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Alternate wetting and drying modulated physio-biochemical attributes, grain yield, quality, and aroma volatile in fragrant rice

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Abstract

Alternate wetting and drying (AWD) has been recognized as a water-saving technology in rice production systems; however, pre- and post-flowering AWD could induce changes in yield, quality and aroma biosynthesis in fragrant rice. In the present study, two fragrant rice cultivars (Guixiangzhan and Nongxiang-18) were subjected to AWD till soil water potential reached −25 to −30 kPa during vegetative stage (VS), reproductive stage (RS), and both stages (VS + RS). The AWD did not affect net photosynthesis and gas exchange significantly, while malondialdehyde (MDA), H_2O_2 and electrolyte leakage (EL) were higher than in control plants. The AWD treatments variably affected soluble sugars, proline and protein accumulation as well as the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and reduced glutathione (GSH) contents. Moreover, filled grain percentage and 1000-grain weight in AWD treatments were found to be statistically similar ($p > 0.05$) to control, except grains panicle^{-1} under AWD-VS + RS that was reduced by 11% and 14% for Guixiangzhan and Nongxiang-18, respectively. On average, yield and related attributes in Guixiangzhan remained higher than in Nongxiang-18. In addition, the grain aroma volatile (2-acetyl-1-pyrroline, 2-AP) content increased by 8.79%, 14.45%, and 6.87% and 7.95%, 14.02%, and 5.04% under AWD-VS, AWD-RS, and AWD-VS + RS treatments, for Guixiangzhan and Nongxiang-18, respectively. Overall, AWD treatments, either at VS or RS, could promote rice aroma in terms of accumulation of 2AP, which might be linked with enhanced endogenous proline contents (a precursor for 2AP biosynthesis) without any severe consequences on rice yield and quality.

1 | INTRODUCTION

Fragrant rice is well-recognized as a "special type of rice" having a perfumed, nutty or popcorn-like smell and is world-famous due to its unique character (Li et al., 2016). It is widely grown in Asia and the Middle East and sold all around the globe, including Europe and USA (Pinson, 1994). Fragrant rice is becoming more and more popular in international markets (Singh et al., 2000; Verma & Srivastav, 2018). Mostly, fragrant rice is cultivated and marketed in India, Pakistan, and Thailand, whereas Indian and Pakistani "Basmati" and Thai "Jasmine" are popular fragrant rice types lobally. Besides, some other aromatic rice cultivars, such as Della, Texamati and Kasmati (USA), Sadri (Iran), and Siamati (Thailand), are also important fragrant rice cultivars (Singh et al., 2000). Currently, research is being done on aromatic rice development and breeding in China (Ashraf & Tang, 2017; Mo et al., 2019; Yang et al., 2012).

Rice quality and aroma are important characteristics of fragrant rice that are largely affected by both internal and external plant factors. The aromatic compound 2-acetyl-1-pyrroline (2AP) is recognized as the single most important compound responsible for aroma in fragrant rice, over aliphatic alcohols, aromatics, aliphatic aldehydes, terpenoids, and aliphatic ketones (Goufo et al., 2010). Water application dynamics is one of the main external factors that regulate plant physio-biochemical mechanisms, yield and quality, as well as aroma biosynthesis in fragrant rice (Tian et al., 2010; Wang et al., 2013). Normally, drought conditions induce oxidative stress by excessive production of reactive oxygen species (ROS), which causes oxidative damage in plants; nevertheless, plants possess a well-sophisticated antioxidative defense system to overcome the damaging effects of ROS (Farooq, Wahid, Ito, et al., 2009).

Water stress during rice growth cycle causes regulations in rice aroma, for instance Bhattacharjee et al. (2002) reported that water stress during grain filling or ripening stages is conducive to aroma accumulation in fragrant rice, while some others researchers have found that water stress during vegetative and/or tillering stage could result in high aroma content in fragrant rice (Tang & Pan, 2014; Wang et al., 2013). However, severe water stress at any stage could result in yield loss in rice. Thus, alternate wetting and drying (AWD) may be a better option to induce rice aroma with a low risk of yield loss and quality deterioration. Previous studies reported that AWD in rice substantially enhanced the agronomic attributes of rice with substantial improvements in water use efficiencies (Sandhu et al., 2017; Ishfaq, Farooq et al., 2020). Moreover, AWD within safe limits was found equally or even more productive than flooding, required less water and yielded better grain quality (Bao et al., 2018). Ren et al. (2017) suggested that AWD at vegetative and reproductive (booting and flowering) stages substantially enhanced the yield and grain quality attributes in different fragrant rice cultivars. Furthermore, AWD may enhance rice yield up to 7%–9% compared with continuous flooding depending upon edaphic and climatic factors, AWD duration and crop growth stage (Ishfaq, Akbar et al., 2020). Mild AWD could potentially improve the grain quality characteristics with improved head rice recovery and reduced grain chalkiness (Ishfaq et al., 2021).

Though AWD-induced alterations in rice yield attributes are well reported, the AWD at pre- and post-flowering in rice and its effects on rice physiology, grain quality and biosynthesis of aroma volatile, specially 2-AP, were rarely reported. Therefore, the present study was conducted to explore the role of AWD at the vegetative stage (VS), reproductive stage (RS), and both VS + RS in regulating the physio-biochemical mechanism, yield and quality, especially grain aroma in terms of 2-AP contents in fragrant rice.

2 | MATERIALS AND METHODS

2.1 | Experimental details

A pot experiment was conducted in a rain-protected wirehouse from Mid-July to Mid-November 2015 at Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou, China (23°09'N, 113°22'E, and 11 m above the sea level). The climatic conditions of this region are subtropical. During the experiment (July–November), the mean monthly temperatures lie between 22°C and 29°C (temperature falls towards the end of the growing season) with average relative humidity (RH) between 75% and 85%. The rice grown in this season is regarded as "late-season rice" of the double rice cropping system in South China.

The seeds of two aromatic rice cultivars ("Guixiangzhan" and "Nongxiang-18") were soaked in water for 48 h at 25–30°C and shade-dried afterward. Both rice cultivars have almost similar growth periods and are widely grown by the farming community owing to their special aromatic character. The germinated seeds were sown on July 15, 2015 in soil-containing (3–4 cm layer) PVC trays placed on a well-leveled paddy field, and covered with transparent polythene sheets. The seedlings of both rice cultivars were transplanted on August 5, 2015. Generally, rice planted in the late season (July–November) grows more vigorously than the early season rice (March–July) due to the natural climatic conditions of this region.

Twenty days before seedling transplantation, the pots (25 cm in height and 32 cm in diameter) were filled with 10 kg of air-dried sandy loam soil collected from a paddy field. The soil comprised of 1.802 g kg^{-1} total N, 0.862 g kg^{-1} total P, 17.80 g kg^{-1} total K, 92.31 mg kg^{-1} available N, 8.63 mg kg^{-1} available P, 128.22 mg kg^{-1} available K, and 20.80 g kg^{-1}organic matter. The pH of the soil was 5.83. Uniform and well-grown seedlings were transplanted in pots with 3–4 seedlings per hill and five hills per pot, whereas the broken and damaged seedlings were replaced with healthy seedlings till 1 week after transplanting. Each pot was fertilized with urea (46% N), superphosphate (12% P_2O_5), and potassium chloride (60% K_2O) at 2.20, 3.35, and 1.30 g with 70% as basal dose and 30% at 15 days after transplanting. Flooded conditions were maintained during the first 15 days of growth and then plants were divided into four sets: (1) control: a water layer of 2–3 cm was maintained from transplanting till physiological maturity of the crop (soil water potential 0 kPa), (2) AWD-VS: pots were flooded with 2–3 cm water layer and then allowed to decrease gradually till the soil water potential reached −25 to −30 kPa, and then pots were re-watered to make the soil water potential 0 kPa during vegetative stage (VS) (from 16 to 50 days of growth), (3) AWD + RS: the similar water treatment conditions as in "AWD-VS" but during reproductive stage (RS) (from 51 to 70 days of growth), and (4) AWD-VS + RS: continued alternate wetting and drying from 16 to 70 days of growth at the same water potential, i.e., −25 to −30 kPa. Except during the treatment application phase, the pots were watered regularly till physiological maturity. The soil water potential was assessed by a phenotypic vacuum negative pressure type soil moisture meter hermetically sealed with a vacuum gauge (5 M/HT4-TEN-30, Chinese Academy of Soil Sciences, Nanjing, China) with the sensor immersed at a depth of 10–15 cm below the soil surface (Figure 1). The plants were monitored regularly throughout the growing season and standard agronomic practices were followed as recommended by the province to avoid any insect/pest damage.

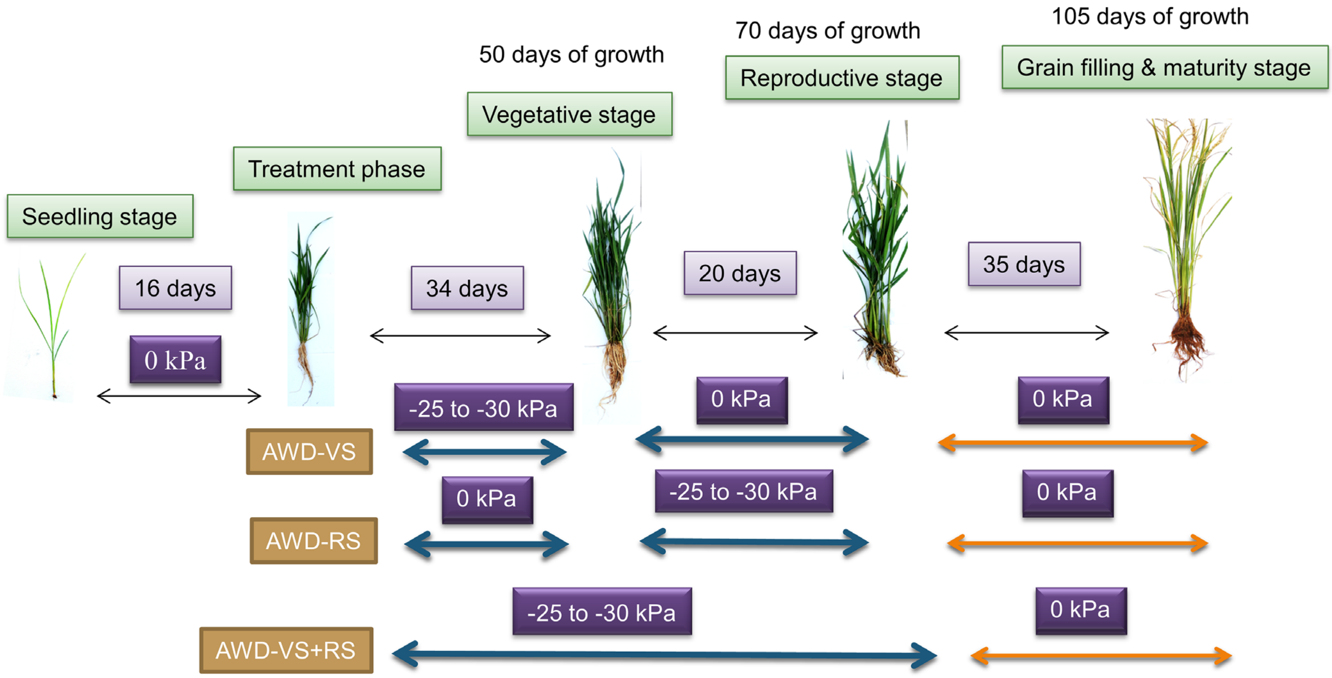


FIGURE 1 Description of experimental treatments. Alternate wetting and drying (AWD) treatments were monitored through soil moisture meter sealed with a vacuum gauge.

2.2 | Sampling and data collection

The net photosynthesis and leaf gas exchange were measured in flag leaves of both rice cultivars at the end of the reproductive stage. For physio-biochemical analyses, plants were sampled at the end of VS (50 days of growth) and RS (70 days of growth) as well as at physiological maturity (105 days of growth) and stored at −80°C after washing with tap water and then with distilled water, while yield and quality attribute were recorded at physiological maturity. To estimate the 2-AP contents, fresh rice panicles were harvested at physiological maturity and stored at −20°C till determination.

2.3 | Net photosynthesis and gas exchange

Net photosynthetic rate and gas exchange attributes, that is, stomatal conductance, intercellular CO_2, and transpiration rate were determined in three plants from each pot and nine pots from each treatment by using a portable photosynthesis system (LI-6400, LI-COR) between 09:00 and 11:30 h with the following adjustments: photosynthetically active radiation at leaf surface was up to 1200 μmol m^{-2} s^{-1}, the molar flow of air per unit leaf area was about 500 μmol s^{-1}, ambient CO_2 concentration almost 400 μmol mol^{-1}, the air temperature was about 28–30°C with 70%–85% RH. Water use efficiency (WUE) and instantaneous water use efficiency (WUEi) were calculated as the ratio of net photosynthesis with transpirational rate and stomatal conductance, respectively.

2.4 | Physio-biochemical assays

The malondialdehyde (MDA) contents were estimated according to Hodges et al. (1999). Briefly, fresh leaves (0.2 g) were homogenized in thiobarbituric acid (0.5%) and trichloroacetic acid (10%) and boiled in water bath for 30 min. The samples were then centrifuged after coolings and absorbances were read at 532, 600, and 450 nm and the MDA contents were expressed as μmol g^{-1} fresh weight (FW). The hydrogen peroxide (H_2O_2) was estimated according to Velikova et al. (2000). Briefly, fresh leaves (0.2 g) were homogenized with trichloroacetic acid (0.1%) and centrifuged at 12,000 *g* for 15 min. The reaction mixture comprised potassium phosphate buffer (0.5 ml), potassium iodide (1 ml), and an aliquot of the leaf extract and the absorbance was read at 390 nm. The H_2O_2 contents were expressed as μmol g^{-1} FW. The electrolyte leakage (EL) was measured according to Valentovic et al. (2006). Briefly, fresh leaves were cut into small pieces and put into vials for 24 h at room temperature and initial electrical conductivity (EC_1) was measured. The samples were then boiled at 90°C for 30 min, and final EC was recorded (EC_2) and the EL was reocrded: $EL = (EC_1/EC_2 \times 100)$.

Soluble sugars were determined according to Leyva et al. (2008). Fresh leaf samples (0.5 g) were put into 10 ml distilled water and boiled in a water bath and cooled in an ice bath. Boiled samples (0.5 ml) and anthrone reagent (0.5 ml) were vortexed. The absorbance of the mixture was read at 620 nm and expressed as mg g^{-1} FW. Proline contents were estimated according to Bates et al. (1973) by using ninhydrin. The amount of proline was estimated by comparison with a standard curve and expressed as μg g^{-1} FW. Protein contents were estimated according to Bradford (1976) using G-250 and the absorbance of the reaction mixture was recorded at 595 nm in triplicate.

2.5 | Determination of antioxidants activities and GSH contents

Fresh leaves samples (0.3 g) of both rice cultivars were crushed in liquid N_2 and homogenized in 6 ml of 50 mM sodium phosphate buffer (pH 7.8). The homogenate was then centrifuged at 10,000 *g* for 20 min at 4°C and the supernatant was used to estimate the enzymatic activities and GSH content.

Superoxide dismutase (SOD, EC 1.15.1.1) was determined according to Zhang, Zhang, Raziuddin, et al. (2008b); Zhang, Zhang, Yang, et al. (2008a) by following the inhibition of photochemical reduction due to nitro blue tetrazolium (NBT) and the absorbance was read at 560 nm. The SOD activity per unit was the amount of enzyme required to inhibit NBT photochemical reduction to 50% as an activity unit (U). The activity of peroxidase (POD, EC 1.11.1.7) was estimated as described by Zhou and Leul (1999) by using the guaiacol method and the absorbance was recorded at 470 nm. One unit of POD activity was the amount of enzyme that caused the decomposition of 1 μg substrate at 470 nm. The catalase activity (CAT, EC 1.11.1.6) was calculated according to Aebi (1984) and the absorbance of the reaction mixture was read at 240 nm. One unit of enzyme activity (U) was the decomposition of 1 M H_2O_2 at A_{240} within 1 min in 1 g of fresh leaves samples. The activity of ascorbate peroxidase (APX, EC 1.11.1.11) and content of reduced glutathione (GSH) were estimated by using ready-made kits purchased from Nanjing Jiancheng Bioengineering Institute, China (www.njjcbio.com).

2.6 | Yield and quality attributes

Total tillers per pot were counted from five random pots and averaged. For the estimation of productive tillers per pot, the panicle-bearing tillers were counted from the same pots at the panicle heading stage. Grains were separated from panicles from each pot and averaged to estimate the grains per panicle and filled grain percentage. Three samples of 1000-grains were taken from from random plants (from different pots) weighed to get 1000-grain weight (g). For estimating the yield per pot, all plants from each pot were harvested, threshed and weighed to get yield per pot (g). The harvest index was calculated as: 100 × (grain yield/plant dry biomass).

The grains were taken from a stored seed lot for grain quality assessment. Brown rice (containing bran layer and not polished) was obtained with the help of a rice huller (Jiangsu, China) and the brown rice rate was calculated as: {(brown rice weight / paddy weight) × 100}. The brown rice was milled with the help of Jingmi testing rice miller (Zhejiang, China) and the milled rice rate was estimated as: {(milled rice weight / original weight) × 100}. Whole milled grains were separated from 100 grains and the head rice rate was calculated on a percentage basis. Grain chalkiness was evaluated with the help of an SDE-A lightbox (Guangzhou, China). Grain amylose and protein contents were determined with the help of Infratec 1241 (FOSS-TECATOR) grain analyzer.

2.7 | Extraction and estimation of aroma volatile (2-AP)

Grain 2-AP content was extracted using the method of Jezussek et al. (2002) with some modifications. Fresh rice grain samples (0.5 g) were ground in pestle and mortar with liquid N_2. Grain powder (≈0.3 g) was mixed with 10 ml of dichloromethane and the mixture was kept in a water bath at 40°C for 4 h followed by the addition of Na_2SO_4 (anhydrous) until the moisture present in the mixture is absorbed. The 2-AP contents were quantified by gas chromatography–mass spectrometry (GCMS)-QP 2010 Plus (Shimadzu Corporation). The measuring conditions were as follows: the gas chromatograph fixed with silica capillary column (30 m × 0.32 mm × 0.25 μm) of Restek Rxi-5 ms (Shimadzu) and helium as a carrier gas with a flow rate of 2.0 ml min^{-1} (99.99%, Guangzhou Gases Co., Ltd.) (Mo et al., 2015). The grain 2-AP content was expressed as ng g^{-1} fresh weight (FW).

2.8 | Experimental design and statistical analyses

The pots were arranged in a completely randomized design (CRD) with 10 pots per treatment. All parameters were recorded in triplicate and the values were averaged. The recorded data were analyzed by using statistical software "Statistix 8" (Analytical software), while the least significant difference (LSD) test at 5% probability level was used to calculate differences among treatment means. SigmaPlot 9.0 (Systat Software Inc.) was used for the graphical representation of the dataset.

3 | RESULTS

3.1 | Net photosynthesis and gas exchange

The AWD affected net photosynthesis and gas exchange attributes of both rice cultivars. Compared with control, the AWD-VS + RS led to reductions in net photosynthesis, stomatal conductance, inter-cellular CO_2, transpiration rate, water use efficiency by 2.42%, 12.83%, 6.94%, 1.09%, and 0.62% in Guixiangzhan and 2.75%, 2.22%, 2.65%, 1.80%, and 0.84% in Nongxiang-18 (Figure 2A–F).

3.2 | Malondialdehyde (MDA), H_2O_2 and electrolyte leakage (EL)

AWD induced regulations in MDA contents across different growth stages in both rice cultivars. For Guixiangzhan, compared with control,

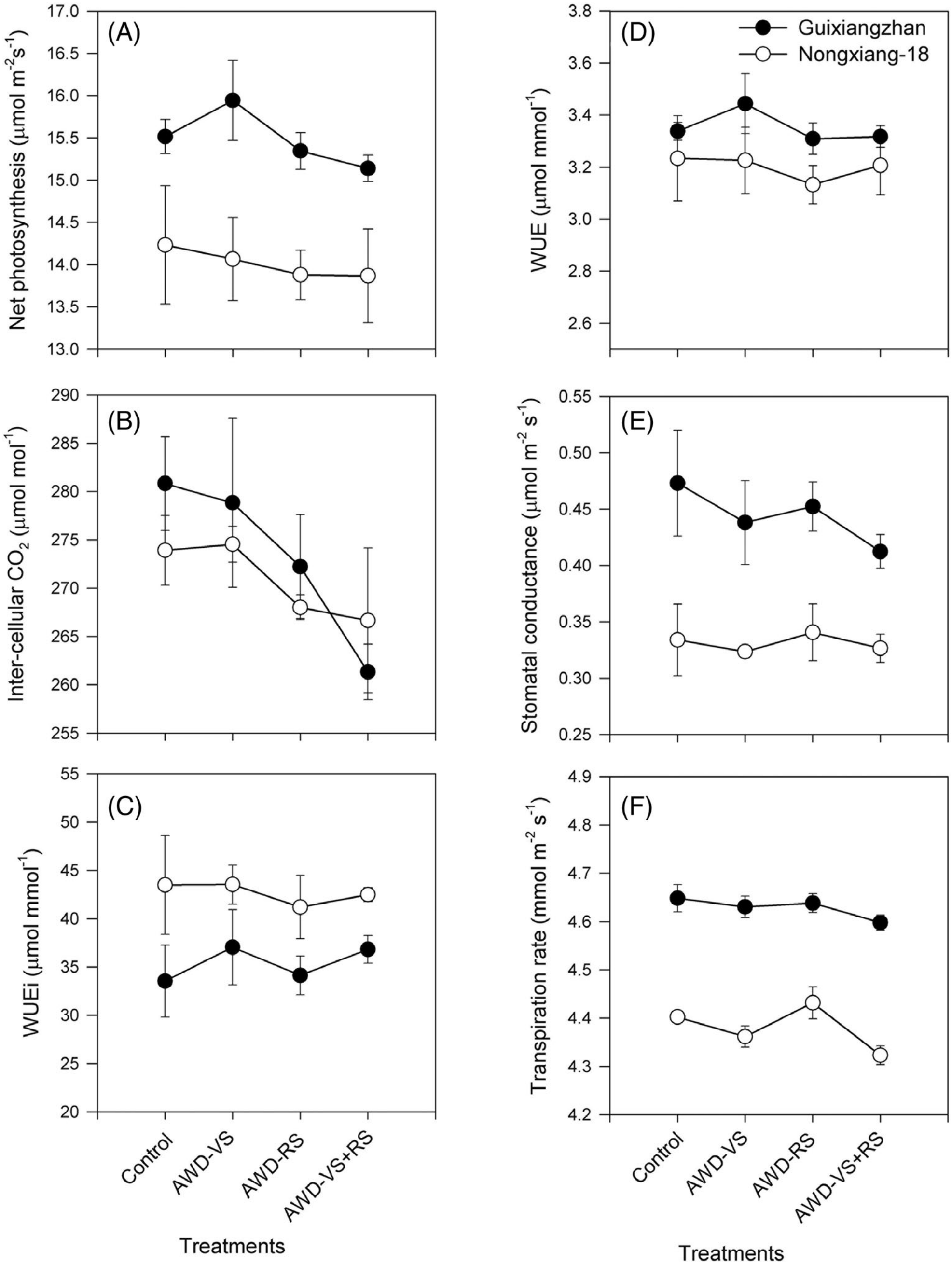


FIGURE 2 Alternate wetting and drying (AWD) induced regulations in (a) net photosynthesis, (B) inter cellular CO_2, (C) instantaneous water use efficiency (WUEi), (D) water use efficiency, (E) stomatal conductance, and (F) transpiration rate in Guixiangzhan and Nongxiang-18. Capped bars above means are the S.E. of three replicates. Control, water potential 0 kPa; AWD-VS, AWD at vegetative stage; AWD-RS, AWD at reproductive stage; AWD-VS + RS, AWD at both vegetative and reproductive stages.

the MDA contents increased up to 15.31% (for AWD-VS) at VS, and 121.91% and 47.33% (for AWD-VS + RS) at RS and MS, respectively. For Nonxiang-18, compared with control, the MDA contents increased up to 18.49% (for AWD-VS) at VS, and 21.84% and 8.73% (at AWD-RS) at RS and MS, respectively. The H_2O_2 contents were also higher in AWD treatments than control. Across cultivars, the H_2O_2 contents were increased by 10.98%–64.59% at VS, 58.39%–99.48% at RS and 66.24%–92.30% at MS for AWD-VS + RS, compared with control. The EL was also higher in AWD treatments than control. At VS, EL remained the highest for AWD-VS (increased by 58.32% and 80.77%), whereas at RS and MS, EL was for AWD-VS + RS (increased by 63.50% and 84.24%, and 36.91% and 59.55%, respectively), as compared with control. (Figure 3A–C).

3.3 | Protein, proline, and soluble sugars

The AWD treatments substantially affected the soluble sugars, protein and proline contents in both rice cultivars. Specifically, soluble sugars, protein and proline contents increased up to 58.89%, 34.72%, and 45.99% in Guixiangzhan under AWD-VS + RS as compared with control across all growth stages. For Nongxiang-18, the soluble sugars, protein and proline contents increased up to 56.89% (for AWD-VS) at VS, and 48.05% and 37.82% (for AWD-RS) both at RS and MS, as compared with control. On average, the contents of soluble sugars, proline, and protein were higher in Guixiangzhan than in Nongxiang-18 except for protein contents at VS, which was 5.85% higher in Nongxiang-18 (Figure 4A–C).

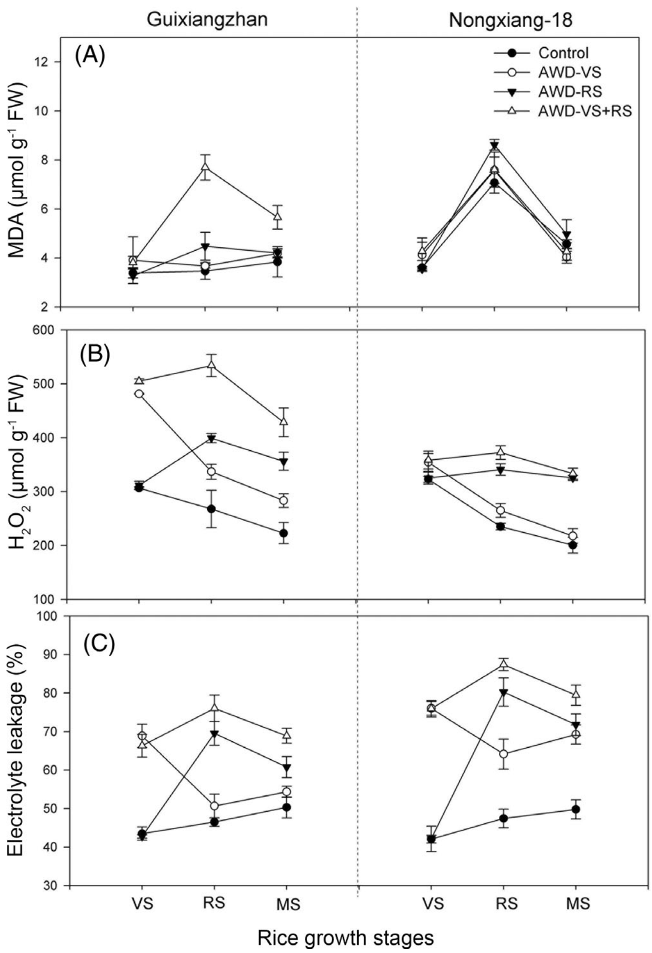


FIGURE 3 Alternate wetting and drying (AWD) induced regulations in (a) malondialdehyde (MDA), (B) H_2O_2, and (C) electrolyte leakage (EL) in Guixiangzhan and Nongxiang-18. Capped bars above means are the S.E. of three replicates. Control, water potential 0 kPa; AWD-VS, AWD at vegetative stage; AWD-RS, AWD at reproductive stage; AWD-VS + RS, AWD at both vegetative and reproductive stages. VS, vegetative stage; RS, reproductive stage; MS, maturity stage.

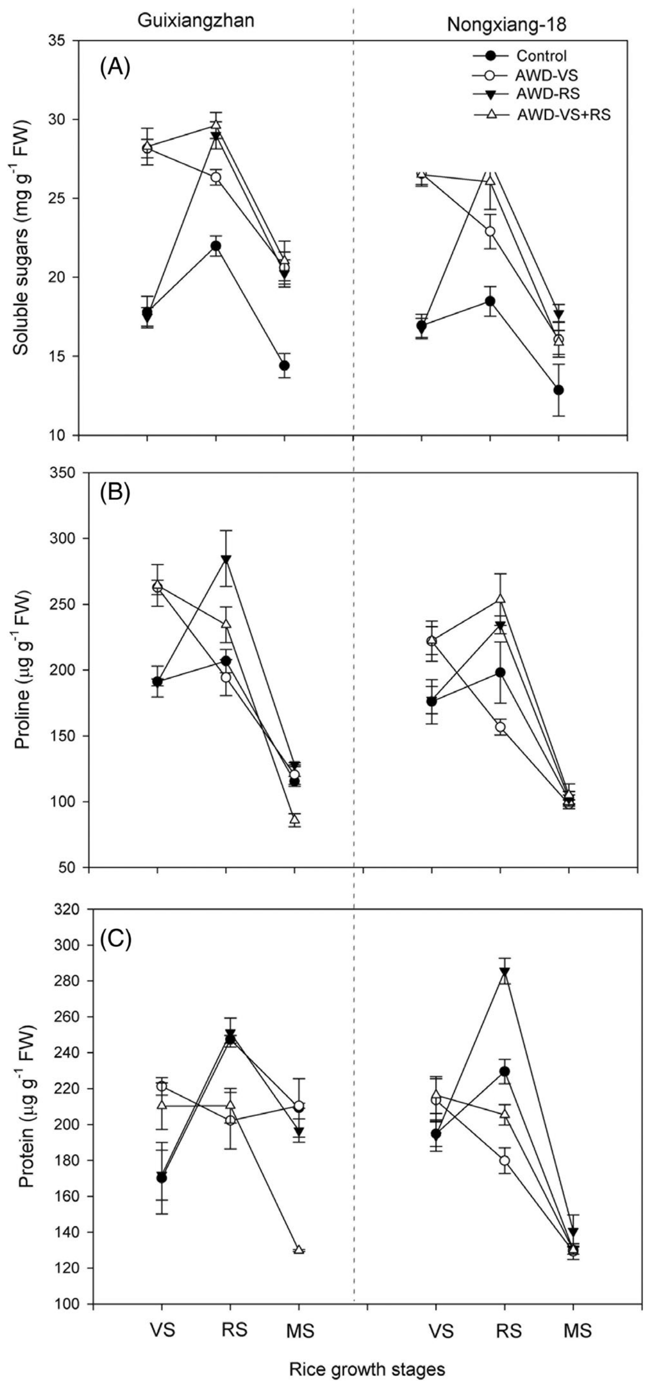


FIGURE 4 Alternate wetting and drying (AWD) induced regulations in (a) soluble sugars, (B) proline, and (C) protein contents in Guixiangzhan and Nongxiang-18. Capped bars above means are the S.E. of three replicates. Control, water potential 0 kPa; AWD-VS, AWD at vegetative stage; AWD-RS, AWD at reproductive stage; AWD-VS + RS, AWD at both vegetative and reproductive stages. VS, vegetative stage; RS, reproductive stage; MS, maturity stage.

3.4 | Activities of antioxidants and reduced glutathione (GSH) contents

Compared with control, the SOD activity in Guixiangzhan increased up to 24.07% (for AWD-VS) and 52.67% (AWD-RS) at VS and RS, respectively. For Nongxiang-18, the maximum reduction in SOD activities was noticed for AWD-VS + RS at all VS, RS, and MS. The POD activity was increased for AWD-VS and AWD-VS + RS at VS and remarkably reduced in all AWD treatments at RS and MS, as compared with control. Moreover, for Guixiangzhan, the CAT activity increased up to 47.17% (for AWD-VS) at VS, 45.57% (for AWD-RS) at RS and 32.53% (for AWD-VS + RS) at MS, as compared with control. For Nongxiang-18, the CAT activities increased up to 25.10% (for AWD-VS) at VS, and 42.93% (for AWD-RS) at RS, while the CAT activity was severely reduced for all AWD treatments at MS compared with control. Compared with control, the APX activity increased up to 5.40%, 24.79%, and 40.29% for Guixiangzhan and 13.57%, 42.19%, and 10.20% for Nongxiang-18 under AWD-VS, AWD-RS, and AWD-VS + RS, respectively, across different growth stages.

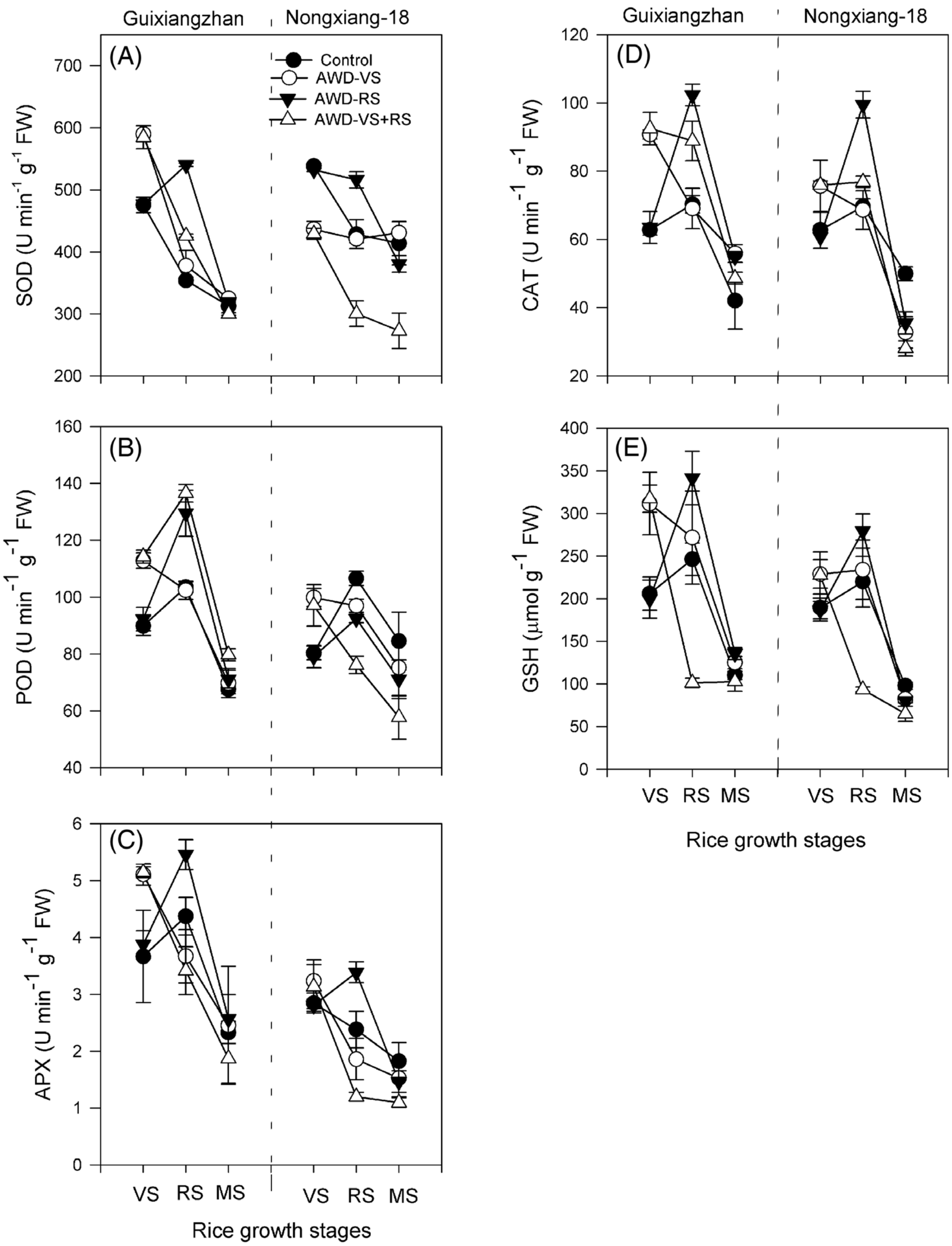


FIGURE 5 Alternate wetting and drying (AWD) induced regulations in the activities of (a) superoxide dismutase (SOD), (B) peroxidase (POD), (C) ascorbate peroxidase (APX), (D) catalase (CAT), and (E) reduced glutathione (GSH) contents in Guixiangzhan and Nongxiang-18. Capped bars above means are the S.E. of three replicates. Control, water potential 0 kPa; AWD-VS, AWD at vegetative stage; AWD-RS, AWD at reproductive stage; AWD-VS + RS, AWD at both vegetative and reproductive stages. VS, vegetative stage; RS, reproductive stage; MS, maturity stage.

Compared with control, the GSH contents increased up to 54.06% and 20.77% (for AWD-VS) at VS, and 38.63% and 27.07% (for AWD-RS) at RS in Guixiangzhan and Nonxiang-18, respectively. At MS, the AWD-VS and AWD-RS resulted in 13.60% and 23.10% higher GSH contents in Guixiangzhan than control. For Nongxiang-18, at MS, the GSH contents decreased by 15.88%, 18.48%, and 33.63% under AWD-VS, AWD-RS, and AWD-VS + RS, respectively, compared with control (Figure 5A–E).

3.5 | Yield and related attributes and above-ground dry biomass

AWD treatments variably affected the yield and yield components of both rice cultivars. For example, compared with control, the AWD at VS reduced tillers pot^{-1} up to 10%–12% in both rice cultivars. Compared with control, the productive tillers pot^{-1} were marginally reduced in AWD treatments for both rice cultivars; with the exception of AWD-VS + RS for Guixiangzhan that was reduced by 12%. Furthermore, the AWD treatments did not significantly affect ($p > 0.05$) the grains panicle^{-1}, filled grain percentage, and 1000-grain weight compared with control, except grains panicle^{-1} under AWD-VS + RS, which was reduced by 11% and 14% in Guixiangzhan and Nongxiang-18, respectively. Similarly, grain yield and harvest index of both rice cultivars in all AWD treatments also remained statistically similar ($p > 0.05$) with the control, except AWD-VS + RS where grain yield was reduced by 25% and 27% in Guixiangzhan and Nongxiang-18, respectively. On average, all yield and related attributes remained higher in Guixiangzhan than Nongxiang-18 after AWD treatments (Table 1). In addition, AWD affectedmore the plant dry biomass in

TABLE 1 Alternate wetting and drying-induced regulations in yield and yield components of Guixiangzhan and Nongxiang-18

Treatments	Tillers pot^{-1}	Productive tillers pot^{-1}	Grains panicle^{-1}	Filled grain (%)	1000-grain weight (g)	Grain yield pot^{-1}	Harvest index (%)
Guixiangzhan							
Control	38.59 ± 0.72 a	29.91 ± 0.94 a	173.15 ± 3.87 a	83.09 ± 3.57 a	18.27 ± 0.17 a	78.62 ± 4.75 a	74.50 ± 0.92 a
AWD-VS	34.84 ± 0.58 b	28.20 ± 0.59 ab	168.92 ± 2.12 a	83.93 ± 1.05 a	18.42 ± 0.30 a	73.66 ± 2.45 a	73.08 ± 4.49 a
AWD-RS	37.98 ± 0.81 a	28.04 ± 0.16 ab	165.51 ± 3.79 a	83.06 ± 1.64 a	18.26 ± 0.28 a	70.38 ± 2.01 a	70.83 ± 3.21 a
AWD-VS + RS	34.74 ± 0.50 b	26.47 ± 0.62 b	154.45 ± 2.47 b	80.70 ± 0.41 a	17.97 ± 0.34 a	59.21 ± 0.65 b	62.27 ± 3.53 a
Means	36.54	28.16	165.51	82.69	18.23	70.46	70.17
Nongxiang-18							
Control	35.08 ± 1.02 a	27.52 ± 0.64 a	164.55 ± 4.89 a	82.08 ± 3.83 a	18.58 ± 0.16 a	68.88 ± 2.16 a	67.76 ± 8.54 a
AWD-VS	30.93 ± 0.46 b	26.84 ± 0.38 a	160.52 ± 4.23 a	81.13 ± 2.48 a	18.12 ± 0.45 a	63.17 ± 0.56 a	57.90 ± 2.21 a
AWD-RS	34.26 ± 0.71 a	25.86 ± 1.06 a	156.78 ± 2.44 ab	81.39 ± 2.01 a	18.09 ± 0.28 a	59.69 ± 2.96 ab	54.56 ± 2.73 a
AWD-VS + RS	31.74 ± 0.92 b	25.10 ± 0.89 a	141.42 ± 5.32 b	79.33 ± 4.86 a	17.89 ± 0.14 a	50.35 ± 3.87 b	55.33 ± 4.70 a
Means	33.00	26.33	155.82	80.98	18.17	60.52	58.89
Treatments (T)	**	*	**	NS	NS	**	NS
Cultivars (C)	**	**	**	NS	NS	**	**
T × C	NS	NS	NS	NS	NS	NS	NS

Note: Values are the means of three replicates ± SE. *Significant at $p < 0.05$; **significant at $p < 0.01$.
Abbreviations: AWD-RS, AWD at reproductive stage; AWD-VS, AWD at vegetative stage; AWD-VS + RS, AWD at both vegetative and reproductive stages; C, rice cultivars; Control, water potential 0 kPa; ns, nonsignificant; T, treatments.

Nongxiang-18 than Guixiangzhan. For both rice cultivars, the AWD-VS + RS resulted in a more substantial reductions in plant dry biomass than AWD-VS and AWD-RS; however, the plant dry biomass presumably increased from VS to RS and then reduced towards maturity for both rice cultivars (Figure 6A).

3.6 | Grain quality attributes and grain 2-AP contents

The AWD treatments variably affected the grain quality attributes of both rice cultivars. For example, non-significant variations ($p > 0.05$) were noticed regarding the brown rice rate of both rice cultivars, while the milled rice rate reduced significantly (15.48%) in Nongxiang-18 under AWD-VS + RS compared with control. Likewise, significant reductions ($p < 0.05$) in the head rice rate (16.11% and 15.66%, respectively) were also recorded in Guixiangzhan and Nongxiang-18 under AWD-VS + RS. The AWD treatments induced rice chalkiness where the chalkiness rate followed the following trend: AWD-VS + RS > AWD-RS > AWD-VS > AWD-VS + RS > control. No significant variations ($p > 0.05$) were found in grain amylose and protein contents for Guixiangzhan between AWD treatments and control, whereas grain amylose and protein contents were higher in Nongxiang-18 upon AWD-VS + RS than the control (Table 2).

All AWD treatments considerably improved grain aroma in terms of 2-AP contents in both rice cultivars, with an increase of 6.87%–14.45% and 5.04%–14.02% for Guixiangzhan and Nongxiang-18, respectively. For both rice cultivars, the effectiveness of AWD treatments regarding grain 2AP contents was observed as: AWD-RS > AWD-VS > AWD-VS + RS > control. Hence in both rice cultivars, the highest grain 2-AP contents were detected in Guixiangzhan (120.62 ng g^{-1}) and Nongxiang-18 (112.83 ng g^{-1}) under AWD-RS (Figure 6B).

4 | DISCUSSION

The AWD is a promising water-saving technique for rice production systems and has been widely used to replace continuous flooding without compromising yield (Carrijo et al., 2017). The AWD has been found to regulate the photosynthesis and gas exchange and remobilization of photo-assimilates in rice (Zhang, Zhang, Raziuddin, et al., 2008b; Zhang, Zhang, Yang, et al., 2008a). In the present study, AWD treatments regulated the net photosynthesis and gas exchange attributes in both rice cultivars; however, the effects remained non-significant ($p > 0.05$) (Figure 2A–F). Rice yield is closely related to photosynthesis; however, water deficit conditions can reduce net photosynthesis and leaf gas exchange in rice (Centritto et al., 2009; Gu et al., 2012). Our findings are in contradiction to Song et al. (2020),

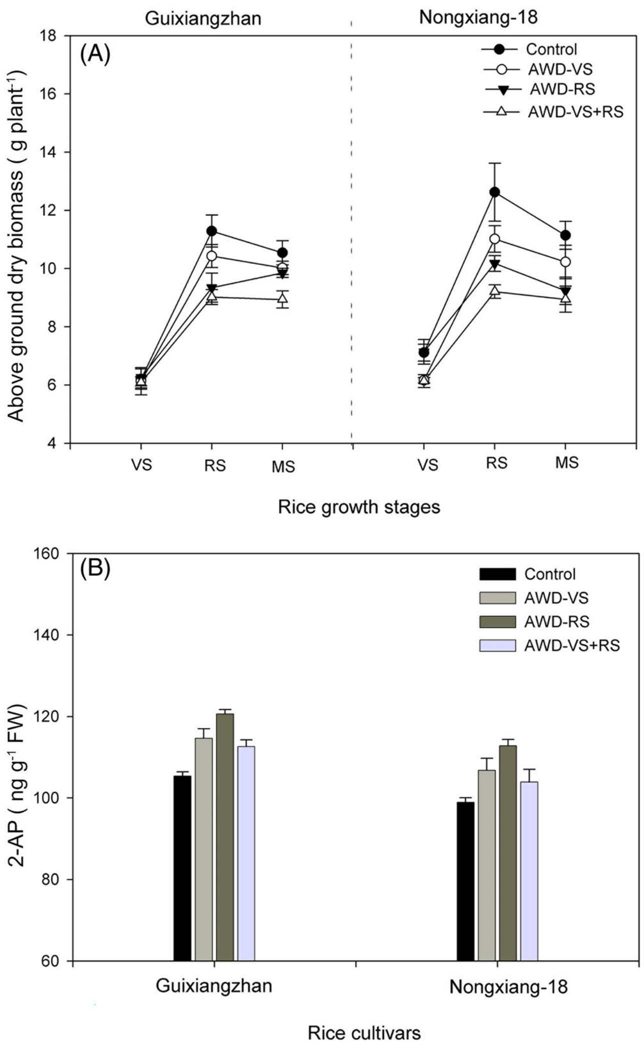


FIGURE 6 Alternate wetting and drying (AWD) induced regulations in (a) above ground dry biomass, and (B) grain 2-AP contents in Guixiangzhan and Nongxiang-18. Capped bars above means are the S.E. of three replicates. Control, water potential 0 kPa; AWD-VS, AWD at vegetative stage; AWD-RS, AWD at reproductive stage; AWD-VS + RS, AWD at both vegetative and reproductive stages. VS, vegetative stage; RS, reproductive stage; MS, maturity stage.

who reported that AWD (soil water potential reaching −15 kPa) significantly reduced the photosynthetic rate, stomatal conductance, transpiration rate, and increased the water use efficiency in rice. Non-significant changes in net photosynthesis and gas exchange attributes may be due to the self-protective mechanism of both rice cultivars to avoid the excessive absorption of sunlight under limited water application. In addition, rice cultivars having a higher potential for CO_2 uptake even under water-deficit conditions show their potential to maintain higher rates of net photosynthesis to achieve maximum yields (Adachi et al., 2013; Scafaro et al., 2011). However, higher gas exchange capacities under AWD can be accompanied towards less adaptability/suitability to grow actively under moisture deficit conditions (Manickavelu et al., 2006). Furthermore, possible improvements in rice yield can be employed through maximizing water use efficiency (WUE), with crops with higher stomatal conductance being the most suited for maintaining photosynthesis and yield even under water deficit conditions in rice (Lauteri et al., 2014).

The AWD enhanced the production of MDA (an indicator of lipid peroxidation), H_2O_2, and EL, while the oxidative damage was higher in AWD-VS + RS than in the other two AWD treatments in both rice cultivars (Figure 3A–C). Higher levels of MDA in plants under AWD treatments suggested that AWD irrigation regimes (low soil water potential) could induce oxidative stress through the overproduction of reactive oxygen species (ROS). The H_2O_2 production at lower levels may play a signaling role against oxidative stress, but over-production of H_2O_2 may result in severe oxidative damage (Ashraf et al., 2015; Hossain et al., 2015; Ashraf et al., 2017). Higher MDA, H_2O_2 and EL levels in Nongxiang-18 might be due to its higher sensitivity to water-deficit conditions than Guixuangzhan. Previous studies also reported that low soil moisture levels could result in the excessive production of H_2O_2 and oxidative stress in rice (Cunha et al., 2016; Iseki et al., 2014). In addition, Ruíz-Sánchez et al. (2011) argued that drought conditions in rice exacerbate lipid peroxidative and reduce rice growth.

Osmo-regulation and/or osmotic adjustment are important physiological responses of plants to water deficit conditions: plants synthesize and accumulate various types of proteins and osmolytes/solutes to maintain osmotic potential as well as to protect the cellular structure and their functions (Bartels & Sunkar, 2005; Seki et al., 2007). It was found that AWD treatments led to an increase in soluble sugars, proline, and protein in both rice cultivars(Figure 4A–C). The nhancement of soluble sugars, proline and protein in both rice cultivars indicated that these solutes were responsible for negating the adverse effects of water deficit conditions (as in AWD treatments) by regulating cell turgor and other physiological mechanisms. Previously, Farooq, Wahid, Kobayashi, et al. (2009) stated that cytosolic accumulation of different osmolytes is involved in osmotic adjustment in rice grown under water-scarce conditions. In addition to its extraordinary role in osmoregulation, proline is also involved in the protection of thylakoid membranes by quenching ROS (Mika et al., 2005). Furthermore, Dasgupta et al. (2015) reported that AWD (terminal soil water potential up to −110 kPa) improved proline contents and maintained low EL in rice (Dasgupta et al., 2015).

Significant effects of AWD treatments were observed on antioxidants (i.e., SOD, and CAT) as well as reduced glutathione (GSH) but both cultivars responded differently to AWD treatments in this regard. Further, activities of POD and APX were recorded statistically similar (p > 0.05) to control whereas activities of all antioxidants increased from VS to RS, and then decreased towards MS (Figure 5A–E). The antioxidant defense system helps plants to confer catastrophic effects of oxidative stress. Higher anti-oxidative activities might be induced by excess energy captured by photosynthetic machinery; however, low soil water potential under AWD could stimulate the antioxidant defense system. In general, SOD is a front-line enzyme to detoxify super-oxide radicals, whereas POD and CAT are involved in scavenging H_2O_2 (Sharma et al., 2012). Furthermore, APX

TABLE 2 Alternate wetting and drying-induced regulations in quality traits of Guixiangzhan and Nongxiang-18

Treatments	Brown rice rate (%)	Milled rice rate (%)	Head rice rate (%)	Chalkiness rice rate (%)	Amylose contents (%)	Protein contents (%)
Guixiangzhan						
Control	79.15 ± 4.98 a	71.80 ± 2.14 a	70.33 ± 3.84 a	26.33 ± 0.88 c	19.32 ± 0.41 a	6.97 ± 0.38 a
AWD-VS	78.52 ± 1.76 a	72.49 ± 2.89 a	69.67 ± 1.45 a	29.33 ± 1.76 bc	19.10 ± 0.31 a	7.17 ± 0.34 a
AWD-RS	78.69 ± 1.11 a	70.79 ± 3.44 a	64.67 ± 2.40 ab	32.67 ± 1.45 ab	19.90 ± 0.25 a	7.46 ± 0.28 a
AWD-VS + RS	75.23 ± 1.60 a	67.93 ± 1.29 a	59.00 ± 0.58 b	36.33 ± 1.86 a	19.70 ± 0.85 a	7.13 ± 0.34 a
Means	77.90	70.75	65.92	31.17	19.50	7.18
Nongxiang-18						
Control	74.50 ± 2.30 a	69.66 ± 2.79 a	66.00 ± 3.00 a	32.00 ± 3.00 b	17.20 ± 0.35 c	6.13 ± 0.19 b
AWD-VS	71.74 ± 3.04 a	64.00 ± 1.90 ab	61.33 ± 4.10 ab	31.33 ± 2.40 b	18.07 ± 0.43 bc	6.23 ± 0.09 b
AWD-RS	72.79 ± 0.56 a	64.44 ± 0.98 ab	60.67 ± 0.33 ab	44.33 ± 2.96 a	18.77 ± 0.13 ab	6.57 ± 0.12 ab
AWD-VS + RS	69.06 ± 0.61 a	58.87 ± 1.17 b	55.67 ± 2.60 b	48.00 ± 2.08 a	19.73 ± 0.32 a	6.76 ± 0.07 a
Means	72.02	64.24	60.92	38.92	18.44	7.18
Treatments (T)	NS	*	**	**	*	NS
Cultivars (C)	**	**	*	**	**	**
T × C	NS	NS	NS	NS	NS	NS

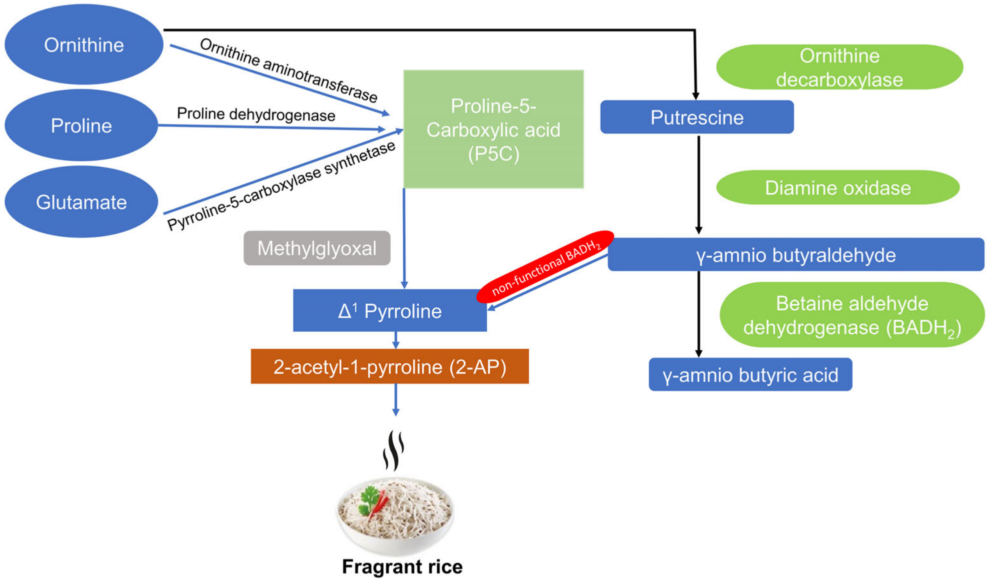
Note: Values are the means of three replicates ± SE. *significant at $p < 0.05$; **significant at $p < 0.01$.
Abbreviations: AWD-RS, AWD at reproductive stage; AWD-VS, AWD at vegetative stage; AWD-VS + RS, AWD at both vegetative and reproductive stages; C, rice cultivars. Control, water potential 0 kPa; ns, non-significant; T, treatments.

is a key enzyme of the ascorbate–glutathione cycle, catabolizes ascorbic acid to dehydroascorbate and helps to quench H_2O_2 to H_2O in the chloroplast (Asada, 1992). In this study, all AWD treatments were found statistically similar ($p > 0.05$) regarding APX activities. Moreover, the non-enzymatic antioxidant GSH contents were higher in AWD-VS and AWD-RS for both rice cultiars at VS and RS than MS. Generally, the GSH contributes to the non-enzymatic ROS-quenching system by detoxifying super-oxide and hydroxyl radicals. Dynamics in the activities of both enzymatic and non-enzymatic antioxidants under AWD have also been previously reported in rice (Hussain et al., 2016; Ashraf et al., 2018).

Moreover, AWD treatments caused differential effects on yield and dry biomass accumulation in fragrant rice. In both rice cultivars, all yield and related components remained lower in AWD-VS + RS than in the other two AWD treatments and control (Table 1). For both rice cultivars, AWD-VS + RS caused more severe reductions in above-ground plant dry biomass than AWD-VS and and AWD-RS; however, percentage reductions were higher in Nongxiang-18 than Guixiangzhan (Figure 6A). Generally, continued water deficit conditions until the reproductive stage may result in a loss of physiological activity and water absorption, which may have severe consequences on rice yield formation. Increased soil water deficit from 0 to −60 kPa reduced grain numbers and seed setting rate (higher sterility percentage) in rice (Oliveira et al., 2016). Thus, variations in water regimes at anthesis and/or grain filling stages possibly reduced the filled grain %, 1000-grain weight, and yield in rice. In addition, enhanced MDA production and reduced SOD activities are also linked with yield loss in rice under water-deficit conditions (Wu et al., 2011). On the other hand, Jahan et al. (2013) reported that AWD can be used as an alternative to flooding in rice without yield penalty. Previously, Guimarães et al. (2016) reported that withholding water at the beginning of the reproductive stage (R5) was more severe than low soil water potential imposed at the panicle exertion (R3) stage. They further argued that irrigation withholding at early and late reproductive stages often results in reduced evapotranspiration and has a linear relation with yield loss in rice. In contrast, Chu et al. (2014) found no significant difference between continuous flooding (CF) and AWD treatments for dry biomass accumulation in drought resistance rice cultivars.

In addition, AWD treatments variably affected the grain quality of both Guxiangzhan and Nongxiang-18. Among all AWD treatments, the AWD-VS + RS resulted in substantial reductions in milled rice rate (in Nongxinag-18) and head rice rate (in both cultivars) with increased grain chalkiness rate compared with control. Furthermore, amylose and protein contents did not differ significantly for Guixiangzhan, while they increased considerably in Nongxiang-18 under AWD-VS + RS as compared with control (Table 2). Under AWD, the water deficit conditions at reproductive and/or grain filling periods could result in remobilization of the carbon reserves, which may result in plant senescence and/or may accelerate grain filling. Overall, water deficit at reproductive stages may change the grain filling periods and patterns, which could have a significant influence on the grain quality attributes of rice. Renmin and Yuanshu (1989) reported that milled rice recovery and protein contents in brown rice increased, while grain amylose contents decreased under soil moisture deficit conditions (60% of the saturated soil moisture contents at flowering) in *indica* rice. Our results also corroborate with Zhang et al. (2004), who concluded that a moderate AWD (soil water stress level − 20 kPa) could

FIGURE 7 Schematic representation of the mechanism of aroma volatile (2-AP) formation in fragrant rice.



regulate the milling, appearance and nutritional quality characteristics of rice while intense moisture stress (−40 kPa) could impart the grain quality characters. Furthermore, a soil water potential of −60 to −65 kPa could decrease the eating qualities in terms of increased amylose, crude protein and fatty acid contents which correlates negatively with rice eating qualities (Zheng et al., 2004). In addition, Dong et al. (2011) argued that a soil water potential between −15 and −30 kPa during the grain filling period could improve rice grain qualities, while the moisture stress beyond this level could reduce the grain quality attributes of rice significantly. Hence, in addition to genetic factors, the external environment (especially water dynamics at various growth stages) severely affects the grain quality attributes in rice.

The formation and accumulation of aroma volatile (i.e., 2-AP) in fragrant rice are affected by various factors, from complicated mechanisms at the cell/molecular level to simple agronomic/management and environmental factors (Mo et al., 2016; Sakthivel et al., 2009). It was observed that AWD treatments at AWD-VS, AWD-RS, and AWD-VS + RS improved the rice aroma by enhancing 2-AP accumulation in grains. All AWD treatments resulted in higher 2-AP contents than control (Figure 6B), but the 2-AP contents were substantially higher in AWD-RS at VS than other AWD treatments. Higher grain 2-AP contents in AWD treatments may be related to the higher proline contents (the precursor of 2-AP formation) in both rice cultivars under water deficit conditions. The production and conversion rate of proline to Δ^1-pyrroline-5-carboxylic acid (P5C) (through the activity of proline dehydrogenase) and then to 2-AP via enzymatic (acetyl Co-A group) or non-enzymatic (methylglyoxal) pathways may be accelerated under AWD treatments (Bao et al., 2018). Furthermore, AWD could also enhance endogenous P5C, which in turn converts into 2-AP contents in aromatic rice (Huang et al., 2008; Tian et al., 2010). In the present study, lower levels of leaf proline contents at MS represent the higher aroma volatile (i.e., 2-AP) contents in rice grains due to its conversion to 2-AP via the above-mentioned phenomena. Recently, Luo et al. (2020) reported that exogenous proline application substantially improved the grain 2-AP contents by up-regulating the endogenous P5C and △1-pyrroline contents, proline dehydrogenase (ProDH) activity and transcription level of *PRODH* gene. Yoshihashi et al. (2002) also found increased 2AP and/or aroma in seedlings and developing callus of fragrant rice. In addition, Bao et al. (2018) showed that mild drought conditions influenced the rice aroma volatiles by regulating the proline biosynthesis while significant and positive associations were noticed between rice aroma and proline contents under rice-duck co-culture conditions (Li et al., 2019). The schematic aroma volatile formation process has been described in Figure 7.

Furthermore, water regulations at early or late growth stages may influence aroma volatiles (e.g., grain 2-AP) contents in fragrant rice. For example, Bhattacharjee et al. (2002) stated that low soil water potential at post-anthesis improved the aroma volatiles in aromatic rice, whereas reduced irrigations at tillering (Tang & Pan, 2014; Wang et al., 2013) and grain filling (Tian et al., 2010) stages resulted in higher 2-AP contents in fragrant rice. It is interesting to note the findings of Zheng et al. (2005) and Yang et al. (2012), who reported that fragrant rice cultivars are rich in aroma volatiles but low in yield as compared with non-fragrant rice cultivars, showing that there may be some competitive mechanisms involved in the biosynthesis of aroma volatiles and yield formation process in fragrant rice. However, there is still a need to unravel these intricate pathways and relationships of aroma volatile and yield formation for a better understanding of these phenomena. On the other hand, soil water potential for AWD should be optimized under field conditions for its safe application without compromising rice yields.

5 | CONCLUSION

Overall, the AWD (soil water potential reached −25 to −30 kPa) applied at either vegetative or reproductive stage promoted morpho-physiological, yield and quality attributes, especially grain aroma volatiles in terms of 2-AP contents. However, continued AWD throughout the growth period (AWD-VS + RS) caused substantial yield loss in both rice cultivars. Further, the AWD-RS at reproductive stage was noticed more effective than at the vegetative stage regarding aroma volatile in fragrant rice. In the future, optimization of rice aroma volatiles, especially grain 2-AP contents, with sustainable rice yields through AWD (with reduced water consumption than conventional flooding) could be of great interest to farmers.

AUTHOR CONTRIBUTIONS

Umair Ashraf and Xiangru Tang conceived the idea, conducted the experiments, data acquisition, interpretation and wrote the manuscript. Umair Ashraf and Zhaowen Mo contributed in data collection and lab analyses. Saddam Hussain and Muhammad Naveed shahid helped in data analyses. Umair Ashraf, Shakeel A. Anjum, and Motohiko Kondo critically reviewed the manuscript, edited and improved the initial and final draft. All authors read and approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as all new created data is already contained within this article

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