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Regulation of sucrose synthase and its association with grain filling in spermine-treated rice plant under water deficit

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ABSTRACT

Spermine (SPM) was utilized to investigate its impact on the accumulation of sucrose synthase (SUS) and the role of SUS as a predictor of sink strength of rice under cyclic water stress. Treatments which consisted of control and SPM were arranged in a randomized complete block design. Biochemical analyses showed significantly higher contents of sucrose, starch and carbohydrate in SPM-treated panicles which pointed to increased loading of sucrose into the grains or conversion of sucrose into starch. Besides, the expression of SUS gene was up-regulated in both inferior and superior spikelets at twenty and three fold, respectively. Correspondingly, SUS enzyme showed an increase in its activity. The high expression of SUS3 during grain filling is linked to the increased capacity for starch synthesis. Grain weight and grain filling rate of SPM-treated spikelets improved due to their large sink capacity and increased number of spikelets per panicle.

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KEYWORDS
Grain filling; rice; sink strength; spermine (SPM); sucrose synthase (SUS); water stress

Introduction

Water deficit stress or drought-related stresses are yield limiting factors for all plants including rice, *Oryza sativa* L. which is a major cereal crop. It is consumed as a staple food by at least 3 billion of the human population especially in the Asian region (Fairhurst and Dobermann 2002). Rice in this region is predominantly grown using flooded irrigation system whereby water is the main limiting factor for increased production of rice. It was estimated that more than 200 million tonnes of rice yield per year are lost due to different abiotic stress factors (Chen and Murata 2002). In addition, the cultivated area in Asia is likely to decrease due to increased demand for non-agricultural use, while the population keeps increasing. As a result, the demand for food especially rice will increase tremendously every year but hectarage of paddy parcel and planted area for wetland paddy would still remain the same or probably reduced due to land usage competition with other sectors (Ministry of Agriculture and Agro-Based Industry, Malaysia 2014).

Water scarcity is a major limitation in plant productivity as it affects physiological processes associated with growth and yield. The effect of water stress may vary with the variety, degree and duration of water stress and the growth stage of the rice crop (Adejare and Unebesse 2008). Drought stress at critical period such as booting stage for instance, can cause serious reduction in yield and quality of rice (Pantuwan et al. 2002). It has been shown that moisture stress of the rice reduced tillering and thereby reduced yield. When moisture stress was extended into reproductive phase, yield loss was significant (Sokoto and Muhammad 2014) which might be due to acceleration of flag leaf senescence (Kariali et al. 2012)

Polyamines (putrescine, spermine and spermidine) are plant growth regulators that are made of organic molecules containing amino groups (Ahmad et al. 2012). In plants, spermine (SPM) have been associated with regulating many physiological processes, such as organogenesis, embryogenesis, floral initiation and development, leaf senescence, fruit development and ripening, and abiotic and biotic plant stress responses (Alcázar et al. 2011; Ahmad et al. 2012). Exogenous application of polyamines was found to be effective in mitigating harmful effects of drought stress in some plant species (Saruhan et al. 2006; Zeid and Shedeed 2007; Ashraf et al. 2008).

Drought stress simultaneously affects many traits through morphological, physiological and metabolic modifications in all plant organs, finally leading to decreased net photosynthesis rate and grain yield, especially during grain filling period (Thameur et al. 2012). Growth enhancers have been implicated as key components in water deficit induced responses, including those triggered by drought, salinity, and low temperature. Growth enhancers not only altered stomatal opening but also increased root hydraulic conductivity (Hose et al. 2000). In addition to their role in protecting plants against potentially lethal stresses, growth enhancers help to maintain near-homeostasis of leaf water status when plants are subjected to mild water deficits or to changes in evaporative demand (Borel et al. 2001).

In order to achieve higher grain yield in rice, higher sink strength is one of the most important yield determining traits. Sink strength is the ability of sink organs to attract or import carbohydrate and in rice it is the filling up of the grains (Counce and Gravois 2006). Carbohydrate is primarily transported as sucrose in rice and most other higher plants (Duan and Sun 2005). Sucrose is actively loaded into the developing rice grains and subsequently converted into starch by a series of enzymatic steps (Mohapatra et al. 2011). It has been reported that the low activities of starch synthesis-related

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enzymes, such as sucrose synthase enzyme (SUS), ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), and starch branching enzyme (SBE), are closely associated with the inferior grain weight (Kato et al. 2007; Panda et al. 2009; Tang et al. 2009; Sekhar et al. 2015). Meanwhile, some of these enzymes have been shown to be differentially expressed at the transcript level in both inferior and superior caryopses during grain filling (Ishimaru et al. 2005). In general, caryopses spatially located on the apical primary branches (superior spikelets) flower earlier, show the highest grain filling, and achieve larger and heavier grains on a panicle, whereas caryopses located on the proximal secondary branches (inferior spikelets) flower relatively later and are either sterile or fill slowly (Ishimaru et al. 2005). In seeds, the most rapidly filling sink, SUS has the highest activity (Emes et al. 2003; Tetlow et al. 2004). Since SUS catalyzes the conversion of sucrose into UDP Glucose and Fructose (Panigrahi et.al., 2019) in the cytosol of endosperm cells of developing rice grains for subsequent starch synthesis, its activity can be an excellent indicator of sink strength (Sung et al. 1989: Fu et al. 2011).

To understand grain filling processes, the ability of a sink organ to attract or import carbohydrate in rice must be explored. Thus, this study was undertaken to elucidate the response of SPM on sink strength and sucrose synthase (SUS) enzyme in relation to the development of rice under water stress condition in a controlled environment. It could help in the understanding of the grain filling process that involves current assimilates from photosynthesis and assimilates redistributed from reserve pools in vegetative tissue.

Materials and methods

Experimental site and soil

An experiment was conducted between the months of August to November 2014 under the rain shelter facilities at Rice Research Centre, Field number 10, Faculty of Agriculture, Universiti Putra Malaysia. During the experimental period, monthly average maximum and minimum temperatures and relative humidity ranged from 25 to 29 °C and 70–85%, respectively. The 'Tok Yong' soil series having a clay loam texture (20.23% sand, 46.72% silt and 33% clay) with pH 5.3 and 2.2% organic carbon was used as cultivation medium. The soil was composed of 0.21% total N, 36 mg kg^{-1} available P and 67 mg kg^{-1} available K. The soil was obtained from rice growing area of Kampung Hutan Buloh, Melor, Kemubu Agricultural Development Authority (KADA) Kelantan, Malaysia (East Coast of Peninsular Malaysia).

Experimental design and treatments

The plants were arranged in a Randomized Complete Block Design (RCBD) with four replications. This experiment consisted of control and spermine (SPM) foliar treatments. The control treatment was prepared by adding 1% of Tween 20 (Sigma-Aldrich) to distilled water while the stock solution of SPM was diluted with distilled water to reach a final concentration of 70 µM of SPM according to the method described by Farooq et al. (2009). Spermine solution was then added with 1% Tween 20 (Sigma-Aldrich) that acted as a surfactant. These solutions were stored at 5°C before the time of application.

Neighbouring plants were shielded from the spray solution with large sheets of plastics. An average of about 100 ml of SPM solution was applied per hill by spraying the plants uniformly to the point of run-off using a Stihl type hand sprayer (USA) with constant flow. Both foliar treatments were applied at 35 and 55 days after sowing (DAS). The treatments were applied early morning (9.00-11.00 am) when the stomata were fully open and have sufficient time to absorb the treatment before light recommences (turgid state). The water-stress treatment was started on the 30th DAS for seven cycles (lasted 10 days in each where rewatering was done on the first day of each cycle) (Puteh et al. 2013; Nurul Amalina and Mohd Razi 2015).

Planting materials and plant establishment

The seeds of MR219 rice variety were obtained from Seri Merbok Sdn. Bhd., Kangkong, Alor Setar, Kedah, Malaysia. The seeds were soaked in water containing seed priming product from ZAPPA-PLUS (PeladangTech, Bangi, Selangor, Malaysia) and spread on wet tissue in a flat tray overnight. After two days of soaking, four seeds were sowed in each pot (390 width × 390 diameter × 350 mm height) containing approximately 17 kg of soil by direct seeding technique.

Plant maintenance

Fertilizer applications

Compound fertilizer that contained N:P:K in equal ratio was applied at 1.5 g pot^{-1} at 15 DAS while urea was applied at 0.4 g pot^{-1} at 35 DAS. In addition, at 50 and 70 DAS, N:P:K blue (12:12:17:2TE) was applied at 0.7 g pot^{-1} (MADA 2015).

Pests and disease management

Plant protection measures were necessary to avoid yield loss due to weeds, pests and diseases. In this experiment, visual inspection was carried out regularly while weeding was done manually by hand. The rain shelter wire mesh with the addition of hard wire mesh surrounding the rice plant served as barrier against the entry of rodents and birds. The water level was maintained at 5 cm to reduce weed infestation. The details of fungicides and insecticides used during the experiment are shown in Table 1.

Data collection

A total of 315 panicles that flowered on the same day were chosen and tagged in each treatment (Figure 1). Three hundred of these tagged panicles were sampled at 3, 6, 9, 12 and 15 days after post anthesis (DPA) for determination of starch content,

Table 1. Details of fungicides and insecticides used during the experiment in the rain shelter.

Days after sowing (DAS)	Fungicide	Rate/class	Insecticide	Rate/class
30	Top-sin (Thiohanate-methyl)	28 g/10 liter, Class IV	Decis (Deltamethrin)	20 ml/10 liter, Class III
40	Kencozeb (Mancozeb)	25 g/10 liter, Class IV	Armada (Chlorpyrifos)	10 ml/10 liter, Class II
50	Thiram (Thiram)	20 g/10 liter, Class III	Confidor (Imidacloprid)	25 ml/10 liter, Class III
60–120	Fungicide and pesticide applied repeatedly every 10 days			

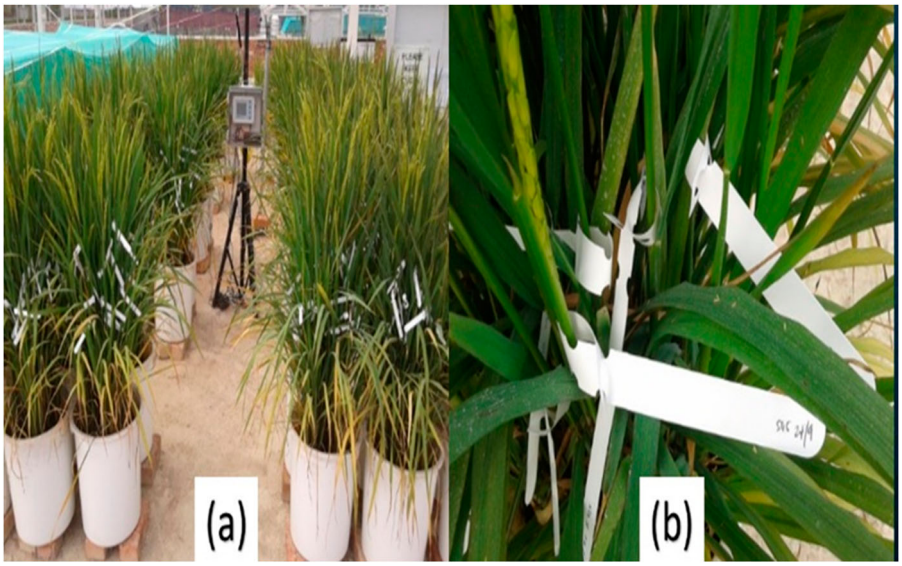

Figure 1. General steps involved in the assays of sucrose, starch content, SUS activity and SUS3 gene expression (a) glasshouse conditions (b) Tagging panicles.

sucrose content, sucrose synthase enzyme activity and grain dry weight (together with grain filling rate) from superior spikelets. Seventy-five panicles were used for each of these quantification. Superior spikelets were collected from upper halves (3 halves from upper spikelets) of each panicle as described by Mohapatra et al. (2011). The remaining 15 tagged panicles were sampled at 15 DPA for SUS 3 gene expression in superior and inferior spikelets (3 halves from upper and lower spikelets as described by Mohapatra et al. 2011). The samples were immediately frozen in liquid nitrogen and stored at –80° C until use, except for the samples used for determination of starch content, sucrose content and grain dry weight (together with grain filling rate) which were assayed and analyzed immediately after sampling.

Soil water content

The soil water content (HH2 Moisture Meter, Delta-T, UK) and stomatal resistance (SC-1 Leaf Porometer, Decagon, USA) obtained from inverse of stomatal conductance were taken continuously for 10 days at reproductive stage.

Starch content

The starch content was determined acccording to the method described by Thayumanavan and Sadasivam (1984). The panicles were dried at 60°C for 3 days before starch assay. The dry superior spikelets were detached from the panicles and ground to powder under liquid nitrogen. Briefly, the ground powder (0.5 g) was extracted with 10 ml of 80% ethanol (Merck) at 80°C for 30 min. Supernatants of the same treatment were pooled and used for soluble sugar analysis. The residue was digested with 52% perchloric acid (Across) and used for estimation of starch at 630 nm (Shimadzu UV-1800 Spectrophotometer). Glucose was used as a standard while starch content was expressed as mg glucose per gram of dry weight sample (mg g^{-1} dwt).

Sucrose content

Sucrose content was determined according to the method of Edward (1954). The ground powder (0.5 g) was extracted with 4 ml of Anthrone reagent (Merck) at 100°C for 10 min in a water bath. The absorbance of the sample was measured at 620 nm using a spectrophotometer (Shimadzu UV-1800 Spectrophotometer) where sucrose was used as the standard. The sucrose in the grain was expressed as mg sucrose per gram of dry weight sample (mg g^{-1} dwt).

Sucrose synthase enzyme activity

Collected samples for the assay were immediately frozen in liquid nitrogen and stored at –80 °C until needed. The frozen superior spikelets were detached from the panicles and then powdered in liquid nitrogen for SUS activity assay. Enzyme extract was prepared as described by Huber (1986) and Counce and Gravois (2006). About 500 mg of powdered spikelets were homogenized in a pre-cooled mortar containing 4 ml of extraction buffer which consisted of 50 mM HEPES-NaOH (pH 7.5), 5 mM $MgCl_2$, 2.5 mM dithiothreitol (DTT), 1 mM EDTA, 1% bovine serum albumin, and 0.6% (w/v) insoluble polyvinylpyrrolidone. After centrifugation at 12 000 g for 10 min, the supernatant was used for the assay of SUS enzyme. An aliquot of 0.4 ml supernatant was incubated with 0.4 ml of 50 mM HEPES-NaOH (pH 7.5), 14 mM $MgCl_2$, 0.1 ml uridine diphosphate (UDP)-glucose, 0.1 ml 100 mM fructose for one hour at 30°C and the reaction was terminated by adding 1N NaOH in boiling water. The mixture was then centrifuged at 8000 rpm for 5 min. Another 0.4 ml of aliquot was added to 1% of ethanolic resorcinol and 1.5 ml 30% HCl and incubated at 80°C for 10 min. The absorbance of the sample was measured at 520 nm (Shimadzu UV-1800 Spectrophotometer). The enzyme activity was expressed in milimoles of sucrose synthase per gram fresh weight per minute (mmolgfwt^{-1} min^{-1}).

Grain dry weight, grain filling and grain filling rate

Grain weight per grain was weighed manually after drying for 3 days at 60°C by using a digital balance (QC 35EDE-S Sartorius, Germany). The percentage of filled grains per panicle was derived from the ratio of the number of fully ripened grains (filled grains) to the total number of grains per panicle per average hill. The grain filling rate was computed by dividing the maximum grain weight by the duration of the grain filling period (Fu et al. 2011). All parameters were determined at successive growth stages from 3 to 15 DPA at 3 days intervals.

Quantitative real-time PCR (qPCR)

Fifteen tagged panicles were sampled at 15 DPA for the expression of sucrose synthase 3 gene (EC 2.4.1.13 SUS) or *SUS3* gene. Spikelets were immediately frozen in liquid nitrogen and stored in −80°C freezer. Frozen superior and inferior spikelets were detached from the panicles and finely powdered under liquid nitrogen. Total RNA was isolated and transcribed with oligo (dT) primers using Super Script first-strand synthesis system according to the manufacturer's instruction (KAPPA BIOSYSTEMS, USA). The optimum temperature and standard curve for each gene was analyzed by Thermal Cycler T100 (Bio-Rad, USA). Transcript levels of selected genes were measured by CFX384 Touch Real-Time PCR Detection System (Bio-Rad, USA) with KAPA SYBR® Fast qPCR kits (KAPA BIOSYSTEMS, USA). The data were normalized to the amplification of the rice *ACTIN2* and 18S rRNA genes. For each sample, the mean value from three qRT-PCRs was used to calculate the transcript abundance. The primer details were as follows: *SUS3* (*Forward: 5′ CGGTGAAAAGAATGGGCAATG 3′ and reverse: 5′CCATGAAAAGGCCAGAGCAT 3′*), 18S rRNA (*Forward: 5′ TCCATTGAAGGGCAAGTCTGG 3′ and reverse: 5′ CTTGGCAAATGCTTTCGCAG 3′*) and ACTIN2 (*Forward: 5′ CAATGTGCCAGCTATGTATGTCGCC 3′ and reverse: 5′ TTCCCGTTCAGCAAGTGGTAGTGAAG 3′*) (Yamakawa et al. 2007; Zhu et al. 2011). Comparative method of CT was used to calculate the relative expression of gene ($2-^{\Delta\Delta CT}$; Schmittgen and Livak 2008).

Carbohydrate content

Total carbohydrate content was measured according to method described by AOAC (1990) based on the grain and biomass at maturity, whilst quantification of carbohydrate is carried out by proximate analysis. The sample was ground and sieved through a 20 micron mesh then dried in an oven at 98–100°C. The moisture content was determined by drying the samples at 105°C for 8 h. The nitrogen and protein contents were calculated by multiplying percentage of N by the factor of 6.25. To evaluate the lipid content, moisture free samples were run for 2 h until fat was extracted. The ash content was determined by placing samples in pre-weighed crucible and then placed in a muffle furnace at 550°C for 4 h. Percentage of carbohydrate was determined by the following formula:

$$(100 - \%\text{moisture} - \%\text{protein} - \%\text{lipid} - \%\text{mineral}).$$

Yield attributes

At 115 DAS, all plants were harvested. The grain weight and yield components were determined after drying (72 h, 60°C). The grain yield was based on the weight of filled grains per hill and expressed in grain per hill (g hill^{-1}). The grain yield was determined using digital balance (QC 35EDE-S Sartorius, Germany). Panicle per hill, grain number per panicle and percentage of filled grain per panicle were counted and calculated manually. The thousand grains weight (g) was also obtained using the same balance.

Ten panicles bearing tillers from each treatment were sampled. Prior to weighing the grains, fully filled grains were separated from the unfilled grains manually. The percentage of filled grains per panicle was derived from the ratio of the number of fully ripened grains (filled grains) to the total number of grains per panicle per average hill. Thus, the total percentage of filled grains was calculated using the following formula (1):

$$\text{Percentage of filled grain per panicle} = \frac{\text{Number of filled grains}}{(\text{Filled} + \text{Unfilled grains})} \times 100\%. \quad (1)$$

The partitioning of dry matter between grains and vegetative part is indicated by harvest index. Harvest index was calculated as the ratio of grain dry weight to the total weight (2);

$$\text{Harvest Index} = \frac{\text{Grain dry weight (economic yield)}}{\text{Total dry weight of plant (biological yield)}}. \quad (2)$$

The function for determination of both percentage of filled grain and harvest index was followed accordingly to methods by Yoshida (1981).

Statistical analysis

All data were statistically analyzed using SAS software (Windows version 9.1, SAS Institute, Cary, NC, USA). Two-sample *t*-test at $P \leq 0.05$ was used to test significant differences among treatments. Relationships between parameters were determined using Pearson's simple correlation test.

Results

Soil water content and stomatal resistance during reproductive stage

The soil water steadily decreased from 80 to 30% during the 10 days of water stress inducement at reproductive stage (Figure 2(a)). Meanwhile, stomatal resistance in both treatments ranged from 2.0–5.0 sm^{-1} as recorded from the inverse data of stomatal conductance (Figure 2(b)). At day 10 of the treatment, SPM reduced the resistance by 37%.

Starch content

SPM significantly increased the starch content by 13, 33, 39, 6 and 31% compared to control at 3, 6, 9, 12 and 15 DPA, respectively (Figure 3).

Sucrose content

SPM significantly ($P \leq 0.05$) increased the sucrose contents in rice grain at 3, 12 and 15 DPA (Figure 4). However, no significant increment was recorded at 6 and 9 DPA. The treatment resulted in relatively higher sucrose contents with 22, 19 and 48% increment at 3, 12 and 15 DPA, respectively indicating that more sucrose load directed to the grains or got converted into starch.

Sucrose synthase enzyme assay

SPM treatment significantly increased the concentration of SUS enzyme activity in rice grain by 11.9 and 13.6 mmol gfwt^{-1} min^{-1} compared to control which were 3.29 and 11.2 mmol gfwt^{-1} min^{-1} at 15 and 35 DPA, respectively (Figure 5). There were no differences in the SUS activities between the control and SPM measured at 3–12 DPA.

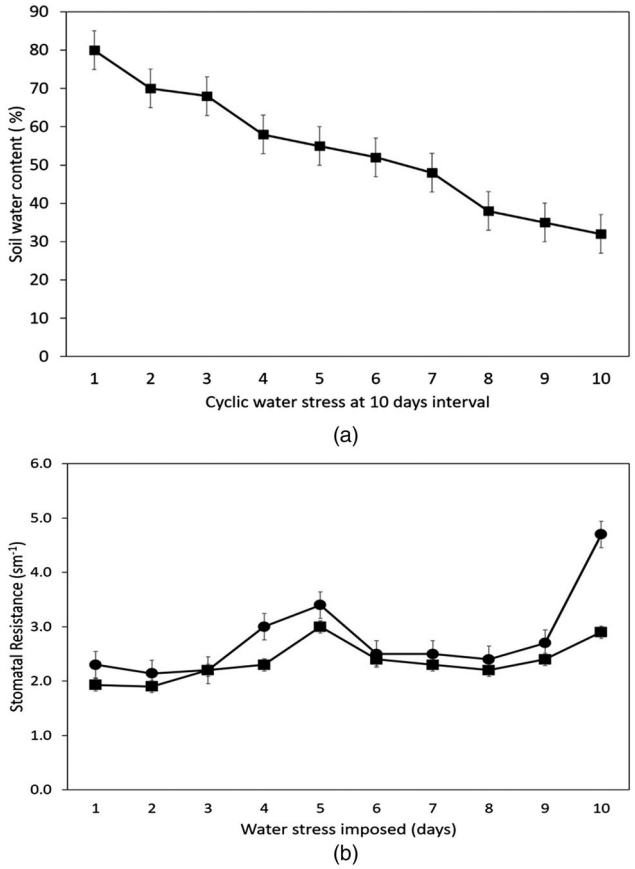

Figure 2. (a) Changes in soil water content and (b) stomatal resistance in the uppermost leaf during 10 days water stress inducement at reproductive stages (80-90 DAS). Symbols represent: ● Control and ■ Spermine.

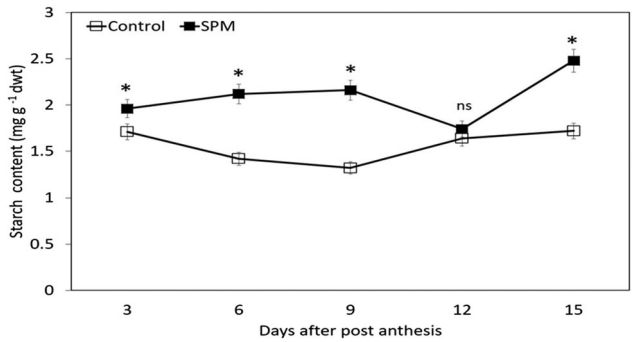

Figure 3. Effect of SPM on starch content at 3, 6, 9, 12 and 15 DPA in the rice grains. SPM: spermine, *: Significant at $P \leq 0.05$ according to Fisher's least significant difference (LSD) and ns: not significant.

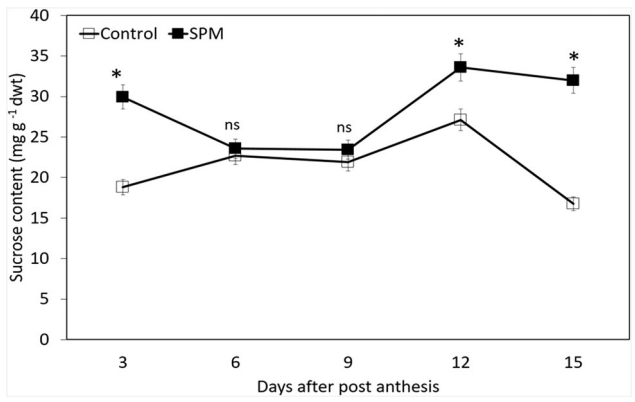

Figure 4. Effect of SPM on sucrose content at 3, 6, 9, 12 and 15 DPA in the rice grains. SPM: spermine, *: Significant at $P \leq 0.05$ according to Fisher's least significant difference (LSD) and ns: not significant. Vertical bar represents standard deviation of mean.

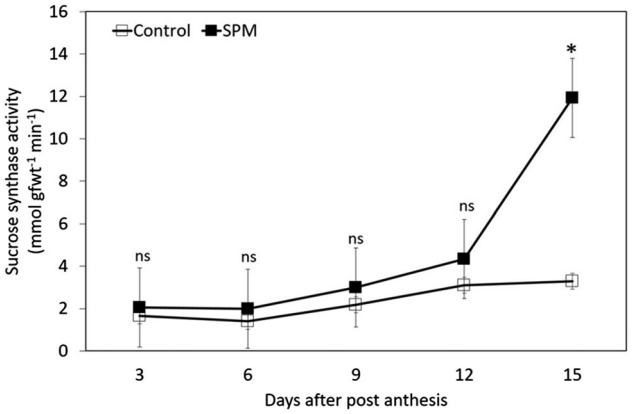

Figure 5. Effect of SPM on sucrose synthase enzyme activity at 3, 6, 9, 12 and 15 DPA in the rice grains. SPM: spermine, *: Significant at $P \leq 0.05$ according to Fisher's least significant difference (LSD) and ns: not significant.

Grain dry weight

Grain dry weight was significantly ($P \leq 0.05$) increased under the influence of SPM treatment as shown in Figure 6. The grain dry weight increased proportionally with the advancement of DPA in both control and SPM-treated plants. Dry weight of grains treated with SPM increased by 6, 39, 37 and 27% at 6, 9, 12 and 15 DPA respectively compared to control, indicating that the development of grain showed positive increment of grain weight for prevailing water stress.

Grain filling rate

There were significant differences ($P \leq 0.05$) in grain filling rate between treatments whereby the SPM significantly increased grain filling rate at 6, 9, 12 and 15 DPA by 26, 49, 35 and 25%, respectively compared to control (Figure 7). Apparent grain filling acceleration was obtained by SPM treatment particularly at 3–15 DPA. An exponential phase was clearly seen at 12–15 DPA in both the untreated and SPM-treated samples. Reduction of grain filling rate was obviously seen at 3–9 DPA in the control plant but such trend was absent in SPM treatment.

Total carbohydrate content in the grain and biomass at maturity

The total carbohydrate content was significantly higher in the grain treated with SPM (74.4%) and lowest in the biomass

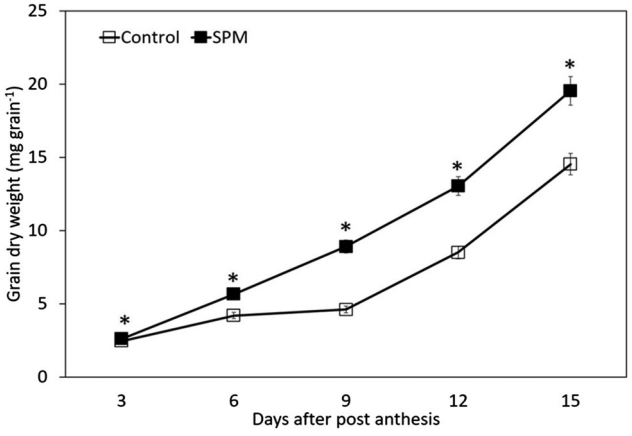

Figure 6. Temporal changes in grain dry weight at 3, 6, 9, 12 and 15 DPA in the rice grains. SPM: spermine and *: Significant at $P \leq 0.05$ according to Fisher's least significant difference (LSD).

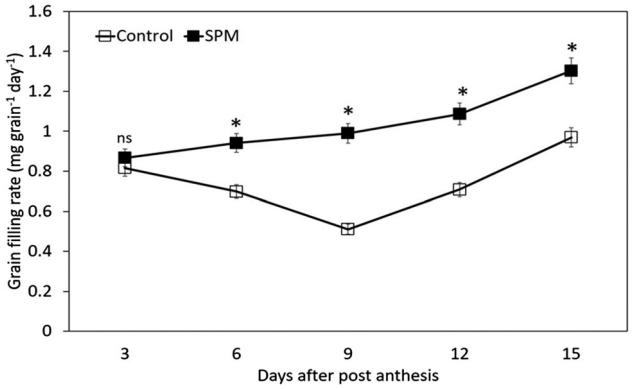

Figure 7. Changes in grain filling rate in the control and SPM-treated plants at 3, 6, 9, 12 and 15 DPA. SPM: spermine, *: Significant at $P \leq 0.05$ according to Fisher's least significant difference (LSD) and ns: not significant.

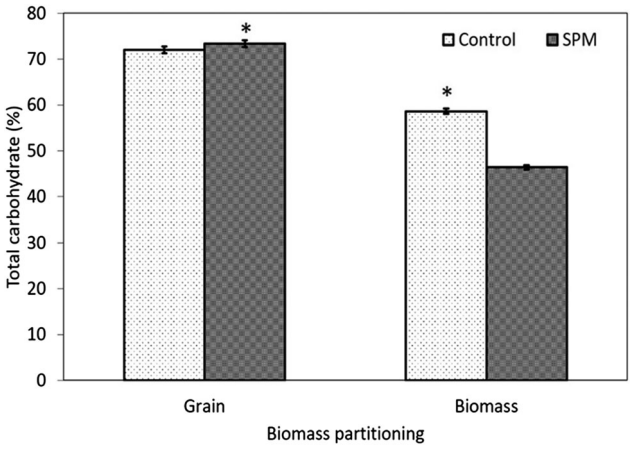

Figure 8. Total carbohydrate content in the grain and biomass of rice plants at maturity. SPM: spermine and *: Significant at $P \leq 0.05$ according to Fisher's least significant difference (LSD).

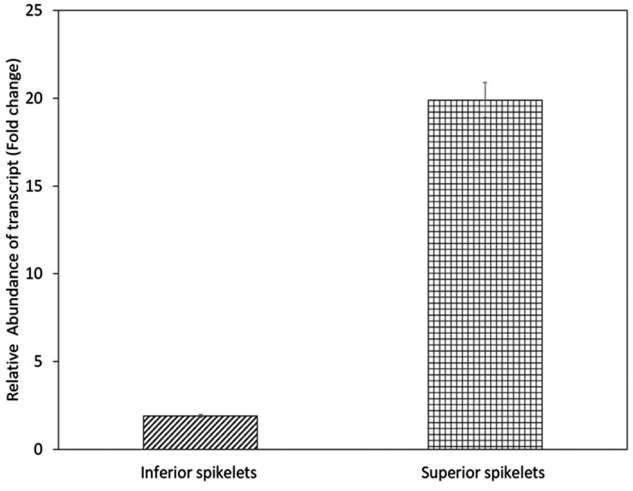

Figure 9. Fold change in inferior and superior spikelets in SPM treatments at 15 DPA in the rice grains for SUS3 gene expression.

(46.4%) compared to control (Figure 8). In addition, 2% increment was observed in the grain and 21% reduction in the biomass production with SPM treatment compared to the control.

Gene expression of sucrose synthase enzyme 3 gene (SUS3)

The gene expression of SUS3 in superior spikelets in SPM treatments was significant ($P \leq 0.05$) at the ripening stage (Figure 9) compared to the inferior spikelets. Transcripts of SUS3 in superior spikelets of SPM-treated grains increased by twenty folds as compared to control due to an increase in starch and carbohydrate content. The high expression of SUS3 during grain filling in SPM-treated grains could be closely linked to the increased capacity for starch synthesis.

Yield attributes

The grain weight of SPM-treated plant (14.7 g per hill) was remarkably higher than control (13.1 g per hill) with an increment of 11% (Table 2). There were also significant increases in the number of grains per panicle (15%) and grain filling percentage (2.3%) under SPM treatment. The thousand grain weight of SPM-treated plants showed 15% increase as compared to control, which weighed at 20.6 g only. Tiller number and biomass per hill in both treatments showed minimal differences. Harvest index improved under SPM treatment, indicating greater yield production over biomass partitioning with a 12% increment. Figure 10 shows the relatively superior morphological features of the panicle in SPM-treated plants grown under water stress condition.

Table 3 shows that SUS, sucrose, starch, carbohydrate contents, grain filling rate and grain weight at maturity were all significantly correlated under SPM treatment. In general, higher biochemical compositions such as sucrose and starch would be manifested into greater grain filling rate as well as grain weight. All the parameters showed significant positive relationship.

Discussion

Water deficit stress or water shortage due to unpredictable weather has made it increasingly difficult to realize the potential yield of high yielding rice varieties while negatively impacting photosynthesis, thus rice production. Under water deficit, cell enlargement decreases, leading to growth inhibition and reproductive failure (Lisar et al., 2012). Stomata closure is the first responsive event of plants to water deficiency that is commonly regulated by growth enhancers (Farooq et al. 2009; Ashraf 2010). Water stress simultaneously affects many traits through morphological, physiological and metabolic modifications in all plant organs leading to decreased grain yield (Thameur et al. 2012). Since breeding programmes are long and exhaustive, short-term and cost effective measures are absolutely necessary to mitigate water stress.

Table 2. Morphological features of the panicle and biomass at maturity.

Treatments	Tiller no. (hill^{-1})	Grain number (panicle^{-1})	Filled grains (%)	1,000 grain weight (g)	Grain weight (ghill^{-1})	Biomass (ghill^{-1})	Harvest Index
Control	11ns	118	93.3	20.6	13.1	30.5ns	0.30
SPM	12ns	138*	95.5*	24.2*	14.7*	30.2ns	0.34*

Notes: Mean ± standard deviation ($n = 3$) SPM: spermine, *: Significant at $P \leq 0.05$ according to Fisher's least significant difference (LSD) and ns: not significant.

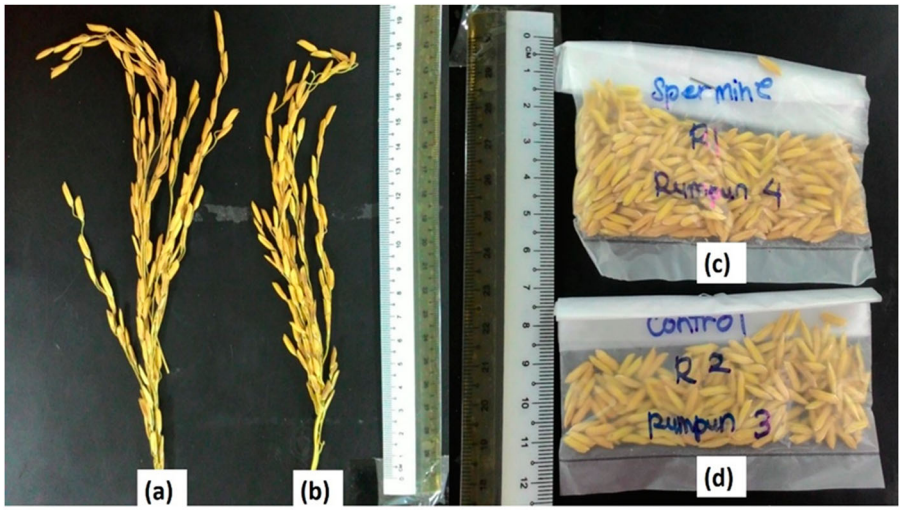

Figure 10. Morphological comparison of the panicles in (a) SPM-treated and (b) control and samples of filled grain per hill in (c) SPM-treated and (d) control.

Foliar application of growth enhancers that are capable of altering physiological and biochemical processes are most preferred for they could improve growth and yield of rice plant relatively quicker. The maintenance of turgor is in fact crucial for maintaining normal cell activity under water stress conditions (Ashraf 2010; Lisar et al., 2012). Spermine regulates stomatal responses by reducing their aperture and inducing closure (Alcázar et al. 2011). The ability of growth enhancers to alleviate water stress by stomatal closures and increased stomatal resistance to conserve water were reported by Farooq et al. (2009) and Zhang et al. (2008) which corroborated with results obtained in the current study.

Farooq et al. (2009) discovered that exogenous application of polyamines improved drought tolerance of rice plants, as measured by total dry mass and photosynthetic capacity under water stress. Assimilation rates in photosynthetic leaves increased due to enhanced photosynthetic metabolites and enzyme activity which directly improved grain productions (Lisar et al., 2012). In this study, SPM proved to be an effective growth enhancer as it increased the activity of SUS enzyme (Figure 5) which translated into enhanced grain filling and improved yield attributes under water stress which was in agreement with other studies (Cai et al. 2004; Liu et al. 2008; Sekhar et al. 2015).

Water deficit stress, especially at reproductive stage mostly affects filled grains and ultimately yield by increasing empty grains which may result in a lower yield of rice (Mohapatra et al. 2011; Ashraf et al. 2011). Grain filling or development of rice grains (fertilized ovaries develop into caryopses) depends on current assimilates from photosynthesis and assimilates reallocated from storage pools in vegetative tissue (Thameur et al. 2012). Poor filling of spikelets is due to source limitation (Murty and Murty 1982), sink size limitation (Kato 2004), imbalance hormonal levels (Yang et al. 2006; Zhang et al. 2008), low activities and/or gene expressions of enzymes involved in sucrose to starch conversion (Ishimaru et al. 2005; Wang et al. 2014) and assimilate transportation impediment (Yang and Zhang 2010). Remobilization and relocation of reserve assimilates in vegetative tissues to the grain in monocarpic plants such as rice and wheat involve the initiation of whole plant senescence (Asli and Houshmandfar 2011). Normally, water stress during grain filling period encourages early senescence and reduces the time for grain filling but enhances the remobilization of assimilates from straw to grains (Asseng and Van Herwaarden 2003; Thameur et al. 2012). Slowly filled grains can frequently be related to the delay of whole-plant senescence (Mi et al. 2002; Mohapatra et al. 2011).

Starch in rice grains contributes to about 90% of the final dry weight of an unpolished grain and sucrose is the main transported form of assimilates from source organs to sink organs (Yoshida 1972). In addition, sucrose is the major solute of phloem which is transported to the developing rice seeds. Sucrose partitioned into the endosperm is stored in the form of starch through starch biosynthesis pathway which is unique in cereal endosperm as the isoforms of enzyme required for starch biosynthesis in rice are not found in other cereal tissues or non-cereal plants (Geigenberger 2011). Additionally, optimal activity of enzymes processing the incoming sucrose for starch synthesis is required for grain filling. Grain filling and the grain quality of the inferior spikelets of the rice panicle can be affected by the activity of the enzymes of sucrose metabolism in the endosperm, and the enzyme action can determine the sink strength of the endosperm. Sucrose synthase enzyme (SUS) acts on the incoming sucrose as the first step of starch synthesis (Kato et al. 2007; Mohapatra et al. 2011). In rice, endosperm SUS activity is considered as a potential indicator of

Table 3. Pearson's correlation coefficients matrix among sucrose synthase enzyme, sucrose, starch, carbohydrate, grain filling rate and grain weight at maturity.

Variable	SUS	Sucrose	Starch	Carbohydrate	GFR	GW
SUS	1.0					
Sucrose	0.94**	1.0				
Starch	0.91*	0.85*	1.0			
Carbohydrate	0.89*	0.99**	0.81	1.0		
GFR	0.96**	0.99**	0.87*	0.98**	1.0	
GW	0.96**	0.99**	0.87*	0.98**	0.98**	1.0

Notes: SUS: Sucrose synthase enzyme activity, GFR: Grain filling rate, GW: Grain weight, $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.

high grain yield (Counce and Gravois 2006). Activity of the enzyme is low in the inferior spikelets compared to the superior spikelets (Mohapatra et al. 2011), and it is positively linked to the strength of the developing grain to accept sucrose (Yang et al. 2003)

The increase in SUS enzyme activity in the present study corroborated with increased expression of SUS gene which was in accordance with the findings of Cai et al. (2004), Liu et al. (2008) and Sekhar et al. (2015). Rice is formed mainly through the synthesis and accumulation of starch and protein. Interestingly, the increase in sucrose and starch accumulation in SPM treatments produced better hydrolysis of starch hence increased carbon remobilization which attributed to the enhanced sink activity by regulating key enzymes (SUS3) involved in sucrose to starch conversion (Counce and Gravois 2006; Yang and Zhang 2010; Stella et al. 2016).

Ishimaru et al. (2005) and Zhu et al. (2011) found that the patterns of gene expression of SUS in superior spikelets were higher from those of inferior spikelets which were evident in our study of SPM-treated plants. The results indicated that higher sink activity may have contributed to faster grain filling and increased grain weight for superior spikelets of rice whereas poor sink strength of inferior spikelets resulted in either small sink size or low activity especially for control plant. Therefore, sink size is mainly determined by the number of cells and the cell size of the endosperm. The results showed that the difference in endosperm cell weight was significant among treatments in superior and inferior spikelets.

In addition, we found that foliar application of SPM led to higher SUS activity in the grains at the earlier stage of grain filling in rice (Figure 5) that was closely related to a higher grain filling rate and starch accumulation. On the other hand, the inadequate supply of assimilate in control plant consequently delayed sucrose and starch accumulation whereas sucrose availability for translocation to the grains was reduced. These is postulated to be the main inhibitor for the slow and poor grain filling of the inferior spikelets as reported by Mohapatra et al. (2011), Dong et al. (2012), Lemoine et al. (2013) and Sekhar et al. (2015), particularly due to water stress. The synthesis of sucrose and starch as well as SUS activity during grain filling were totally interrupted when water stress was imposed which was in agreement with other studies (Zhu et al. 2011 and Sekhar et al. 2015). When re-watering took place, spikelets started to re-accumulate starch and sucrose, thus producing higher number of seeds. Similar finding was reported by Puteh et al. (2013) who worked with weedy rice.

Furthermore, the sucrose, starch and carbohydrate contents, SUS enzyme activity, grain weight and grain filling rate of SPM-treated spikelets improved due to their large sink capacity which was evident from the increased number of spikelets per panicle. The duration of measurements (15 days) was sufficient as it covered the rapid grain-filling phases and extended till maturation phase (Yang et al. 2000a; Yang et al. 2000b; Murchie et al. 2002). However, due to the biphasic nature of grain filling in MR219 variety, the spikelets of plants treated with SPM completed grain filling faster than control plants.

Moreover, the carbohydrate contents in grain was higher due to demand of larger sink in the grains (Figure 8) thus indicating that the increase in pre-anthesis carbohydrate reserves in the biomass could improve grain filling at maturity (Fu et al. 2011). Dai et al. (2009) and Jiang et al. (2003) reported that superior spikelets of wheat had higher starch accumulation rates and activities of enzymes including SUS which consequently produced much higher grain weight. It is proven that SPM does increase SUS activity and SUS3 expression. The increased SUS activity is the key to greater grain filling percentage. Since SUS is the main enzyme that is involved in sucrose cleavage in the rice grains, its activity is regarded as biochemical marker of sink activity. At maturity, the control plant had higher carbohydrate content in the stem but lower in the grains (Figure 8) due to early senescence that reduced photosynthetic rate and leaf area compared to SPM treatment. Similar results were reported by Kuanar et al. (2010), Ji et al. (2012) and Sekhar et al. (2015).

Greater harvest index with SPM treatment indicated that rice plant managed to overcome the impact of water stress through improvement of photosynthesis and better assimilates distribution. In addition, results above also supported previous findings (Tang et al. 2009; Kuanar et al. 2010; Fu et al. 2011) which showed that significant enhancement of the activities of starch biosynthesizing enzymes in rice panicles do influence the grain weight accumulations and also grain filling characteristics. The characteristic of grain filling depends on the supply of assimilates from source organs, as well as on the demand for assimilates within the embryonic tissues (sink activity), whereby both maternal and embryonic factors contribute to the maintenance of seed growth even under water stress (Lemoine et al. 2013).

Interestingly, Pearson's correlation coefficients matrix analysis showed that SUS enzyme production was highly correlated with the presence of sucrose, grain filling rate and grain weight. They are significantly associated to each other for better contribution to grain yield production. These results suggest the ability of SPM application to improve grain filling and SUS enzyme production whilst experiencing water stress at reproductive stage during grain filling periods.

Conclusion

The study has shown that the application of exogenous SPM promoted grain filling and increased grain weight. Spermine-treated plants was relieved of water stress by regulation of assimilate partitioning (sink strength) and relatively higher SUS enzymatic activity, bigger sink size, enhanced grain filling and yield production.

Disclosure statement

No potential conflict of interest was reported by the authors.

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