PAB
Pesquisa Agropecuária Brasileira

Genetics/ Original Article

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Physiological and phenotypical effects of the overexpression of the *OVP1* gene in Japonica rice

Dhiôvanna Corrêia Rocha[1], Thaís Ignez da Cruz[2], João Augusto Vieira de Oliveira[3], Isabela Pavanelli de Souza[4], Beata Dedicova[5], Alexandre Siqueira Guedes Coelho[4], Rosana Pereira Vianello[2] and Claudio Brondani[2] ✉

[1] Instituto Agronômico de Campinas, Rodovia Anhanguera, Km 158, CEP 13490-970 Cordeirópolis, SP, Brazil. E-mail: dhiovannarocha@gmail.com

[2] Embrapa Arroz e Feijão, Rodovia GO-462, Km 12, Fazenda Capivara, Zona Rural, Caixa Postal 179, CEP 75375-000 Santo Antônio de Goiás, GO, Brazil. E-mail: thais_ignez@hotmail.com, rosana.vianello@embrapa.br, claudio.brondani@embrapa.br

[3] Advanced Bacterial Sciences Ltd., Consultancy Team & Molecular Laboratory, Unit 3, Northgate Business Park, White Lund Industrial Estate, Morecambe, LA3 3BJ, Great Britain. E-mail: augusto.joao@outlook.com

[3] Universidade Federal de Goiás, Escola de Agronomia, Avenida Esperança, s/nº, Campus Samambaia, CEP 74690-900 Goiânia, GO, Brazil. E-mail: isabela.pavanelli@gmail.com, asgcoelho@gmail.com

[4] Swedish University of Agricultural Sciences, Department of Plant Breeding, Box 101, Sundsvägen 10, SE 230 53 Alnarp, Sweden. E-mail: beata.dedicova@slu.se

✉ Corresponding author

Received
October 28, 2022

Accepted
April 20, 2023

How to cite
ROCHA, D.C.; CRUZ, T.I. da; OLIVEIRA, J.A.V. de; SOUZA, I.P. de; DEDICOVA, B.; COELHO, A.S.G.; VIANELLO, R.P.; BRONDANI, C. Physiological and phenotypical effects of the overexpression of the *OVP1* gene in Japonica rice. **Pesquisa Agropecuária Brasileira**, v.58, e03167, 2023. DOI: https://doi.org/10.1590/S1678-3921.pab2023.v58.03167.

Abstract – The objective of this work was to evaluate the physiological, phenotypic, and gene expression parameters in genetically modified (GM) rice plants that overexpress the *Oryza sativa Vacuolar H⁺-Pyrophosphatase* 1 (*OVP1*) gene, compared with non-genetically modified (NGM) rice. GM and NGM plants of the BRSMG Curinga cultivar were evaluated in two experiments, in a laboratory and greenhouse, in a randomized complete block design, with four replicates. Agronomic traits of interest were estimated, and transcriptome analysis and gene expression quantification were carried out. GM plants showed a 31 and 21% higher number of spikelets per panicle and total number of grains per panicle, respectively, in comparison with NGM plants. Physiological changes occurred during the grain-filling stage, in which GM plants presented a photosynthetic rate and carboxylation efficiency 61 and 89% higher than those of NGM plants, respectively. The overexpression of the *OVP1* gene favors the upregulation of some photosynthesis genes and the increase in the number of spikelets and in the photosynthetic rate, but does not favor the increase in grain yield.

Index terms: *Oryza sativa*, genetic engineering, grain yield components, RNAseq.

Efeitos fisiológicos e fenotípicos da superexpressão do gene *OVP1* em arroz japonica

Resumo – O objetivo deste trabalho foi avaliar os parâmetros fisiológicos, fenotípicos e de expressão gênica em plantas de arroz geneticamente modificadas (GM) que superexpressam o gene *Oryza sativa Vacuolar H⁺-Pyrophosphatase* 1 (*OVP1*), em comparação ao arroz não geneticamente modificado (NGM). Plantas GM e NGM da cultivar BRSMG Curinga foram avaliadas em dois experimentos, em laboratório e casa de vegetação, no delineamento de blocos ao acaso, com quatro repetições. Foram estimados caracteres agronômicos de interesse, e realizadas análise de transcritoma e quantificação da expressão gênica. Plantas GM apresentaram número de espiguetas por panícula e número total de grãos por panícula 31 e 21% maiores, respectivamente, em comparação às plantas NGM. Ocorreram alterações fisiológicas durante a fase de enchimento de grãos, em que as plantas GM apresentaram taxa fotossintética e eficiência de carboxilação 61 e 89% mais altas do que as das plantas NGM, respectivamente. A superexpressão do gene *OVP1* favorece a indução de alguns genes da fotossíntese e o aumento do número de espiguetas e da taxa fotossintética, mas não favorece o aumento da produtividade de grãos.

Termos para indexação: *Oryza sativa*, engenharia genética, componentes de produtividade, RNAseq.

Introduction

Rice (*Oryza sativa* L.) Brazilian production for the 2021/2022 cycle was estimated to be 10.8 million tons, representing a reduction of 8.4% when compared with the 11.8 million tons of the previous cycle, which was expected due to the lower planted area of 3.6% and lower crop yield of 4.9% (Conab, 2022). Overall, rice production and consumption are similar, possibly indicating food insecurity, which, combined with the low international trade in rice in relation to the total produced, shows the need of significantly increasing rice production to meet consumer demands (Mohidem et al., 2022). In this scenario, biotechnology is an alternative to increase yield, expanding the productive potential of rice.

One of the most important components of rice yield to be considered for plant improvement is the grain-filling rate, which, to be high, requires a balanced distribution, throughout the panicle, of the carbohydrates produced during photosynthesis (Chen et al., 2019). These carbohydrates are transported over long distances to the sink organs in the form of sucrose, which needs to be carried to the phloem, transported, and released in order to reach the panicle (Scholz-Starke et al., 2019). Sucrose is loaded into phloem companion cells through sucrose/H^+ symport pumps, a function that requires an electrochemical potential gradient in the protons between these cells and the apoplast in order to occur; this gradient is generated by an electrogenic proton pump, H^+-ATPase (Scholz-Starke et al., 2019).

Protein H^+-PPases, encoded by the *Oryza sativa Vacuolar H^+-Pyrophosphatase* 1 (*OVP1*) gene, act in the plasma membrane of the phloem companion cells, synthesizing cellular pyrophosphate (Primo et al., 2019), which is used to produce adenosine triphosphate (ATP), used by H^+-ATPases to maintain the proton gradient necessary for sucrose loading into the phloem. In this way, H^+-PPases contribute to the distribution of sucrose between source and sink organs and, consequently, to grain filling (Scholz-Starke et al., 2019). In the tonoplast (membrane of cell vacuoles), H^+-PPases transport H^+ from the cytoplasm to the interior of the vacuole through the cleavage of inorganic pyrophosphate, which causes several metabolic and morphological changes in plants transformed to overexpress or silence the *OVP1* gene (Schilling et al., 2017). As a result, plants genetically modified with this gene have a higher biomass and are tolerant to saline stress, water deficit, heat, cold, and soils with a low phosphorus content (Esmaeili et al., 2019).

Therefore, the *OVP1* gene, due to its role in several metabolic pathways, should be evaluated through genetic engineering, considering its overexpression may generate useful and nonexistent phenotypic modifications in the gene pool of cultivated rice, resulting in a phenotype of interest for obtaining superior lines and cultivars. For this, it is essential to evaluate the physiological changes caused by this overexpression, as well as its effects on important traits for the genetic improvement of rice.

The objective of this work was to evaluate the physiological, phenotypic, and gene expression parameters in genetically modified (GM) rice plants that overexpress the *OVP1* gene, compared with non-genetically modified (NGM) rice.

Materials and Methods

The BRSMG Curinga cultivar of upland rice was transformed according to the method described by Dedicova et al. (2015), using the *EHA* 105 *Agrobacterium* strain, resulting in 16 transformation events overexpressing the *OVP1* gene. The used plasmid (p7i2x-Ubi) contains both the coding sequence (2,349 bp) of the rice *OVP1* gene (LOC_Os06g43660) driven by the maize ubiquitin promoter and the *bar* selection gene driven by the CaMV35S promoter (Dedicova et al., 2015). The 16 transformation events were evaluated throughout generation advances, and the most promising one (*OVP1*-E4) was selected for the analysis.

Two experiments were carried out to compare GM rice plants of the *OVP1*-E4 transformation event with NGM plants of the same cultivar, under laboratory and greenhouse conditions, at Embrapa Arroz e Feijão, in the municipality of Santo Antônio de Goiás, in the state of Goiás, Brazil (16º28'S, 49º17'W, at 779 m of altitude).

The first experiment was conducted on the SITIS Automated Plant Phenotyping Platform (Pereira et al., 2017) between October 2018 and March 2019, in randomized blocks with four replicates for each genotype. Seeds belonging to the T4 generation were sown in PVC columns (40 cm height × 30 cm

in diameter) filled with soil classified as Latossolo Vermelho Ácrico típico (Santos et al., 2018), i.e., a Rhodic Ferralsol (IUSS Working Group WRB, 2015). The second experiment was carried out in a greenhouse between October 2019 and March 2020, in randomized complete blocks with four replicates for each genotype. In this case, the seeds from the T5 generation were sown in plots consisting of pots filled with approximately 9.0 kg soil.

In both experiments, irrigation occurred once a day to replenish 80% of soil field capacity. Using a brush, a solution consisting of 2.0% of the Liberty glufosinate-ammonium herbicide (BASF, Ludwigshafen, Germany) with 0.1% Tween-20 was applied to the adaxial part of the leaves of the GM plants of the T4 generation, in order to certify that all plants were GM. In addition, flag leaf size was estimated based on the average length and width of three leaves per plot during physiological grain maturation. The number of tillers and panicles was counted in the same period. The average number of spikelets, the number of sterile spikelets, and the total number of grains per panicle were obtained by counting the grains of three panicles per plant. All spikelets were manually separated and weighed on a precision digital scale to obtain the total weight of grains per plant (grain yield). To evaluate the data from the two experiments, a joint analysis of variance was performed using the RStudio, v.4.0.2, software (RStudio Team, 2020), followed by Tukey's test, at 5% probability.

In the first experiment, the physiological parameters were measured in one plant per plot during the following stages described by Counce et al. (2000): R3, panicle formation; R6, grain filling; and R8, physiological maturation with at least one brown grain developed. In the second experiment, the measurements were taken in a greater number of stages: R2, flag leaf formation; R4, flowering; R6, grain filling; R7, beginning of the physiological maturation of the grains with at least one yellow grain; and R9, end of the physiological maturation of the grains with panicles containing the brownest grains. The LCpro-SD infrared gas analyzer (ADC BioScientific Ltd., Hoddesdon, United Kingdom) was used to determine the following physiological parameters: photosynthetic rate (*A*), expressed in μmol m^{-2} s^{-1}; transpiration rate (*E*), in mmol m^{-2} s^{-1}; stomatal conductance (*gs*), in mol m^{-2} s^{-1}; and concentration of internal carbon (Ci), in vpm. The active light photon flux density used was 1,200 μmol [quanta] m^{-2}, and the leaf area was 3.8 cm^2. From these data, efficiency (*A*/Ci), instantaneous water use efficiency (iWUE) obtained by *A*/*E* (μmol m^{-1}), and intrinsic water use efficiency (WUEintr) calculated by *A*/*gs* (μmol m^{-1}) were estimated.

For the gene expression analysis, leaf tissue from GM and NGM plants was collected simultaneously to obtain physiological data. In the first experiment, the expression of the *OVP1* gene was quantified in the developmental stages in which the leaves were harvested, i.e., in R3, R6, and R8. In the second experiment, quantification via RT-qPCR was carried out in stages R2, R6, and R7. Immediately after harvested, the leaves were stored in an ultra-freezer at -80°C and, subsequently, macerated in liquid nitrogen to a fine powder. From 100 mg tissue, total RNA was extracted using the RNeasy kit according to the manufacturer's instructions (QIAGEN, Hilden, Germany); the enzyme used for the process was DNase I (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). RNA quality was estimated based on the 260/280 and 260/230 ratios provided by the UV-visible NanoVue Plus spectrophotometer (GE Healthcare, Chicago, IL, USA). The integrity of total RNA was verified with the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and quantification was performed using the Qubit 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). cDNA synthesis was performed according to the manufacturer's instructions using random primers of the GOScript Reverse Transcription System kit (Promega, Madison, WI, USA). Amplification reactions (RT-qPCR) were performed in triplicate using three reference genes: actin (ACT), GenBank ID X15865 (Zhang et al., 2009); eukaryotic elongation factor-1α (eEF-1α), GenBank ID AK061464 (Zhang et al., 2009); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), GenBank ID AK064960 (Kim et al., 2003). The 7500 Real-Time PCR thermal cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) was used to achieve the following thermocycling conditions: 50°C for 2 min (incubation of uracil-DNA glycosylases), 95°C for 2 min (denaturation), and 40 cycles of 95°C for 15 s and 60°C for 1 min (annealing), followed by the dissociation curve starting at 95ºC for 15 s and continuing at 60ºC for 1 min, 95ºC for 30 s, and 60ºC for 15 s.

Initial data analysis was performed using the Sequence Detection System (SDS), v2.05, software (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). After obtaining the SDS results, the stability value of the reference genes was determined by the Normfinder software, and the two most stable genes were used to calculate relative expression (relative quantification) by the ΔΔCt method of Livak & Schmittgen (2001) through the DataAssist, v3.01, software (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The control were plants of the BRSMG Curinga cultivar. The statistical packages used were readxl (Wickham & Bryan, 2019), lattice (Sarkar, 2008), and agricolae (Mendiburu & Yaseen, 2020). The physiological parameters were evaluated by a simple analysis of variance using packages ExpDes (Ferreira et al., 2014) and easyanova (Arnhold, 2013).

In the first experiment, the plant that presented the highest number of spikelets in each genotype was analyzed in duplicate by transcriptome sequencing (RNAseq) on the HiSeq 2000 platform (Ilumina Inc., San Diego, CA, USA). Three micrograms of total RNA from the leaves collected during the R6 stage were stored in RNAstable tubes (Sigma Aldrich, Merck & Co. Inc., Rahway, NJ, USA) and lyophilized under vacuum. After obtaining the sequencing data, quality filters were applied using the Trimmomatic software (Bolger et al., 2014), and the transcriptome was reassembled with the Trinity software (Haas et al., 2013). Assembly quality, called representativeness of the reads, was evaluated from the alignment of the filtered fragments in the obtained transcriptome using the Bowtie2 software (Langmead et al., 2019). Additionally, to determine the degree of coverage (completeness) of the genes, the assembled transcriptome was aligned to the rice reference transcriptome, *Oryza sativa* ssp. *Japonica* var. Nipponbare of MSU Rice Genome Annotation Project Release 7 (Kawahara et al., 2013), using the BLAST tool (Altschul et al., 1990).

Once the quality of the assembly was verified, the genes were quantified through the joint action of the RSEM (Li & Dewey, 2011) and Bowtie2 (Langmead et al., 2019) software, which used the transcriptome itself as a reference for quantification. Then, the differential expression analysis was performed with the EdgeR software (Robinson et al., 2010), which only considered as significant the differentially expressed genes with a p-value for a false discovery rate lower than 0.001 and a fold change higher than 4. The open reading frames encoding at least 100 amino acid residues were obtained with the TransDecoder software (Haas, 2023). Then, the polypeptide sequences were aligned in the Uniprot database (Uniprot Consortium, 2019) with the BLASTp tool and used to predict the conserved domains of the putative proteins present in the Pfam database (Finn et al., 2014). Data annotation continued with BLASTx from transcriptome, BLASTp from protein data, and a new search for conserved protein domains. Trinotate (Bryant et al., 2017) was used to develop a database to store all the obtained results and to determine Gene Ontology (GO) according to Mi et al. (2019). The differential analysis of the genes and the Trinity-enriched pathways were performed using GO. In addition, the MapMan software was used to visualize the metabolic maps (Usadel et al., 2005), and a GO plot pipeline from the RStudio software was used to plot the GO categories (Walter et al., 2015).

Results and Discussion

The joint analysis of the two experiments showed that the GM plants were superior to the NGM ones due to a 31% higher number of spikelets per panicle, 21% higher total number of grains produced per panicle, and 9.0% longer flag leaf length, indicating that the rice metabolism was benefited by the increase in the amount of *OVP1* transcripts (Table 1).

The higher number of spikelets shows a more balanced distribution of photoassimilates in this part of the GM plants, possibly due to the action of H^+-PPases in the phloem, together with H^+-ATPases and sucrose/

Table 1. Trait values obtained from the joint analysis of the experiments for rice (*Oryza sativa*) plants of cultivar BRSMG Curinga non-genetically modified (NGM) and genetically modified (GM) with the *OVP1* gene.

Trait	NGM	GM	p-value
Tiller number	38.50	36.00	0.41
Panicle number	38.50	36.00	0.41
Flag leaf width (mm)	1.66	1.75	0.33
Flag leaf length (cm)	25.32	27.86	0.01
Number of spikelets per panicle	174.05	228.21	0.001
Number of sterile spikelets per panicle	39.39	30.29	0.39
Number of total grains per panicle	213.44	258.50	0.004
Grain yield (gram per plant)	91.03	91.85	0.86

H^+, which contributed to increasing sugar transport to the panicles (Primo et al., 2019; Scholz-Starke et al., 2019).

Regarding total number of grains, Regmi et al. (2020) also observed an increase in wheat (*Triticum aestivum* L.) plants overexpressing the *Arabidopsis Vacuolar Pyrophosphatase* I (*AVP1*) gene, a finding that may be related to the action of auxin during panicle formation, i.e., in the R3 stage (Zhang et al., 2016b). In *Arabidopsis thaliana* (L.) Heynh. plants transformed with *AVP1*, Asaoka et al. (2016) concluded that H^+-PPases can alter auxin displacement by altering intracellular and extracellular pH, causing several morphological changes, such as an increased leaf area.

As to flag leaf, according to Lee et al. (2017), a longer length is interesting for rice plants since this leaf is the main supplier of photoassimilates to the panicle and, therefore, plays an important role in grain yield.

When comparing GM and NGM plants, most physiological parameters only differed significantly in the R6 stage (Table 2). During grain filling, GM plants showed an increase of 61.4% in photosynthetic rate, a reduction of 23% in internal carbon concentration, and an increase of 89.6% in carboxylation efficiency. Other authors also found the highest photosynthetic rate during grain filling due to the overexpression of the *OVP1* gene in *A. thaliana* (Khadilkar et al., 2016), rice (Kim et al., 2014), cotton (*Gossypium hirsutum* L.) (Pasapula et al., 2011), and sugarcane (*Saccharum officinarum* L.) (Raza et al., 2016). However, unlike in cotton and sugarcane, the overexpression of the *OVP1* gene in the GM rice plants evaluated in the present study did not show significant changes in stomatal conductance and transpiration rate.

GM plants also presented an increase of 49.8 and 76.1% in iWUE and WUEintr, even under normal irrigation conditions (Table 2). This is an indicative that they may be more suitable for shorter critical periods regarding water availability, such as in the reproductive stage.

Therefore, the relative expression of the *OVP1* gene was evaluated during the reproductive stage of GM rice, considering that the manipulation of specific genes can contribute to obtaining cultivars with a greater yield potential, even though this trait is quantitative. However, as the used promoter is constitutive, GM plants were able to regulate *OVP1* and to only overexpress it during the R6 stage in the first experiment, a result that was confirmed by qPCR (Figure 1). Furthermore, due to the expression of the *OVP1* gene in GM plants, there was only a positive correlation with the photosynthetic rate (r^2=0.96; $p<0.05$) and carboxylation efficiency (r^2=0.99; $p<0.05$) measured in the R6 stage in the first experiment. In the

Table 2. Physiological data during the R6 stage, obtained from the joint analysis in 2018/2019 and 2019/2020, for rice (*Oryza sativa*) plants of cultivar BRSMG Curinga non-genetically modified (NGM) and genetically modified (GM) with the *OVP1* gene.

Physiological parameter[(1)]	NGM	GM	p-value
Photosynthetic rate (*A*)	8.15	13.15	0.002
Concentration of internal carbon (Ci)	315.71	256.76	0.001
Carboxylation efficiency (*A*/Ci)	0.029	0.055	0.003
Stomatal conductance (*gs*)	0.182	0.178	0.822
Transpiration rate (*E*)	3.18	3.11	0.802
iWUE (*A*/*E*)	2.59	3.88	0.001
WUEintr (*A*/*gs*)	41.53	73.15	0.001

[(1)]iWUE, instantaneous water use efficiency; and WUEintr, intrinsic water use efficiency.

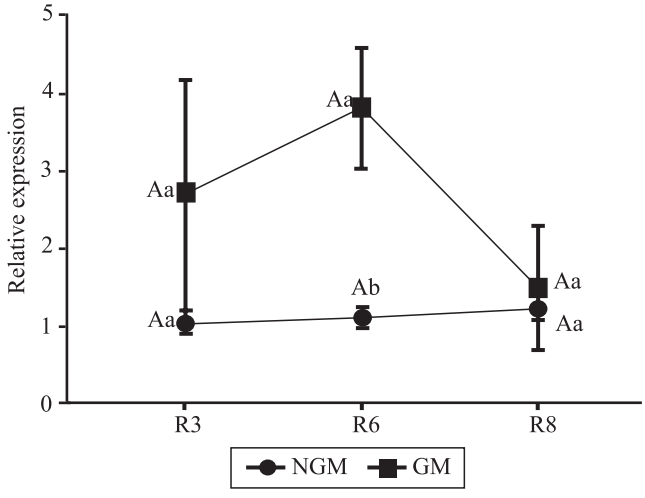

Figure 1. Relative expression of the *OVP1* gene in genetically-modified (GM) rice (*Oryza sativa*) plants of cultivar BRSMG Curinga compared with non-genetically modified (NGM) plants in 2018/2019. Equal lowercase letters do not differ between genotypes, and equal uppercase letters do not differ between developmental stages by Tukey's test, at 5% probability (n=4).

second, there was no significant difference between both genotypes regarding any of the analyzed stages.

From the RNAseq analysis of the GM and NGM plants from the first experiment, the transcriptome sequencing of the four libraries generated from 41.94 to 82.62 million reads. On average, 93.8% of the sequenced bases had a Phred-value greater than 30 (Q30), whereas the contigs obtained had an average of 1,199.89 bases, with a N50 of 2,370 bases.

As to representativeness, the libraries presented more than 99% of the readings aligned in the transcriptome, indicating the high quality of the assembly. Moreover, the completeness analysis showed that 74% of the genes had more than a 50% coverage. In total, 126 differentially-expressed genes between the two genotypes were identified, of which 51 were upregulated and 75 were downregulated in the *OVP1* event. Among the upregulated genes that may be related to the overexpression of *OVP1*, ion transporters and those involved in the photochemical and biochemical stage of photosynthesis were identified. Finally, genes related to plant development and defense were highlighted, such as transcription factors and those belonging to metabolic routes of auxins and flavonoids (Table 3).

Most genes that were downregulated in GM plants were encoding transcription factors (LOC_Os01g70270.4 and LOC_Os02g04810.1) and proteins acting in signal transduction pathways (Table 4). The majority of the GO hierarchical biological categories enriched in GM plants belong to the classification of biological processes, followed by the classification of molecular function and cellular components. Among these categories, there are those involved in homeostasis and in the transport of ions, such as zinc, iron, and manganese, across membranes.

Among the genes that were upregulated in GM plants, those involved in the photochemical (LOC_Os08g40160.1) and biochemical (LOC_Os06g04270.1) stages, in the transport of cations (LOC_Os05g39540.1) and proteins (LOC_Os09g28610.1), and in the transport of toxic compounds through the efflux protein (LOC_Os08g37432.2) of the MATE family were identified (Table 3). Considering transcription factors and genes that encode proteins that act in signal transduction pathways and that had their expression upregulated

Table 3. Differentially expressed genes upregulated in rice (*Oryza sativa*) plants of cultivar BRSMG Curinga genetically modified (GM) with the OVP1 gene, compared with non-genetically modified (NGM) plants.

Related function	Gene ID	Description[1]	$\log_2$ (fold change)[2]
Photosynthesis	LOC_Os06g04270.1	Transketolase, chloroplast precursor	11.29
	LOC_Os08g40160.1	Thylakoid lumen protein, chloroplast precursor	11.98
Carbon metabolism	LOC_Os01g11054.2	Phosphoenolpyruvate carboxylase	11.43
Transporters	LOC_Os06g43660.1	Inorganic H^+ pyrophosphatase	3.41
	LOC_Os05g39540.1	Metal cation transporter	10.43
	LOC_Os07g34006.1	Transporter family protein	10.44
	LOC_Os09g28610.1	Transporter family protein	10.41
	LOC_Os08g37432.2	MATE efflux family protein	11.62
Flavonoids	LOC_Os09g07450.1	Flavonol synthase	10.41
	LOC_Os10g41020.1	Flavonol synthase/flavanone 3-hydroxylase	11.66
Transcription factors and signal transduction pathways	LOC_Os02g43750.1	Transcription initiation factor	10.54
	LOC_Os03g47200.1	bZIP transcription factor domain	11.80
	LOC_Os01g59850.1	GTPase-activating protein	11.98
	LOC_Os03g29250.1	SPX domain-containing protein	11.55
	LOC_Os09g17190.1	OsFBX320 domain-containing protein	12.85
	LOC_Os08g09720.1	OsFBX272 domain-containing protein	10.90
	LOC_Os05g05320.2	PPR repeat domain-containing protein	12.30
	LOC_Os03g09170.1	Ethylene-responsive transcription factor	2.52
	LOC_Os01g68160.3	ZOS1-22 – C_2H_2 zinc finger protein	2.04
	LOC_Os01g49830.1	B3 DNA binding domain	10.53
	LOC_Os05g16740.1	Receptor-like kinase	10.48

[1]According to MSU Rice Genome Annotation Project Release 7 (Kawahara et al., 2013). [2]$\log_2$ values show the increase of the expression of transcripts in GM plants due to the OVP1 gene, in comparison with NGM plants.

in the *OVP1* event, the transcription factor of the bZIP family (LOC_Os02g43750.1) and a protein with SPX domain (LOC_Os03g29250.1) were identified. According to the differential expression analysis, the *OVP1* (LOC_Os06g43660) gene was upregulated in GM plants with a fold change value (log2) equal to 3.41.

The LOC_Os08g40160.1 gene, which was upregulated in GM plants, is responsible for encoding a structural protein of the PsbP family, a component of photosystem II (PSII) (Quero et al., 2021). This protein is involved in the oxidation of the water molecule carried out by PSII, which has the function of removing electrons from the water initiating electron transport (Mentewab et al., 2014). The ortholog of LOC_Os08g40160.1 was downregulated with a low growth rate in *A. thaliana* (AT1G76450 gene) mutant plants (Zhang et al., 2016a) and with a reduced photosynthetic rate in maize (*Zea mays* L.) plants under water deficit (Zhang et al., 2018). These examples indicate that the PsbP protein plays an essential role in carrying out photosynthesis and, consequently, in the normal development of plants. The LOC_Os06g04270.1 gene, which was also upregulated in GM plants, encodes a transketolase that acts in the regeneration stage of the Calvin-Benson cycle, in two steps: production of erythrose-4P from fructose-6P and of xylulose-5P from sedoheptulose-7P (Rocha et al., 2014). This route may be related to the greater carboxylation efficiency of GM plants, which did not show a high stomatal conductance, but probably had a greater amount of regenerated ribulose-1,5 bisphosphate available for CO_2 fixation. The ortholog of this gene (Sb06g004280), together with others of the Calvin-Benson cycle, was upregulated in sorghum [*Sorghum bicolor* (L.) Moench] plants that accumulated sugars during saline stress (Sui et al., 2015), showing the importance of transketolase for the production and accumulation of carbohydrates. Therefore, it is likely that the induction of the two genes that participate directly in photosynthesis, together with the increase in the photosynthetic rate, contributed to the higher number of spikelets in GM rice plants. However, despite this higher number of spikelets, the grain yield of GM plants was not significantly higher than that of the NGM ones.

Table 4. Differentially expressed genes downregulated in rice (*Oryza sativa*) plants of cultivar BRSMG Curinga genetically modified (GM) with the OVP1 gene, compared with non-genetically modified (NGM) plants.

Related function	Gene ID	Description[1]	log_2 (fold change)[2]
Transcription factors and signal transduction pathways	LOC_Os01g49529.4	OsWAK10d – OsWAK receptor-like cytoplasmic kinase	12.09
	LOC_Os02g58520.1	CAMK – calcium/calmodulin-dependent protein kinase	11.14
	LOC_Os08g37800.1	CAMK KIN1/SNF1/Nim1_like_AMPKh.4	10.70
	LOC_Os01g73530.1	ABC transporter, ATP-binding protein	11.86
	LOC_Os02g46970.1	AMP-binding domain-containing protein	4.71
	LOC_Os10g40780.3	CCR4-Not complex component, Not1domain- containing protein	11.32
	LOC_Os12g06340.3	BEL1-like homeodomain transcription factor	11.07
	LOC_Os01g51300.2	WD domain, G-beta repeat domain-containing protein	11.03
	LOC_Os05g25430.1	Receptor-like protein kinase At3g46290 precursor	10.85
	LOC_Os03g19870.1	ATP-binding protein	11.88
	LOC_Os01g72330.1	OsRR4 type-A response regulator	2.45
	LOC_Os02g35180.1	OsRR2 type-A response regulator	2.82
	LOC_Os02g45600.1	rhoGAP domain-containing protein	11.00
	LOC_Os07g12590.1	OsFBX225 – F-box domain-containing protein	11.33
Auxin	LOC_Os01g70270.4	Auxin response factor	11.74
	LOC_Os02g04810.1	Auxin response factor 5	11.83
	LOC_Os09g29940.2	Auxin-independent growth promoter protein	10.95
Development	LOC_Os05g29930.1	Late embryogenesis abundant protein	12.32
	LOC_Os12g01200.3	Senescence-induced receptor-like serine/threonine-protein kinase precursor	11.95
	LOC_Os09g25490.1	CESA9 – cellulose synthase	8.39

[1]According to MSU Rice Genome Annotation Project Release 7 (Kawahara et al., 2013). [2]log_2 values show the increase of the expression of transcripts in GM plants due to the OVP1 gene, in comparison with NGM plants.

Huang et al. (2011) highlighted that the number of spikelets is one of the components of rice yield that also depends on the number of panicles per area. For this reason, further studies are necessary to evaluate GM and NGM plants in the field, since no significant differences were observed for number of panicles under greenhouse conditions in the present study. If an increase in yield is identified, it will pave the way for the development of superior rice cultivars.

Conclusions

1. The overexpression of the *Oryza sativa Vacuolar H^+-Pyrophosphatase* 1 (*OVP1*) gene favors the upregulation of some photosynthesis genes and the increase in the number of spikelets and in the photosynthetic rate of genetically modified (GM) rice (*Oryza sativa*) plants.

2. The overexpression of the *OVP1* gene in GM rice plants does not favor the increase in grain yield when compared with non-genetically modified plants.

Acknowledgments

To Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for grants to the seventh (process number 310935/2019-9) and eighth (process number 313688/2021-4) authors; to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes, Finance Code 001), for grant to the first author; and to CNPq (process number 482724/2010-2) and to Empresa Brasileira de Pesquisa Agropecuária (Embrapa, process number 03.10.04.001.00.00), for financial support.

References

ARNHOLD, E. Package in the R environment for analysis of variance and complementary analyses. **Brazilian Journal of Veterinary Research and Animal Science**, v.50, p.488-492, 2013. DOI: https://doi.org/10.11606/issn.1678-4456.v50i6p488-492.

ALTSCHUL, S.F.; GISH, W.; MILLER, W.; MYERS, E.W.; LIPMAN, D.J. Basic local alignment search tool. **Journal of Molecular Biology**, v.215, p.403-410, 1990. DOI: https://doi.org/10.1016/S0022-2836(05)80360-2.

ASAOKA, M.; SEGAMI, S.; FERJANI, A.; MAESHIMA, M. Contribution of PP_i-hydrolyzing function of vacuolar H^+-pyrophosphatase in vegetative growth of *Arabidopsis*: evidenced by expression of uncoupling mutated enzymes. **Frontiers in Plant Science**, v.7, art.415, 2016. DOI: https://doi.org/10.3389/fpls.2016.00415.

BOLGER, A.M.; LOHSE, M.; USADEL, B. Trimmomatic: a flexible trimmer for Illumina sequence data. **Bioinformatics**, v.30, p.2114-2120, 2014. DOI: https://doi.org/10.1093/bioinformatics/btu170.

BRYANT, D.M.; JOHNSON, K.; DITOMMASO, T.; TICKLE, T.; COUGER, M.B.; PAYZIN-DOGRU, D.; LEE, T.J.; LEIGH, N.D.; KUO, T.-H.; DAVIS, F.G.; BATEMAN, J.; BRYANT, S.; GUZIKOWSKI, A.R.; TSAI, S.L.; COYNE, S.; YE, W.W.; FREEMAN JR., R.M.; PESHKIN, L.; TABIN, C.J.; REGEV, A.; HAAS, B.J.; WHITED, J.L. A tissue-mapped axolotl de novo transcriptome enables identification of limb regeneration factors. **Cell Reports**, v.18, p.762-776, 2017. DOI: https://doi.org/10.1016/j.celrep.2016.12.063.

CHEN, L.; DENG, Y.; ZHU, H.; HU, Y.; JIANG, Z.; TANG, S.; WANG, S.; DING, Y. The initiation of inferior grain filling is affected by sugar translocation efficiency in large panicle rice. **Rice**, v.12, art.75, 2019. DOI: https://doi.org/10.1186/s12284-019-0333-7.

CONAB. Companhia Nacional de Abastecimento. **Produção de grãos é estimada em 272,5 milhões de toneladas com clima favorável para as culturas de 2ª safra**. Avaliable at: <https://www.conab.gov.br/ultimas-noticias/4684-producao-de-graos-e-estimada-em-272-5-milhoes-de-toneladas-com-clima-favoravel-para-as-culturas-de-2-safra>. Acessed on: Oct. 14 2022.

COUNCE, P.A.; KEISLING, T.C.; MITCHELL, A.J. A uniform, objective, and adaptive system for expressing rice development. **Crop Science**, v.40, p.436-443, 2000. DOI: https://doi.org/10.2135/cropsci2000.402436x.

DEDICOVA, B.; BERMUDEZ, C.; PRIAS, M.; ZUNIGA, E.; BRONDANI, C. High-throughput transformation pipeline for a Brazilian *japonica* rice with bar gene selection. **Protoplasma**, v.252, p.1071-1083, 2015. DOI: https://doi.org/10.1007/s00709-014-0741-x.

ESMAEILI, N.; YANG, X.; CAI, Y.; SUN, L.; ZHU, X.; SHEN, G.; PAYTON, P.; FANG, W.; ZHANG, H. Co-overexpression of *AVP1* and *OsSIZ1* in *Arabidopsis* substantially enhances plant tolerance to drought, salt, and heat stresses. **Scientific Reports**, v.9, art.7642, 2019. DOI: https://doi.org/10.1038/s41598-019-44062-0.

FERREIRA, E.B.; CAVALCANTI, P.P.; NOGUEIRA, D.A. ExpDes: an R package for ANOVA and experimental designs. **Applied Mathematics**, v.5, p.2952-2958, 2014. DOI: https://doi.org/10.4236/am.2014.519280.

FINN, R.D.; BATEMAN, A.; CLEMENTES, J.; COGGILL, P.; EBERHARDT,R.Y.;EDDY,S.R.;HEGER,A.;HETHERINGTON, K.; HOLM, L.; MISTRY, J.; SONNHAMMER, E.L.L.; TATE, J.; PUNTA, M. Pfam: the protein families database. **Nucleic Acids Research**, v.42, p.D222-D230, 2014. DOI: https://doi.org/10.1093/nar/gkt1223.

HAAS, B.J. **TransDecoder**. Available at: <https://github.com/TransDecoder/TransDecoder>. Accessed on: Aug. 28 2023.

HAAS, B.J.; PAPANICOLAOU, A.; YASSOUR, M.; GRABHERR, M.; BLOOD, P.D.; BOWDEN, J.; COUGER,

M.B.; ECCLES, D.; LI, B.; LIEBER, M.; MACMANES, M.D.; OTT, M.; ORVIS, J.; POCHET, N.; STROZZI, F.; WEEKS, N.; WESTERMAN, R.; WILLIAM, T.; DEWEY, C.N.; HENSCHEL, R.; LEDUC, R.D.; FRIEDMAN, N.; REGEV, A. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. **Nature Protocols**, v.8, p.1494-1512, 2013. DOI: https://doi.org/10.1038/nprot.2013.084.

HUANG, M.; ZOU, Y.-B.; JIANG, P.; XIA, B.; MD, I.; AO, H.-J. Relationship between grain yield and yield components in super hybrid rice. **Agricultural Sciences in China**, v.10, p.1537-1544, 2011. DOI: https://doi.org/10.1016/S1671-2927(11)60149-1.

IUSS WORKING GROUP WRB. **World Reference Base for Soil Resources 2014**: international soil classification system for naming soils and creating legends for soil maps: update 2015. Rome: FAO, 2015. (FAO. World Soil Resources Reports, 106).

KAWAHARA, Y.; de la BASTIDE, M.; HAMILTON, J.P.; KANAMORI, H.; MCCOMBIE, W.R.; OUYANG, S.; SCHWARTZ, D.C.; TANAKA, T.; WU, J.; ZHOU, S.; CHILDS, K.L.; DAVIDSON, R.M.; LIN, H.; QUESADA-OCAMPO, L.; VAILLANCOURT, B.; SAKAI, H.; LEE, S.S.; KIM, J.; NUMA, H.; ITOH, T.; BUELL, C.R.; MATSUMOTO, T. Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. **Rice**, v.6, art.4, 2013. DOI: https://doi.org/10.1186/1939-8433-6-4.

KHADILKAR, A.S.; YADAV, U.P.; SALAZAR, C.; SHULAEV, V.; PAEZ-VALENCIA, J.; PIZZIO, G.A.; GAXIOLA, R.A.; AYRE, B.G. Constitutive and companion cell-specific overexpression of *AVP1*, encoding a proton-pumping pyrophosphatase, enhances biomass accumulation, phloem loading, and long-distance transport. **Plant Physiology**, v.170, p.401-414, 2016. DOI: https://doi.org/10.1104/pp.15.01409.

KIM, B.-R.; NAM, H.-Y.; KIM, S.-U.; KIM, S.-I.; CHANG, Y.-J. Normalization of reverse transcription quantitative-PCR with housekeeping genes in rice. **Biotechnology Letters**, v.25, p.1869-1872, 2003. DOI: https://doi.org/10.1023/A:102698032009.

KIM, Y.S.; KIM, I.S.; CHOE, Y.H.; BAE, M.J.; SHIN, S.Y.; PARK, S.K.; KANG, H.G.; KIM, Y.H.; YOON, H.S. Overexpression of the Arabidopsis vacuolar H^+-pyrophosphatase AVP1 gene in rice plants improves grain yield under paddy field conditions. **Journal of Agricultural Science**, v.152, p.941-953, 2014. DOI: https://doi.org/10.1017/S0021859613000671.

LANGMEAD, B.; WILKS, C.; ANTONESCU, V.; CHARLES, R. Scaling read aligners to hundreds of threads on general-purpose processors. **Bioinformatics**, v.35, p.421-432, 2019. DOI: https://doi.org/10.1093/bioinformatics/bty648.

LEE, S.; JEONG, H.; LEE, S.; LEE, J.; KIM, S.-J.; PARK, J.-W.; WOO, H.R.; LIM, P.O.; AN, G.; NAM, H.G.; HWANG, D. Molecular bases for differential aging programs between flag and second leaves during grain-filling in rice. **Scientific Reports**, v.7, art.8792, 2017. DOI: https://doi.org/10.1038/s41598-017-07035-9.

LI, B.; DEWEY, C.N. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. **BMC Bioinformatics**, v.12, art.323, 2011. DOI: https://doi.org/10.1186/1471-2105-12-323.

LIVAK, K.J.; SCHMITTGEN, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. **Methods**, v.25, p.402-408, 2001. DOI: https://doi.org/10.1006/meth.2001.1262.

MENDIBURU, F.; YASEEN, M. **agricolae**: statistical procedures for agricultural research. R package v.1.4.0. 2020. Available at: <https://myaseen208.github.io/agricolae/https://cran.rproject.org/package=agricolae>. Accessed on: Apr. 20 2023.

MENTEWAB, A.; MATHESON, K.; ADEBIYI, M.; ROBINSON, S.; ELSTON, B. RNA-Seq analysis of the effect of kanamycin and the ABC transporter AtWBC19 on *Arabidopsis thaliana* seedlings reveals changes in metal content. **PLoS ONE**, v.9, e109310, 2014. DOI: https://doi.org/10.1371/journal.pone.0109310.

MI, H.; MURUGANUJAN, A.; EBERT, D.; HUANG, X.; THOMAS, P.D. PANTHER version 14: more genomes, a new PANTHER GO-slim, and improvements in enrichment analysis tools. **Nucleic Acids Research**, v.47, p.D419-D426, 2019. DOI: https://doi.org/10.1093/nar/gky1038.

MOHIDEM, N.A.; HASHIM, N.; SHAMSUDIN, R.; CHE MAN, H. Rice for food security: revisiting its production, diversity, rice milling process and nutrient content. **Agriculture**, v.12, art.741, 2022. DOI: https://doi.org/10.3390/agriculture12060741.

PASAPULA, V.; SHEN, G.; KUPPU, S.; PAEZ-VALENCIA, J.; MENDOZA, M.; HOU, P.; CHEN, J.; QIU, X.; ZHU, L.; ZHANG, X.; AULD, D.; BLUMWALD, E.; ZHANG, H.; GAXIOLA, R.; PAYTON, P. Expression of an Arabidopsis vacuolar H^+-pyrophosphatase gene (*AVP1*) in cotton improves drought- and salt tolerance and increases fibre yield in the field conditions. **Plant Biotechnology Journal**, v.9, p.88-99, 2011. DOI: https://doi.org/10.1111/j.1467-7652.2010.00535.x.

PEREIRA, R.C.; GUIMARÃES, C.M.; HEINEMANN, A.B.; LANNA, A.C.; LOPES JUNIOR, S.; NARCISO, M.G.; STONE, L.F.; VIANELLO, R. P.; CASTRO, A. P. SITIS - Plant Phenotyping Platform. In: LATIN-AMERICAN CONFERENCE ON PLANT PHENOTYPING AND PHENOMICS FOR PLANT BREEDING, 2., 2017, São Carlos. **Proceedings**. São Carlos: Embrapa Instrumentação, 2017. p.47. Editores: Paulo Sérgio de Paula Herrmann Junior, Paulino Ribeiro Villas Boas.

PRIMO, C.; PIZZIO, G.A.; YANG, J.; GAXIOLA, R.A.; SCHOLZ-STARKE, J.; HIRSCHI, K.D. Plant proton pumping pyrophosphatase: the potential for its pyrophosphate synthesis activity to modulate plant growth. **Plant Biology**, v.21, p.989-996, 2019. DOI: https://doi.org/10.1111/plb.13007.

QUERO, G.; BONNECARRÈRE, V.; SIMONDI, S.; SANTOS, J.; FERNÁNDEZ, S.; GUTIERREZ, L.; GARAYCOCHEA, S.; BORSANI, O. Genetic architecture of photosynthesis energy partitioning as revealed by a genome-wide association approach. **Photosynthesis Research**, v.150, p.97-115, 2021. DOI: https://doi.org/10.1007/s11120-020-00721-2.

RAZA, G.; ALI, K.; ASHRAF, M.Y.: MANSOOR, S.: JAVID, M.: ASAD, S. Overexpression of an H^+-PPase gene from Arabidopsis in sugarcane improves drought tolerance, plant growth, and photosynthetic responses. **Turkish Journal of Biology**, v.40, p.109-119, 2016. DOI: https://doi.org/10.3906/biy-1501-100.

REGMI, K.C.; YOGENDRA, K.; FARIAS, J.G.; LI, L.; KANDEL, R.; YADAV, UP.; SHA, S.; TRITTERMANN, C.; SHORT, L.; GEORGE, J.; EVERS, J.; PLETT, D.; AYRE, B.G.; ROY, S.J.; GAXIOLA, R.A. Improved yield and photosynthate partitioning in *AVP1* expressing wheat (*Triticum aestivum* L.) plants. **Frontiers in Plant Science**, v.17, art.273, 2020. DOI: https://doi.org/10.3389/fpls.2020.00273.

ROBINSON, M.D.; McCARTHY, D.J.; SMYTH, G.K. EdgeR: a bioconductor package for differential expression analysis of digital gene expression data. **Bioinformatics**, v.26, p.139-140, 2010. DOI: https://doi.org/10.1093/bioinformatics/btp616.

ROCHA, A.G.; MEHLMER, N.; STAEL, S.; MAIR, A.; PARVIN, N.; CHIGRI, F.; TEIGE, M.; VOTHKNECHT, U.C. Phosphorylation of *Arabidopsis transketolase* at Ser^{428} provides a potential paradigm for the metabolic control of chloroplast carbon metabolism. **Biochemical Journal**, v.458, p.313-322, 2014. DOI: https://doi.org/10.1042/BJ20130631.

RSTUDIO TEAM. **RStudio**: integrated development environment for R. Boston: RStudio, PBC, 2020.

SANTOS, H.G. dos; JACOMINE, P.K.T.; ANJOS, L.H.C. dos; OLIVEIRA, V.Á. de; LUMBRERAS, J.F.; COELHO, M.R.; ALMEIDA, J.A. de; ARAÚJO FILHO, J.C. de; OLIVEIRA, J.B. de; CUNHA, T.J.F. **Sistema brasileiro de classificação de solos**. 5.ed. rev. e ampl. Brasília: Embrapa, 2018. 356p.

SARKAR, D. **Lattice**: multivariate data visualization with R. New York: Springer, 2008. DOI: https://doi.org/10.1007/978-0-387-75969-2.

SCHILLING, R.K.; TESTER, M.; MARSCHNER, P.; PLETT, D.C.; ROY, S.J. AVP1: one protein, many roles. **Trends in Plant Science**, v.22, p.154-162, 2017. DOI: https://doi.org/10.1016/j.tplants.2016.11.012.

SCHOLZ-STARKE, J.; PRIMO, C.; YANG, J.; KANDEL, R.; GAXIOLA, R.A.; HIRSCHI, K.D. The flip side of the *Arabidopsis* type I proton-pumping pyrophosphatase (AVP1): using a transmembrane H^+ gradient to synthesize pyrophosphate. **Journal of Biological Chemistry**, v.294, p.1290-1299, 2019. DOI: https://doi.org/10.1074/jbc.RA118.006315.

SUI, N.; YANG, Z.; LIU, M.; WANG, B. Identification and transcriptomic profiling of genes involved in increasing sugar content during salt stress in sweet sorghum leaves. **BMC Genomics**, v.16, art.534, 2015. DOI: https://doi.org/10.1186/s12864-015-1760-5.

UNIPROT CONSORTIUM. UniProt: a worldwide hub of protein knowledge. **Nucleic Acids Research**, v.47, p.D506-D515, 2019. DOI: https://doi.org/10.1093/nar/gky1049.

USADEL, B.; NAGEL, A.; THIMM, O.; REDESTIG, H.; BLAESING, O.E.; PALACIOS-ROJAS, N.; SELBIG, J.; HANNEMANN, J.; PIQUES, M.C.; STEINHAUSER, D.; SHEIBLE, W.-R.; GIBON, Y.; MORCUENDE, R.; WEICHT, D.; MEYER, S.; STITT, M. Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of coresponding genes, and comparison with known responses. **Plant Physiology**, v.138, p.1195-1204, 2005. DOI: https://doi.org/10.1104/pp.105.060459.

WICKHAM, H.; BRYAN, J. **readxl**: read Excel files. v.1.3.1. 2019. Available at: <https://CRAN.R-project.org/package=readxl>. Accessed on: Apr. 20 2023.

ZHANG, J.; YUAN, H.; YANG, Y.; FISH, T.; LYI, S.M.; THANNAUSER, T.W.; ZHANG, L.; LI, L. Plastid ribosomal protein S5 is involved in photosynthesis, plant development, and cold stress tolerance in Arabidopsis. **Journal of Experimental Botany**, v.67, p.2731-2744, 2016a. DOI: https://doi.org/10.1093/jxb/erw106.

ZHANG, J.L.; LIU., D.-H.; WANG, J.-H.; YU, C.; CAO, J.-H.; WANG, C.-T.; JIN, D.-M. Expression pattern of *GS3* during panicle development in rice under drought stress: quantification normalized against selected housekeeping genes in real-time PCR. **Asian Journal of Plant Sciences**, v.8, p.285-292, 2009. DOI: https://doi.org/10.3923/AJPS.2009.285.292.

ZHANG, W.; CAO, Z.; ZHOU, Q.; CHEN, J.; XU, G.; GU, J.; LIU, L.; WANG, Z.; YANG, J.; ZHANG, H. Grain filling characteristics and their relations with endogenous hormones in large- and small-grain mutants of rice. **PLoS ONE**, v.11, e0165321, 2016b. DOI: https://doi.org/10.1371/journal.pone.0165321.

ZHANG, X.; LEI, L.; LAI, J.; ZHAO, H.; SONG, W. Effects of drought stress and water recovery on physiological responses and gene expression in maize seedlings. **BMC Plant Biology**, v.18, art.68, 2018. DOI: https://doi.org/10.1186/s12870-018-1281-x.