Therefore, these QTLs and marker information could be validated further and utilized in the marker-assisted selection to improve nutritional trait (zinc) in rice.

**Keywords:** Biofortification, genetic enhancement, grain zinc genetic variation, QTL mapping, rice.

## Introduction

Rice (*Oryza sativa* L.) occupies prime place among the food crops cultivated around the world and source of calories for more than one third of the world's population (Brar and Khush 1997). It accounts for 35-60 per cent of the caloric intake of three billion Asians (Guyer et al. 1998) and 40 per cent of protein consumed on an average in Asia. While brown rice is a good source of nutrients and vitamins, polished rice which is devoid of most of the important nutrients is the most preferred form of consumption (Nachimuthu et al. 2015; Pradhan et al. 2020; Rao et al. 2020). Most of the modern high-yielding rice varieties are reported to be poor in nutrient content after polishing (Anandan et al. 2011; Swamy et al. 2016). Micronutrient malnutrition has been designated as the most serious challenge to humanity (Horton et al. 2008) as two-third of the world's population is at risk of deficiency in one or more essential mineral elements (Cakmak 2002; White and Broadley 2009; Stein 2010). Among the micronutrients, iron (Fe) and zinc (Zn) deficiencies are the most widespread in developing countries (Johnson et al. 2011).

Zinc deficiency is the most common cause of malnutrition (Ronaghy 1987). About 25% of the world's population is at risk of zinc deficiency (Maret and Sandstead 2006). Nearly 500600 million people particularly in Asia and Africa are at risk for low zinc intake (Harvest Plus 2010). The dependency on cereal-based diets may induce zinc deficiency related health problems in humans, such as impairments in physical development, immune system, brain function, pneumonia, weight loss, growth retardation and delayed puberty in adolescents, poor appetite, delayed wound healing (WHO 1996; Cakmak 1 2008). The mean intake of zinc in males ranged from 9.32 mg in 75 years and older and 13.53 mg in persons of 15–18 years.

Rice being worlds most consumed staple food in most parts of the Asia and Africa, serves as potential source to meet zinc deficiency (Jiang et al. 2008. However, biofortification, the process aims at enrichment of food stuffs with vital micronutrients and thus, improving micronutrient content including Zn in grains of rice through plant breeding has the potential to ameliorate health problems induced by its deficiency (Graham et al. 1999; WHO 2002). Once rice is biofortified with vital nutrients, the farmer can grow the crop indefinitely without any additional input to produce nutrient packed rice grains in a sustainable way (Nagesh et al. 2012).

The nutritional concentrations of grain are determined by polygenes and their interaction with the environmental factors. Thus, a deeper insight into genetic basis genetic basis, associations with one another, and interactions with agronomic traits is essential for developing successful rice varieties with enhanced nutritive value (Swamy 1 et al. 2016). QTL analysis for complex traits can detect association between markers and traits that can lead to elucidation of the genetic control of complex traits. Therefore, the use of markers closely linked to QTL will allow rice breeders to impose positive selection on essential elements and negative selection on potentially toxic elements in grain through marker assisted selection (MAS). With this background a set of 190 RILs along with parents were phenotyped to understand the relationship between yield, yield attributing traits and zinc content. The mapping population along with parents were also genotyped to identify the *QTLs* associated with yield related traits and grain zinc, which assist in faster development of biofortified rice varieties through marker-assisted breeding (MAB).

## Materials and methods

### *Plant materials and experimentation*

The recombinant inbred lines or populations (RILs) were derived from the cross between a popular rice cultivar MTU1010 a short duration popular variety with long slender grain type, high yielder, tolerant to blast, BPH but low in grain zinc (Zn = 14.4 ppm) and donor BR2655, a long duration variety tolerant to blast, sheath blight with high grain zinc (Zn=23.7 ppm) were used in the present study.

The experiment was carried out at ICAR-Indian Institute of Rice Research (IIRR), Hyderabad, during wet season (WS) or *kharif* 2016 and dry season (DS) or *rabi* 2016-17. The seeds of 190 RILs along with parents were sown in a nursery bed and 25 days old seedlings were transplanted to the main field following a spacing of 20 cm × 15 cm in a Randomized Complete Block Design with three replications. All the recommended packages of rice crop production and protection practiced to raise a good crop.

Phenotyping was done in both the seasons, WS 2016 and DS, 2016-17. Seven yield related traits namely, days to 50% flowering (DFF), plant height (PH), panicle length (PL), number of productive tillers per plant (NPT), number of filled grains per panicle (NFG), 1000-grain weight (TW) and grain yield per plant (GY) in 190 RILs and parents.

### *Estimation of grain zinc*

The rice samples from both the parents and 190 RILs were collected dried to a moisture content of 13% and 20g from each sample during WS 2016 and DS 2016-17 were dehusked using non-ferrous dehuller. Five grams of brown rice sample of each RIL and parents were subjected to Energy Dispersive X-ray Fluorescence (ED-XRF) available at Biofortification Laboratory, Indian Institute of Rice Research, Rajendranagar, Hyderabad. Samples are presented for analysis in cuvettes on a sample carousel, enabling multiple samples to be analyzed in a single run (Paltridge et al. 2012).

### *Statistical analysis*

The mean data of the RILs and parents were used for statistical analysis. Genetic parameters such as genotypic, phenotypic coefficients of variation (Falconer 1981), genetic advance, heritability (Johnson et al. 1955) analyzed using INDOSTAT software. Correlation coefficients were depicted in the form of correlogram and frequency distribution plots were also drawn using R software (R Core Team 2018).

### *Genomic DNA extraction*

The genomic DNA was extracted from fresh and tender leaves by Cetyl Tri Methyl Ammonium Bromide (CTAB) method (Doyle and Doyle 1987). Quality and quantity assessment of extracted DNA was done using Nanodrop (Thermo Fisher Scientific, Wilmington, USA). DNA samples (30-50 ng) were amplified in 10µL of volume containedprimers 0.5 µm each, 1× PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.5 mM MgCl2 and 0.01 mg $mL^{-1}$ gelatin), 0.5 mm dNTP mix, and 1unit of TaqDNA polymerase (GeNei). The amplified PCR products were analyzed by electrophoresis using 3% agarose gel (Sigma USA) and stained with 0.5 µg/ml ethidium bromide, visualized under UV light and was documented in a gel documentation system (Alpha Innotech, USA) with the help of standard ladder/ DNA marker (GeneRuler 100 bp ladder) (Suman et al. 2020).

### *Genotyping of RIL population*

A total of 948 SSR markers were screened for parental polymorphism, 119 were found polymorphic between the parents and used for genotyping the population. On selective genotyping, 60 were found polymorphic between parents and RILs. These 60 markers were further utilised for construction of linkage map and QTL detection. Those fragments which had unambiguous and clear amplification were considered for scoring with 100 bp ladder. The scoring of the population was done based on the band corresponding to MTU1010"A type"and BR2655"B type"

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and then marker data was generated for 60 polymorphic markers using the given codes A and B.

### *Construction of SSR based genetic linkage map and QTL mapping*

Inclusive composite interval mapping method (ICIM) deployed for the construction of SSR based linkage QTL map using QTL IciMapping software ver. 4.0.1 (Wang 2009; Meng et al. 2015). QTL nomenclature was followed as described by (Mc Couch et al. 1997). For QTL mapping, the Kosambi map function was used and the number of permutations for the determination of significance levels (at p = 0.05) was set to 1,000 and the LOD threshold was set to 2.5. At significant LOD peak (i.e., at greater than or equal to 2.5), QTL mapping in terms of QTL effects namely the log-likelihood ratio (LOD), additive effect of the identified loci and phenotypic variation explained (PVE) were estimated. The LOD test statistic used was – 2ln (L0/L1), where L0/ L1 is the ratio of the likelihood under the null hypothesis (indicating the absence of QTL) and the alternative hypothesis (indicating the presence of QTL) (Fiyaz, 2016). Each of the scored traits along with phenotypic means was subjected to QTL mapping.

## Results

### *Phenotypic data*

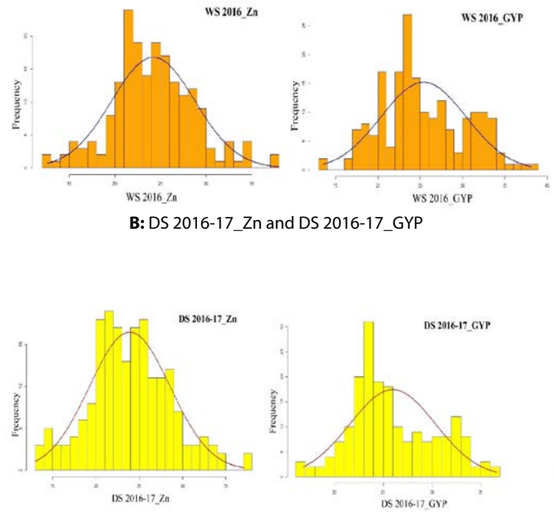
The mean, range, variability, heritability and genetic advance for grain zinc concentrations in RILs and parents during both seasonswere estimated and presented in Table 1, details are described in (Ramya et al. 2020). In WS 2016, the mean values of grain Zn concentration ranged from 12.6 to 38.0 ppm with an average of 24.1 ppm and grain yield had a range from 13.5 g to 38.1 g with a mean of 25.0 g. InDS2016-17, the mean values of grain Zn concentration ranged from 13.0 to 37.1 ppm with an average of 23.9 ppm and grain yield had a range from 13.5 to 36.6 g with a mean of 25.1 g (Table 1, Fig. 1 and Fig. 2). The genotypic coefficient variance (GCV) and phototypic coefficient of variance (PCV) were high during both seasons indicating existence of higher variability among RILs. Over both the seasons, NFG, GY and grain zinc concentration recorded higher PCV and GCV. Moderate PCV and GCV was observed for TW, low PCV, GCV for PL and DFF during WS 2016 and DS 2016-17. High PCV and GCV was noted during WS 2016 and moderate during DS 2016-17 for PH and NPT. In both the seasons high heritability coupled with high genetic advance was recorded for plant height, number of productive tillers per plant, number of filled grains per panicle, 1000-grain weight, grain yield per plant and grain zinc concentration indicating minor influence of environment on these traits and these traits are governed by additive gene action Across seasons the correlation analysis of RILs showed that Zn has moderate positive correlation with DFF and TW. Grain zinc concentration has negative significant correlation with grain yield per plant (Fig. 2).

**Table 1.** Estimates of range, variability, heritability, genetic advance for yield attributing traits and nutritional trait in RILs of rice during WS 2016 and DS 2016-17

| S. No. | Trait | Season | Mean | Range | | Coefficient of variation (%) | | Heritability | Mean of Gen. Adv % (at 5%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Min | Max | Phenotypic (PCV) | Genotypic (GCV) | $(h^2)_{bs}$% | |
| 1 | Zn | WS 2016 | 24.2 | 12.6 | 38.0 | 20.4 | 20.1 | 99.8 | 37.9 |
| | | DS 2016-17 | 23.9 | 13.1 | 37.1 | 20.3 | 20.2 | 99.3 | 39.4 |
| 2 | NFGP | WS 2016 | 207.0 | 75.0 | 371.0 | 30.7 | 30.6 | 99.2 | 58.2 |
| | | DS 2016-17 | 205.0 | 75.0 | 365.0 | 29.9 | 29.8 | 99.3 | 61.1 |
| 3 | GYP | WS 2016 | 25.1 | 13.5 | 38.1 | 20.8 | 20.1 | 88.5 | 37.7 |
| | | DS 2016-17 | 25.1 | 13.6 | 36.6 | 20.7 | 18.8 | 82.3 | 35.1 |
| 4 | TW | WS 2016 | 24.0 | 14.3 | 32.4 | 18.0 | 15.8 | 91.8 | 28.6 |
| | | DS 2016-17 | 23.9 | 13.7 | 32.7 | 14.2 | 12.8 | 81.2 | 23.8 |
| 5 | DFF | WS 2016 | 99.0 | 80.0 | 106.0 | 3.6 | 3.5 | 93.0 | 6.9 |
| | | DS 2016-17 | 93.0 | 70.0 | 101.0 | 6.7 | 6.6 | 96.2 | 13.3 |
| 6 | PH | WS 2016 | 105.0 | 63.0 | 154.5 | 21.8 | 21.0 | 76.6 | 45.6 |
| | | DS 2016-17 | 102.0 | 61.3 | 145.7 | 18.8 | 18.8 | 68.3 | 38.6 |
| 7 | NPT | WS 2016 | 12.0 | 9.0 | 17.0 | 21.3 | 20.5 | 69.6 | 26.8 |
| | | DS 2016-17 | 11.0 | 7.0 | 19.0 | 16.3 | 13.5 | 61.3 | 23.0 |
| 8 | PL | WS 2016 | 25.3 | 16.5 | 31.4 | 8.4 | 7.5 | 79.6 | 13.8 |
| | | DS 2016-17 | 23.9 | 16.0 | 30.0 | 10.3 | 8.6 | 70.1 | 14.9 |

Zn=Grain zinc concentration (ppm); GYP=Grain yield per plant (g); NFGP=Number of filled grains per panicle; TW=1000-grain weight (g); DFF=Days to 50 per cent flowering; PH=Plant height (cm); NPT=Number of productive tillers per plant and PL=Panicle length






**B:** DS 2016-17_Zn and DS 2016-17_GYP

**A:** WS 2016_Zn and WS 2016_GYP

**Fig. 1. Frequency distribution graphs of WS 2016 & DS 2016-17 for grain zinc concentration (Zn) and grain yield per plant (GYP).**

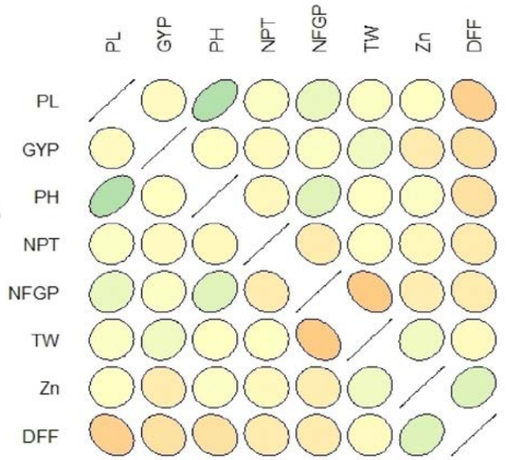



**Fig. 2. Correlogram-correlation matrix's for RILs.Zn-Grain zinc concentration; PH-Plant height; NPT-Number of productive tillers per plant; DFF-Days to 50% flowering in days; GYP-Grain yield per plant**

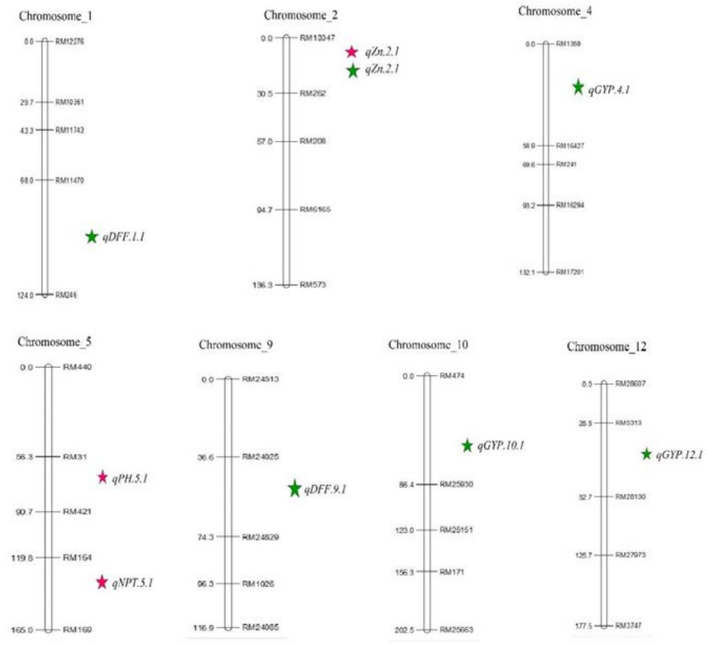



**Fig. 3. Identified *QTLs* for WS 2016 (Red colour *QTLs*) and DS 2016-17 (Green colour *QTLs*) for grain Zn concentration, yield and yield related QTLs in RILs of MTU1010/BR2655 using IciMapping v4.2.**

### *Parental polymorphism and selective genotyping*

Parental polymorphism survey between MTU1010 and BR2655 was studied using 948 Rice Microsatellite (RM) markers chosen based on their distribution throughout genome and 119 markers (12.55%) were polymorphic between the parents were employed for selective genotyping. A total of 60 markers (five each) found polymorphic between parents and RIL population and these markers were distributed across 12 chromosomes of rice. A representation of the banding pattern of polymorphic SSR marker across the RILs along with parents has been given in represented in Supplementary Fig. S1.

### *Linkage map construction*

The linkage map was constructed utilizing 60 polymorphic SSR markers (Fig. 3) through IciMapping v4.1 following Meng et al. (2015). The length

**Table 2.** *QTLs* identified for yield and yield related traits and grain zinc concentration in RILs of MTU1010/BR2655 during WS2016

| Trait | QTL | Chr. | Flanking markers | Position (cM) | LOD | PVE (%) | Additive effect | Allele |
|---|---|---|---|---|---|---|---|---|
| Zn | *qZn-2.1* | 2 | RM13347-RM262 | 26 | 3.37 | 7.74 | -1.99 | BR 2655 |
| PH | *qPH-5.1* | 5 | RM31-RM421 | 78 | 3.24 | 6.90 | 12.13 | MTU 1010 |
| NPT | *qNPT-5.1* | 5 | RM164- RM169 | 128 | 2.7 | 7.54 | 0.44 | MTU 1010 |

**Table 3**. *QTLs* identified for yield and yield related traits and grain zinc concentration in RILs of MTU1010/BR2655 during DS 2016-17

| Trait | QTL | Chr. | Flanking markers | Position (cM) | LOD | PVE (%) | Additive effect | Allele |
|---|---|---|---|---|---|---|---|---|
| Zn | *qZn-2.1* | 2 | RM13347-RM262 | 26 | 4.13 | 10.20 | -2.17 | BR 2655 |
| DFF | *qDFF-1.1* | 1 | RM11740-RM246 | 90 | 2.85 | 3.6 | -3.73 | BR 2655 |
| | *qDFF-9.1* | 9 | RM24025- RM24829 | 56 | 3.20 | 3.18 | -3.71 | BR 2655 |
| GYP | *qGYP-4.1* | 4 | RM1359- RM16427 | 40 | 3.52 | 1.34 | 3.79 | MTU 1010 |
| | *qGYP-10.1* | 10 | RM474- RM25930 | 53 | 3.11 | 1.35 | 3.69 | MTU 1010 |
| | *qGYP-12.1* | 12 | RM5313- RM2810 | 53 | 3.29 | 1.33 | 3.75 | MTU 1010 |

Zn=Grain zinc concentration; PH=Plant height; NPT=Number of productive tillers per plant; DFF=Days to 50% flowering in days; GYP=Grain yield per plant; LODlogarithm of the odds; PVE=phenotypic variance explained.

**Table 4.** Candidate genes in the *qZn-2.1* QTL region flanked between RM13347 and RM262 (19.37–20.79 Mb region)

| S.No | Locus_ID | Gene name | Physical Position |
|---|---|---|---|
| 1 | *Os02g0530100* | Heavy metal-associated domain containing protein, expressed | 19.47 Mb |
| 2 | *Os02g0550800* | Ammonium transporter protein, putative, expressed | 20.73 Mb |

of the linkage map ranged from 124.04 (chromosome 1) to 202.47 (chromosome 10) Cm with mean of 1874.09 cM distance. The linkage map was created by using Kosambi function (Kosambi, 1944) resulting in an average marker interval of 156.17 cM. Among the 60 markers used (five markers on each chromofome), 41 markers showed segregation distortion to varied degrees on all the chromosomes. The segregation distortion of the markers was mostly skewed towards the parent BR2655. Map Chart v.2.3 was used for visualization of the QTLs (Voorrips, 2002).

### QTL mapping

QTL analysis was carried out separately for two seasons, during WS 2016, a total of three QTLs (*qZn-2.1*on chromosome 2; *qPH-5.1*and*qNPT-5.1*on chromosome 5) were detected with phenotypic variance (PVE) ranged from 6.90 to 7.74%. Out of 3 QTLs, one QTL (*qZn-2.1* with PVE of 7.74%) for grain zinc concentration flanked between RM13347-RM262 and two QTLs (*qPH-5.1*withPVE of 6.90% flanked between RM31-RM421and*qNPT-5.1*with PVE of 7.54% flanked between RM164- RM169on chromosome 5)for yield related traits were identified (Table 2 and Fig. 3).

During DS 2016-17, totally six QTLs with PVE ranged from 1.33 to 10.20% were identified for three traits. Out of 6 QTLs, one QTL (*qZn-2.1* with PVE of 10.20%) for grain zinc concentration flanked between RM13347-RM262, identified consistently in both the seasons. Two QTLs for days to 50 per cent flowering were detected on chromosome 1 (*qDFF-1.1* flanked between RM11740-RM246 at 90 cM distance) and chromosome 9 (*qDFF-9.1* flanked between RM24025-RM24829 at a distance of 56 cM) with phenotypic variance of 3.60 % and 3.18 % respectively. The additive effect for both QTLs *qDFF-1.1* (-3.73) and *qDFF-9.1* (-3.71) found with negative sign indicates alleles for days to 50 per cent contributed from donor parent BR2655 (Table 3 and Fig. 3). Three QTLs *viz., qGYP-4.1* with PVE of 1.34% flanked between RM1359-RM16427 on chromosome 4, *qGYP-10.1* with PVE of 1.35% flanked between RM474-RM25930 on chromosome 10 and *qGYP-12.1* with PVE of 1.33% flanked between RM5313-RM2810 on chromosome 12, were identified for grain yield per plant (Table 3, Table 4 and Fig. 3).

In the present study, consistent QTL for grain zinc concentration (*qZn-2.1*) located in the 19.37–20.79 Mb region flanked between RM13347 and RM262 was identified on chromosome 2 for WS 2016 with PVE 7.74% and *DS* 2016-17 with PVE 10.20% (Tables 2, 3 and Fig. 3).

### *Candidate gene analysis in the identified QTLs*

Considering only onecommon QTL for grain zinc concentration, in the identified QTL region out of 206 candidate genes, single transporter and heavy metal associated genes were observed (https://rapdb.dna.affrc.go.jp/). The identified putative candidate genes associated with metal metabolism were selected from the annotated candidate genes in the QTL region. The role of two candidate genes in the grain zinc concentration metabolism was evaluated using Knetminer (http://knetminer.rothamsted.ac.uk/Oryza_sativa/)

Network analysis of KnetMiner software, the two candidate genes in the common QTL region (*qZn-2.1*), *viz.*, *Os02g0530100* encoding heavy metal-associated domain containing protein, expressed to be positioned within 19.47 Mb; and *Os02g0550800* encoding ammonium transporter



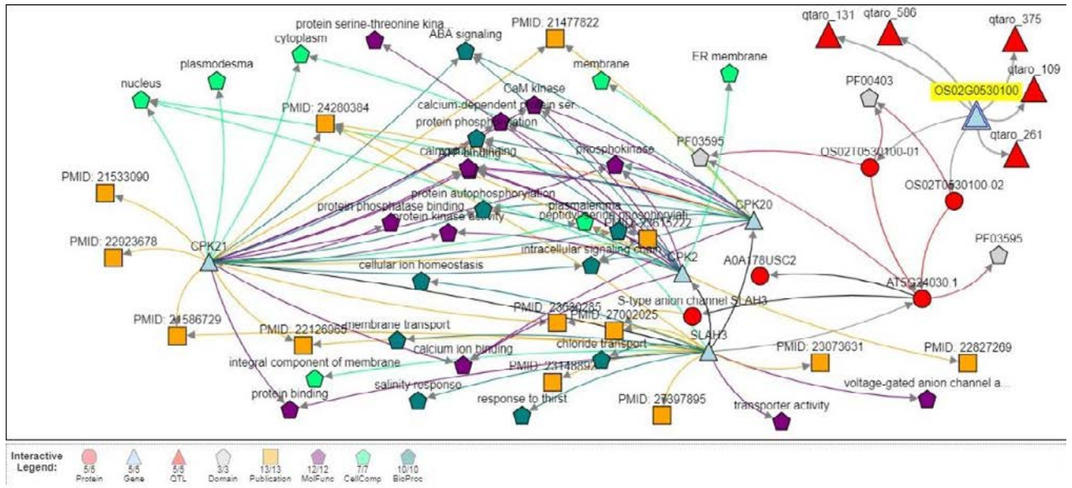



**Fig. 4. Network analysis of candidate gene (*Os02g0530100*, encoding heavy metal-associated domain containing protein) in QTL*qZn-2.1* using Knetminer**

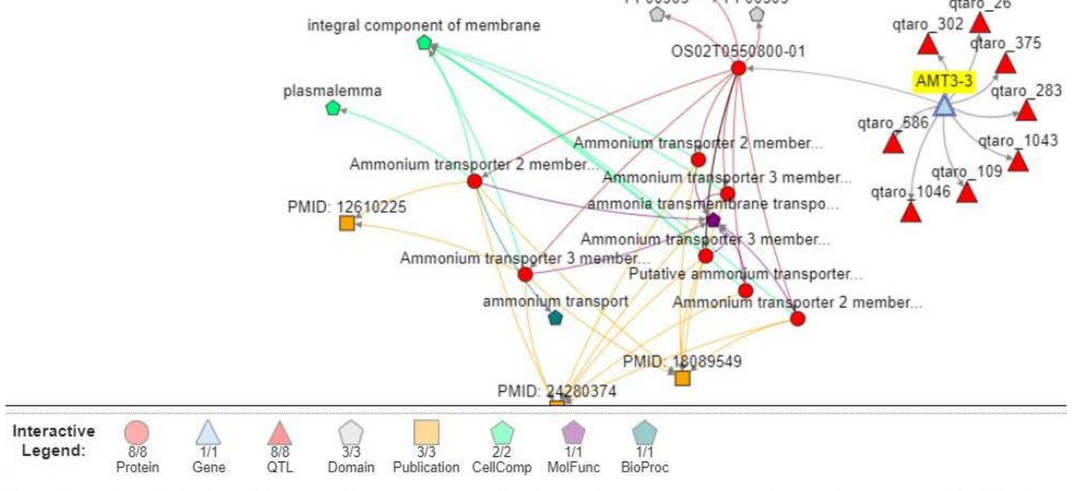



**Fig. 5. Network analysis of candidate gene (*Os02g0550800*encoding Ammonium transporter protein, putative, expressed) in QTL *qZn-2.1* using Knetminer**

protein, putative, expressed to be positioned within 20.73 Mb from the QTL region of *qZn-2.1* (19.37–20.79 Mb) on chromosome 2 (Table 4).

The *Os02g0530100*was found to be linked with 4 genes, 5 proteins and 12 molecular functions (Fig. 4). The *Os02g0550800*was found to be linked with 25 genes, 8 proteins, nine QTLs, nine phenotype traits and 1 molecular function (Fig. 5). These two candidate genes were originated to be closely linked with identified QTL being further investigated.

## Discussion

One third of the world's population was reported to lack sufficient Zn nutrition (White 1 and Broadley 2011). For a staple food crop like rice, any increase of micronutrients would have impact on the malnutrition affecting most

of the developing countries with rice as principal calorie food. Enhancing micronutrient density in the staple crops, especially in cereal crops has been established through release of several biofortified varieties across the world. In rice, a few biofortified varieties with high grain Zn concentration have been developed (Harvest Plus and FAO 2019). The released Zn biofortified rice varieties were however developed using conventional breeding strategy wherein the selection was only based on phenotyping for grain Zn concentration and also yield and its attributes (Nakandalage et al. 2016; Khan et al. 2019). Hence, the present focuses on accelerating the breeding process, for instance use of marker assisted selection program for identification of QTLs associated with grain Zn concentration could be helpful in hastening the release of biofortified varieties (Mahender et al. 2016). Based on the requirement and bioavailability of grain Zn concentration, the recommended target content has been enhanced to 28 ppm (Bouis and Saltzman 2017). To meet the enhanced levels of high grain Zn concentration in rice, identification and deployment of QTLs would be of great use to increase the efficiency of the breeding program and expedite the development of biofortified rice varieties with high grain Zn concentration.

In the present study, QTL for grain zinc concentration *qZn-2.1* was consistently detected on chromosome 2 as (Fig. 3), which accounts for phenotypic variance of 7.74% and 10.20% during WS 2016 and DS 2016-17, respectively. QTL *qZn-2.1* was flanked by RM13347-RM262 SSR loci at a distance of 26 cM. indicating that this genomic region involved in controlling the grain zinc concentration. It is reported that grain Zn content in rice is governed by a number of QTLs located on different regions of the chromosome with different phenotypic effects (Biradar et al. 2007; Lu et al. 2008; Garcia-Oliveira et al. 2009; Zhang et al. 2011). Identified common major QTL (qZBR.2.1 and qZPR.2.1) on chromosome 2 for grain Zn content across SSR and SNP maps (Suman 1 et al. 2021). Three QTLs were identified by Garcia-Olivera et al. (2009) qZN-5, qZN-8 and qZn-12 for Zn content in 85 backcross population (ILs) obtained by crossing of Teqing (*Oryza sativa* ssp. *indica*) and elite wild rice (*O. rufipogon* Griff.) using 179 SSR markers. Grain zinc concentration was estimated in recombinant inbred lines (RIL) derived from IRRI38 X Jeerigesanna and validation of putative candidate gene markers with rice accessions. Single marker analysis revealed that four (*OsNAC, OsZIP8a, OsZIP8c and OsZIP4b*) candidate gene markers showed significant variation among RIL population with a phenotypic variation of 4.5, 19.0, 5.1 and 10.2% respectively by (Gande et al. 2014). QTLs for the contents of seven mineral elements in milled rice using recombinant inbred lines of the indica rice cross Zhenshan 97/Milyang 46, Co-localizations of QTLs for multiple traits were observed, of which the *qP3/qMg3/qZn3* region was shown to have the largest effects for the contents of phosphorus, magnesium, zinc and the *qK6.1/qCa6/qZn6/qMn6/qCu6* region was found to be responsible for five of the seven traits (Yu et al. 2015). Analysed, grain Zn concentration in 60 $F_7$ RILs derived from Swarna x Moroberekan and three markers were associated to Zn content in rice grain concentration on chromosome 2, 3 and 11 (Indurkar et al. 2016). Total of 22 QTLs for the concentrations of Cd, Cu, Fe, Mn and Zn in brown rice with 204 recombinant inbred lines, distributed on chromosomes 1, 2, 4, 5, 7, 8, 10 and 11 were identified (Huang 2018). Identified and mapped QTLs for average Fe/Zn concentration and AOC (advantage over control) for Fe/Zn concentration in milled rice grains using $F_5$ population of 166 recombinant inbred lines derived from the cross, MTU1010 x IR94033. Out of 6 QTLs, one QTL for iron concentration was located in chromosome 8 (*qFE8.1*) and 5 QTLs (*qZN1.2, qZN2.1, qZN5.2, qZN7.1* and *qZN10.1*) for zinc concentration were located on chromosomes 1, 2, 5, 7 and 10. QTLs together for grain zinc concentration explained 7.8% phenotypic variance. (Swamy 2 et al. 2018a) identified QTLs for Fe and Zn concentrations in two $BC_2F_3$ mapping population derived from the crosses of *O. sativa* cv. Swarna with two different accessions of *O. nivara*. In all, 8 QTLs were identified for grain Zn concentrations in population 1 whereas 5 QTLs were identified in population 2, respectively. Three QTLs for Zn explained more than 15% phenotypic variance either in interval or composite interval mapping. Identified 20 QTL susing doubled haploid (DH) population, PSBRc82 x Joryeongbyeo and PSBRc82 x IR69428 for agronomic traits and 59 QTLs for a number of biofortification traits, of the 79 QTLs, 12 were large-effect QTLs (>25% PVE), nine QTLs were consistent across the seasons in either of the population, and one QTL was identified in both population (Swamy et al. 2018b). $BC_2F_5$ population from cross between RP-Bio226 and Sampada was used to localize genomic region(s)/QTL(s) for grain Zn concentration together with yield and yield-related traits (Dixit et al. 2019). Comparative analysis across the two seasons has revealed four consistent QTLs for Fe (*qFe1.1*, *qFe1.2*, *qFe6.1* and *qFe6.2*) and two QTL for Zn content (*qZn1.1* and *qZn6.2*). Two doubled-haploid populations derived from the crosses IR64 × IR69428 and BR29 × IR75862 revealed 15 QTL for agronomic traits and 50 QTL for grain element concentration (Empleo et al. 2019).

Three QTLs *qGYP-4.1*, *qGYP-10.1* and *qGYP-12.1* identified on chromosome number 4, 10 and 12 respectively for grain yield per plant. The phenotypic variance explained by these QTLs such as *qGYP-4.1 qGYP-10.1* and *qGYP-12.1* was 1.34%, 1.35% and 1.33, respectively. QTL *qGYP-4.1* flanked by RM1359- RM16427 markers, whereas QTL *qGYP-10.1* was flanked by RM474- RM25930 microsatellite loci and *qGYP-12.1* was flanked by RM5313-RM2810 markers at a distance of 40 cM, 53 cM and 53 cM respectively. Favourable allele for *qGYP-4.1*, *qGYP-10.1* and *qGYP-12.1* had positive additive effect of 3.79, 3.69 and 3.75, respectively revealed the enhancing alleles are contributed by MTU1010 parent. As these QTLs *qGYP-4.1*, *qGYP-10.1* and *qGYP-12.1* identified in the present study are having very low phenotypic variance which infers these are minor QTLs. Earlier reports (Marri et al. 2005) indicated single plant yield QTLs on chromosome 2 and 9 with a phenotypic variation ranging between 7.05 % and 23.2 %. Number of productive tillers per plant found to have one QTL (*qNPT-5.1*) on chromosome 5, which is flanked by RM164- RM169 microsatellite loci. The magnitude of 7.54 % phenotypic variance explained by the QTL which infers it as a minor QTL. The additive effect (0.44) found to be positive, which indicate positive alleles for number of productive tillers per plant contributed from recipient parent MTU1010. Previous reports (Marri et al. 2005) revealed QTLs for number of productive tillers on chromosomes 2 and 5 with a phenotypic variation of 11.11 % and 5.9 %, respectively and (Anuradha 1 et al. 2013) identified QTLs for number of productive tillers on chromosomes 3, 6 and 10 with a phenotypic variation of 25.9 to 34.5 %. For plant height, one QTL (*qPH-5.1*) located on chromosome 5 was detected using CIM accounted for 6.90% phenotypic variation and 3.24 of LOD score in *WS* 2016. QTL *qPH-5.1* was flanked by RM31-RM421 markers at 78 cM distance with a positive additive effect of 12.13. The favourable alleles for plant height were contributed by MTU1010. Previously two QTLs for plant height was reported by (Marri et al. 2005) on chromosome 1, phenotypic variation explained by two QTLs was 17.48 % and 6.82 %. (Anuradha et al. 2012b) reported 5 QTLs on chromosome 1 with the phenotypic variation ranging between 19-62.7 %. In present study, the successfully mapped and identified consistent QTL *qZPR.2.1* was found across the seasons for grain Zn concentration. Four RILs *Viz*., J146, J153, J19, J130 produced higher yield in combination with high grain zinc concentration (>30 ppm grain Zn concentration and >30grams grain yield/plant) over seasons and these four RILs can be utilised in future breeding program.QTL for grain zinc concentration(*qZn-2.1*)need to be validated in alternate population for future use in marker assisted selection and fine mapping. The potential of the two candidate genes (*Os02g0530100* and*Os02g0550800*) in the QTL region (*qZn-2.1*) were confirmed by network analyses offers span for their deployment in rice biofortification and breeding programme in rice.

## Supplementary material

Three Supplementary figures are supplied.

## Authors' contributions

Conceptualization of research (RR, NCN, SLV, SK, MB); Designing of the experiments (RR, NCN, SK, MB, EKB); Contribution of experimental material (NCN, SLV); Execution of field experiments and data collection (RR, SK, MB, NCN); Analysis of data and interpretation (RR, SCD, EKB, SLV, SK); Preparation of the manuscript (RR, NCN, SLV, EKB).



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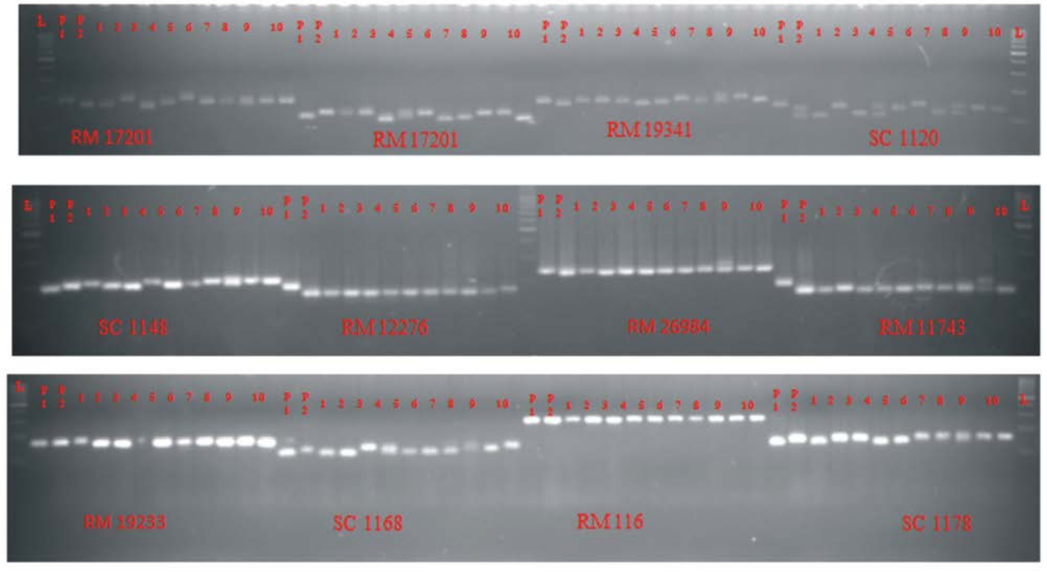
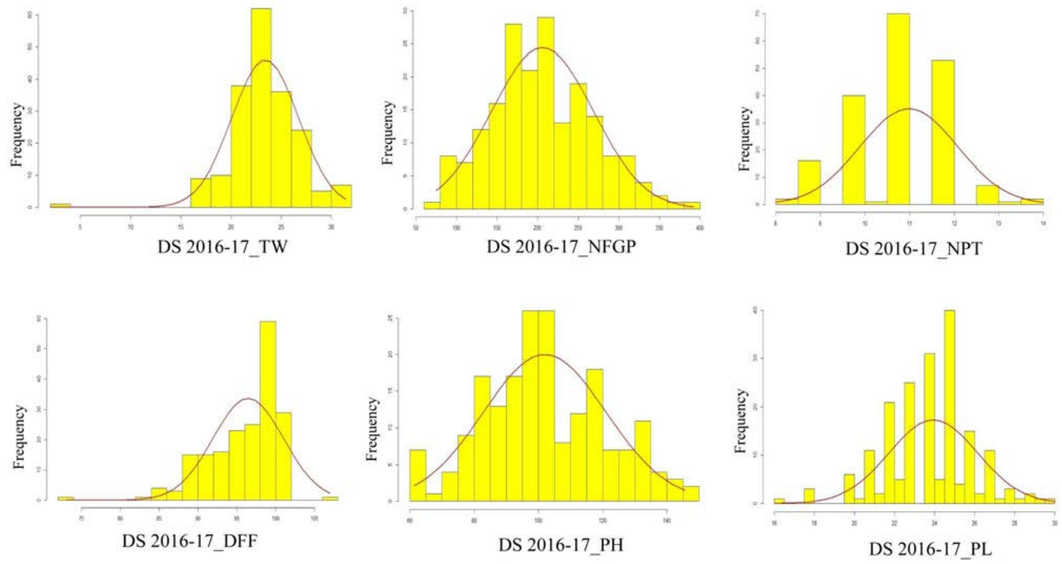
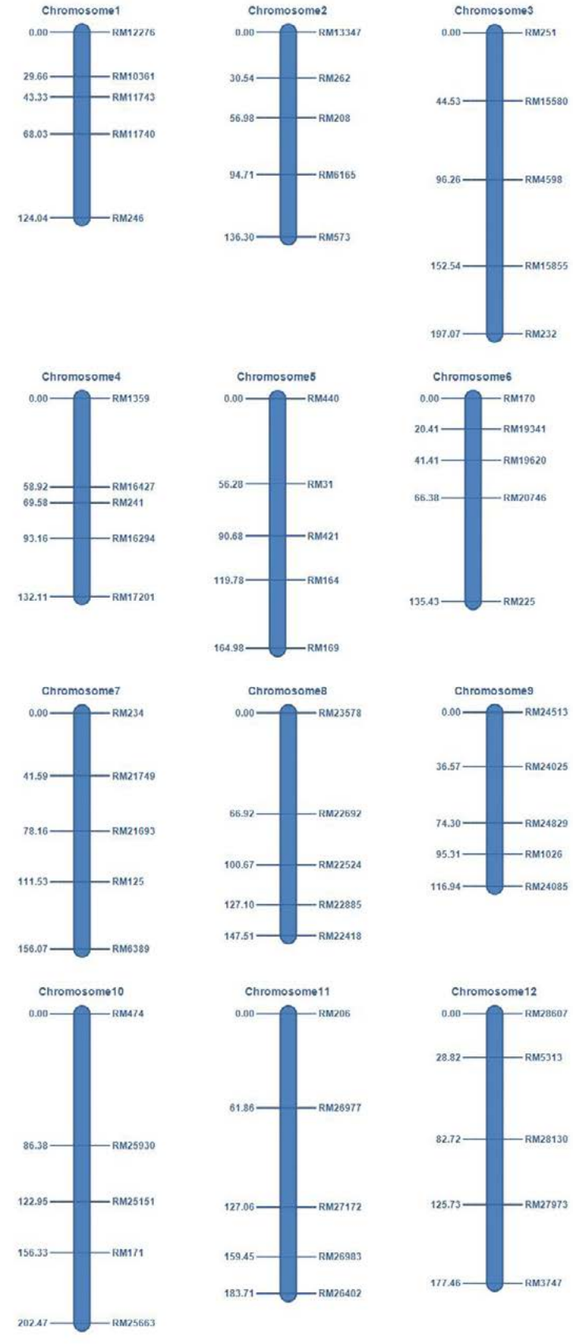



**Supplementary Fig. S1. Frequency distribution of WS 2016_TW; WS 2016_NFGP; WS 2016_NPT; WS 2016_DFF; WS 2016_PH and WS 2016_PL**




**Supplementary Fig. 2. Frequency distribution of DS 2016-17_TW; DS 2016-17_NFGP; DS 2016-17_NPT; DS 2016-17_DFF; DS 2016-17_PH and DS 2016-17_PL.**






**Supplementary Fig. 3. Linkage map constructed in RIL population derived from MTU1010 and BR2655**