Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2022.018996

ARTICLE

Constitutive Activation *OsbZIP62* Improves Plant Height and Yield through Regulating the Expression of Agronomic Traits Related Genes in Rice

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Received: 16 September 2021 Accepted: 17 December 2021

ABSTRACT

Plant height is an important morphological trait that affects crop yield. Several genes related to plant height and yield have been reported in rice (*Oryza sativa* L.), however, the molecular mechanism underlying the regulation of these traits is still not completely understood. VP64 is widely used as a transcriptional activator to investigate the biological function of genes encoding transcription factors. Here, we identified a novel bZIP transcription factor *OsbZIP62* that is involved in modulating agronomic traits in rice. Overexpression of *OsbZIP62-VP64* (*OsbZIP62V*) significantly increases the plant height and yield per plant in rice. RNA-seq analysis showed that some plant height and panicle development related genes (i.e., *OsEATB*, *OsDSS1* and *OsGA3ox2*) were up-regulated in *OsbZIP62V* overexpressing rice plants. Besides, *OsbZIP62* could also bind to the promoters of several putative target genes. These results suggested that *OsbZIP62* plays a role as transcriptional regulator in regulating the expression of genes associated with agronomic traits, and *OsbZIP62* fused with VP64 would be useful in crop genetic modification with improved plant architecture and yield.

KEYWORDS

Rice; *OsbZIP62*; panicle length; plant height; transcription factor; yield

1 Introduction

Rice is not only an important food crop that provides security for more than 50% of the world's population, but also the crop with the largest water demand, accounting for about 70% of the total agricultural water consumption [1,2]. In recent years, climate disasters are increasingly frequent, which have a serious impact on crop growth and production, seriously restricting the long-term goal of high and stable yield of crop breeding [3–5]. In order to meet the growing food demand, it is very important to study genetics and molecular mechanism of rice yield to develop new rice varieties with high and stable yields, and ensure the safety of food production [6–8].

The rice yield is mainly determined by three key factors, which are effective panicle number per plant, grain number per panicle and 1000-grain weight. It is necessary to elucidate the molecular mechanisms underlying these traits for developing high yield rice varieties [9–11]. At present, many genes have been reported to be involved in the regulation of rice agronomic traits. For example, the GRAS family protein single tiller gene *MOC1*, plays an important role in the regulation of rice tiller development [12,13]. The number of tillers and panicles of *MOC1* mutants decreased, which regulated the development of tillers and panicles, and then affected the yield of rice [14]. Early heading date 1 (*Ehd1*) promotes early heading under short sunlight by inducing the expression of FT-like gene, which reduces the number of primary branches, leads to the reduction of grain number per ear, and then affects the field yield of crops [15]. *HD1*, a gene affecting heading, can promote the expression of *Ghd7* under long sunshine, inhibit Ehd1-Hd3a/RFT1 flowering pathway, thus affecting rice yield [16]. Ideal plant architect 1 (*IPA1*) can disrupt the regulation of *OsmiR156* on *IPA1*, resulting in the reduction of tillers, the increase of grain number per panicle and 1000 grain weight, the stout stem, so as to improve the yield [17].

Plant height is another important agronomic trait of rice that directly affects the yield of this crop. It is also a strategy to increase plant height to increase yield. Genes related to plant height were also reported, such as *OsGA3ox2* and *OsEATB*. *OsGA20ox2* is a gene related to GA biosynthesis. Mutation of this site can lead to different degrees of dwarfing in Rice [18]. *OsGA3ox2*, a gibberellin (GA) biosynthesis related gene, is significantly induced by BR. Its mutation can lead to a decrease in GA accumulation, thus inhibiting cell elongation, resulting in plant growth reduction and dwarfing [19,20]. In the process of internode elongation, *OsEATB* inhibits ethylene inducing gibberellin response by down regulating the expression of *ent-kaurene synthase A*, a gene related to gibberellin biosynthesis.

Transcription factors (TFs) play important role in plant development and stress responses [21,22]. Among different types of transcription factors, basic leucine zipper motif (bZIP) transcription factor has been widely studied [23–25]. There are about 89 bZIP transcription factors in rice [25,26]. Studies have shown that several members of the bZIP transcription factors, such as *OsbZIP73*, *OsbZIP75*, *OsbZIP77* and *OsRE1*, are involved in the regulation of rice agronomic traits [24,27–29]. Overexpression of *OsbZIP75* delays the heading date of rice [28,30]. *OsbZIP77* was involved in the regulation of rice heading date through interacting with SAPKs [29,31]. *OsRE1* interacts with *OsRIP1* to modulate the heading stage of rice by fine regulating the expression of *Ehd1* [27].

VP64 is a protein composed of four repeats of an eleven amino acid peptide from the C-terminal of the viral protein 16 (VP16), which exhibits strong activation activity [32]. The transcription activation activities of TFs fused with VP64 can be significantly increased. Therefore, VP64 has been used as a transcriptional activator to investigate the biological function of TFs in rice [33].

Although the functions of several bZIP genes have already been characterized to be involved in modulating agronomic traits in rice, a number of other bZIP genes have not been identified. Previously, we identified a novel bZIP transcription factor, *OsbZIP62*, which was participated in rice drought tolerance [34]. In this study, we reported that constitutive activation of *OsbZIP62* through fused with transactivation domain VP64 significantly improved yield per plant through increasing plant height, panicle length and grain number per panicle in rice. These results showed that *OsbZIP62* is a novel regulator in rice agronomic traits and has potential application value in developing novel rice varieties with improved yield.

2 Materials and Methods

2.1 Transgenic Materials

The full-length cDNA of *OsbZIP62* was the seeds of transgenic japonica rice Kitaake with *OsbZIP62V* were acquired from Fujian Agriculture and Forestry University, and these transgenic rice plants were generated as described previously [33].

2.2 Plant Materials and Growth Conditions

The rice plants (*Oryza sativa* L. *japonica*. cv. Nipponbare) used in this study, including the wild-type plants Kitaake and the relevant transgenic plants were in the experimental fields at the Shanghai Agrobiological Gene Center in Shanghai during the natural growing seasons.

2.3 Phenotype Determination

Agronomic traits of WT and *OsbZIP62V* transgenic rice plants, including plant height, panicle length, primary branches number, secondary branches number, grain number per panicle and 1000-grain weight, were manually measured after plants were harvested. Dry seeds were evaluated for grain width and length *via* morphology analyzer (http://www.jsjeda.com). Water content of seeds determined by AOAC official method 930.15. Ash content was measured by AOAC Official Method 942.05. Protein content was measured using the Kjeldahl method (984.13). Statistics analysis of the phenotypic data was performed using SPSS version 19.0 (IBM).

2.4 RNA Isolation and Gene Expression Pattern Analysis

Total RNA was extracted from the rice leaves using TRNzol-A$^+$ reagent (Tiangen) according to the manufacturer's instructions. First-strand cDNA was synthesized via EasyScript One-Step gDNA Removal and cDNA Synthesis Super Mix (Transgen). Real-time quantitative PCR (qPCR) was performed on an optical 96-well plate with a Bio-Rad CFX96 Real-Time PCR Detection System using TransStart Green qPCR SuperMix (Transgen). The PCR procedure was as follows: 94°C for 30 s, followed by 40 cycles at 94°C for 5 s, 55°C for 15 s and 72°C or 10 s. The rice *OsAct8* (No. AY212324) was used as an internal control to normalize the target gene expression, and relative expression levels were determined as described previously [35].

2.5 RNA Sequencing Analysis

The RNA sequencing data of *OsbZIP62V* transgenic rice plants and wild type plants were obtained previously [34]. We counted the number of differentially expressed genes (DEGs) at different levels of each Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [36], and then determined the metabolic pathways and signaling pathways in which the DEGs were mainly involved.

2.6 Promoters Sequence Analysis of DEGs

Ten DEGs that are related with rice growth and agronomic traits were chosen for further study. The ~2 kb promoter sequences of these genes were downloaded from the rice genome database and the *cis*-elements were analyzed through PlantCARE online software.

2.7 Protein Expression and EMSA

OsbZIP62 was ligated to the prokaryotic expression vector pGEX-6P-1, which was expressed by GST fusion label, transformed into *E. coli* DE3, and was induced by 1 mM IPTG for 18 degrees, and SDS-PAGE was used to detect the expression of GST-OsbZIP62. After induction, the bacteria were collected and GST-OsbZIP62 were purified by Profinia protein purification system. The *cis*-element of ABRE and G-box were labeled with biotin. EMSA was performed according to the instructions of LightShift Chemiluminescent EMSA kit (Thermo Fisher Scientific). The membrane was exposed to a ChemiDoc XRS + imaging system (Bio-Rad).

2.8 Yeast One-Hybrid Assays

We selected 10 genes (i.e., *LOC_Os01g08220*, *LOC_Os10g32980*, *LOC_Os03g04680*, *LOC_Os11g12740*, *LOC_Os02g54600*, *LOC_Os09g16510*, *LOC_Os09g28440*, *LOC_Os07g05360*, *LOC_Os01g41710* and *LOC_Os10g32600*) that were differentially expressed in *OsbZIP62V* overexpression

transgenic plants. The upstream approximately 0.3 kb long promoters of these potential target genes were cloned from genome DNA of a japonica rice (Nipponbare). In yeast one-hybrid experiment, the promoter sequences of these target genes were digested by SmaI and EcoRI and cloned into yeast expression vector pHIS2.1, and then co-transformed into Y187 yeast cells with the pGAD-T7-OsbZIP62. The interaction between DNA and protein was evaluated by transformation growth analysis on SD/−Leu/−Trp/−His plates, which provided 50 mM of 3-AT.

3 Results

3.1 Constitutive Expression of OsbZIP62 Enhanced Plant Height of Rice

In the previous studies, we found that *OsbZIP62* fused with VP64 (*OsbZIP62V*) could enhance rice drought tolerance at the seedling stage [34]. To investigate whether *OsbZIP62* affects the growth of rice plants, we further charactered and investigated agronomic traits of rice plants overexpressing *OsbZIP62V*. First of all, we checked the expression level of *OsbZIP62V* in two independent transgenic lines (OE1, OE2). We found that the expression level of *OsbZIP62* in transgenic plants was significantly higher than that of wild type plants (Fig. 1B). Secondly, we measured agronomic traits (i.e., plant height, panicles length and the number of primary/secondary branches) of *OsbZIP62V*-overexpression rice plants. It was found that *OsbZIP62V* transgenic plants had slightly fewer tillers, whereas the plant height of *OsbZIP62V* transgenic plants is noticeably higher than that of the wild type plants, which increased by 16%–27% (Figs. 1C and 2A; Table 1). *OsbZIP62V* transgenic plants have longer panicles which increased by 25%–31% (Figs. 1D, 1E and 2C; Table 1). Both the numbers of primary and secondary branches of *OsbZIP62V* transgenic plants increased by more than 50% (Figs. 1F, 2E and 2F; Table 1). The grain numbers per panicle of *OsbZIP62V* transgenic plants were also significantly higher compared with WT plants, which increased by 38%–52% (Fig. 2D; Table 1). The yield per plant of *OsbZIP62V* transgenic plants increased by 23–24% (Fig. 2G; Table 1). Finally, we also checked whether *OsbZIP62* mutation affected rice phenotype under normal condition. The result showed that there was no significant difference between *OsbZIP62* mutant plants and wild type plants (Fig. S1). These data indicated that the constitutive expression of *OsbZIP62V* markedly improved the plant height and grain yield of transgenic rice plants.

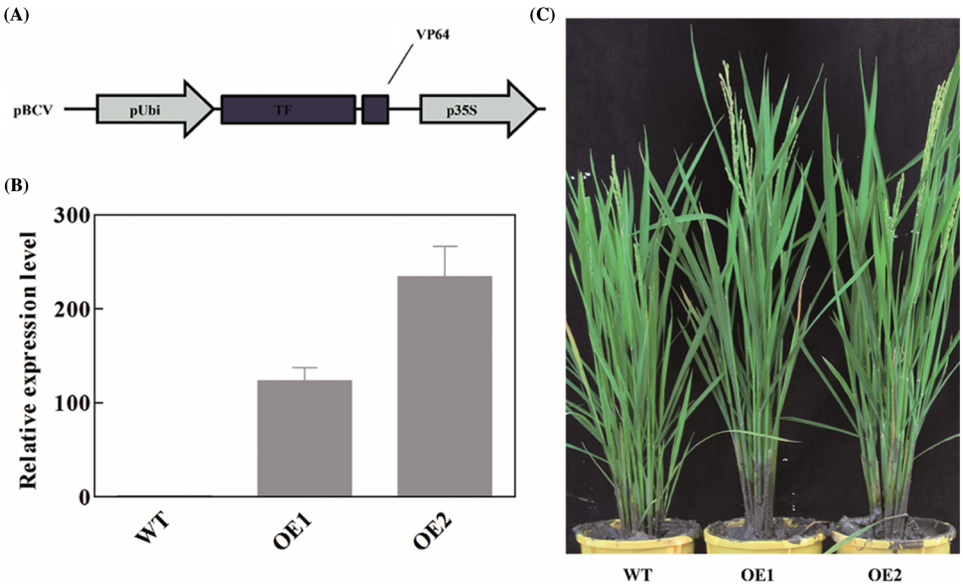


Figure 1: (Continued)

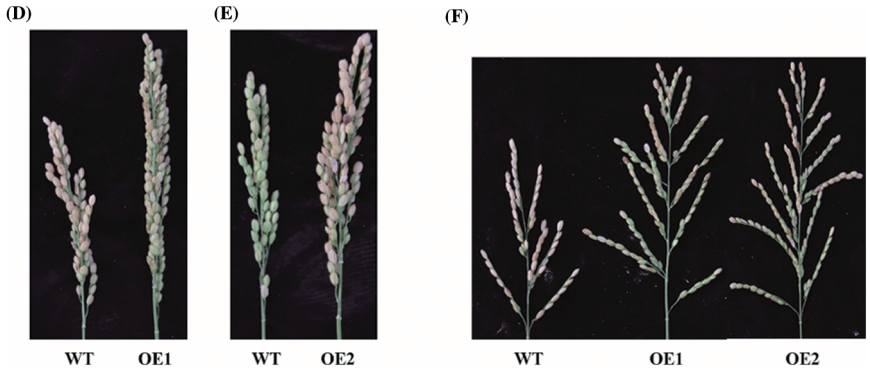


Figure 1: Phenotypic characterization of *OsbZIP62V* transgenic plants. (A) Structure diagram of *OsbZIP62V*. (B) Expression levels of *OsbZIP62V* in Kitaake (WT) and two independent *OsbZIP62V* transgenic plants. Data represents means ± SE (n = 3). (C) Booting phenotype of *OsbZIP62V*. (D, E) Panicle morphology of Kitaake (WT) and two independent *OsbZIP62V* transgenic plants. (F) Primary/ secondary branches per panicle

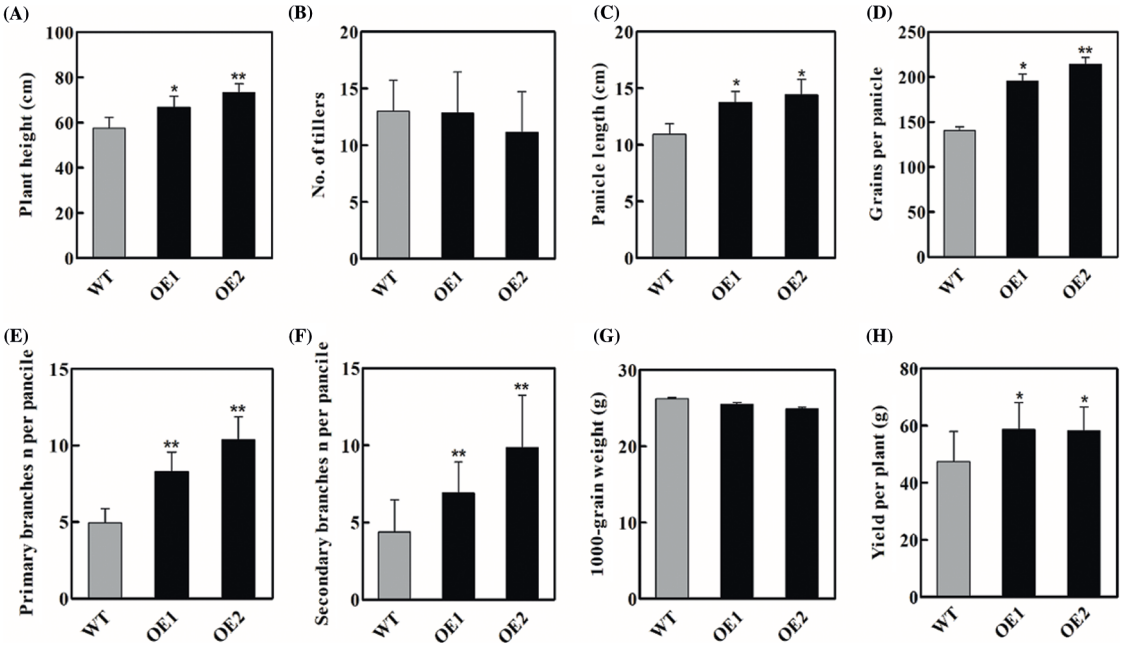


Figure 2: Agronomic traits of WT and *OsbZIP62V* transgenic plants. (A) Plant height. (B) Number of tillers. (C) Panicle length. (D) Grain number per panicle. (E, F) The number of primary and secondary branches. (G) 1000-grain weight. (H) Yield per plant. Data represents means ± SE (n = 8~10), **P* < 0.05, ***P* < 0.01, Student's *t*-test

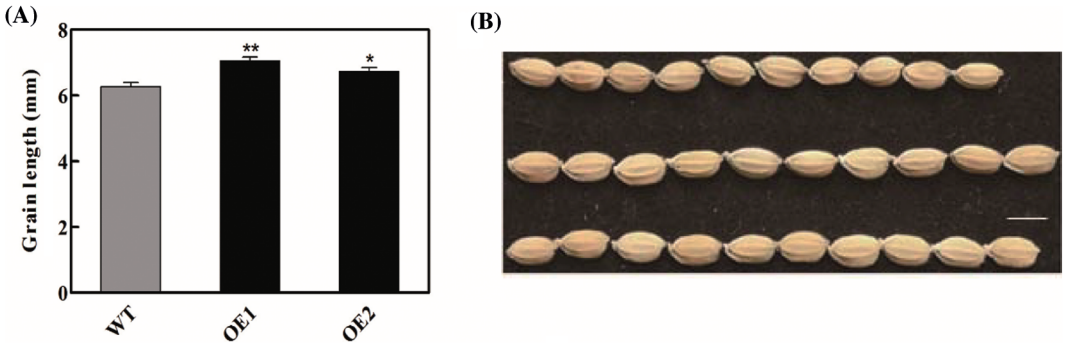
Table 1: Agronomic traits of WT and *OsbZIP62V* transgenic plants

Trait	Kitaake (WT)	*OsbZIP62V* (OE1)	*OsbZIP62V* (OE2)
Plant height, cm	57.52 ± 4.88	66.86 ± 4.97*	73.36 ± 3.94**
No. of tillers	13.01 ± 2.72	12.83 ± 3.62	11.13 ± 3.60
Panicle length, cm	10.94 ± 0.92	13.73 ± 1.01*	14.42 ± 1.33*
Primary branches n per pancile	4.95 ± 0.93	8.31 ± 1.26**	10.36 ± 1.51**
Secondary branches n per pancile	4.41 ± 2.06	6.93 ± 1.99**	9.86 ± 3.41**
Grains per panicle	140.54 ± 4.07	195.00 ± 8.43*	214.18 ± 7.24**
1000-grain weight, g	26.27 ± 0.13	25.52 ± 0.23	24.91 ± 0.19
Yield per plant, g	47.5 ± 10.44	58.71 ± 9.34*	58.2 ± 8.27*

Notes: Agronomic traits are based on Kitaake and two independent *OsbZIP62V* transgenic plants under natural growing seasons. Agronomic traits are presented as mean ± standard deviation (n = 8~10). *$P < 0.05$, **$P < 0.01$ according to Student's *t*-test.

3.2 OsbZIP62 Plays an Important Role in Grain Shape

Overexpression of *OsbZIP62V* increased grains number per panicle and yield per plant of transgenic rice plants. It promoted us to investigate whether it also has an effect on grain shape. The result indicated that the grain length of *OsbZIP62V* transgenic plants was higher, which increased by 8%–13% compared with the wild-type plants (Figs. 3A and 3B). The grain width of *OsbZIP62V* transgenic plants increased by 5%–11% compared with the wild-type control (Figs. 3C and 3D). Moreover, we also want to know whether *OsbZIP62V* changes the grain quality of transgenic rice. The results showed that there was no significantly difference between the grain water, ash and protein content of transgenic plants and those of wild-type plants (Table S1). These results indicated that the overexpression of *OsbZIP62V* affected rice grain shape but did not affect the rice grain quality.



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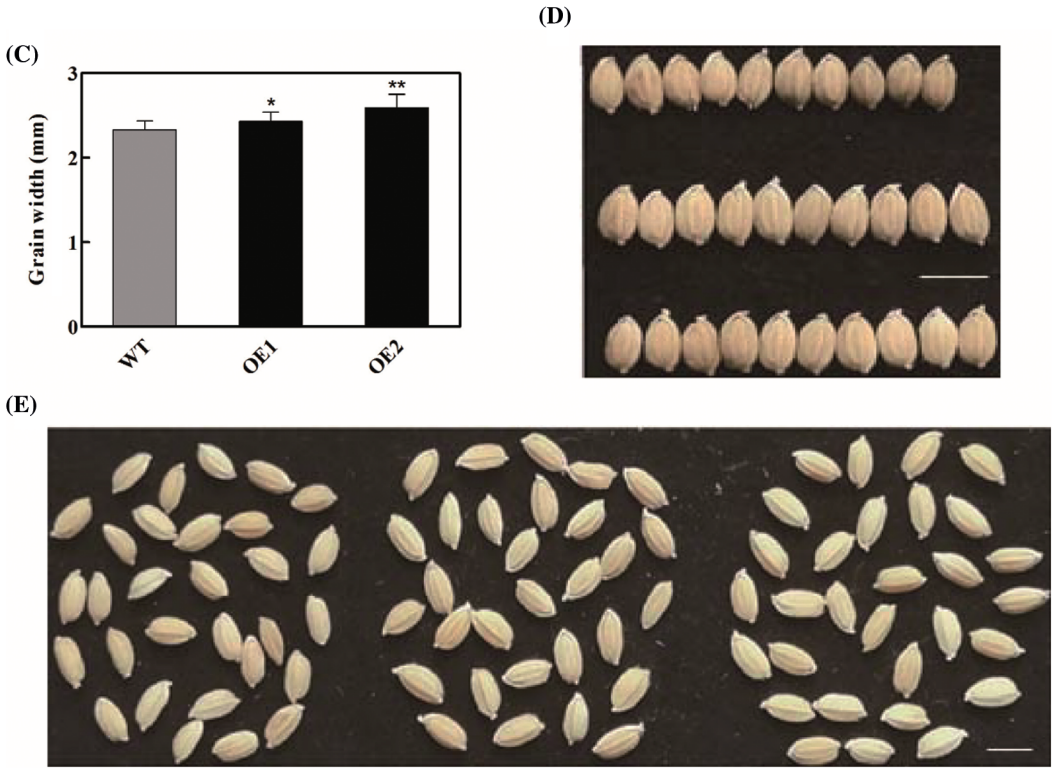

Figure 3: Grain shape was regulated by *OsbZIP62*. (A, B) Grain length of transgenic plants. (C, D) Grain width of transgenic plants. (E) Grains phenotypes of transgenic plants and wild-type, Bar = 5 mm. Data represents means ± SE, n = 10, **P* < 0.05, ***P* < 0.01, Student's *t*-test

3.3 Identification of Putative Target Genes Regulated by OsbZIP62

To elucidate the molecular mechanism of *OsbZIP62* on modulating rice agronomic traits and yield traits, we analyzed transcriptome of *OsbZIP62V* overexpression plants and WT plants through high-throughput sequencing previously [34]. Differentially expressed genes (DEGs) between the transgenic rice and wild plants were analyzed. Many DEGs up-regulated in *OsbZIP62V* transgenic plants were related to agronomic traits including plant height, panicle length, and yield in rice (Fig. 4A). KEGG metabolic pathway enrichment analysis indicated that the DEGs in *OsbZIP62V* are significantly enriched in starch and sugar metabolism, hormone signal transduction, photosynthesis and other metabolic pathways (Fig. 4B).

The expression levels of several DEGs were further checked by qPCR, and the results showed that the expression level of these selected DEGs (i.e., *LOC_Os01g08220*, *LOC_Os10g32980*, *LOC_Os03g04680*, *LOC_Os11g12740*, *LOC_Os02g54600*, *LOC_Os09g16510*, *LOC_Os09g28440*, *LOC_Os07g05360* and *LOC_Os01g41710*) increased in *OsbZIP62V* overexpression transgenic lines (Fig. 5).

We also analyzed the expression of DEGs which encode transcription factors and chlorophyll proteins involved in rice growth and development (i.e., *LOC_Os01g18240*, *LOC_Os09g16510* and *LOC_Os09g28440* encode a transcription factor; *LOC_Os01g41710* and *LOC_Os11g12740* encode chlorophyll synthesis protein and polypeptide transporter, respectively) and the results showed the expression of these DEGs were also increased in *OsbZIP62V* overexpression plants. These results demonstrated that the transcription of these DEGs were affected by *OsbZIP62* and these DEGs might be target genes of *OsbZIP62*.

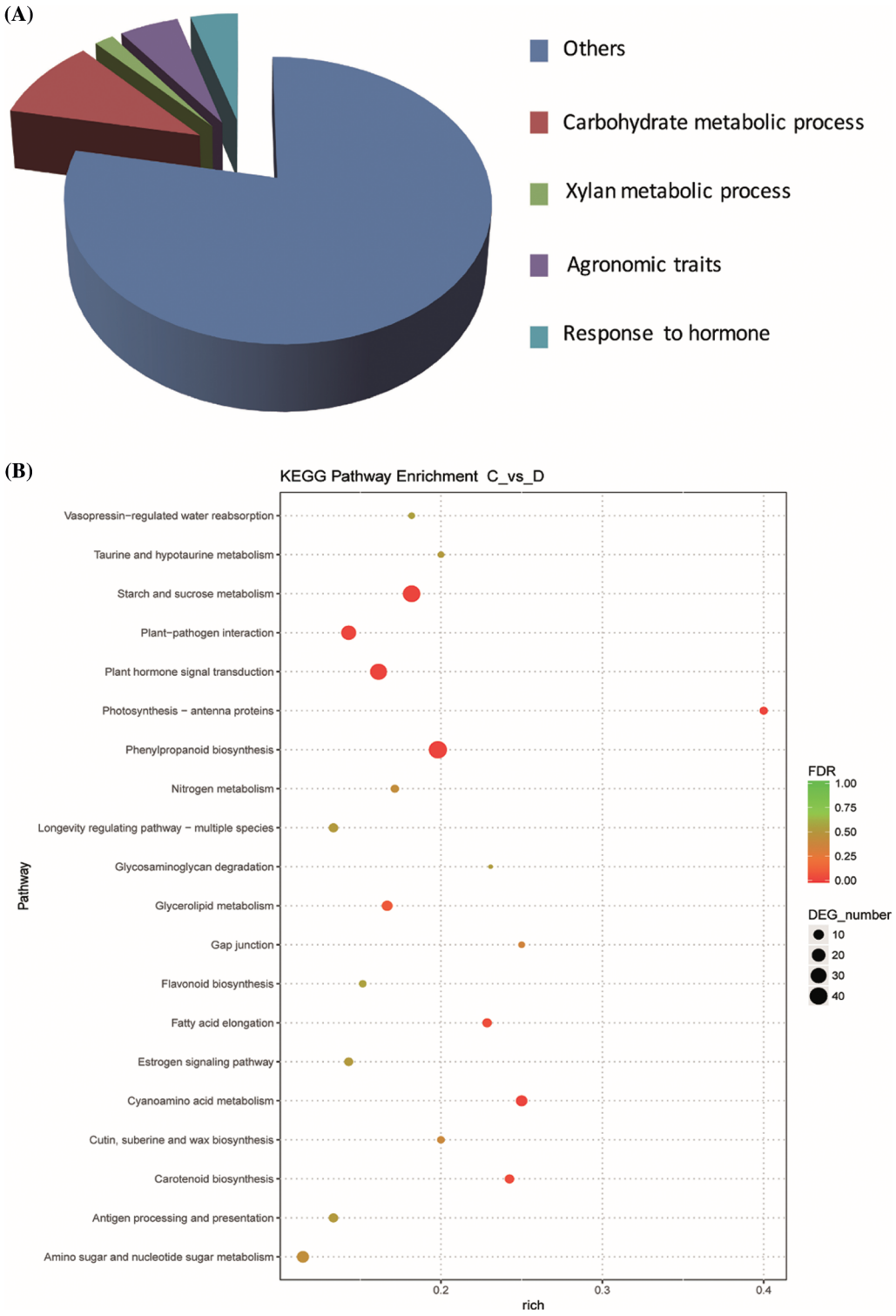

Figure 4: Transcriptome analysis of *OsbZIP62V* plants and WT plants. (A) Classification of upregulated genes in *OsbZIP62V* plants. (B) KEGG Pathway functional enrichment of DEGs in the *OsbZIP62V vs*. WT plants. The x-axis represents the enrichment factor (rich factor). The y-axis shows the pathway names. A larger value of the rich factor indicates a higher enrichment value. The color indicates the *P* value. Point size indicates DEG number and larger dots refer to higher numbers of DEGs

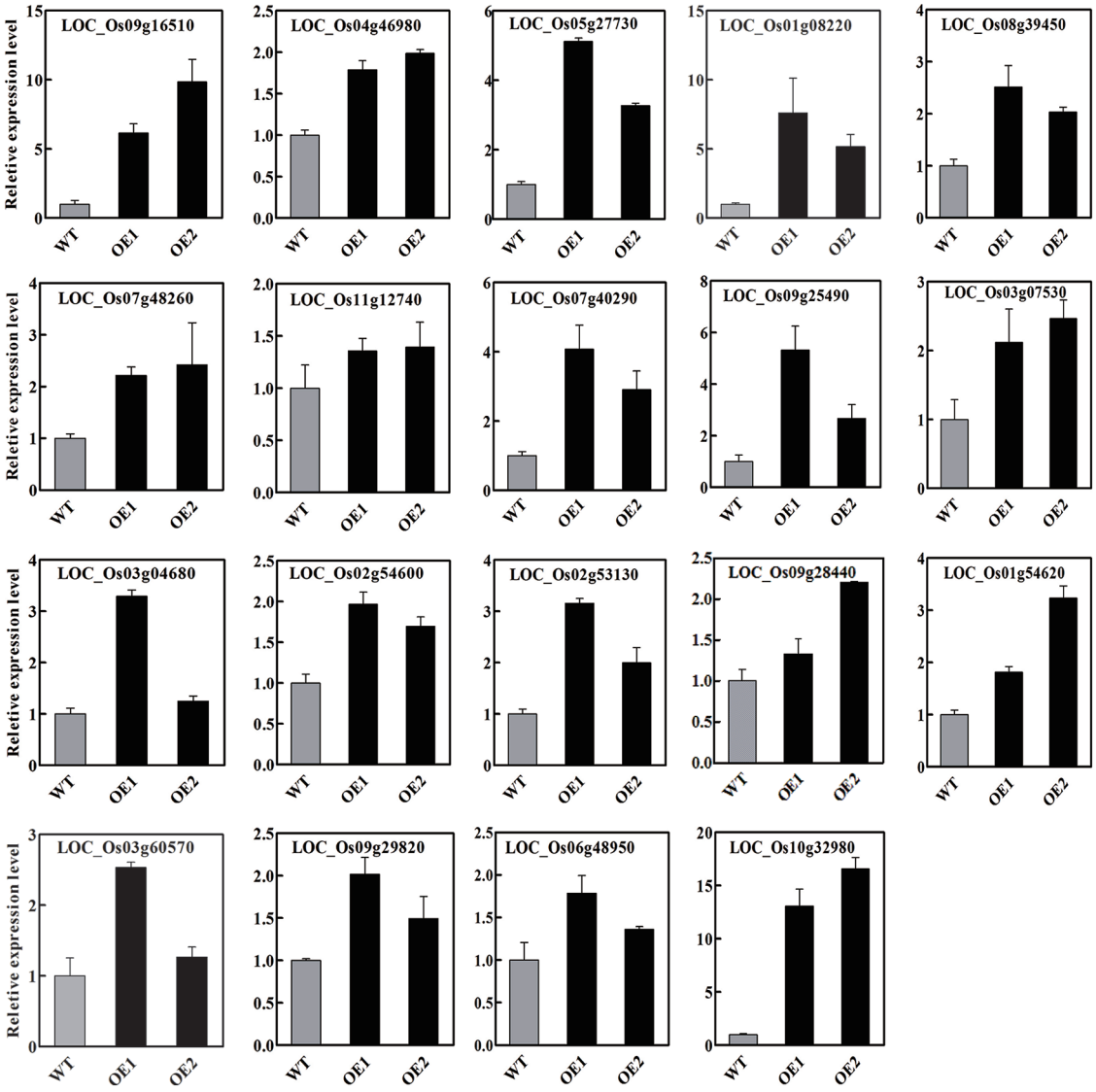

Figure 5: Relative expression level of DEGs involved in agronomic traits in *OsbZIP62V* and WT plants detected through qPCR. Data represents means ± SE (n = 3)

We also investigated the expression of several important genes affect rice agronomic traits such as *Hd1*, *Ehd1*, *Ghd7* and *DTH8* (Fig. S2). The results showed that the expression of these genes in *OsbZIP62V* transgenic rice plants were similar with that of WT plants, except that expression of *Ehd1* in *OsbZIP62V* plants were much lower compared with WT plants.

To clarify the mechanism of *OsbZIP62V* modulating rice agronomic traits, we selected ten DEGs that are related to plant height, rice panicle development and photosynthesis for further study. We analyzed the cis-elements in promoters of these DEGs (i.e., *LOC_Os01g08220* (*OsGA3ox2*), *LOC_Os10g32980* (*OsCesA7*), *LOC_Os03g04680* (*OsDSS1*), *LOC_Os11g12740* (*OsNPF4.1*), *LOC_Os02g54600* (*OsMKK4*), *LOC_Os09g16510* (*OsWRKY74*), *LOC_Os10g32600* (*Ehd1*) and *LOC_Os09g28440* (*OsEATB*)). The results showed that promoters of eight DEGs contain ABREs or G-box *cis*-elements that may be recognized and bond by bZIP transcription factors (Table 2). We firstly investigated whether *OsbZIP62*

bind to ABRE and G-box *cis*-elements through EMSA assay. The results showed that OsbZIP62 protein can bind to the core sequence of ABRE and G-box *in vitro* (Fig. 6A). We further investigated whether the OsbZIP62 protein could bind to the promoters of these putative target genes through a yeast one-hybrid assay. A pGAD-OsbZIP62 plasmid (containing the OsbZIP62 putative DNA domain fused to the GAL4 active domain) and pHIS-cis reporter construct (~0.3 kb promoters of 10 presumed target genes) were co-transformed into yeast strain Y187 (Fig. 6B). As indicated by the activation of the reporter genes, OsbZIP62 could bind to the promoters of several genes (i.e., *LOC_Os01g08220*, *LOC_Os10g32980*, *LOC_Os03g04680*, *LOC_Os11g12740*, *LOC_Os02g54600*, *LOC_Os09g16510*, *LOC_Os07g05360* and *LOC_Os01g41710*), except for two genes (*LOC_Os09g28440* and *LOC_Os10g32600*) (Fig. 6C). These results indicated that OsbZIP62 exhibits DNA binding activity and might directly regulate the expression of these target genes.

Table 2: *Cis*-acting elements (ABRE and G-box) of *OsbZIP62* predicted target genes promoters predicted by PlantCARE software

LOC ID	ABRE	G-box
LOC_Os01g08220	1	1
LOC_Os10g32980	2	2
LOC_Os03g04680	2	2
LOC_Os11g12740	1	1
LOC_Os02g54600	2	1
LOC_Os09g16510	3	2
LOC_Os10g32600	1	1
LOC_Os09g28440	4	4
LOC_Os07g05360	5	1
LOC_Os01g41710	5	4

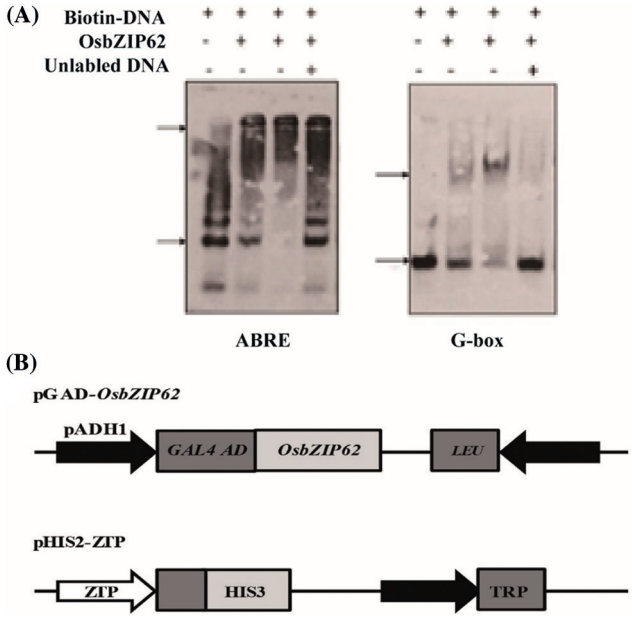

Figure 6: (Continued)

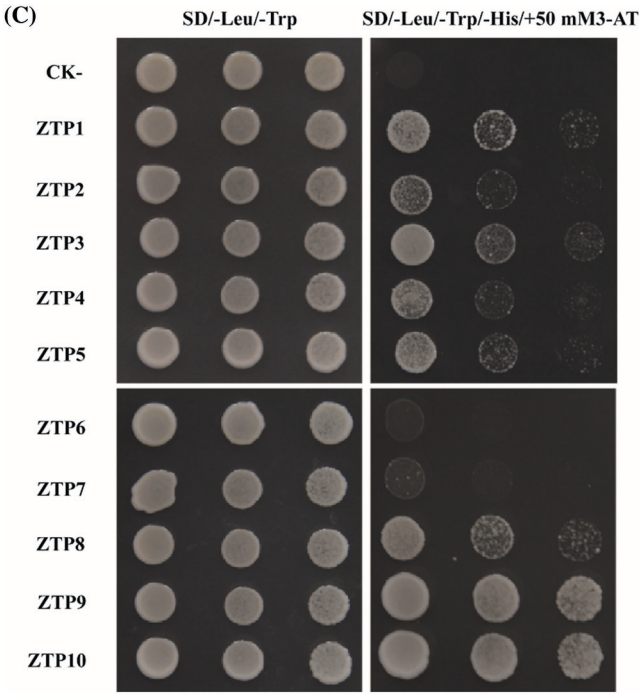

Figure 6: Identification of DNA binding activity of *OsbZIP62*. (A) EMSA assay of *OsbZIP62* and ABRE and G-box. The upper arrow shows the protein-DNA binding complex, while the lower arrow indicates the free DNA. (B) Schematic structure of the yeast one-hybrid expression construct pGAD-*OsbZIP62* and the reporter construct pHIS2.1-ZTP (*OsbZIP62* putative target gene promoter); (C) Growth performance of transformants on SD/−Leu−/Trp/−His medium containing 50 mM 3-AT. ZTP1-ZTP10 indicates the pGAD-*OsbZIP62* plus pHIS2.1-cis (with the promoters of *LOC_Os03g04680*, *LOC_Os07g05360*, *LOC_Os01g08220*, *LOC_Os11g12740*, *LOC_Os09g16510*, *LOC_Os09g28440*, *LOC_Os10g32600*, *LOC_Os10g32980*, *LOC_Os01g41710* and *LOC_Os02g54600* in pHIS2.1, respectively). ck+: positive control, ck–: negative control (pGADT7-rec2-*OsbZIP62* plus p53HIS2)

4 Discussion

Studies have shown that bZIP transcription factor is involved in plant growth and stress response [23,37–40]. In previous studies, we identified the bZIP family transcription factor *OsbZIP62* belonging to the third subfamily of rice bZIP transcription factors [34]. Overexpression of *OsbZIP62V* improved drought tolerance and antioxidant capacity of rice. However, there are few reports on the relationship between bZIP transcription factors and agronomic traits in rice. Here, we found the overexpression of *OsbZIP62V* led to increases in several agronomic traits such as plant height, panicle length, primary branch number, secondary branch number and grain number per panicle, which contributed to enhancing the yield of rice (Figs. 1 and 2; Table 1). In addition, we found that overexpression of *OsbZIP62V* increased the grain length and width, which indicated that the gene had a certain effect on the grain shape (Fig. 3). In contrast, we found that there was no obvious difference between agronomic characters of *OsbZIP62* mutants and that of wild type plants (Fig. S1). Therefore, we speculate that *OsbZIP62* fused with VP64 can improve agronomic traits and yield, and could be used in rice breeding.

Transcription factors and *cis*-elements are the core components of plant gene transcription regulatory networks. Identification of direct downstream target genes is an effective strategy to identify the function of *OsbZIP62* transcription factor. Not surprisingly, *OsbZIP62* altered the expression levels of many genes

[34]. Though *OsbZIP62* did not affect the expression of several important genes that control rice agronomic traits (Fig. S2), several DEGs were found to be related to rice agronomic traits. For example, *Ehd1* expressed lowly in *OsbZIP62V* plants (Fig. S2). *OsbZIP62* was unable to bind to the promoter of *Ehd1* (Fig. 6C), it is possible that the expression of *Ehd1* was indirectly regulated by *OsbZIP62*. In contrast, several DEGs (e.g., *LOC_Os01g08220* (*OsGA3ox2*), *LOC_Os09g28440* (*OsEATB*) and *LOC_Os03g04680* (*OsDSS1*)) related to rice agronomic traits were highly expressed in *OsbZIP62V* plants, and *OsbZIP62* might bind to the promoters of these genes (Fig. 6), suggesting that these DEGs might be targets of *OsbZIP62*.

OsGA3ox2 encodes gibberellin 3β-hydroxylase, and induced expression of *OsGA3ox2* led to an increase of GA levels and plant height [19]. *OsDSS1* is a member of P450 gene cluster and corresponds to the other reported *CYP96B4/SD37* gene. Previous research indicated that dss1 mutants showed dwarfism and reduced grain size, and moderate overexpression *SD37* increased rice plant height [41,42]. *OsbZIP62* may promote the expression of *OsGA3ox2* and *OsDSS1*, lead to an increase the synthesis of gibberellin, and then improve the plant height. *OsEATB* is an ethylene responsive transcription factor, which mainly mediates the interaction between ethylene and gibberellin to mediate internode elongation in rice. Overexpression of *OsEATB* also enhanced the spikelet branching ability [43,44]. In this study, *OsbZIP62V* increased the number of primary and secondary branches might partially attribute to the enhanced expression of *OsEATB*. These findings suggest that the increased expression of agronomic trait-related genes by *OsbZIP62V* contributes to the improvement of plant height and yield of the transgenic rice plants.

In conclusion, we found that *OsbZIP62* could regulate the expression of *OsGA3ox2*, *OsEATB*, and *OsDSS1*, and overexpression of *OsbZIP62V* increased the plant height and grain yield of rice. Combined with the previous studies on the drought resistance function of *OsbZIP62* [34], we speculated that overexpression of *OsbZIP62V* might increase the yield while improving the drought resistance. Our results revealed that fusion of *OsbZIP62* with VP64 would have important application value in rice breeding.

Acknowledgement: We thank Professor Chentao Lin (Fujian Agriculture and Forestry University) for providing *OsbZIP62-VP64* overexpression rice seeds and Professor Yaoguang Liu (South China Agricultural University) for providing the CRISPR/CAS9 system.

Data Availability: The RNA-seq data supporting the results of this article have been submitted to the GEO at NCBI with the Accession No. GSE122887.

Research Involving Human and Animal Rights: The research does not involve human and/or animal experimentation.

Funding Statement: This research was funded by Funding for Basic Research Projects of Taicang (TC2021JC12), School-Level Research Projects of Yancheng Institute of Technology (xjr2021029), Shanghai Agriculture Applied Technology Development Program (2017-02-08-00-08-F00071) and Natural Science Foundation of Shanghai (18ZR1433300).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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Table S1: Grain quality for WT and *OsbZIP62V* transgenic plants

Species	Water content (%)	Ash content (%)	Protein content (%)
WT	11.71	1.79 ± 0.04	9.88 ± 0.19
OE1	10.78	1.66 ± 0.15	9.62 ± 0.45
OE2	12.00	1.73 ± 0.11	9.62 ± 0.30

Note: Grain quality is based on Kitaake and two independent *OsbZIP62V* transgenic plants. Data represents means ± SE (n = 3).

Figure S1: Phenotypic characterization of *OsbZIP62* mutant transgenic plants. NIP: Wild type control; C54 and C56 were two *osbzip62* knockout mutants

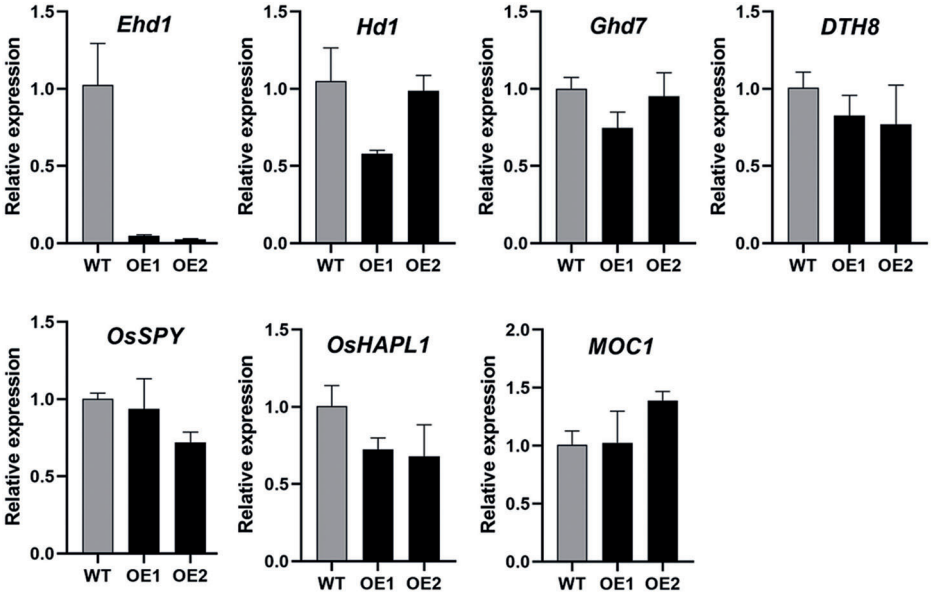

Figure S2: Expression level of important agronomic traits genes in *OsbZIP62V* transgenic plants detected through qPCR. Data represents means ± SE (n = 3). WT: wild-type. OE1 and OE2: *OsbZIP62V* overexpression lines