Agr. Nat. Resour. 53 (2019) 292–297

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Short-term water deficit stress induces anthocyanin accumulation and changes in grain yield and yield components in colored rice grain

Tepsuda Rungrat*, Choosakun Poothab

Department of Agricultural Science, Faculty of Agriculture Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand

Article Info

Article history:
Received 23 March 2018
Revised 1 August 2018
Accepted 21 September 2018
Available online 30 June 2019

Keywords:
Anthocyanin,
Alternate wetting and drying irrigation (AWD),
Colored rice,
Water deficit stress,
Yield components

Abstract

The effect of short-term water deficit stress was studied, on anthocyanin content and yield components in rice using a comparative study of three different water management regimes, continuous flooding (CF), safe-alternate wetting and drying irrigation (AWD) and critical-AWD, with the aim of gaining better understanding of the effectiveness of modified AWD treatments to increase the anthocyanin content in the Riceberry variety. The results showed that both the critical-AWD and safe-AWD conditions promoted a longer period to flowering than the CF condition by 7 d and 4 d, respectively, while plant growth slowed. The average grain yield per pot and the harvest index decreased significantly by decreasing the soil moisture content. The safe-AWD treatment had the highest water use efficiency (0.66 g/m^3) which was not significantly difference to critical-AWD. The total anthocyanin content (TAC) in unpolished colored rice grain progressively increased in response to decreasing water supply. The TAC of safe-AWD and critical-AWD plants was 2-fold and 4-fold, respectively, higher compared to well-watered plants.

Introduction

Rice (*Oryza sativa* L.) is one of the staple food crops worldwide (Li et al., 2004; Vijay and Roy, 2013). White rice has been the most common rice consumed by humans for many years (Li et al., 2004). In recent years, colored rice has become more popular for its health benefit due to its high nutritional value (Chunthaburee et al., 2016). Colored rice contains naturally occurring pigment called anthocyanin, which is one of the bioactive compounds with antioxidant benefit (Khoo et al., 2017). The potential health benefits of anthocyanins are to prevent cardiovascular disease (Rechner and Kroner, 2005; Cassidy, 2018), its anticancer (Wang et al., 2009) and anti-obesity (Kwon et al., 2007) properties and to improve visual health (Shim et al., 2012). The grain-filling stage is the most crucial step in the process of grain development and polyphenol accumulation (Ma et al., 2016). In wheat, anthocyanin levels increase rapidly during grain development and decrease before maturity (Knievel et al., 2009). In Thai colored rice varieties, research revealed that there is a rich source of natural anti-oxidative compounds for beneficial use as an active ingredient in cosmetics, functional foods and pharmaceuticals (Saewan and Vichit, 2015).

Anthocyanins are the primary pigment responsible for red, purple and blue in plants where they are synthesized by the flavonoid biosynthesis pathway (Aizza and Dornelas, 2011; Chunthaburee et al., 2016). It can present in many tissues, such as leaves, flowers, fruits and seeds (Khoo et al., 2017). Anthocyanin can be induced in

* Corresponding author.
E-mail address: tepsudar@nu.ac.th (T. Rungrat)

https://doi.org/10.34044/j.anres.2019.53.3.11

plants by several environmental factors, such as high salinity, excess light intensity, pH level, high or low temperature and soil moisture (Kovinich et al., 2015). Under direct drought stress, an increased anthocyanin level results mainly from photoprotection of chlorophyll by the xanthophyll cycle, reactive oxygen species scavengers and stress signals (Tahkokorpi, 2010; Kovinich et al., 2015). Therefore, it indicated that anthocyanin helps plants to cope with abiotic stress, most importantly preventing crop yield reduction.

A successful irrigation management called alternate wetting and drying irrigation (AWD) has been proven to effectively reduce water used in paddy fields (Price et al., 2013; Linquist et al., 2014; Lampayan et al., 2015). Under AWD, fields are subjected to alternate cycles of continues flooding and drying conditions. Irrigation using AWD is interrupted until the soil water content reaches a certain moisture level, after which the field is re-flooded. However, to date, it has not been widely adopted due to the potential for yield loss. In addition, the effect of short-term water deficit during the vegetative stage on the content of antioxidants (such as anthocyanin) remains unexplored, which could affect the anthocyanin accumulation in colored rice at different developmental stages of the plant. Thus, the current experiment investigated the effects of water stress including alternate wetting and drying irrigation on plant growth, anthocyanin accumulation and yield components in Thai colored rice.

Materials and Methods

Plant materials and growth conditions

Seeds of the Thai colored rice variety, Riceberry, were obtained from the Phitsanulok Rice Research Center, Thailand. The experiment was conducted in the greenhouse at Naresuan University, Thailand. The average air temperature and relative humidity (%RH) were 37 ± 3ºC and 60 ± 5 %RH during the day and 25 ± 2ºC and 85 ± 5 %RH at night. Seeds were sown on moistened filter papers placed on Petri plates. Germinated seedlings were transferred to a seedling tray with the soil water content maintained at 100%. The 21-day-old seedlings were transferred into plastic pots (25.4 cm in diameter and 30 cm high) containing 4 kg of clay soil with one plant per pot. A plastic tube (4 cm in diameter and 30 cm long) with holes around it was installed to observe the water level of each pot.

Five replications of the three irrigation treatments, continues flooding (CF), safe-AWD and critical-AWD, were laid out in a completely randomized design. Three water regimes were modified based on AWD techniques developed by the International Rice Research Institute (IRRI; Price et al., 2013). Plants were well-watered until day 20 after being transferred into pots. Thereafter, the treatments were applied. The CF condition kept the water depth at approximately 5 cm above the soil surface throughout the growing stage. The safe-AWD treatment kept the soil dry until the water depth was 15 cm below the soil surface (by observing the water level inside the tube) and then the pots were re-watered to a standing water depth of approximately 5 cm above the soil surface (Bouman and Lampayan, 2009). The critical-AWD condition was designed according to Carrijo et al. (2017) with some modification. It involved a more severe water deficit compared to safe-AWD. Critical-AWD kept the soil dry before re-watering. Briefly, the soil water level was observed (by checking the water level inside the tube) until it was lower than 15 cm from the surface for a few days or until rolled leaves appeared at level 4 (O'Toole and Moya, 1978). At this stage, the soil water content was 25% compared to the CF condition. The cycles of the AWD treatments were repeated until the appearance of the panicle initiation phase. During and after the flowering period, all plants of all treatments were well-watered to prevent yield reduction (Bouman et al., 2007).

Based on previous observations and measurements in the study of the impact of different irrigation treatments on the soil moisture content, plants under the CF, safe-AWD and critical-AWD treatments were maintained in the greenhouse at soil water contents before re-watering of 100%, 50% and 25%, respectively. The soil moisture content was maintained using the irrigation methods described previously. The irrigated water per pot was recorded to calculate the water use efficiency per pot.

Measuring yield components

Yield components were measured according to an IRRI protocol (Gomaz, 1972) with some modification. Five samples per treatment were selected for collecting data. Plant height was measured at the grain-filling stage. After harvest, the panicle length, number of grains and number of filled grains were manually measured for each panicle sample. The panicle length was measured using a centimeter ruler from the neck to the tip of each panicle. Grain was weighed for the 1,000 grains weight and collected grain yield per pot at 14% seed moisture. The harvest index (HI) and water use efficiency (WUE) per plot were calculated according to Howell et al. (2015), with some modification. The HI calculated as the ratio of grain weight and total biomass weight from each sample. The WUE was calculated from corrected grain weight per plot in grams / the total volume of irrigation water in cubic meters applied per pot in the period from transplanting to harvesting and expressed as grams per cubic meter.

Extraction of rice grain samples

The anthocyanin contents were extracted from the unpolished rice grains according to Pedro et al. (2016), with some modification. Unpolished rice grains were carefully ground into powder using a mortar and pestle. A portion (50 g) of each ground sample was extracted using 100 mL of HCl in ethanol (0.01 mL/mL) at room temperature for 1 hr. The extracts were filtered with Whatman® No. 1 filter paper. The clear extract samples then were stored at 4ºC for further analysis.

Determination of total anthocyanin content

The total anthocyanin content (TAC) was determined using the pH-differential method (Giusti and Wrolstad, 2001). The buffer systems were prepared as 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). Briefly, two sets of rice grain extract (20 μL for each set) were prepared. The first set was mixed with 180 μL of pH 1.0 buffer, and the second set was mixed with 180 μL of pH 4.5 buffer. These mixes were incubated for 30 min in the dark at room temperature before measuring the absorbance at 520 nm and 700 nm using a microplate reader. The TAC measured in milligrams per liter which was converted to milligrams of total anthocyanin content per 1 g of dry grain was calculated using Equation 1:

$$\text{Total anthocyanin content} = (A \times mW \times DF \times 1000) / (\varepsilon \times 1) \quad (1)$$

where, A is the absorbance of the diluted sample = $(A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$; MW is the molecular weight of cyanidin-3-glucoside = 449.2; DF is the dilution factor; 1 is the path length in centimeters, ε is the molar extinction coefficient of cyanidin-3-glucoside = 26,900; and 1,000 provides the conversion from grams to milligrams.

Statistical analysis

Results were expressed as the mean ± SD. Data were analyzed using one-way analysis of variance followed by Duncan's multiple range test at the 95% confidence level ($p < 0.05$). Statistical analysis was performed using the R software (RStudio Team, 2016).

Results and Discussion

Effect of water managements on growth and yield components

Water management significantly influenced plant development and most of the yield component parameters in the Riceberry variety as presented in Table 1. Short-term water deficit treatments (the AWD treatments) significantly affected the reproductive stage of rice. The number of days to flowering (DTF) increased significantly when water availability in the soil was limited at the prior to flowering stage. Under critical-AWD, the flowering time was delayed for 7 d compared to the CF condition. The results indicated that the number of days to flowering in Riceberry was responded dependent on any short-term water deficit stress. The results were consistent with Zhang et al. (2016) who reported that flowering time of all tested *indica* and *japonica* cultivars was significantly delayed by drought, suggesting that drought-related flowering responsive genes may have already existed prior to genetic divergence between *indica* and *japonica*. Whereas most rice cultivars tend to delay flowering time under water deficit stress (Wopereis et al., 1996; Ndjiondjop et al., 2010), some commercial rice varieties exhibit early flowering in order to escape drought (Vikram et al., 2016). The drought-escape mechanism has also been reported in a model plant such as Arabidopsis by rapid flowering and reproduction before soil drying (Yue et al., 2006; Riboni et al., 2013). Taken together, these results highlight the consequence of genotype and environment interactions on the flowering time in rice.

The average number of panicles per hill was significantly affected by water treatments, especially at the panicle initiation stage. Critical-AWD plants had fewer panicles per hill (27), while CF and safe-AWD plants produced more panicles per hill (31 and 29, respectively). Critical-AWD management showed a direct effect on decreasing the panicle length, the number of filled grains per panicle and the 1,000 grains weight.

A significant reduction in the rice grain yield per pot was observed in the AWD treatments (Fig. 1A and 1B), which had short-term water deficit stress. Based on the difference in irrigation methods, regression analysis on the variation in grain yield per pot and soil moisture content levels showed that grain yield was significantly correlated with soil moisture content ($p < 0.05$) as presented in Fig. 1B. The grain yield decreased significantly when the soil moisture content decreased. The safe-AWD treatment (which had 50% lower soil moisture content than CF) decreased the grain yield per pot by 30% compared to the CF plants. Moreover, the critical-AWD treatment (where the soil was dried below 25% of the soil moisture content of the CF condition) resulted in grain yield losses of 51% relative to CF. Most lowland rice varieties can tolerate up to 30% reduction in total irrigation volume without significantly decreasing yield (Richards and Sander, 2014). In contrast, the current finding showed a reduction in the grain yield under safe-AWD, which involved a 50% reduction in total irrigated water compared to CF. It has also been reported that yield loss was mainly due to a reduced growth rate (Devkota et al., 2013) which was caused by limited water availability. The reduced growth rate and grain yield are due to water stress slowing down carbohydrate synthesis by reducing the photosynthetic rate, resulting in a weakened sink strength in the reproductive stages (Kumar et al., 2012; Pascual and Wang, 2017). Thus, it is important to note that applying a short-term water deficit treatment in order to improve the ability of anthocyanin accumulation requires precise timing and duration to prevent yield loss.

Table 1 Yield components under different water management systems, continuous flooding (CF), safe-alternate wetting and drying irrigation (AWD) and critical-AWD

Irrigation	Days to flowering	Number of panicles/hill	Panicle length (cm)	Filled grains/ panicle	Unfilled grains/ panicle	1,000 grains weight (g)
CF	88.00 ± 0.28^{c}	31.33 ± 0.55^{a}	29.94 ± 0.06^{a}	174.22 ± 6.53^{a}	16.56 ± 1.03^{b}	24.70 ± 0.24^{a}
Safe-AWD	92.00 ± 0.48^{b}	29.33 ± 0.55^{a}	26.79 ± 0.43^{b}	131.78 ± 4.17^{b}	24.00 ± 0.73^{ab}	23.86 ± 0.15^{b}
Critical-AWD	95.67 ± 0.28^{a}	27.00 ± 0.48^{b}	25.13 ± 0.35^{c}	102.11 ± 2.01^{c}	30.22 ± 0.48^{a}	23.66 ± 0.20^{b}
Coefficient of variation (%)	3.37	7.21	8.04	29.05	18.16	2.46
F test	***	**	***	***	*	*

Data are mean ± SE of five replicate biological samples.

Means in the same column superscripted with different letters are significant different ($p < 0.05$).

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

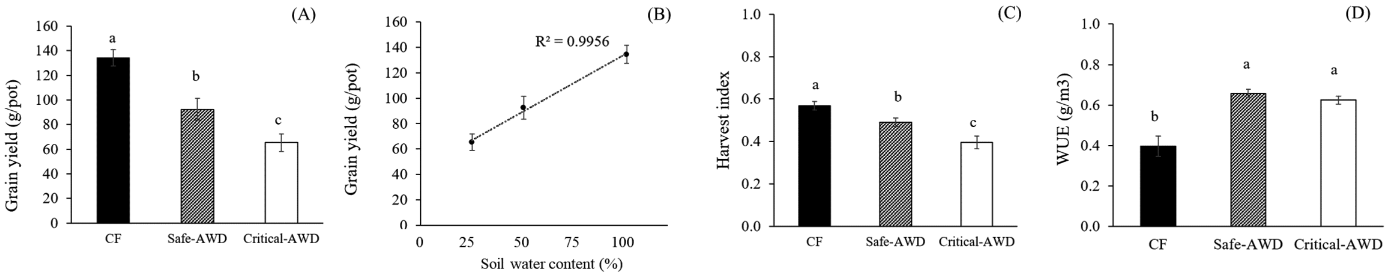

Fig. 1 Effects of water managements on: (A) mean grain yield per pot; (B) correlation between grain yield per pot and soil moisture content; (C) harvest index (HI); (D) water use efficiency (WUE), where data are mean of five replicate biological samples, error bars indicate ± SD and different lowercase letters indicate significant differences ($p < 0.05$) between means

The harvest index (HI) was significantly different among treatments, being significantly lower by 15.5% under the safe-AWD treatment and substantially decreased by 32.7% under the critical-AWD treatment compared to the CF treatment. WUE or water productivity was significantly different between the AWD and CF treatments (Fig. 1D). The highest WUE was observed in the safe-AWD treatment (0.66 g/m^3) followed by the critical-AWD treatment (0.62 g/m^3). There was no significant differences between the two short-term water deficit treatments. The highest grain production loss was 0.66 g/m^3 under the safe-AWD treatment, indicating that 0.66 g of grain was lost for a saving of 1 m^3 of water. CF had the lowest grain production loss (0.40 g/m^3). Although under critical-AWD the water use efficiency was less than for CF, this was also accompanied by reduced growth and grain yield (Carrijo et al., 2017).

Accumulation of total anthocyanin content in response to water stress

Rice with a red, purple and black bran layer is a great source of nutrients and bioactive compounds, such as anthocyanin (Chen et al., 2015). The anthocyanin content is highly unstable depending on genotype (G), environment (E) and G×E interactions (Khoo et al., 2017). Variation in the total anthocyanin content (TAC) of unpolished Riceberry grain under different water managements (Fig. 2) showed that a short-term water deficit during the growing season had a significant influence on anthocyanin production. Under the critical-AWD condition, the anthocyanin content was 4-fold higher than for the well-watered plants (CF) plants. The highest TAC was 15.17 mg/g, for the critical-AWD treatment followed by the safe-AWD treatment (6.82 mg/g), while the CF plants had the lowest TAC (3.61 mg/g).

Environmental stresses, such as excess light, salinity, drought and high or low temperature can affect the production of antioxidant compounds and the grain quality (Steyn et al. 2002; Hatier and Gould 2008; Van den Ende and El-Esawe, 2014). In the current study, the levels of anthocyanin concentration increased with increased stress conditions. Furthermore, the anthocyanin increase was not only caused by the continuous drought condition, but a short-term water deficit in the vegetative stage could also increase anthocyanin levels in rice grain.

A similar study also reported that the capacity of antioxidant compounds varied among different growing conditions (Somsana et al., 2013). They showed that the interaction between genotype and environment plays a major role in the variation in the anthocyanin content in rice grain by 42.79%, and 22.46%, respectively. The findings from their experiment are consistent with the results presented in the current study, which showed that variation in the anthocyanin content was affected by environmental factors, such as short-term water deficit stress. An unfavorable growing condition can stimulate the biosynthesis of phenolic substances, which play an important role in adapting to environmental stress and defense responses. A plant responds to stress by increasing the anthocyanin content because anthocyanin acts as a protective regulator against various biotic and abiotic stresses (Roychoudhury et al., 2008; Chunthaburee et al., 2016). In addition, a certain period and level of water deficit stress could activate the expression of genes involved in anthocyanin biosynthesis, resulting in the accumulation of anthocyanin and improvement in drought resistance in rice (Chen et al., 2015). These results emphasize the importance of water management during the growing period to enhance anthocyanin accumulation in colored rice grain.

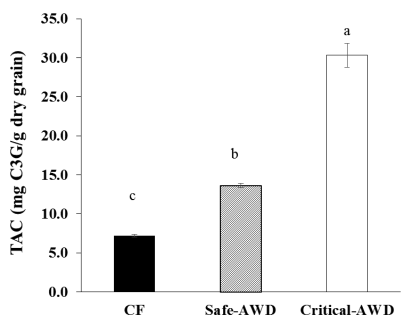

Fig. 2 Effects of water managements on total anthocyanin content (TAC) in unpolished Riceberry grain (mean ± SD); different lowercase letters above bars show significant differences ($p < 0.05$) between means; CF = continuous flooding; AWD = alternate wetting and drying irrigation.

Water management using AWD techniques is a highly effective water-saving irrigation strategy without affecting yield based on several studies, while other studies have reported that AWD could reduce plant growth and yield, as was the result from the current study. The main factors causing yield loss depend on the degree of soil drying (AWD threshold) which seems to have a large effect on yield. Although using this irrigation technique can cause yields to decline by 30 %, it has been demonstrated that safe-AWD can reduce water input by about 50% compared to well-watered method (CF). Despite the effect of AWD on the growth, yield and water use efficiency, AWD triggered the protective mechanisms by increasing the anthocyanin concentration to cope with the stress conditions. However, the degree of the changes in the yield components and anthocyanin content is dependent on many factors, such as timing, duration, plant developmental stage, genotype, soil type and severity of the water deficit stress. In the current study, safe-AWD presented as a promising water management strategy that can increase the levels of anthocyanin content and water productivity. This suggests that factors causing delayed flowering time, yield loss and increased TAC are not only caused by a decreased soil water content, but also relate to the timing, duration and severity of the water deficit application. Hence, further investigations will address the exact timing, duration and severity of applying short-term water deficit stress that can increase anthocyanin accumulation in rice grain without reducing the yield. This is could be a great opportunity to develop nutrient-rich rice products for health benefits.

Conflict of Interest

The authors declare that there are no conflicts of interest

Acknowledgements

The authors acknowledge the Department of Agriculture Natural Resources and Environment for providing the facilities and equipment used in this study and the laboratory staff there provided invaluable assistance.

References

Aizza, L.C.B., Dornelas, M.C. 2011. A genomic approach to study anthocyanin synthesis and flower pigmentation in passion flowers. J. Nucleic Acids. 2011: 1–17. doi: 10.4061/2011/371517.

Bouman, B.A.M., Lampayan, R.M. 2009. Rice Fact Sheet – Alternate Wetting Drying (AWD). International Rice Research Institute, Los Baños, Philippines.

Bouman, B.A.M., Lampayan, R.M., Tuong, T. 2007. Water Management in Irrigated Rice: Coping with Water Scarcity. International Rice Research Institute. Los Baños, the Philippines.

Carrijo, D.R., Lundy, M.E., Linquist, B.A. 2017. Rice yields and water use under alternate wetting and drying irrigation: A meta-analysis. Field Crops Res. 203: 173–180.

Cassidy, A. 2018. Berry anthocyanin intake and cardiovascular health. Mol. Aspects Med. 61: 76–82.

Chen, H., Dai, X.J., Gu, Z.Y. 2015. OsbZIP33 is an ABA-dependent enhancer of drought tolerance in rice. Crop Sci. 55: 1673–1685.

Chunthaburee, S., Sakuanrungsirikul, S., Wongwarat, T., Sanitchon, J., Pattanagul, W., Theerakulpisut, P. 2016. Changes in anthocyanin content and expression of anthocyanin synthesis genes in seedlings of black glutinous rice in response to salt stress. Asian J. Plant Sci. 15: 56–65.

Devkota, K.P., Manschadi, A.M., Lamers, J. P. A., et al. 2013. Growth and yield of rice (*Oryza sativa* L.) under resource conservation technologies in the irrigated drylands of Central Asia. Field Crops Res. 149: 115–126.

Giusti, M.M., Wrolstad, R.E. 2001. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Giusti, M.M., Wrolstad, R.E. (Ed.). Current Protocols in Food Analytical Chemistry. John Wiley & Sons, Inc. New York, NY, USA. pp. F1.2.1–F1.2.13.

Gomaz, K. 1972. Techniques for Field Experiments with Rice. International Rice Research Institute. Los Baños, the Philippines.

Hatier, J.B., Gould, K.S. 2008. Foliar anthocyanins as modulators of stress signals. J. Theor. Biol. 253: 625–627.

Howell, K.R., Shrestha, P., Dodd, L.C. 2015. Alternate wetting and drying irrigation maintained rice yields despite half the irrigation volume, but is currently unlikely to be adopted by smallholder lowland rice farmers in Nepal. Food Energy Secur. 4: 144–157.

Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M. 2017. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr. Res. 61: 1361779. doi: 10.1080/16546628.2017.1361779.

Knievel, D.C., Abdel-Aal, E.S.M., Rabalski, I., Nakamura, T., Hucl, P. 2009. Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (*Triticum aestivum* L.). J. Cereal Sci. 50: 113–120.

Kovinich, N., Kayanja, G., Chanoca, A., Otegui, M. S., Grotewold, E. 2015. Abiotic stresses induce different localizations of anthocyanins in Arabidopsis. Plant Signal. Behav. 10: e1027850. doi: 10.1080/15592324.2015.1027850.

Kumar, S.V., Lucyshyn, D., Jaeger, K.E., Alos, E., Alvey, E., Harberd, N.P., Wigge, P.A. 2012. Transcription factor PIF4 controls the thermosensory activation of flowering. Nature. 484: 242–245.

Kwon, S.H., Ahn, I.S., Kim, S.O., Kong C.S., Chung, H.Y., Do, M.S., Park, K.Y. 2007. Anti-obesity and hypolipidemic effects of black soybean anthocyanins. J. Med. Food. 10: 552–556.

Lampayan, R.M., Rejesus, R.M., Singleton, G.R., Bouman, B.A.M. 2015. Adoption and economics of alternate wetting and drying water management for irrigated lowland rice. Field Crops Res. 170: 95–108.

Li, J.C., Dong, S.L., Zhi, P.S., et al. 2004. Gene flow from cultivated rice (*Oryza sativa*) to its weedy and wild relatives. Ann. Bot. 93: 67–73.

Linquist, B., Anders, M.M., Adviento-Borbe, M.A.A., Chaney, R.L., Nalley, L.L., DaRoda, E.F.F., Van Kessel, C. 2014. Reducing greenhouse gas emissions, water use, and grain arsenic levels in rice systems. Glob. Change Biol. 21: 407–417.

Ma, D., Li, Y., Zhang, J., Wang C., Qin, H., Ding, H., Xie, Y., Guo T. 2016. Accumulation of phenolic compounds and expression profiles of phenolic acid biosynthesis-related genes in developing grains of white, purple, and red wheat. Front. Plant Sci. 7: 528 doi: 10.3389/fpls.2016.00528.

Ndjiondjop, M.N., Cisse, F., Futakuchi, K., Lorieux, M., Manneh, B., Bocco, R., Fatondji, B. 2010. Effect of drought on rice (*Oryza* spp.) genotypes according to their drought tolerance level. In: Second Africa Rice Congress. Bamako, Mali. pp. 151–158.

O'Toole, J.C, Moya T.B. 1978. Genotypic variation in maintenance of leaf water potential in rice. Crop Sci. 18: 873–876.

Pascual, V.J., Wang, U.M. 2017. Utilizing rainfall and alternate wetting and drying irrigation for high water productivity in irrigated lowland paddy rice in southern Taiwan. Plant Prod. Sci. 20: 24–35.

Pedro, A.C., Granato, D., Rosso, N.D. 2016. Extraction of anthocyanins and polyphenols from black rice (*Oryza sativa* L.) by modeling and assessing their reversibility and stability. Food Chem. 191: 12–20.

Price, A.H., Norton, G.J., Salt, D.E. et al. 2013. Alternate wetting and drying irrigation for rice in Bangladesh: Is it sustainable and has plant breeding something to offer? Food Energy Secur. 2: 120–129.

Rechner, A.R., Kroner, C. 2005. Anthocyanins and colonic metabolites of dietary polyphenols inhibit platelet function. Thromb. Res. 116: 327–334.

Riboni, M., Galbiati, M., Tonelli, C., Conti, L. 2013. GIGANTEA enables drought escape response via abscisic acid-dependent activation of the florigens and suppressor of overexpression of constants. Plant Physiol. 162: 1706–1719.

Richards, M., Sander, B.O. 2014. Alternate wetting and drying in irrigated rice. Climate-Smart Agriculture Practice Brief. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS). Copenhagen, Denmark.

Roychoudhury, A., Basu, S., Sarkar, S.N., Sengupta, D.N. 2008. Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars. Plant Cell Rep. 27: 1395–1410. doi: 10.1007/s00299-008-0556-3.

RStudio Team. 2016. RStudio: Integrated Development for R. RStudio, Inc. Massachusetts, USA. http://www.rstudio.com/, 1 March 2016.

Saewan, N., Vichit, W. 2015. Antioxidant activities and cytotoxicity of Thai pigmented rice. Int. J. Pharm. Pharm. Sci. 7: 329–334.

Shim, S.H., Kim, J.M., Choi, C.Y., Kim, C.Y., Park, K.H. 2012. Ginkgo biloba extract and bilberry anthocyanins improve visual function in patients with normal tension glaucoma. J. Med. Food. 15: 818–823. doi: 10.1089/jmf.2012.2241.

Somsana, P., Wattana, P., Suriharn, B., Sanitchon, J. 2013. Stability and genotype by environment interactions for grain anthocyanin content of Thai black glutinous upland rice (*Oryza sativa*). Sabrao J. Breed Genet. 45: 523–32.

Steyn, W.J., Wand, S.J.E., Holcroft, D.M., Jacobs, G. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. 155: 349–361.

Tahkokorpi, M. 2010. Anthocyanins under drought and drought-related stresses in bilberry (*Vaccinium myrtillus* L.). Ph.D. thesis, Faculty of Science, Oulu University, Oulu, Finland.

Van den Ende, W., El-Esawe, S.K. 2014. Sucrose signaling pathways leading to fructan and anthocyanin accumulation: A dual function in abiotic and biotic stress responses? Environ. Exp. Bot. 108: 4–13.

Vijay, D., Roy, B. 2013. Rice (*Oryza sativa* L.). In: Roy, B. (Eds.), Breeding, Biotechnology and Seed Production of Field Crops. New India Publishing Agency. India. pp. 71–122.

Vikram, P., Swamy, B.P.M., Dixit, S., Trinidad, J., Sta Cruz, M.T., Maturan, P.C., Amante, M., Kumar A. 2016. Linkages and interactions analysis of major effect drought grain yield QTLs in rice. Plos One. 11: e0151532. doi: 10.1371/journal.pone.0151532.

Wang, L.S., Hecht, S.S., Carmella, S.G., et al. 2009. Anthocyanins in black raspberries prevent esophageal tumors in rats. Cancer Prev. Res. (Phila) 2: 84–93. doi: 10.1158/1940-6207.CAPR-08-0155.

Wopereis, M.C.S., Kropff, M.J., Maligaya, A.R., Tuong, T.P. 1996. Drought-stress responses of two lowland rice cultivars to soil water status. Field Crops Res. 46: 21–39.

Yue, B., Xue, W., Xiong, L., et al. 2006. Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. Genetics. 172: 1213–1228.

Zhang, C., Liu, J., Zhao, T., et al. 2016. A drought-inducible transcription factor delays reproductive timing in rice. Plant Physiol. 171: 334–343. doi: 10.1104/pp.16.01691.