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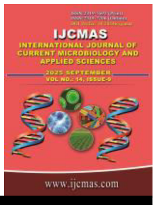

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Grain Filling, and Rice Biofortification Impact on Seedling Vigour

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ABSTRACT

Grain filling is an important part of nutrition delivery to rice grains. It is crucial to provide the grains with adequate carbohydrates, essential mineral elements, and vitamins to the endosperm and aleurone. This review focusses on differences in parts of rice grains containing different amounts of macro- and micro-nutrients. Key focus was also placed on strong culm rice arising out of early seedling vigour. NGS-mediated Bulked segregant analysis for genetic elements responsible for strong culm has been found in Chromosome 12 (23.2 to 25.71 MB). Indel genotyping in agarose plates were performed for validation of the obtained BSA results. Seedling vigour for the canopy establishment and production of increased yield under aerobic circumstances is vital. Iron (Fe) and zinc (Zn) are essential trace elements affront people's energy and stamina worldwide and refrain from nutritional deficiencies arisng from iron/zinc. The biological enrichment of iron, zinc, manganese, and vitamins in rice is economical and accessible to supplement the diet of poor people with this biofortified food. This review discusses progress and strategies to increase grain Iron, Zinc, Manganese and vitamins.

Keywords

Strong Culm, MutMap, Indel genotyping, Biofortification

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Introduction

Minerals such as iron, manganese, copper, and zinc are essential for living organisms and human development. Various sources of iron from food help regulate the functioning of our metabolism. The most important metals, iron (Fe) and zinc (Zn), are unfortunately found in meagre amounts in foods like rice and wheat. However, efforts are ongoing in developing countries like India to fortify the grains with that zinc, iron, and vitamins (Kumar *et al.,* 2019). Iron deficiency is one of the leading causes of disability and death worldwide. Approximately 30% of the world's population is also estimated to suffer from zinc, iron, and vitamin deficiency (Harika *et al.,* 2017). A good mineral diet is also essential for children's growth, immunity, and development. Therefore, iron, zinc, and vitamin deficiency in children and adults have become severe (Hurrell, 2022; Bamji 1983).

Grain-filling is the final stage in the rice life cycle, during which fertilized ovaries develop into caryopses. The filling rates and extent determine the weight of the rice grain and, consequently, grain yield and quality. Considerable demand is for an increase in rice yield and an improvement in grain filling and quality. In general, the top portion of the spikelet is filled faster, followed by the lower one (Chen *et al.,* 2019). The lower grains are either sterile or filled up slowly for grain production, and Apical primary branches are filled faster than proximal secondary branches. Many theories have been proposed that state the reasons for improper grain filling to be because of reduced source size/sink size, imbalance of hormones, reduced gene expression, and reduced activity of enzymes causing sucrose-to-starch conversion result in less efficient to assimilate transport. However, the mechanism responsible for the variation observed because of the rate at which grains are filled at the panicle's top portion and the panicle's lower portion remains to be elucidated. It has been discussed in the following section.

Grain Filling in Rice

The dorsal vascular bundle and aleurone layer results in the nutrient filling under 7 to 18 days after fertilization (DAF). For Ca and K, the contents remained constant from 10 to 30 days after flowering. It was tested that a concentration of 12 elements (nitrogen, potassium, sulfur, calcium, magnesium, manganese, zinc, boron, molybdenum, and arsenic) decreased sharply from 5 to 9-13 days after flowering. RNA-seq revealed genes at 0, 19, and 16 days after flowering related to biosynthesis and auxin metabolism (indole-3-acetic acid, IAA), gibberellin (GA), brassinosteroid (BR), cytokinin (CK), abscisic acid (ABA), and ethylene (ET). Genes like (Rice response regulator) *OsRR3*, *OsRR13*, *OsRR27*, *OsRR33*, *OsYUCCA3, Aux/IAA, GA20ox3*, and *GA20ox6* were upregulated during the development of the fruit. Upregulation in genes related to K transport was *OsHAK19, OsHAK15, OsHAK14, OsHAK12, OsHAK9, OsHAK8, and OsHAK1*. Among the boron genes, *OsBOR1*, *OsBOR2*, and *OsBOR4* coincided with rapid *Ca and B* accumulation at early grain-filling stages.

Reports suggest that high expression of Mn transporter genes (e.g., OsYSL6) at five days after flowering revealed the requirement of Mn in the chloroplast for photosynthetic machinery. SWEET14 gene at different stages of grain development were expressed (5–13 DAF). Later *OsSWEET1a*, *OsSWEET1b*, and *OsSWEET16* were expressed more highly at 21 DAF, suggesting that different apoplastic pathways supply sucrose to the endosperm during the rapid grain-filling stage in rice via SWEET sucrose effluxes. The number of seeds in a panicle represents the plant's sink capacity. Usually, a higher concentration of minerals and nutrients is found in the top part of the panicle (Ren *et al.,* 2023; Ram *et al.,* 2020).

Two rice mutants, (AZU-M) a large-grain mutant, and (ZF802-M) a small-grain mutant, and their consequent wild types were made to grow in the field. Endosperm cell division rate, filling rate, and hormonal levels: idole-3-acetic acid (IAA), zeatin + zeatin riboside (Z+ZR), polyamines (PAs), and abscisic acid (ABA) were experimentally estimated. Results demonstrated the insignificant difference between the filling and endosperm cell division rates. Synchronous rates were observed between superior and inferior spikelets; wherein the superior spikelets fill up faster, and the filling rate is higher, compared to inferior ones. Alterations in the concentration of plant hormones are consistent with the cell's observed division rate and grain filling rate.

Hormones attributed roles: Reduced hormone concentrations for ABA (Abscisic acid), IAA (Indole-acetic acid), and PAs (Polyamines) justified the role of grain filling. The experiments indicate the poor filling of inferior spikelet in rice owing to dampened hormonal concentration. Less accumulation of hormone concentrations, instils a lowered division rate of endosperm cells thus resulting in slow grain filling, and eventually lower grain weight [8]. Demand for rice is increasing exponentially, and important reasons for poor grain filling are: i) low activity of starch biosynthesis enzymes, ii) more excellent ethylene production, thereby negatively controlling starch biosynthesis enzymes, iii) in addition to lower expression of cytokinin and indole-3-acetic acid (IAA), and altered expression of miRNAs unfavourable for grain filling. Recent advancements in terms of i) spikelet-specific downregulation of genes involved in ethylene synthesis; ii) design molecular means for enhanced accumulation of abscisic acid (ABA), cytokinin, and IAA in the caryopses for transport of nutrients to the rice endosperm and embryo, iii) manipulation of expression of transcription factors viz.

MYC, and *OsbZIP58* to drive the expression of starch biosynthesis enzymes; iv) spikelet-specific overexpression of cyclin with the likes of *CycB:1*; and *CycH:1* for promotion of endosperm cell division; and v) targeted enhancement in accumulation of ABA in straw owing to grain filling stage; and v) targeted enhancement in accumulation of ABA in straw owing to grain filling stage for increased carbon resource mobilization to grains. Genes determining panicle compactness may also increase rice yield by changing compact panicle into lax/open panicles (Parida *et al.,* 2022). Under zinc-limited conditions, OsZIP9 is the transporter that helps roots take zinc from the soil (Huang *et al.,* 2020).

Two rice genotypes widely cultivated in China, W1844 and CJ03, had large panicle sizes and the same sink capacity. However, the variations in grain filling rates between the superior and inferior spikelets in W1844 were significantly less pronounced than in CJ03. The sucrose source-to-sink translocation rate during the grain-filling stage was more efficient in W1844 than in CJ03, and the sucrose transporter gene expression level was higher in W1844 than in CJ03. Also, carbon output, transport ratio, and contribution rate from stem, sheath to panicle were higher at the early stages of grain filling than at later stages in W1844. It was concluded that high sink strength and activity lead to an efficient sugar translocation rate at the early grain-filling stage and can improve grain-filling efficiency (Chen *et al.,* 2020; Chen *et al.,* 2019). Similar observations were also noted, and the performance of grain filling rate and starch accumulation in between six cultivars in climatic conditions of Southern Sichuan were in order YXY2115 > CY6203 > FY1 > GY900 > GY188 > CGY6684 higher to lower order (Wu *et al.,* 2022).

In the case of direct seeded rice, chalkiness reduction in three irrigation modes and nitrogen (N) fertilizer management in response to (flooding (W_1), dry–wet alternating (W_2), dry alternating (W_3); three nitrogen application strategies (less than 150 kg ha−1) included a base: tiller: panicle fertilizer application ratio of 30%:50%:20% (N1), 30%:30%:40% (N2), and 30%:10%:60% (N3), with a zero nitrogen control (N0) in the years 2019 and 2020; W2 and the N1 application strategy were utilized. N application approaches (below 150 kg ha−1) consisted of the application ratios of base: tiller: panicle fertilizers as follows: (30%:50%:20% (N1), 30%:30%:40% (N2), and 30%:10%:60% (N3)), with zero N serving as the control (N0) in the years 2019 and 2020. These experiments can further optimize grain filling and reduce grain chalkiness. An increment in mean grain-filling rates during the mid-grain-filling stages in superior grains of primary branches and inferior grains of secondary branches could be achieved by improving water–nitrogen interaction effects, thereby decreasing the chalky behaviour of the grains. Improvements in micro canopy atmosphere (daily avg. temp. difference and daily avg. light intensity difference) during the early-filling stage of inferior grains and mid-grain filling stage of higher grains could reduce grain chalkiness to a large extent (Sun *et al.,* 2022)

Transgenic and Breeding Experiments Concerning Grain Filling

GFR1 gene has been suggested to play essential roles in grain filling by increasing Rubisco's initial activity and causing the production of sucrose and further unloading of sucrose which is mediated by *OsCIN1* coding for cell-wall invertase. *OsCIPK17* has also played a critical role in improving PSII efficiency by favourably regulating starch, sucrose, and amino acid metabolism-related gene expression (Gao *et al.,* 2022). In addition, transgenic experiments in japonica rice expressing NAC family transporter SUPER STARCHY1/ONAC025 also played vital roles in increasing grain filling and negatively affecting vegetative growth (Mathew, 2020). Transgenic rice expressing miR1432 was found to modulate grain yield via enhancement of grain-filling rate in rice and fatty acid metabolism and phytohormone biosynthesis and also found to enhance expression of OsACOT (Acyl-CoA thioesterase) and simultaneously also enhanced auxin and abscisic acid concentration in the panicle during grain filling stages. Another gene, GRAIN INCOMPLETE FILLING 2, was discovered to govern grain filling via regulating starch synthesis during rice caryopsis development (Zhao *et al.,* 2017; Wei *et al.,* 2017).

A Brief Note About Experiment Performed on Strong Culm Rice

Culm of the rice plant originates from plumule and is joined at internodes and bears the rice panicle at the reproductive stage. Strong culm- large culm, lodging resistant rice has been widely associated with having large panicle size. These also have been widely associated to having more structural carbohydrates in the culm that eventually impart to more nutrient filled grains in the culm (Meng *et al.,* 2016; Fu *et al.,* 2011; Wu *et al.,* 2011; Zhang *et al.,* 2010). The hydrostatic pressure enables the nutrients along with non-structural carbohydrates, amino acids and mineral ions to load inside the cells of the seed. In an experiment performed at the Yogi Vemana University, Kadapa, Andhra Pradesh, 690 reciprocal cross population of F_2 between the strong culm mutant TI-17, and BPT-5204 was tested for strong culm related traits-viz. pushing resistance, culm diameter and other yield related traits like panicle weight and number of reproductive tillers; the data is represented (Table S1). The F_1's for the TI-17 cross BPT-5204 were forwarded to the F_2 generation whereby their culm strength and culm diameter parameters and grain weights were measured. The formula for pushing resistance is: {(test reading/40) x 1000}/Number of tillers (Yadav *et al.,* 2017) (Table S1).

The mutant strong culm extreme genotypes and weak culm extreme genotypes were bulked and proceeded for MutMap analysis. Sheath blight resistance measurement of the plant population were also performed according to (Naveenkumar *et al.,* 2023) established protocol. Further, strong culm genotype seeds were forwarded to F3 generation and the plants were undertaken for sheath blight resistance measurement in the advanced population. The plant images and the F_2 population images, correlation analysis, and phenotyping range are provided (Fig. 1).

MutMap data analysis

The MutMap data from extremes sample reads were analyzed using the software package available at http://genome-e.ibrc.or.jp. The raw sequence data have been used. The reference database used was indica reference 93-11 from the NCBI. Raw sequence data were processed and Reads were mapped.

All the sequences (two bulks and parents) were aligned to the indica 93-11 genome sequence as a reference. The strong culm bulk data was mapped to TI-17 data, and the weak culm was mapped to BPT-5204 Next-Generation-Sequencing data. Bulk-segregant-analysis was carried out between BPT-5204, weak culm bulks, strong culm bulks and TI-17. The reads obtained from the mutant extremes were mapped against wild-type extremes, compiled with aligned reads from parents (BPT-5204 and TI-17) were used for bulk-segregation analysis.

Results and Discussion

In the study, we have found out that field culm diameter is proportional to weight/panicle correlation coefficient of 0.610. Pushing resistance is proportional to field culm diameter 0.883. Pushing resistance is also proportional to culm diameter 0.587. These results suggest that pushing resistance and culm diameter is positively correlated to weight per panicle.

In other words, increase in weight of panicle may be attributed to increased thickness of culm of the rice plant and high breaking resistance of the rice plant. The study is influenced by the published works (Ballichatla *et al.,* 2024; Mulsanti *et al.,* 2018; Ookawa *et al.,* 2010).

The significance values obtained in correlation table further suggest that the weight of the panicle is positively dependent on culm diameter (stem diameter), pushing resistance (stem lodging parameter), height of the plant. The obtained values are plotted in form of correlation plot chart in SPSSv29 is represented (Table 1).

The culm diameter distribution graph for the TI17 x BPT-5204 (Fig. 2)

The pushing resistance distribution graph for the TI17 x BPT-5204 is shown (Fig. 3):

MutMap yielded causal Indel variation analysis in advanced F3 population

The MutMap study revealed key SNP variants in genes responsible for the key differences between the two traits i.e transitions and transversions at both the genic and intergenic levels (Table 2). The SNPs obtained in the MutMap analysis were used on the basis of the Indel genotyping to validate the obtained causal SNP.

The Chromosome 12, 23.2 to 25.71 MB was identified in the mutant bulk was held responsible for the culm strength in the population as obtained from MutMap analysis (Fig. 4). To validate the polymorphic InDel markers, six Indels were selected from Mut-Map region of chromosome obtained from NGS sequencing.

To simplify the visualisation of Indel markers on Agarose/PAGE, Indels with lengths more than or equal to 8 were chosen for PCR validation. The results showed that four InDels obtained from Mut-Map/DNA-seq result were amplified successfully and exhibited polymorphisms in two parent genotypes and the selected 94 population. The bulked-segregant polymorphism genotyping results of the four markers in 94 members of F3 population were demonstrated (Fig. 5).

Table.1 Pearson Correlation table

Correlations (N=692)										
		PT reading	DFF in days	Plant height	Field Culm diameter	Nos. of tillers	No. of Reproductive tillers	Pushing resistance	Weight/panicle	SPY
PT reading	Pearson Correlation	1	-0.151**	.147**	.096*	.705**	.721**	.345**	.080*	0.026
	Sig. (2-tailed)		0.000	0.000	0.011	0.000	0.000	0.000	0.036	0.497
DFF in days	Pearson Correlation	-0.151**	1	.089*	-0.090*	-0.046	-0.055	-0.129**	-0.056	-0.009
	Sig. (2-tailed)	0.000		0.019	0.019	0.230	0.145	0.001	0.138	0.819
Plant height	Pearson Correlation	.147**	.089*	1	-0.024	.120**	.126**	0.042	0.027	0.035
	Sig. (2-tailed)	0.000	0.019		0.529	0.002	0.001	0.272	0.475	0.363
Field Culm diameter	Pearson Correlation	.096*	-0.090*	-0.024	1	-0.538**	-0.505**	.883**	.610**	0.211
	Sig. (2-tailed)	0.011	0.019	0.529		0.000	0.000	0.000	0.000	0.768
Nos. of tillers	Pearson Correlation	.705**	-0.046	.120**	-0.538**	1	.989**	-0.384**	-0.369**	0.006
	Sig. (2-tailed)	0.000	0.230	0.002	0.000		0.000	0.000	0.000	0.871
No. of Reproductive tillers	Pearson Correlation	.721**	-0.055	.126**	-0.505**	.989**	1	-0.345**	-0.351**	-0.002
	Sig. (2-tailed)	0.000	0.145	0.001	0.000	0.000		0.000	0.000	0.957
Pushing resistance	Pearson Correlation	.345**	-0.129**	0.042	.883**	-0.384**	-0.345**	1	.587**	0.024
	Sig. (2-tailed)	0.000	0.001	0.272	0.000	0.000	0.000		0.000	0.531
Weight/panicle	Pearson Correlation	.080*	-0.056	0.027	.610**	-0.369**	-0.351**	.587**	1	0.021
	Sig. (2-tailed)	0.036	0.138	0.475	0.000	0.000	0.000	0.000		0.574
SPY	Pearson Correlation	0.026	-0.009	0.035	0.011	0.006	-0.002	0.024	0.021	1
	Sig. (2-tailed)	0.497	0.819	0.363	0.768	0.871	0.957	0.531	0.574	
**. Correlation is significant at the 0.01 level (2-tailed).										
*. Correlation is significant at the 0.05 level (2-tailed).										

Table.2 MutMap data obtained

Reference/Consensus	**CChr Nos.**	**SSNP position**	**Amino acid change**	**Sequence description/Gene**	**Biological process**	**Cellular component**	**Molecular function**	Reference
A / G	12	23326821	p.Ile8Thr	R2R3-MYB	R2R3-MYB transcription factor 85	Nucleus	Regulators of various stresses ——	26, 27, 28 Ballichatla et al. 2024, Guha et al. 2024; Magar et al. 2024
T / C	12	23446314	p.Cys127Arg	OsHsp23.7	Heat shock protein 70	Nucleus and cytosol	Molecular chaperone, stabilizes proteins against denaturation and aggregation	
C / G	12	23460051	p.Ala243Gly	OsDof28	Zn finger protein (Fragment)	Nucleus and cytosol	DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly	
T / C	12	23496899	p.Val338Ala	OsSTA286	Glycoside hydrolase	Intracellular and extracellular enzymes	Metabolism, antibacterial defense, and pathogenesis	
T / C	12	23512617	p.Leu11Ser	OsMT1g	Metallothionein-like protein type 1	Golgi membrane	Regulation of metal homeostasis and controlling physiological heavy metal toxicity, DNA damage, and oxidative stress	
GGCCGCCGCCGCCGCCGCCG / GGCCGCCGCCGCCGCCG	12	23555201	p.Gly332del	OsMYB91	R2R3-type MYB transcription factor	Nucleus	Adaptive growth, Salt tolerance	
T / C	12	23690886	p.Asp70Gly	OsATL10	Amino acid transporter	Organelle membrane	Transmembrane domain containing protein playing roles in nutrient uptake, cell redox balance, cell signaling and regulation of cell volume	
GCTCCCACTCCC / GCTCCC	12	23729289	p.Gly14_Ser15del	Os12g0574800	Bromodomain transcription factor domain containing protein	Nucleus	Regulation of gene transcription	

C / T	12	23805755	p.Gly510Ser	OsPAP1d	Metallophosphoesterase domain containing protein	Cell wall localization under phosphate starvation	Acts as an endoplasmic reticulum-exit signal, regulates GPI-anchored proteins transport in the early secretory pathway	
C / T	12	23813016	p.Glu198Lys	OsPAP1c	Similar to Diphosphonucleotide phosphatase 1 precursor	Nucleus	Regulate reproductive growth of rice	
T / G	12	24037803	p.Cys5Gly	OsPUT2	Polyamine uptake transporter	Plasma membrane	Regulation of intracellular PA levels, which are essential for cell growth	
C / T	12	24066542	p.Ala52Val	OsPP2C78	Protein phosphatase 2C-like protein	Membranes, cytoplasm and nucleus	Stress signalling	
TGGCGG / TGGCGGCGGAGGCGGCGG	12	24114702	p.Thr5_Ala6insAlaAlaSerAla	OsSCP66	Similar to Serine carboxypeptidase family protein	Vacuole	Removes C-terminal amino acids from proteins and peptides, fascilitates stress response, growth, development and pathogen defense	
G / C	12	24203246	p.Val162Leu	OsERF#004	Similar to AP2 domain containing protein, expressed	Nucleus	Transcription factor for flower and seed development	
GGGCGGCGGCGGC / GGGCGGCGGCGGCGGCGGCGGC	12	24237912	p.Lys115_Pro116insProProPro	RZF71	TFIIIA-type zinc finger protein	Nuclear transcription factors	Tolerance to salt and drought stresses	
G / A	12	24360384	p.Arg47Trp	OsFbox670	Cyclin-like F-box domain containing protein	Component of the ubiquitin-26S proteasome pathway	Growth and development	
C / T	12	24486824	p.Arg145Cys	osACA2	Plasma membrane Type IIB Ca2+-ATPase	Plasma membrane and endomembranes	Maintains homeostasis by controlling Ca2+ efflux from the cytosol to organelles and/or to the apoplast	
T / A	12	24621269	p.Met160Leu	CycD5	Cyclin D domain containing protein	Nucleus	Regulate the cell cycle G1/S transition therby involves in cell cycle progression	

C / T	12	24638439	p.Met160Leu	OsAPT1	Similar to Adenine phosphoribosyltransferase	Cytoplasm	Catalyzes cytokinin conversion from nucleobases to nucleotides	
T / G	12	24646582	p.Ser31Ala	hsa1(t)	FLOWERING LOCUS T (FT)-Like homolog	Phloem companion cell of the leaf ▶ sieve tube system ▶ shoot apical meristem (SAM)	FT-Like1 homologous to Arabidopsis Flowering Locus T gene, Promotion of flowering	
G / C	12	24916666	p.Gly520Ala	OsWD40-198	WD40 repeat-like domain containing protein	Cytoplasm and nucleus	Function as molecular “hubs” mediating supramolecular interactions	
A / G	12	25076704	p.Ile69Val	OsM12	MATH domain containing protein, expressed	Proteasome, cytosol, nucleus	Ubiquitin ligase, protein degradation	
G / T	12	25298269	p.Lys68Asn	Os_F0685	Leucine Rich Repeat family protein, expressed	Transmembrane repeat domain containing protein; contains pathogen specific determinants	Pathogen recognition, contains nucleotide binding site and leucine rich repeat, disease resistance	
A / G	12	25335868	p.Met177Val	OsbZIP88	BZIP transcription factor family protein, expressed	Cytoplasm and nucleus	Crucial role in plant growth, development and mediate biotic and abiotic stresses	
TCGCCGCCGCCGCCGCCGC / TCGCCGCCGCCGCCGCCGCCGC	12	25446171	p.Ser13_Gly14insGly	OsCIPK04	CBL-interacting protein kinase 4	Cytoplasm and nucleus	Mediating responses to various extracellular cues, including biotic and abiotic stresses	
A / T	12	25577652	p.Arg158Trp	OsL55	3-methylcrotonyl CoA carboxylase biotin-containing subunit (Fragment)	Mitochondria	Prevents the accumulation of toxic intermediaries, facilitates the generation of branched chain fatty acids	
G / A	12	25759099	p.Ala301Val	OsFbox676	F-box domain containing protein, expressed	Component of the ubiquitin-26S proteasome pathway	Growth and development	
G / C	12	25823002	p.Asp52Glu	OsRLCK373	Serine/threonine-protein kinase receptor	Associated with the plasma membrane and the cytoplasmic and nuclear fractions	Regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation	
AAA / AAATAAATAA	12	25838674	p.Phe516fs	OspPLAIIIzeta	Patatin-like phospholipase family protein,	Parenchyma	Acyl hydrolysis	

					expressed			
G / C	12	25963974	p.Ala56Gly	OsCAT10	Amino acid permease family protein	Plasma membrane of mesophyll cells	Maintain uptake, transport, and distribution of amino acids	
A / G	12	25975041	p.Val195Ala	OsSET43	Histone-lysine N-methyltransferase, H3 lysine-4 specific SET1	Nucleus	Methyl transferase, histone methylation at H3-H4	
G / A	12	25980171	p.Ala142Val	SIP17	Superoxide dismutase, copper/zinc binding; NPH3	Mitochondria, chloroplast, glyoxysome, peroxisome, cytosol	Act as an antioxidant enzyme, lowering the steady-state concentration of superoxide	
G / A	12	25990416	p.Arg81Gln	BIP132	SRP40, C-terminal domain containing protein	Nucleolus	Targets signal peptide-bearing proteins to the eukaryotic endoplasmic reticulum membrane for secretion or membrane insertion	
T / C	12	26003893	p.Thr644Ala	OsARF6b	Auxin response factor 25, Target gene of miR167d	Cytoplasm and nucleus	Regulation of flower opening and stigma size	
T / C	12	26073373	p.Val17Ala	OsRLCK374	Serine/threonine protein kinase domain containing protein	Associated with the plasma membrane and the cytoplasmic and nuclear fractions	Regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation	
G / A	12	26088566	p.Val279Met	OsWAK128	Protein kinase domain containing protein, expressed	Associated with the plasma membrane and the cytoplasmic and nuclear fractions	Regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation, involved in sheath blight tolerance	
G / A	12	26094708	p.Gly37Ser	OsWAK129	Protein kinase domain containing protein, expressed	Associated with the plasma membrane and the cytoplasmic and nuclear fractions	Regulation of cell proliferation, programmed cell death (apoptosis), cell differentiation, involved in sheath blight tolerance	
A / G	12	26956819	p.Ser9Pro	TOND1	Thaumatin protein	Plasma membrane and endomembranes	Provides tolerance to nitrogen deficiency	
C / T	12	27054621	p.Val330Il	STS90	Phytoene dehydrogenase-like	Chloroplast	Involved in the production of carotenoids	

			e					
A / C	12	27110217	p.Lys364Thr	HAIKU2	Receptor-like protein kinase 7	Associated with the plasma membrane and the cytoplasmic and nuclear fractions	Negative regulation of resistance to planthoppers, Regulation of rice growth and development	
C / T	12	27146486	p.Glu353Lys	OsPR1#121	Pathogenesis-related protein PR-1 precursor	Cell walls and intracellular spaces	Release of salicylic acid as defense response to fungal pathogens and disease resistance	
G / T	12	27197752	p.Ala33Glu	OsbZIP89	bZIP transcription factor	Cytoplasm and nucleus	bZIP-1 domain containing protein, maintain crucial role in plant growth, development and mediate biotic and abiotic stresses	

Figure.1 Figure representing experimental outcome a. Pearson correlation between traits b. Population distribution bar graphs for sheath blight score c. bar graphs for culm diameter range d. plant images BPT-5204 and TI-117 e. the F_2 population images, correlation analysis, and phenotyping range

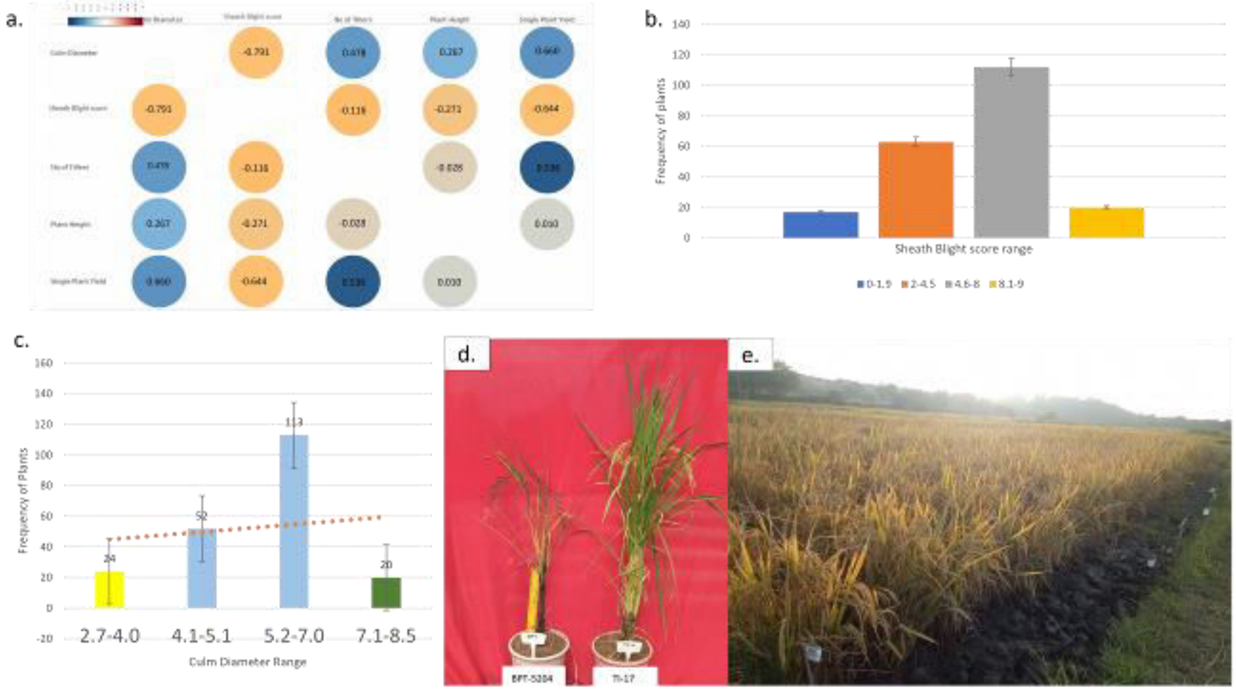


Figure.2 Culm diameter -frequency table and bar graph X-axis: Frequency of plants; Y-axis: Culm diameter range

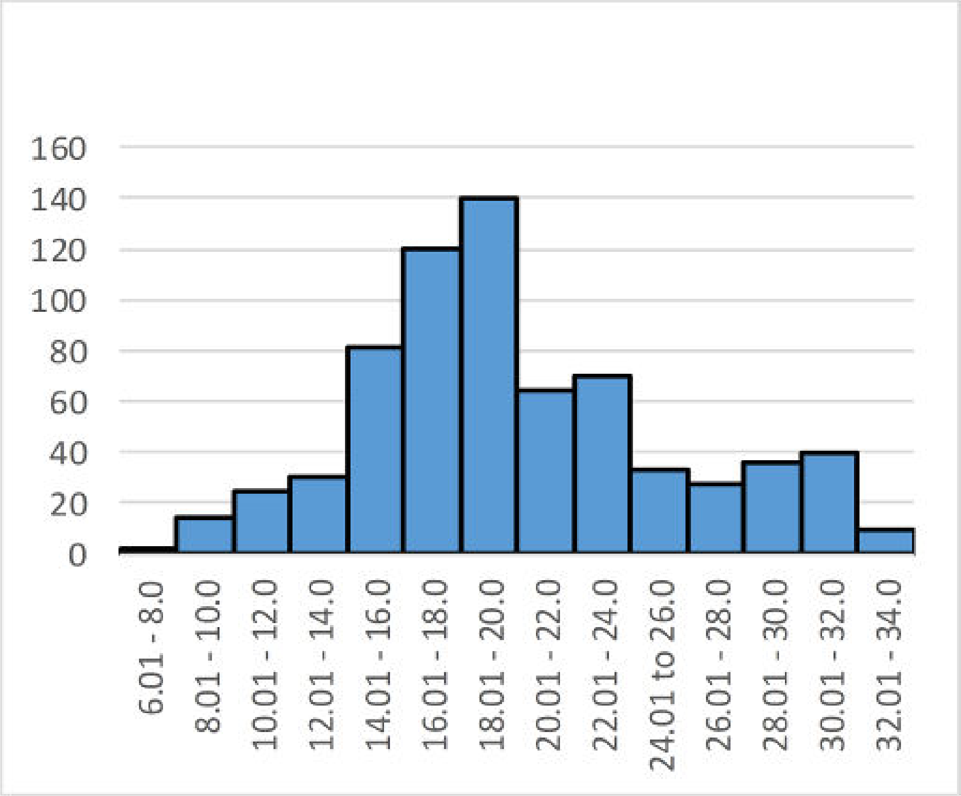


Figure.3 Pushing resistance -frequency of pushing resistances

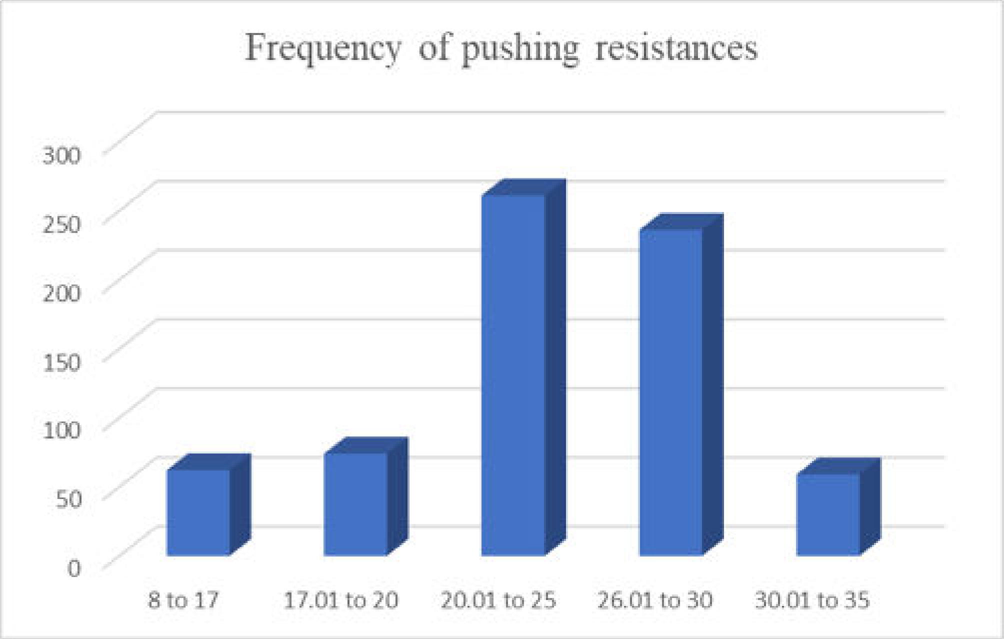
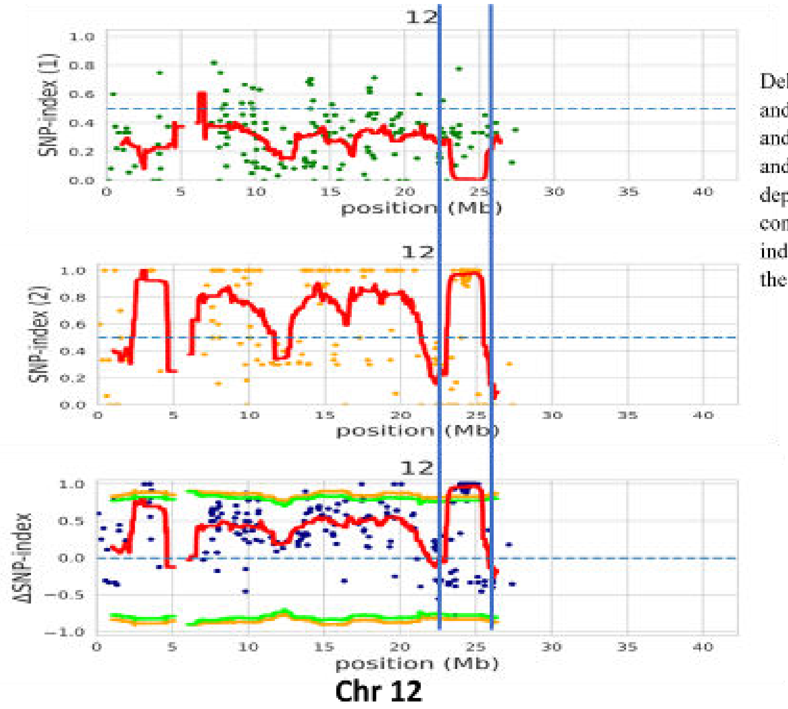


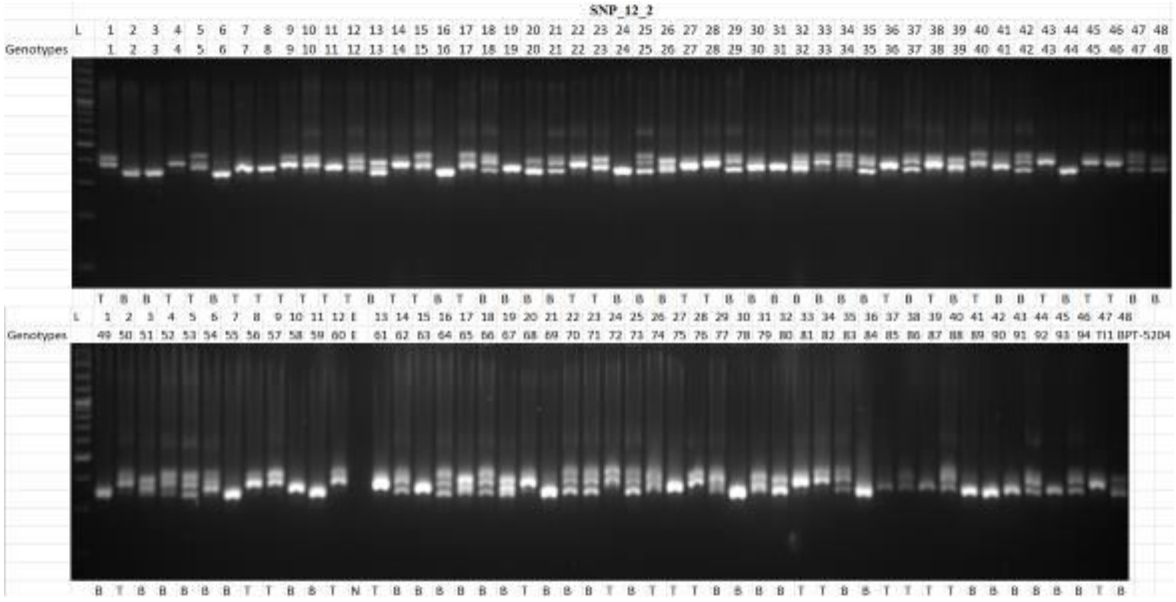
Figure.4 The causal region for genetic variation in Chr. 12 23.2 to 25.71 MB



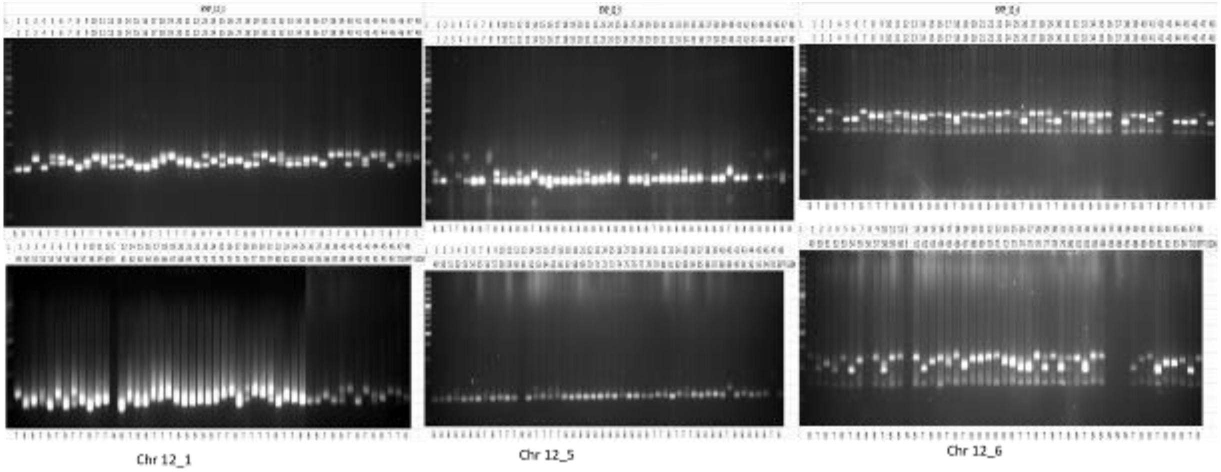
Delta SNP index between the high strong culm bulk and low strong culm bulk for causal SNPs. The X and Y axes depict the chromosomal position (Mb) and delta SNP index respectively. The red line depicts mean SNP-index, orange line depicts 99% confidence interval of the simulated delta SNP-index, green lie depicts 95% confidence interval of the simulated delta SNP-index.

Chr 12 Mut Map region 23.2 to 25.71 MB

Figure.5 Indel genotyping marker for Chr. 12_2 and Chr. 12_1, Chr. 12_5, Chr. 12_6



B=BPT-5204, T=TI17, N=No amplification, L= Ladder



Consequently, the 4 polymorphic Indel genotyping markers were used to demonstrate recombination frequencies at the loci. The F_3 population demonstrated majorly weak culm-sheath blight susceptibility as was the genotyping results for the Indel genotyping markers tested on the F3 population. The designed primers (Table S2) and the recombination frequency for each allele is presented and tabulated (Table S3).

The recombination frequency for the mutant loci for Chr_12_2 (SNP_12_2) was 10.2% and is the best Indel polymorphic marker that has the prerequisite recombination frequency for the target strong culm trait. According to MutMap Obtained genes, CBL-interacting protein kinase 4 (OsCIPK04) has a role in Strong Culm and Sheath Blight resistance via modulating ROS homeostasis. Mutation in the protein should have led to changes in culm diameter and Sheath Blight resistance in mutant (Dos Santos *et al.,* 2023). Serine/threonine-protein kinase receptor is involved in cell programmed death. Pathogenesis-related protein PR-1 precursor (OsPR1#121) is a defence related gene. It provides strong culm and Sheath Blight as per reports and also reported in other transgenic experiments (Kushwaha *et al.,* 2016). Leucine Rich Repeat family protein (Os_F0685) act as the first line of defence against pathogen attack recognition and forms part of innate immunity of plants. R2R3-MYB transcription factors are involved in Sheath Blight resistance by modulation of lignin biosynthetic pathway as per reports (Molla *et al.,* 2010). In our case the R2R3-MYB85, OsMYB91 are also involved in providing Strong Culm and Sheath Blight resistance to TI-17. WD40 repeat-like domain containing proteins like OsWD40-198 are involved in providing plant biotic and abiotic stress resistance (Rose and Raymond 2020). bZIP transcription factor (OsbZIP89) involved in maintenance of plant growth and development may also play role in pathogen defence responses including Sheath Blight. MATH domain containing proteins are for long known to be involved in biotic stress responses (Brar *et al.,* 2021). In our study the OsM12 is also found responsible for providing Strong Culm and Sheath Blight resistance to mutants. Serine/threonine protein kinases (OsRLCK374, WAK128, WAK129) are involved in culm diameter maintenance and pathogen-defence signalling mechanisms and are also involved in SB resistance (Mahender *et al.,* 2015). Thus, the interesting physiology of grain -filling may also be vary also with the stronger fit plants or the plants having more culm strength and culm diameter. However, in support of the concept no analysis of trace mineral elements in rice seeds were performed in the present experiment in its backing.

In conclusion, the rice plant has internal mechanisms to safeguard valuable mineral concentrations in the rice grain iron, zinc, and manganese. The need of the present time is for strong culm- biofortified rice with infusion of vitamins. The strong culm nature also aids in inculcation of sheath blight resistance. Finally in the presence of Next generation sequencing technologies and advanced breeding endeavours it is necessary to improve efficient grain filling, and micronutrient filling in rice plants. The strong culm trait would also be essential in ensuring food security even in growing climatic conditions. The presence and uptake ability of valuable minerals in the soil controls the seed vigour of rice grains. Also, the grain-filling stages are very important in determining the yield hence the grain-filling stages should be made more efficient by adding various mechanisms for increasing the yield.

Authors Contribution

Pritam Kanti Guha: Formal analysis, Methodology, Writing – original draft, Writing – review & editing, idea conceptualization. Gopalakrishna Kadambari: Writing – original draft, Writing – review. Akila Chandra Sekhar: Methodology. Durbaka Vijaya Raghava Prasad – Writing – review & editing. Maganti Sheshu Madhav – Writing – review & editing. Degati Vijaya Lakshmi – Supervision, Investigation, Conceptualization, Writing – original draft, Writing – review & editing.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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