Australian Journal of Crop Science AJCS
AJCS 6(11):1579-1586 (2012) ISSN:1835-2707

Expression levels of some starch metabolism related genes in flag leaf of two contrasting rice genotypes exposed to salt stress

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Abstract

An objective of this investigation was to compare the transcriptional expression of starch metabolism involving genes, soluble sugar, physiological changes and yield components in flag leaf of two contrasting *indica* rice genotypes, Homjan (HJ; salt-tolerant) and Pathumthani 1 (PT1; salt-sensitive), in response to 150 mM NaCl salt stress. *AGPL1*, *SBEIIb* and *GWD* starch involved genes were up-regulated in salt stressed PT1, leading to accumulation of soluble starch, glucose fructose and total soluble sugars. In HJ, expression levels of *AGPL1 AGPS2b*, *SBEIIb* genes in salt stressed plants were higher than in control plants while the soluble sugar level in flag leaf was unchanged. Also, an expression level of some starch related genes i.e. *AGPL1*, *SSI*, *SBEIIb*, *ISA2a*, and *GWD* in HJ salt stressed plants was lower than that in PT1. Water use efficiency in salt stressed PT1 was significantly dropped for 35.42% whereas that in HJ was unchanged. Chlorophyll b (Chl_b) and total chlorophyll (TC) contents in salt stressed PT1 were significantly degraded for 61.76% and 44.93%, respectively, leading to reduce net photosynthetic rate (P_n). The starch metabolism and sugar accumulation in PT1 were clearly unrelated with salt tolerant ability. In contrast, the starch degradation, photosynthetic abilities and sugar accumulation in salt stressed HJ may play a key role as osmoregulation salt defense mechanism, leading to maintain productivity when subjected to salt stress.

Key words: gene expression, salinity stress, soluble starch, soluble sugar, yield components.
Abbreviations: ADPase_ADPglucose pyrophosphorylase; BAM_*β*-amylase; DE_debranching enzyme; DPE_disproportionating enzyme; Chl_a_chlorophyll a; Chl_b_chlorophyll b; F_v/F_m_maximum quantum yield of PSII; GWD_glucan water dikinase; HJ_Homjan; PT1_Pathumthani 1; P_n_net photosynthetic rate; PWD_phosphoglucan-water dikinase; qP_photochemical quenching; Φ_{PSII}_photon yield of PSII; PPFD_photosynthetic photon flux density; SBE_starch branching enzyme; SS_starch synthase; C_{x+c}_total carotenoids.

Introduction

Soil salinity is one of the major abiotic stresses affecting plant growth and development and leading to a reduction of crop productivity, especially in rice (Wang et al., 2003). Rice has been classified as a glycophyte species, which is very sensitive to saline stress at both seedling and reproductive developmental stages (Abdullah et al., 2001; Zeng et al., 2001). 'Pokkali' and 'Homjan' genotypes of *indica* rice have been identified as salt-tolerant, whereas 'IR29', 'KDML105' and 'PT1' cultivars have been classified as salt susceptible (Gregorio et al., 2002; Cha-um et al., 2009 a). The complex salt-tolerance defense mechanisms, including osmoregulation, salt-exclusion, membranes stabilization, antioxidants and hormonal systems, have been characterized in halophyte species (Hasegawa et al., 2000). In reproductive stage, toxic ions delay the transition process, induce sterility of flowering and reduce the number of spikelets per panicle, leading to a reduction in grain yield (Abdullah et al., 2001; Khan and Abdullah, 2003). In general, starch biosynthesis occurs in the plastids of both photosynthetic and non-photosynthetic cells (Zeeman et al., 2010). Two major starch components, the linear α-polyglucan amylose and the branched *α*-polyglucan amylopectin, have been well investigated (Ohdan et al., 2005). The *α*-1,4-glucosidic link chains of both amylose and amylopectin are elongated by the addition of ADP-glucose, which is regulated by ADPglucose pyrophosphorylase (ADPase), starch synthase (SS) and starch branching enzyme (SBE). During starch degradation, the starch granule is subsequently cleaved into small molecules of glucan by a number of enzymes, viz., glucan water dikinase (GWD), phosphoglucan-water dikinase (PWD), debranching enzyme (DE), *β*-amylase (BAM) and disproportionating enzyme (DPE). GWD plays a role as phosphate (P*i*) transferring to glycosyl moieties of amylopectin. PWD functions in a manner similar to GWD. Moreover, these enzymes have multiple isoforms, depending on the plant species (Ohdan et al., 2005; Zeeman et al., 2010). Interestingly, soluble sugars derived from photosynthesis and starch metabolism in 'Pokkali' salt tolerant cultivar have been reported as novel targets relating to salt tolerance defense mechanisms (Theerawitaya et al.,

2012). Soluble sugars play key role not only in osmoregulation, including controlling water potential and osmotic potential in plant cells (Pattanagul and Thitisaksakul, 2008; Cha-um et al., 2009b; Siringam et al., 2012), but also in detoxification as a chelating agent to bound Na^+ with starch granules (Kanai et al., 2007). However, the details regarding the transcriptional profiling of starch metabolism genes under salt stress are largely lacking. We therefore conducted investigated the transcriptional expression of starch metabolism related genes, soluble sugar, physiological changes and productivity in two contrasting *indica* rice genotypes, HJ (salt-tolerant) and PT1 (salt-sensitive) in response to salt stress.

Results and discussion

Gene expression in starch metabolism and soluble sugar levels

In starch biosynthesis, expression levels of ADP-glucose pyrophosphorylase large subunit 1 (*AGPL1*) in both HJ and PT1 were up-regulated when exposed to salt stress for 1 and 3 days. However, expression of *AGPL1* in PT 1 salt-sensitive variety was down-regulated in plants subjected to 150 mM NaCl for 7 days (Fig. 1a, Fig. 3a-b). ADP-glucose pyrophosphorylase small subunit 2b (*AGPS2b*) in salt-stressed HJ was up-regulated to 4.92, 7.33 and 1.53-folds at 1, 3 and 7 days after exposure, respectively, whereas that in salt-stressed PT 1 was unchanged (Fig. 1b). The expression levels of starch synthase I (*SSI*), starch branching enzyme I (*SBEI*) and starch debranching enzyme isomerase II (*ISA2a*) in both HJ and PT1 were unchanged (Fig. 1c, d, f). A regulation of starch branching enzyme II (*SBEIIb*) in salt stressed HJ was reached to 2.00 and 1.81-folds when exposed to salt stress at 1 and 3 days, respectively, whereas in salt-stressed PT 1 it was unchanged (Fig. 1e). In starch degradation, expression pattern of glucan water-dikinase (*GWD*) in salt-stressed PT1 was significantly up-regulated to 2.83, 3.06 and 1.55-folds when plants were subjected to salt stress for 1, 3 and 7 days, respectively (Fig. 2a and Fig. 3c-d). It may play a key role on starch degradation in salt stressed PT1 to produce soluble sugar. In contrast, the expression levels of phosphogluco-water dikinase (*PWD*), starch phosphorylase (*PhoH*) and disproportionnating enzyme II; (*DPE2*) in both HJ and PT1 were unchanged under salt stress (Fig. 2b-d). Soluble starch content in HJ declined in a time-dependent manner upon salt exposure, whereas in salt stressed PT1 it was unchanged when compared to control plants (Fig. 4a). Insoluble starch contents in salt stressed HJ and PT1 were significantly dropped over that in the control (Fig. 4b). Moreover, sucrose level in salt stressed HJ was significantly dropped when plant were exposed to 150 mM NaCl for 7 day (Fig. 4c). Glucose, fructose and total soluble sugar contents in the flag leaf tissues of salt stressed PT1 were significantly enhanced especially 3 day of salt exposure (Fig. 4d-f). The enhanced soluble sugar in the salt stressed PT1 may have been due to increased starch degradation process. The results obtained in our study are corroborated by earlier finding. For example, transcriptional expression levels of *AGPL1*, *PWD* and *PhoH* starch metabolism genes under salt stress condition in Pokkali rice seedling were up-regulted (Theerawitaya et al., 2012). The expression of *AGPL1* and *AGPS1* in salt-stressed tomato was increased, leading to enriched starch content in tomato fruit during the early fruit development stages (Yin et al., 2010). In japonica rice (cv. Tainung 67), an expression of granule-bound starch synthase (*GBSS*) in starch biosynthesis was down-regulated when exposed to 200 mM NaCl for 24 h, leading to decreased starch content, whereas the activity of ADP-glucose pyrophosphorelase (AGPase), soluble starch synthase (SSS) and starch branchin enzyme (SBE) were unchanged (Chen et al., 2008). In contrast, the starch level in Pokkali (salt-tolerant) was increased whereas that in KDML105 (salt-sensitive) was decreased under salt stress (Pattanagul and Thitisaksakul, 2008). In cowpea, the starch content in the salt stressed "Pitiuba" salt tolerance was enhanced in plant subjected to 75 mM NaCl for 10 days (Praxedes et al., 2011). In our study, during starch degradation, an expression of *GWD* gene in salt stressed PT1 was up-regulated, while *PWD*, *PhoH* and *DPE2* genes were unchanged (Fig. 2). *GWD* is required not only in the hydrolytic pathway, which releases carbon from starch in chloroplast and converts to sucrose, but also in the phosphorolytic pathway that utilizes carbon from starch (Weise et al., 2006; Weise et al., 2011). *GWD* has also been involved in starch degradation and correlates positively to freezing tolerant abilities in *Arabidopsis thaliana* (Yano et al., 2005). Soluble sugars, including sucrose, glucose and fructose, have been reported as compatible solutes, played an important role in osmoregulation during salinity stress (Dubey and Singh, 1999; Kerepesi and Galiba, 2000; Watanabe et al., 2000; Pattanagul and Thitisaksakul, 2008; Amirjani, 2011). In the seedling stage, soluble sugar levels in HJ and PT1 rice are accumulated under 342 mM NaCl and the enrichment is greater in leaf tissue than in root tissue (Cha-um et al., 2009b). In KDML105, the starch content in rice seedlings declined significantly, whereas the sugar concentration enhanced greatly under salt stress (Pattanagul and Thitisaksakul, 2008). In contrast, the starch, glucose and fructose concentrations in salt sensitive "TVu" cultivar of cowpea increased when exposed to 75 mM NaCl for10-17 days (Praxedas et al., 2011).

Water use efficiency and photosynthetic abilities

Photosynthetic pigments, including chlorophyll a (Chl_a), chlorophyll b (Chl_b) total chlorophyll (TC) and total carotenoids, in salt-stressed PT1 declined by 25.6%, 61.8%, 44.9% and 27.9%, respectively (Table 1). In contrast, ratio of Chl_a/Chl_b in salt-stressed PT1 was significantly increased. In HJ rice, Chl_b, TC and Chl_a:Chl_b were unchanged when exposed to 150 mM NaCl for 7 days. Maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) in both of HJ and PT1 were insignificant among treatments (Table 2). Water use efficiency in salt-stressed HJ was maintained whereas that in salt-stressed PT1 declined significantly (Table 2). Likewise, net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (E) were decreased by 84.2%, 75.6% and 75.5%, respectively, in salt-stressed PT1 (Table 2). In the present, study, we observed photosynthetic pigment degradation, WUE limitation and reduction in net photosynthetic rate in salt stressed PT1. Degradation of Chl_a, Chl_b, TC and C_{x+c} in salt stressed plants depends on the salt-tolerant ability (Cha-um et al., 2009a; Amirjani 2011). The Chl_b, TC and C_{x+c} levels in PT1 declined significantly when subjected to salt stress, in contrast to those of HJ, which were maintained, leading to high photosynthetic ability. It is paralleled by earlier finding that the photosynthetic pigments in Pokkali (salt-tolerant genotype) can be stabilised better than those in IR29 (salt-sensitive) salt stressed seedlings (200 mM NaCl) (Theerawitaya et al., 2012). Therefore, the accumulation of total soluble sugars, such as glucose and fructose, during the salt-stress treatment of HJ was positively related to net photosynthetic rate and starch degradation. In contrast, the enrichment of total soluble sugar in salt-stressed

Table 1. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoid (C_{x+c}) concentrations and Chl_a/Chl_b ratio in HJ and PT 1 rice genotypes at booting stage when grown under 0 and 150 mM of NaCl salt stress for 7 days.

Varieties	NaCl (mM)	Chl_a (μg g^{-1}FW)	Chl_b (μg g^{-1}FW)	TC (μg g^{-1}FW)	C_{x+c} (μg g^{-1}FW)	Chl_a: Chl_b
HJ	0	691.8b	753.9b	1,445.7b	254.2b	0.92b
	150	812.9a	759.3b	1,572.2b	289.9a	1.07b
PT 1	0	797.1a	913.8a	1,711.0a	292.7a	0.87b
	150	592.7c	349.4c	942.2c	211.0c	1.52a
ANOVA		**	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT). ** is represented a significant difference at $p \leq 0.01$.

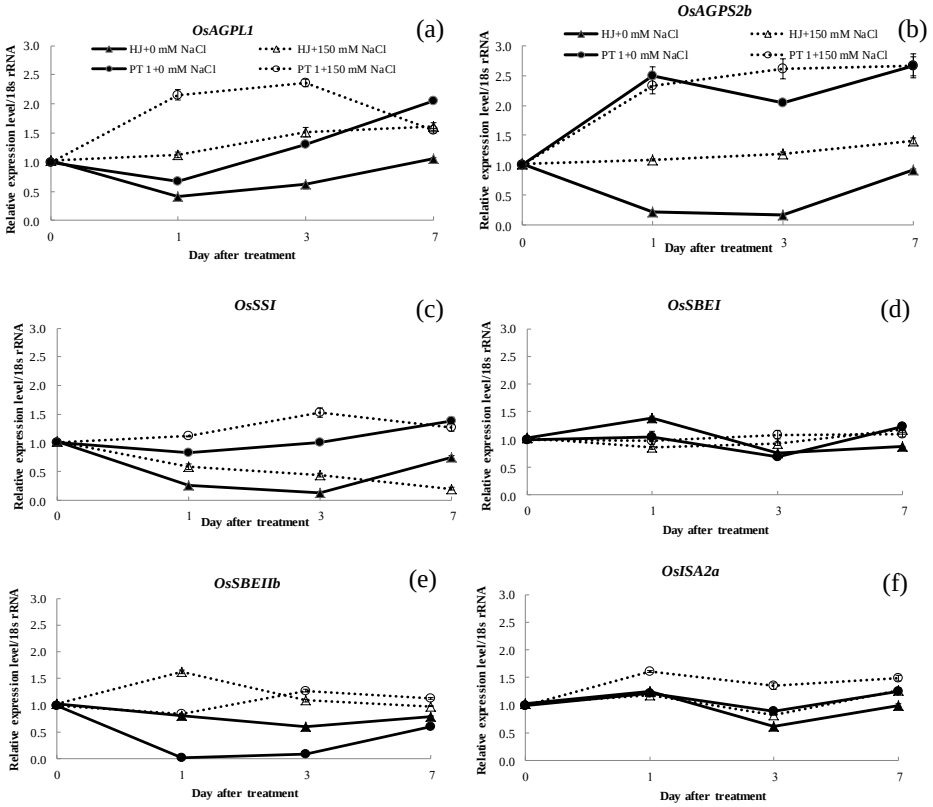

Fig 1. Expression profile of ADP-glucose pyrophosphorylase (*AGPase*) large subunit 1 (a) and small subunit 2b (b), starch synthase (c), starch branching enzyme I and IV (d-e), and starch debranching enzyme isomerase II (f) at booting stage of HJ and PT 1 rice genotypes when exposed to 0 and 150 mM NaCl for 0, 1, 3 and 7 days. Error bars represent ±SE.

PT1 may only derive from starch degradation early in the salt treatment.

Growth characteristics and yield traits

Plant height of salt-stressed HJ and PT1 were reduced by 9.8% and 23.0%, respectively. Seed fertility and seed grain weight in both HJ and PT1 were significantly dropped when plant were grown under 150 mM NaCl (Table 3), whereas one-hundred seed weight was unchanged. The seed fertility and seed grain weight of HJ declined by 19.3% and 50.0%, respectively, when exposed to salt stress. On the other hand, seed fertility and seed grain weight of PT1 declined sharply by 53.6% and 65.6%, respectively. The critical period during reproductive stage of rice plant include booting and panicle initiation stage (Zeng et al., 2001). Delayed flowering and reduction in number of productive tillers, the number of fertile florets per panicle, the weight per grain and grain yield are well known symptoms when rice crop grown under salinity soil (Khatun et al., 1995). Likewise, a reduction in panicle weight, panicle length, primary branches per panicle, filled seeds per panicle, unfilled seeds per panicle, total seeds weight per panicle, 1000-seed weight and total seed weight per plant in salt stressed rice, particularly salt-susceptible cultivars, has been noticed (Abdullah et al., 2001). Furthermore, the reduction of seed set and total seed grain weight have been linked to the diminished carbohydrate translocation from leaf (source) to floral organ (sink) (Zeng et al., 2001).

Materials and Methods

Plant materials and salt stress treatment

Seeds of Homjan (HJ), a salt-tolerant (GS No. 04371) and Pathumthani 1 (PT1), a salt-sensitive (PTT90071-93-8-1-1) variety of *indica* rice (*Oryza sativa* L. subsp. *indica*) were procured from Pathumthani Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand). These were germinated in the plastic tray and 14-day-old seedlings were transplanted to Earthenware pots (15 cm in diameter × 30 cm in height) containing 2 kg of clay soil (Electric Conductivity = 2.687 dS m^{-1}; pH=5.5; organic matter=10.36%; total

Table 2. Maximum quantum yield of PSII (F_v/F_m), photon yield of PSII (Φ_{PSII}), net photosynthetic rate (P_n), stomatal conductance (g_s) transpiration rate (E) and water use efficiency (WUE) in HJ and PT 1 rice genotypes at booting stage when grown under 0 and 150 mM of NaCl salt stress for 7 days.

Varieties	NaCl (mM)	F_v/F_m	Φ_{PSII}	P_n (μmol m^{-2} s^{-1})	g_s (μmol H_2O m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	WUE (mmol CO_2 mol^{-1} H_2O)
HJ	0	0.807	0.793	10.28a	0.05a	2.46a	4.18a
	150	0.826	0.738	3.73b	0.02b	0.85b	4.39a
PT 1	0	0.841	0.800	9.90a	0.05a	2.05a	4.84a
	150	0.804	0.760	1.57b	0.01b	0.50b	3.12b
ANOVA		NS	NS	**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT). NS and ** are represented non significant and significant difference at $p \leq 0.01$, respectively.

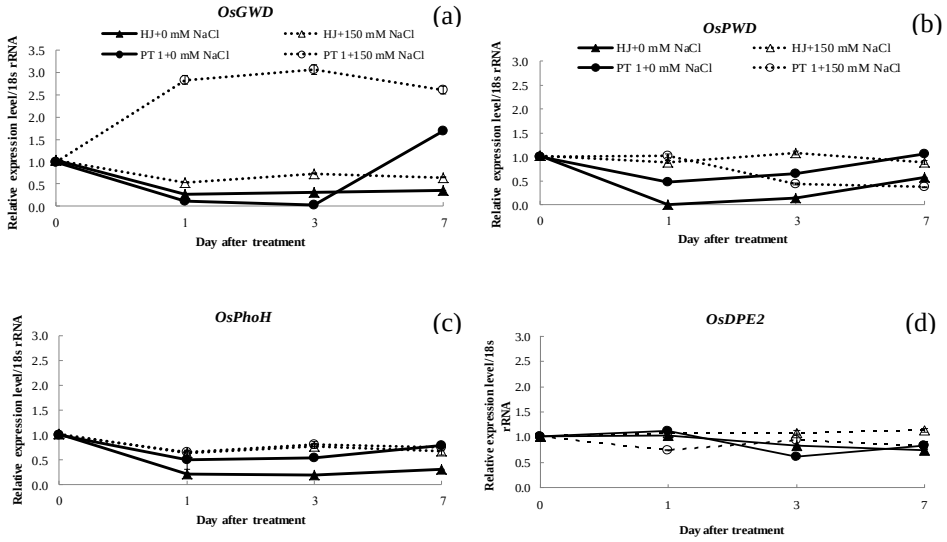

Fig 2. Expression profile of glucan water-dikinase (a), phosphogluco-water dikinase (b), starch phosphorylase H (c) and disproportionnating enzyme II (d) in flag leaf tissues at booting stage of HJ and PT 1 rice genotypes when exposed to 0 and 150 mM NaCl for 0, 1, 3 and 7 days. Error bars represent ±SE.

nitrogen=0.17%; total phosphorus=0.07%; total potassium=1.19%) in 50% shaded light intensity (acclimatization) and grown until booting stage. The salt solution in the pot culture was adjusted to 0 or 150 mM NaCl for 0, 1, 3 and 7 days. Starch content, total soluble sugars, photosynthetic pigments, photosynthetic abilities in flag leaf tissues were analyzed. Plant height, seed fertility 100-seed weight and total seed grain were recorded at the harvest after 120 days.

RNA extraction and cDNA preparation

Flag leaf tissues from the rice plants at booting stage were collected at 0, 1, 3 and 7 days after salt treatment and immediately frozen at –80°C, prior to the extraction of total RNA. Total RNA was pooled and extracted by the Guanidine hydrochloride method (Sambrrok et al., 1989). Flag leaf tissues of rice plants were homogenized in Guanidinium Thiocyanate (Bio Basic Inc.) solution (0.75 M Sodium Citrate (Fisher Scientific) at pH 7.0, 10% N-Lauryl Sarcosine Sodium Salt (Merck) and 2 M 2-β-mercaptoethanol (Amersham Bioscience), Sodium Acetate (Bio Basic Inc.) (pH 4.0) and phenol-chloroform solution. After chilling on ice for 15 min, the homogenate was centrifuged at 10,000×g for 20 min at 4°C. The aqueous phase was separated and mixed with 1×vol isopropanol, then kept at –20°C for 1 h before centrifuging at 10,000×g for 15 min at 4°C. The pellet was completely dissolved in 0.3 mL Guanidinium thiocyanate solution and precipitated with absolute ethanol. Contaminant DNA in the RNA preparations was then treated with RQ1 RNase-Free DNase (Promega) and total RNA was purified by phenol-chloroform extraction. First-stranded DNA was synthesized with 3 µg total RNA per sample, using ImPromp-II TM Reverse Transcriptase (Promega) and oligo-dT15 primer (Promega).

Primer design

Conserved regions of nucleotide related to ADP-glucose pyrophosphorylase small subunit 2b (*OsAGPS2b*; Accession number D50317), ADP-glucose pyrophosphorylase large subunit I (*OsAGPL1*; Accession number AK103906), starch synthase I (*OsSSI*; Accession number D16202), starch branching enzyme I (*OsSBEI*; Accession number EF122471), starch branching enzyme IIb (*OsSBEIIb*; Accession number D16201), starch debranching enzyme isomerase II (*OsISA2a*; Accession number AC132483), starch phosphorylase H (*OsPhoH*; Accession number EF576564), disproportionnating enzyme II (*OsDPE2*; Accession number NM001067082), glucane-water dikinase (*OsGWD*; Accession number AK103463), and phosphoglucane-water dikinase (*OsPWD*; Accession number FN179404) genes were designed as primers. Also, 18s rRNA primer

Table 3. Plant height, seed fertility, 100-seed weight and total seed grain in HJ and PT 1 rice genotypes at booting stage when grown under 0 and 150 mM NaCl salt stress prior to seed harvesting.

Varieties	NaCl (mM)	Plant height (cm)	Seed fertility (%)	100-seed weight (g)	Total seed grain (g)
HJ	0	94.0a	71.5a	2.6	14.4b
	150	84.8b	57.7b	2.3	7.2d
PT 1	0	88.0b	55.8c	2.3	27.0a
	150	67.8c	25.9d	2.2	9.3c
ANOVA		**	**	^{NS}	**

Different letters in each column show significant difference at $p \leq 0.01$ by Duncan's New Multiple Range Test (DMRT). ^{NS} and ** are represented non significant and significant difference at $p \leq 0.01$, respectively.

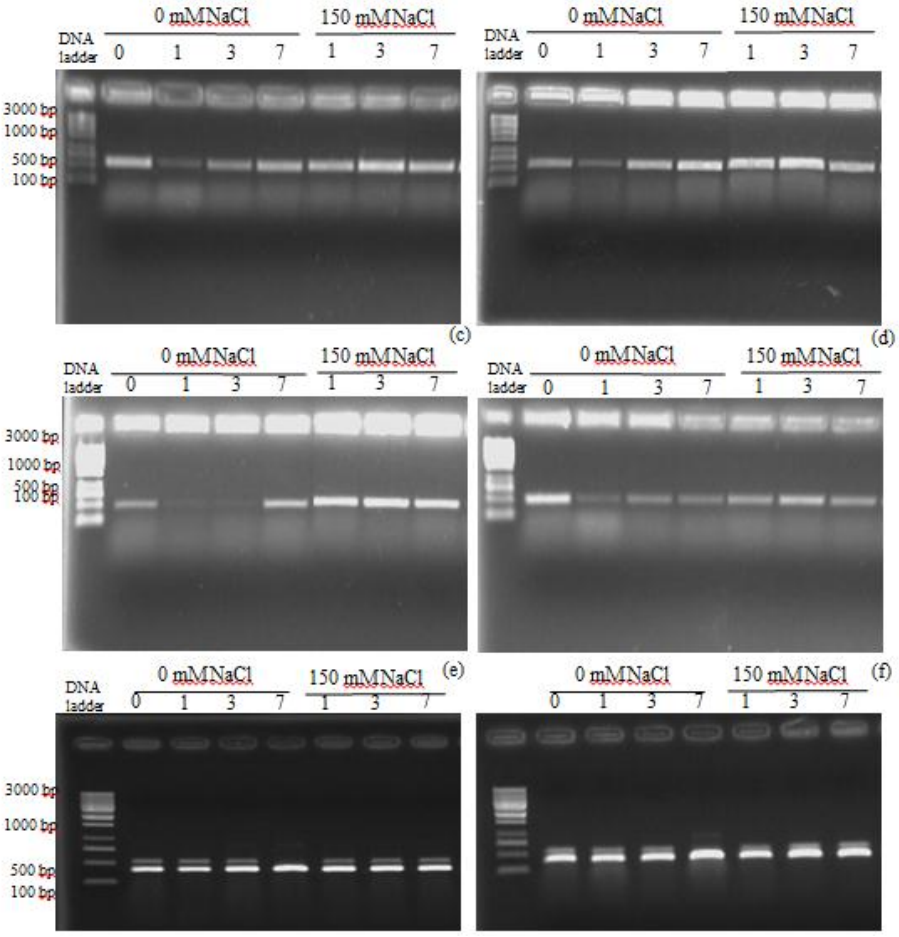

Fig 3. Semi-quantitative RT-PCR results of ADP-glucose pyrophosphorylase (*AGPase*) large subunit 1 in HJ (a) and PT1 (b), glucan water-dikinae (*GWD*) in HJ (c) and PT1 (d), and 18s *r*RNA in HJ (e) and PT1 (f) rice genotypes at booting stage when exposed to 0 and 150 mM NaCl for 0, 1, 3 and 7 days.

(Accession number AK105009) was use as house keeping gene (Table 1 Supplementary).

Gene expression analysis by semi-quantitative PCR

The PCR reaction was performed using a Veriti® Thermal Cycler (Applied Biosystems, CA, USA). All primer sequences and their annealing temperature are given in Table 1 (Supplementary). The PCR reaction was performed with 70-100 ng total RNA, 10 pM primer and EmeraldAmp® GT PCR Master Mix (Takara, Japan). The PCR profiles using conditions were: 94°C for 3 min, 18-37 cycles of 94°C for 30 s, 56-67°C for 30s, 72°C for 30s and 72°C for 5 min. The conditions and cycle numbers were determined in order to avoid saturation of DNA amplification. The DNA obtained was subjected to agarose gel electrophoresis and stained with ethidium bromide. The signal intensity of the stained bands was photographed with a Gel Doc image analysis system (Bio-Rad, Hercules) and the data were analysed using GeneTools™ (Syngene, Cambridge, UK) analysis software.

Starch and total soluble sugars determination

Starch content in flag leaf tissues were determined with an EnzyChrom assay kit (BioAssay Systems, Hayward, CA), using an enzymatic colorimetric method, which was a 2-phase method to quantify the concentration of both soluble and resistant starch (McCleary and Monaghan, 2002). One hundred milligrams of leaf sample was ground and the sugars extracted with 1 mL of 90% ethanol at 60°C for 5 min with triple repeats. After ethanol evaporation, soluble and insoluble starch was dissolved in 0.5 mL water at 60°C for 5 min and then mixed with 0.2 mL DMSO at 60°C for 5 min. Concentrations of soluble and insoluble starch were

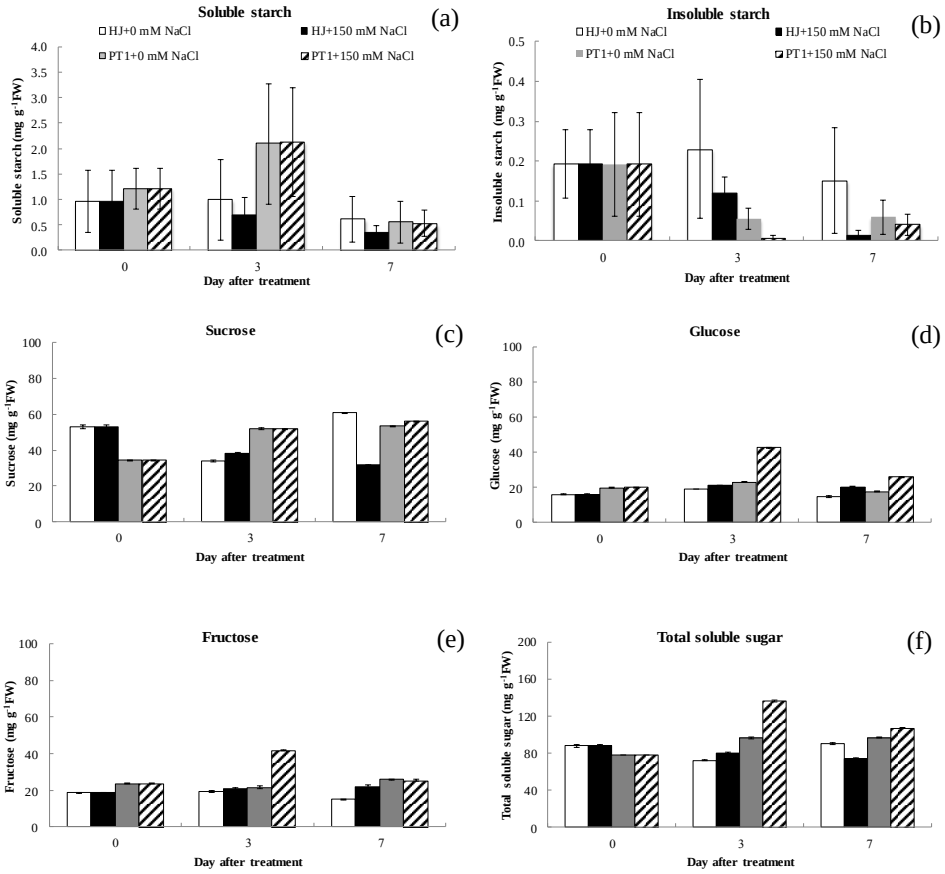

Fig 4. Soluble starch (a), insoluble starch (b), sucrose (c), glucose (d), fructose (e) and total soluble sugars (f) in booting stage of HJ and PT 1 rice genotypes when exposed to 0 and 150 mM NaCl for 0, 3 and 7 days. Error bars represent ±SE.

determined by colorimetric measurement of glucose residue after digestion using amyloglucosidase and α-amylase. Total soluble sugars (sucrose, glucose and fructose) in flag leaf tissues were analyzed according to the modified method of Karkacier et al. (2003). In a pre-cooled mortar, 100 mg fresh weight tissue was ground with liquid nitrogen, extracted with 1 mL nanopure water, vigorously shaken for 15s, sonicated for 15 min and then centrifuged at 12,000 rpm for 15 min. The supernatant was filtered through a 0.45 µm membrane filter (VertiPrure™, Vertical®) and stored at –20°C, prior to the measurement of total soluble sugar content using high performance liquid chromatography (HPLC). A volume of 40 µL crude extracts was automatically injected into the HPLC system fitted with a Waters 600 pump (Water, Milford, MA, USA). The analytical column was a MetaCarb 87C equipped with a guard column Deionized water was used as the mobile phase with a 0.5 mL min^{-1} flow rate. Online detection was performed using a Waters 410 differential refractometer detector and the data analyzed by Empower® software. Sucrose, glucose and fructose (Fluka) were used as standards.

Physiological characteristics, growth performances and yield components

Chlorophyll a (Chl_a), chlorophyll b (Chl_b), and total chlorophyll (TC) concentrations were determined following the method of Shabala et al. (1998), and total carotenoid (C_{x+c}) concentrations were determined following the method of Lichtenthaler (1987). One hundred milligrams of flag leaf material was placed in a 25 mL glass vial, along with 10 mL of 95.5% acetone, and blended using a homogenizer. The glass vials were sealed with parafilm® to prevent evaporation and then stored at 4°C for 48 h. The extract was centrifuged at 14,000 ×*g* for 5 min. The absorbance of the supernatant was read at 662 nm and 644 nm (for Chl_a and Chl_b) and at 470 nm (for C_{x+c}), against a blank of 95.5% acetone, using a UV visible spectrophotometer (DR/4000, HACH, Loveland, CO, USA). Chlorophyll fluorescence emission from the adaxial surface of the flag leaf was monitored with a fluorescence monitoring system (FMS2, Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini et al. (1999). A leaf, adapted to dark conditions for 30 min using leaf-clips, was initially exposed to the modulated measuring beam of a far-red light (LED source with typical peak at wavelength 735 nm). The original (F_0) and maximum (F_m) fluorescence yields were measured under weak modulated red light (<0.5 µmol $m^{-2}s^{-1}$) with 1.6s pulses of saturating light (>6.8 µmol $m^{-2}s^{-1}$ PAR) and autocalculated using FMS software. The variable fluorescence yield (F_v) was calculated by the equation of F_m–F_0. The ratio of variable to maximum fluorescence (F_v/F_m) was calculated as maximum quantum yield of PSII photochemistry. The photon yield of PSII (Φ_{PSII}) in the light was calculated as $\Phi_{PSII} = (F_m'\text{-}F)/F_m'$ after 45 sec of illumination, when a steady state was achieved (Maxwell and

Johnson, 2000). The net photosynthetic rate (P_n; μmol $m^{-2}s^{-1}$), transpiration rate (E; mmol $m^{-2}s^{-1}$), stomatal conductance (g_s; μmol H_2O $m^{-2}s^{-1}$) and water use efficiency (WUE) were measured using a Portable Photosynthesis System with an Infra-Red Gas Analyzer (Model LI 6400, LI-CORR Inc., Lincoln, Nebraska, USA). E and g_s were measured continuously by monitoring the content of the air entering and existing in the IRGA headspace chamber according to Cha-um et al. (2007). Plant height, seed fertility, 100-seed weight and total seed weight were recorded according to Cha-um et al. (2010).

Experimental design

The experiment was arranged as 2×2 factorial in a completely randomized design with 5 replicates (*n*=5). The mean values obtained were subjected to ANOVA and then compared by Duncan's New Multiple Range Test (DMRT) using SPSS software (ver.: 10.0, SPSS Inc., Illinois, USA).

Conclusions

The present study concludes that in HJ genotypes enhance of starch metabolism related genes, *AGPL1*, *AGPS2b* and *SBEIIb*, and photosynthetic abilities act as trigger resulting in sugar accumulation for salt defense mechanism, and leading to maintain water use efficiency, growth and yield traits under salt stress. In contrast, the expression of *AGPL1*, *AGPLS2b*, *SBEIIb*, and *GWD* genes in salt-stressed PT1 was up-regulated relating to enriched soluble sugar level. Therefore, the photosynthetic abilities in salt-stressed PT1 declined significantly leading to growth inhibition and yield reduction.

Acknowledgements

The authors are grateful to National Center for Genetic Engineering and Biotechnology (BIOTEC) for funding the research. Mr Thanaphol Boriboonkaset is thankful to Thailand Graduate Institute of Science Technology (TGIST; Grant number TG-22-14-52-005D) for as a PhD scholarship.

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