DROUGHT STRESS AT FLOWERING STAGE REGULATES PHOTOSYNTHESIS, AROMA AND GRAIN YIELD IN FRAGRANT RICE

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Abstract. Fragrant rice is widely consumed worldwide due to its pleasant aroma and better cooking qualities, however external plant factors such as drought could their yield and quality characteristics of fragrant rice. Drought-induced reductions in morphological and yield attributes of rice were previously reported, nevertheless effects of drought stress imposed at flowering stage on 2-acetyl-1-pyrroline (2AP) contents, grain yield, and related morphophysiological attributes of fragrant rice were rarely investigated. The present study, two fragrant rice cultivars, Yuxiangyouzhan and Guixiangzhan, were exposed to two water levels i.e., (i) well-watered treatment (CK), during the whole plant growth period with a 2-4 cm water layer was maintained until one week before harvest, and (ii) drought stress treatment (Drought) imposed only at 50% heading of the main stem for one week. Results indicated that drought stress substantially improved the grain the 2AP content. The grain yield was decreased by 15.02-20.49% in both rice cultivars owing to substantial reduction in morphological and biochemical attributes, total dry weight, relative water contents, leaf weight per unit area, photosynthesis, and antioxidant activities. Overall, development of drought resistant fragrant rice cultivars is necessary to get grains with strong aroma without compromising grain yield under water deficit conditions.
Keywords: *physiological measurement, drought stress, fragrant rice, yield*

Introduction

Fragrant rice (*Oryza sativa* L.) is globally famous for its unique fragrance and 2-acetyl-1-pyrroline (2AP) was recognized as the key compound responsible for perfumed smell in fragrant rice (Wakte et al., 2017). Physiologically, various enzymes and precursors such as proline, proline dehydrogenase, $^{\Delta}$1-pyrroline are actively involved in 2AP biosynthesis which have been reported in previous studies (Mo et al., 2015, 2019, 2019a; Liu et al., 2020; Xie et al., 2020). Moreover, the enzymes and precursors involved in 2AP formation such as proline, $^{\Delta}$1-pyrroline-5-carboxylate synthetase, $^{\Delta}$1-pyrroline-5-carboxylate, were also involved in the metabolic pathway of plants in response to adverse environments (Xie et al., 2020).

Drought and/or water-deficit conditions not only affect the rice growth and yield traits but also the grain aroma contents in fragrant rice. For example, occurrence of drought during rice growth period could result in poor seed development and yield loss in rice

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(Yang et al., 2013). The inhibition of photosynthetic carbon metabolism under drought stress conditions could make the conditions more adverse in rice (Ambavaram et al., 2014). Previous studies suggested that mild drought (within safe limits) could promote the accumulation of 2AP in fragrant rice without yield loss, whilst shallow-water irrigations/ alternate wetting and drying at tillering, booting stage and grain filling stage could improve the grain 2AP contents (Wang et al., 2013a,b), however, the effects of mild drought stress at flowering stage, the most critical phase for rice yield formation in related to the changes in agronomical and physiological processes under drought stress (Yang et al., 2013; Hinge et al., 2016) on aroma, grain yield, gas exchange parameters and antioxidant activities in fragrant rice have not been well reported. Hence, present study was conducted to evaluate the effects of drought stress at flowering stage on 2AP accumulation in the leaves and grains as well as the gas exchange attributes, antioxidant capacities and grain yield of elite Chinese fragrant rice cultivars.

Materials and methods

Experimental details

A pot experiment was conducted in greenhouse at Experimental Research Farm, South China Agricultural University, Guangzhou, China during 2015. Two regionally popular and widely grown fragrant rice cultivars, i.e., Yuxiangyouzhan and Guixiangzhan having growth periods of 128 and 118 days for early season (March-July), were exposed to two water levels i.e., (i) well water treatment (CK), during the whole plant growth period with 2-4 cm water layer was maintained till one week before harvest, and (ii) drought stress treatment (Drought) imposed only at 50% heading of the main stem (Yang et al., 2013) for one week and then re-watering at the same time before and after treatment. The soil moisture content was measured by oven-drying method. The ratio of the water mass lost to the mass of the dried soil when the samples were dried to a constant weight at 110°Cand expressed as percentage. The pots were arranged in completely randomized design (CRD) with 24 pots per treatment. Rice seedlings (20-days-old) were transplanted into the pots (31 cm in diameter and 29 cm in height), with five hills per pot. All treatments kept shallow water layer 1cm when transplanting, in addition, drought treatment was strictly controlled as required, and all treatments were cut off water one week before harvest. The experimental soil was sandy loam containing 20.45 g kg^{-1} soil organic matter, 1.09 g kg^{-1} total nitrogen, 1.03 g kg^{-1} total phosphorus, 19.68 g kg^{-1} total potassium. The drought was imposed by with-holding irrigation to reduce the water contents at flowering stage and the respective changes of relative soil water content and plant growth of aromatic rice in 7 days after drought stress treatment was shown in (*Figure 1*).

Plant sampling and measurements

The leaves and grains 2AP content were extracted according to (Mo et al., 2019) whereas extract was added for sodium sulfate, filtered with 0.22 μm filter paper (Shimadzu, Japan) and then directly used to detected the 2AP content by using GC-MS QP 2010 Plus (Shimadzu Corporation, Japan) by following the methods of (Hinge et al., 2016). The 2AP content was expressed as μg kg^{-1}. We sampled 6 of 24 pots from each treatment to determine 2AP content.

Yield and yield related traits were estimated according to (Mo et al., 2017). At maturity, grain yield was measured from six pots in each treatment, threshed manually,

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and then sun dried (adjusted to moisture content of ~14%). Panicle number per pot was measured by counting the panicle numbers of each hill in six different pots in each treatment and averaged. Five random samples from filled grains were counted, weighed and averaged. The plant height and total dry weight were measured at maturity stage and the harvest index was calculated as the dry yield weight divided by the total dry aboveground biomass. The leaf weight per unit area was also calculated according to the following formula: specific leaf weight =leaf weight/leaf area.

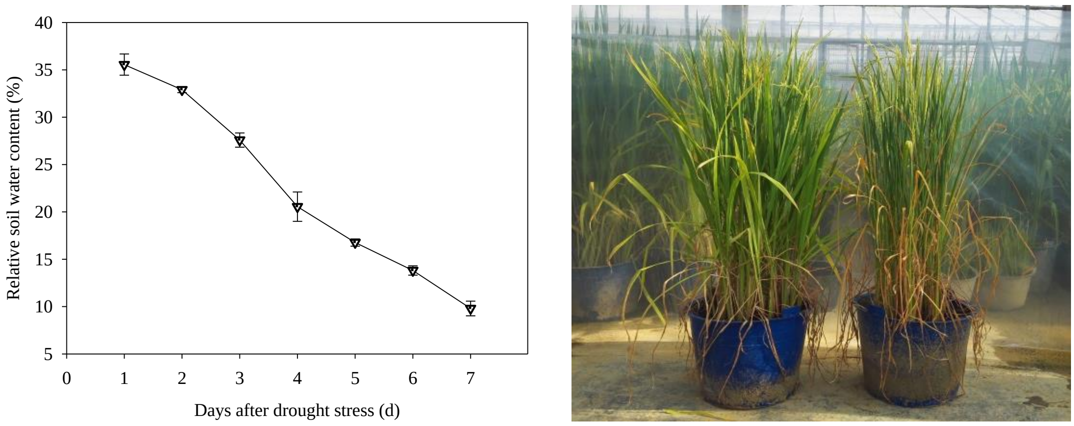

Figure 1. *Changes of the relative soil water content within 7 days after drought stress treatment and the plant growth of aromatic rice*

Net photosynthetic rate and gas exchange attributes i.e., stomatal conductance, intercellular CO_2 and transpirational rates was determined in three plants from each pot and nine from each treatment by using portable photosynthesis system (LI-6400, LI-COR, USA). Photosynthesis and gas exchange attributes were measured at 09:00-11:00 am after treatment application and after 5 days of drought recovery with the following adjustments: photosynthetically active radiation at leaf surface was up to 1200 μmol m^{-2} s^{-1}, molar flow of air per unit leaf area was about 500 μmol s^{-1}, ambient CO_2 concentration almost 400 μmol mol^{-1} (Pan et al., 2016).

In addition, it was cut from after treatment and after 5 days of recovery fresh leaf samples (0.3 g) were homogenized in 6 ml of 50 mM sodium phosphate buffer (pH 7.8) with a mortar and pestle in an ice bath and homogenate were centrifuged at 10000 rpm for 20 min at 4°C and an aliquot of the supernatant was used to record the enzymatic activities. Superoxide dismutase (SOD) was determined according to (Zhang and Kirkham, 1996) by following the inhibition of photochemical reduction due to nitro blue tetrazolium (NBT) and the absorbance was read at 560 nm. SOD activity per units was the amount of enzyme required to inhibit NBT photochemical reduction to 50% as an activity unit (U) and expressed as U g^{-1} protein min^{-1}. Peroxidase (POD) activity was estimated according to Zhang and Kirkham (1996) by using guaiacol method and the absorbance was read at 470 nm. One unit of POD activity was the amount of enzyme that caused absorption variation at 470 nm and expressed as U g^{-1} protein min^{-1}. Catalase activity (CAT) was measured according to the protocols of Zhang and Kirkham (1996). The absorbance was read at 240 nm. One unit of enzyme activity (U) was calculated as the absorption changed at A240 and express as U g^{-1} protein min^{-1}. All were determined by (Shimadzu, Japan UV2600 UV) spectrophotometer.

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Protein contents were estimated according to Bradford (1976) using G-250. The absorbance of the reaction mixture was read at 595 nm in triplicate. The Malondialdehyde (MDA) contents were estimated according to methods advised by Zhang and Kirkham (1996). It was cut from after treatment and after 5 days of recovery fresh leaf samples (0.2 g) were homogenized in 2 ml of 0.5% thiobarbituric acid (TBA) solution in 10% trichloroacetic acid (TCA) and boiled in the water bath at 100°C for 30 min. The boiled samples were then cooled down in an ice bath and centrifuged at 4000 rpm for 15 min. The absorbance of the reaction mixtures was read by spectrophotometer and the final contents were expressed as μmol g^{-1}.

The relative water contents (RWC) of the leaves were determined according to Nauš et al. (2016). To determine the total nitrogen content in after treatment the leaves and grains, the dried samples (0.3 g) were digested using the 5 ml of H_2SO_4 and a few drops of $HClO_4$ to clear the solution. The total N contents were determined by the Kjeldahl method with a 2300 Kjeltec Analyzer Unit (Foss Tecator AB, Sweden).

Statistical analysis

The pots were arranged in completely randomized design (CRD) with 24 pots per treatment. Analyses of variances (ANOVA) were performed by Statistix version 8 (Statistix 8, Analystical, Tallahassee, Florida, USA). Comparisons of means among different treatments were separated according to the least significant difference (LSD) test at the 5% probability level. The figures were made by using the SigmaPlot for windows version 10. 0 and Microsoft Excel 2010.

Results

2AP content in grains and leaves

The 2AP contents for both rice cultivars i.e., Guixiangzhan and Yuxiangyouzhan were substantially enhanced after drought treatment. For instance, the 2AP content in grains were enhanced by 298.84% and 20.52% for Guixiangzhan and Yuxiangyouzhan, respectively, whereas the 2AP contents in leaves were enhanced by 33.46% of Guixiangzhan whereas a marginal increase was noted in the leaves of Yuxiangyouzhan at the end of treatment only. Moreover, no significant increase in 2AP content in leaves of both rice cultivars was detected after 5 days of drought recovery (*Figure 2*).

Agronomic traits

Compared to CK, drought decreased plant height, grain yield and total dry weight in Guixiangzhan by 4.88%, 15.02%, and 14.03%, respectively, as well as by 4.81%, 22.99%, and 20.49% in Yuxiangyouzhan, respectively. No significant effect of drought on harvest index was recorded for both fragrant rice cultivars (*Figure 3*).

Photosynthetic rate and gas exchange parameters

Drought substantially reduced photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate in both fragrant rice cultivars (*Figure 4*). The photosynthetic rate, stomatal conductance and transpiration rate for Guixiangzhan was reduced even after 5 days of recovery. Drought significantly decreased the photosynthetic rate in Yuxiangyouzhan whereas the intercellular CO_2 concentration for both fragrant rice cultivars were enhanced under after 5 days of recovery stress. Moreover, photosynthetic

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rate, stomatal conductance and transpiration rate were remained higher under CK (except intercellular CO_2 concentration) (*Figure 5*).

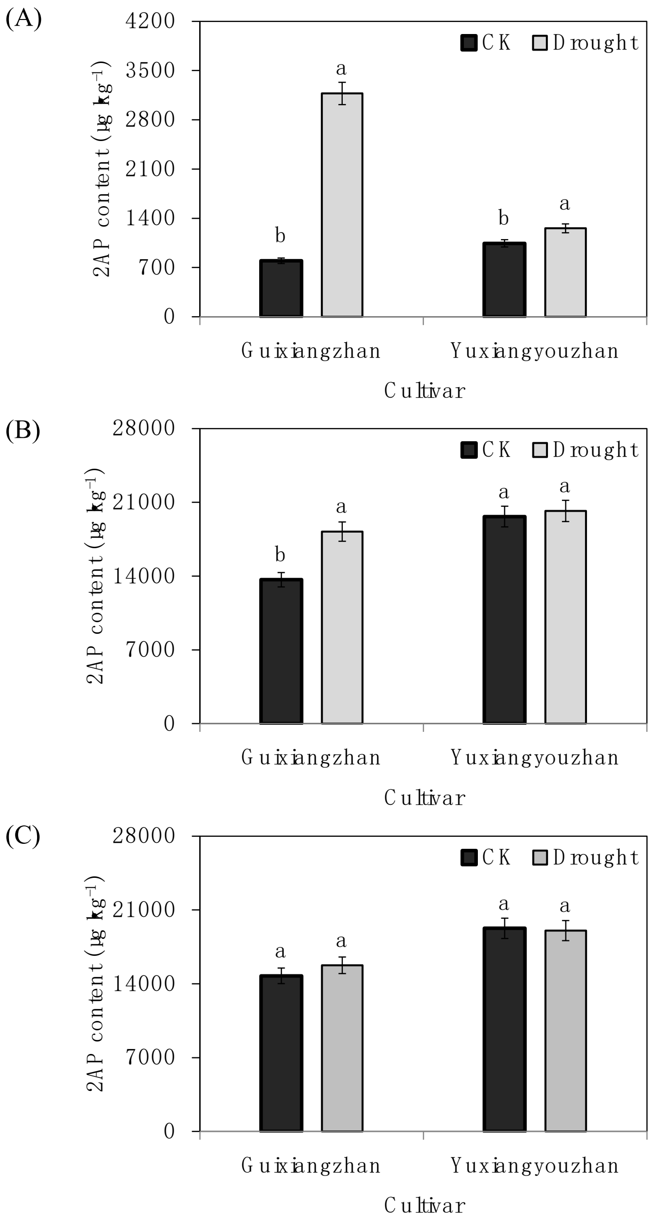

Figure 2. *Effects of drought stress on 2AP content in grains (A) and leaves at the end of treatment(B) and after 5 days recovery(C) of two fragrant rice cultivars. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=3).CK:Well water treatment*

Physio-biochemical attributes and leaf N contents

The protein contents in the leaves were substantially increased after drought but decreased significantly after 5 days of recovery. No significant difference in terms of MDA concentration after drought was found, however, the MDA concentration was remained noticeably higher after 5 days of recovery (*Figure 6*).

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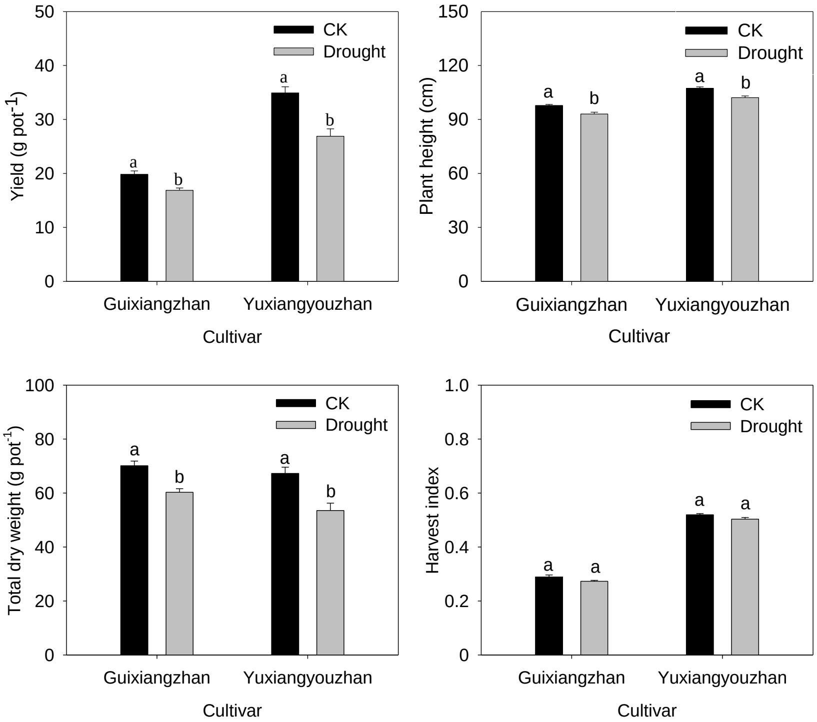

Figure 3. *Effects of drought stress on yield, plant height, total dry weight and harvest index of two fragrant rice cultivars. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=6). CK: Well water treatment*

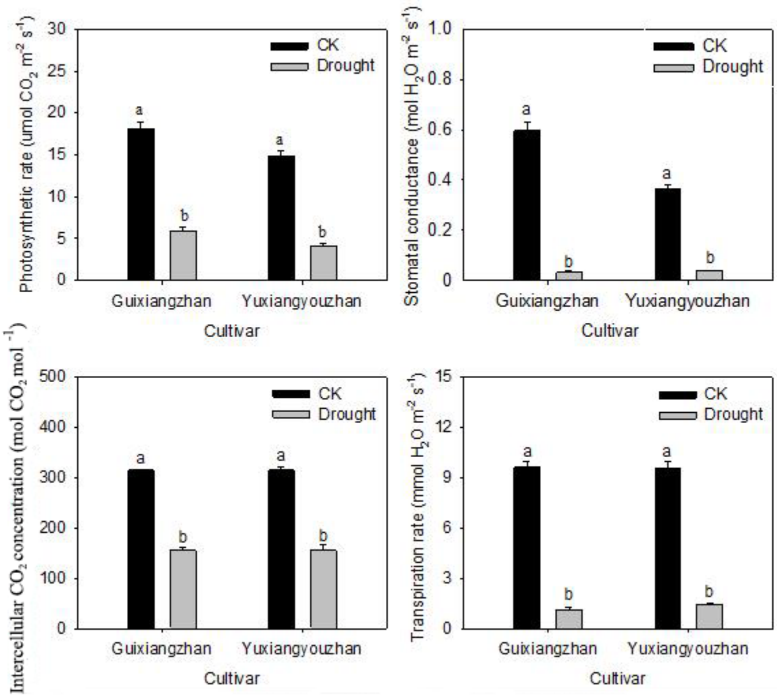

Figure 4. *Effects of drought stress treatment on photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of two fragrant rice cultivars at the end of treatment. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=6). CK: Well water treatment*

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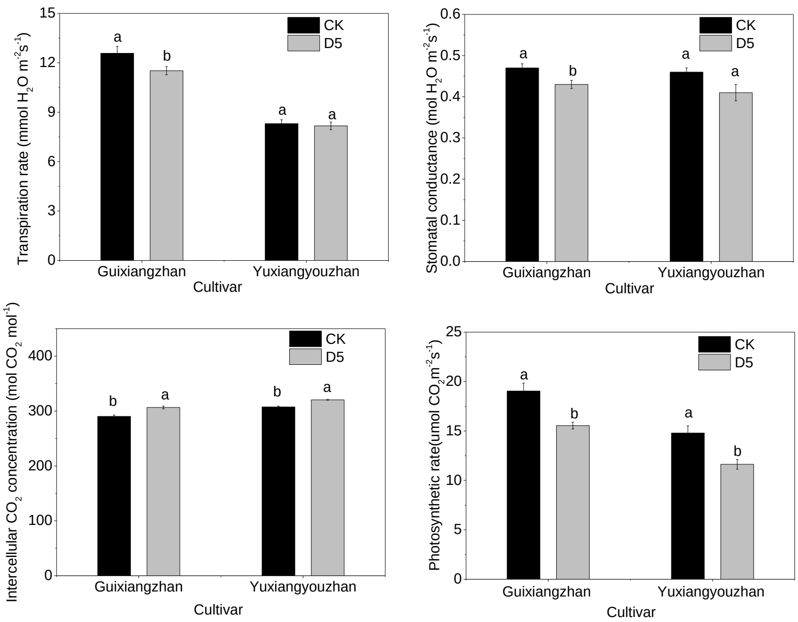

Figure 5. *Effects drought stress treatment on photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of two fragrant rice cultivars after 5 days recovery. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=6).CK: Well water treatment. D5: After 5 days of recovery*

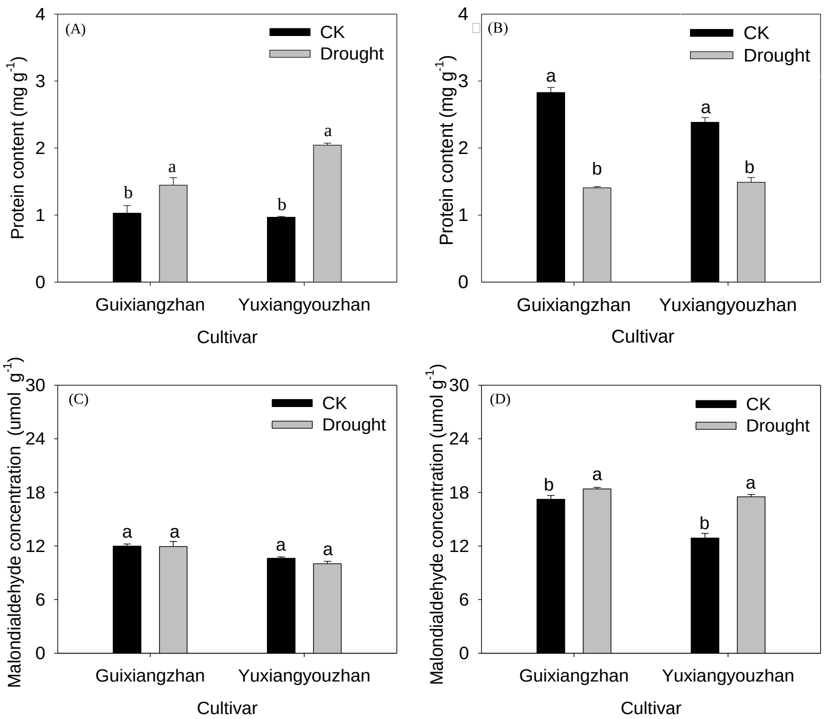

Figure 6. *Effects of drought stress treatment on protein content and malondialdehyde concentration of two fragrant rice cultivars. A, C, sampling at the end of drought stress treatment and B, D, sampling at 5 days after recovery. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=6) CK: Well water treatment*

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Drought stress decreased the activities of SOD, POD, and CAT activities by 31.22%, 32.86%, and 35.70%, respectively in the leaves of Guixiangzhan whilst decreased by 48.71%, 34.03%, and 33.49% for Yuxiangyouzhan under drought stress treatment. After 5 days of recovery, SOD, POD and CAT activities were remained higher under drought stress than CK. Moreover, the activities of SOD, POD, and CAT activities were increased by 42.39%, 64.97%, and 67.65% for Guixiangzhan and 33.04%, 54.49%, and 47.13% for Yuxiangyouzhan (*Figure 7*).

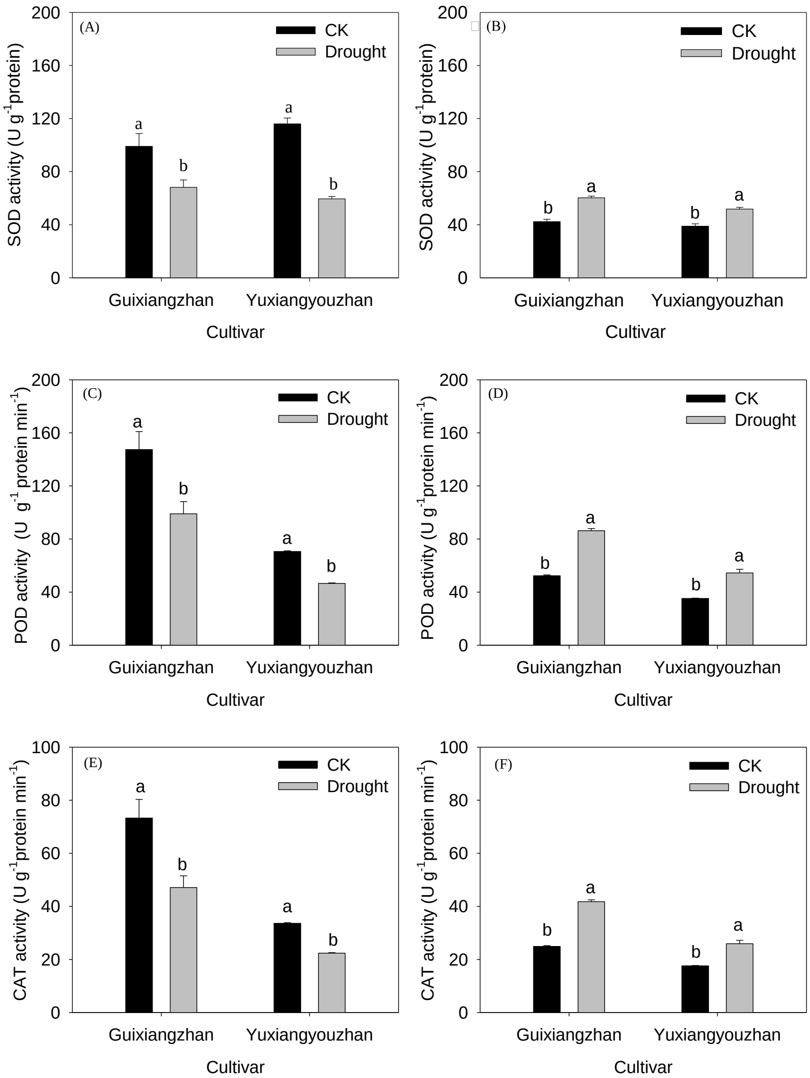

***Figure 7.** Effects of drought stress treatment on SOD, POD and CAT activity of two fragrant rice cultivars. A, C, E sampling at the end of drought stress treatment and B, D, F sampling at 5 days after recovery. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=6). CK: Well water treatment*

The leaf RWC and the leaf weight per unit area of both fragrant rice cultivars were noticeably decreased under drought stress (*Figure 8*). Likewise, the total nitrogen contents in leaves were decreased by 10.19% for Yuxianyouzhan whilst no significant effect of drought was observed on total nitrogen content in leaves for Guixiangzhan (*Figure 9*).

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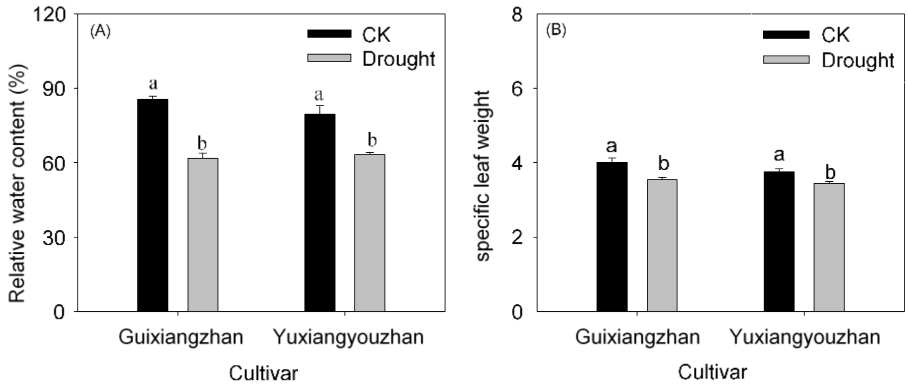

Figure 8. *Effects of drought stress treatment on relative water content and specific leaf weight of two fragrant rice cultivars. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=10). CK: Well water treatment*

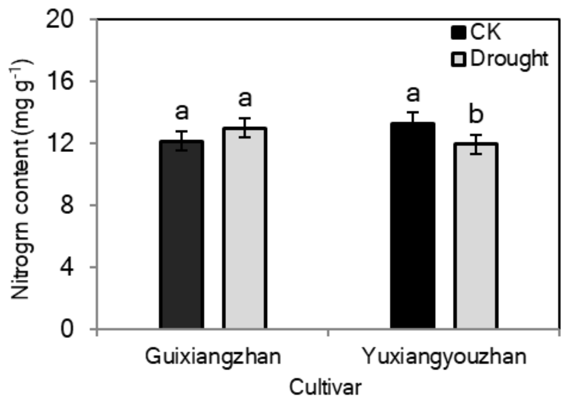

Figure 9. *Nitrogen content in leaves after drought stress treatment of two fragrant rice cultivars. Vertical bars with different lower case letters above are significantly different at P<0.05 by LSD tests. Capped bars represent S.D (n=6). CK: Well water treatment*

Analysis of variance for the investigated parameters

Cultivars (C) were remained statistically different regarding plant height, grain yield, harvest index, photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and protein and MDA contents as well as for POD and CAT activity in the leaves after drought treatment. Drought significantly affected plant height, grain yield, total dry weight, harvest index, photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, protein content, POD, SOD and CAT activities, relative water contents, leaf weight per unit area in leaves as well as photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, protein and MDA contents, and the activities of POD, SOD and CAT in the leaves after 5 days of recovery. For C×T interaction was significantly affected the grain yield, stomatal conductance, intercellular CO_2 concentration, protein and nitrogen contents and SOD activity in the leaves after drought treatment and the protein and MDA contents, POD and CAT activity in leaves after 5 days of recovery whereas no significant difference was observed for other parameters (*Table 1*).

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Table 1. *Analysis of variance of the investigated parameters*

Investigated parameters	Cultivar (C)	Water treatment (T)	C×T
Yield	164.72**	64.03**	13.46**
Plant height	340.72**	27.80**	0.04^{ns}
Total dry weight	2.25^{ns}	38.56**	1.08^{ns}
Harvest index	2255.23**	8.99*	0.00^{ns}
Photosynthesis rate in leaves after treatment	15.78*	485.84**	1.66^{ns}
Stomatal conductance in leaves after treatment	51.75**	561.45**	40.11**
Intercellular CO_2 concentration in leaves after treatment	16.31**	292.92**	41.84**
Transpiration rate in leaves after treatment	0.46^{ns}	705.94**	0.45^{ns}
Protein content in leaves after treatment	9.17*	170.14**	33.05**
MDA concentration in leaves after treatment	9.21*	1.88^{ns}	1.37^{ns}
POD activity in leaves after treatment	51.20**	26.96**	3.06^{ns}
SOD activity in leaves after treatment	0.45^{ns}	80.80**	6.92*
CAT activity in leaves after treatment	48.43**	27.82**	4.41^{ns}
Photosynthetic rate in leaves after 5 days of recovery	27.16**	30.40**	0.08^{ns}
Stomatal conductance in leaves after 5 days of recovery	5.98^{ns}	9.43*	0.27^{ns}
Intercellular CO_2 concentration in leaves after 5 days of recovery	71.04**	89.26**	1.22^{ns}
Transpiration rate in leaves after 5 days of recovery	100.97**	6.64*	3.90^{ns}
Protein content in leaves after 5 days of recovery	30.07**	223.38**	11.57**
MDA concentration in leaves after 5 days of recovery	24.85**	72.64**	25.87**
POD activity in leaves after 5 days of recovery	136.00**	258.02**	19.93**
SOD activity in leaves after 5 days of recovery	29.12**	57.64**	1.57^{ns}
CAT activity in leaves after 5 days of recovery	152.10**	252.16**	29.10**
Relative water content in leaves after treatment	0.82^{ns}	86.87**	2.88^{ns}
Specific leaf weight after treatment	3.70^{ns}	27.37**	1.00^{ns}
Nitrogen content in leaves after treatment	0.04^{ns}	0.93^{ns}	17.97**
2AP content of grains after treatment	338.44**	1462.8**	1031.36.00**
2AP content of leaves after treatment	0.08^{ns}	0.01^{ns}	2.85^{ns}
2AP content of leaves after 5 days of recovery	507.74**	3.36^{ns}	6.48^{ns}

*, significant at $P<0.05$; **, significant at $P<0.01$; ns, no significant at $P<0.05$ level

Discussion

Cultivar, drought stress and their interactive effect on fragrant rice have been detected in this study (*Table 1*). Previously, Fitzgerald et al. (2010) reported that the fragrance in rice was related to yield reduction under salt treatment. In this study, drought stress significantly increased 2AP content in mature grain, and enhanced the 2AP content in leaves (*Figure 2*) whereas the 2AP of Guixiangzhan and Yuxiangyouzhan was remained higher than CK under drought stress. Moreover, the yield of both rice cultivars was significantly higher under CK than drought treatment, which indicates that the content of 2AP is negatively correlated with the yield under drought stress. In addition, it can also

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be inferred that the yield of Yuxiangyouzhan under drought conditions is higher than that of Guixiangzhan, which is mainly related to its higher harvest index (*Figure 3*).

In addition, the 2AP possibly transports from leaves and other above ground plant parts to grains (Mo et al., 2016) whilst the grain 2AP content is related to the nitrogen content in plants (Mo et al., 2018, 2019a). Thus, higher 2AP content in leaves could result in high 2AP content in mature grains. Furthermore, the regulations in the N content, Na content, rice yield, shoot dry weight and tiller number are linked to the dynamics in grain 2AP contents (Funsueb et al., 2016). In this study, the agronomic parameter such as grain yield, N content, relative water content, gas exchange parameters, protein content and antioxidative capacities of the fragrant rice were regulated by drought stress (*Figures 2-11*). The gas exchange parameters contribute to rice photosynthesis that lead to dry matter accumulation in fragrant rice thus could affect the morpho-physiological and yield traits under drought stress. Moreover, the physiological traits such as protein, relative water contents, as well as antioxidant activities regulated the drought-resistant metabolism in fragrant rice, which affects rice yield as well as the aroma accumulation. Overall, these results suggested that changes in 2AP content in leaves, total dry weight, plant height, harvest index, gas exchange parameters, protein content, relative water content, N content and antioxidant activities were related to 2AP in grains under drought stress.

Rice yield formation substantially affected in response to drought stress at flowering stage as a consequence of the change of the agronomic and physiological attributes (Fu et al., 2010). Drought stress at flowering stage decreased photosynthetic rate in rice (Yang et al., 2013). In this study, drought stress substantially reduced photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate in both fragrant rice cultivars after drought stress treatment (*Figure 4*). The photosynthetic rate, stomatal conductance and transpiration rate for Guixiangzhan was substantially lower even after 5 days of recovery. Drought stress treatment significantly decreased the photosynthetic rate in Yuxiangyouzhan whereas intercellular CO_2 concentration for both fragrant rice cultivars were enhanced under after 5 days of recovery (*Figure 5*). Therefore, yield reduction in fragrant rice is related to alteration in photosynthesis and gas exchange attributes under drought stress at flowering stage.

Drought stress at flowering stage led to excessive accumulation of reactive oxygen species (ROS) that may cause oxidative damage and thus lead to substantial reduction in photosynthetic rate (Zhou et al., 2015). The ROS are scavenged through the enzymatic antioxidant defense system i.e., SOD, POD, and CAT and non-enzymatic components i.e., ASA, GSH, vitamin (Ashraf et al., 2015, 2017a; Ashraf and Tang, 2017). In general, antioxidants such as SOD, POD and CAT play an important role in eliminating ROS in plant cells (Ashraf et al., 2017b, 2018). Our results indicated that the activities SOD, POD, CAT were decreased under drought stress treatment (*Figure 7A,C,E*), which indicated that the water-deficit conditions might damage antioxidant defense system, however, after 5 days of drought recovery, SOD, POD, CAT activity have been improved. Dynamics in the activities of antioxidants under water deficit conditions were previously reported (Yang et al., 2014; Anjum et al., 2016). Besides, the changes in protein synthesis and degradation are regarded as direct responses of plants to drought stress (Shanker et al., 2014). The protein contents were substantially increased under drought treatment and was reduced after 5 days of recovery (*Figure 6A,B*), which implicated that the trial cultivars may maintain water balance via altered protein contents to adapt the irreversible condition. Interestingly, no significant difference in terms of MDA concentration after

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drought stress treatment was found whereas MDA concentration was remarkably increased after 5 days of recovery (*Figure 6C,D*). Thus, morpho-physiological changes and regulations in 2AP contents of fragrant rice in response to drought stress at flowering stage were quite prominent. Therefore, close relationships exist among the investigated parameters in fragrant rice such as the rice yield, gas exchange parameters and antioxidative capacities, and all those parameters were also associated with aroma in fragrant rice. In addition, limited water application and/or water deficit conditions could substantially reduce the rice yield as compared to normal irrigation application which might be due to drought stress induced the closure of leaf stomata and gaseous exchange between leaf mesophyll cells and the external environment (Du et al., 2020). Hence, reduced photosynthetic rate could result in reduced yield production under drought conditions. No doubt, drought stress often results in yield reduction in rice. Some studies have shown that appropriate application of nitrogen can promote the development of rice root system and improve the rice yield under drought conditions (Cao et al., 2017). In this experiment, under drought treatment, the content of total nitrogen in leaves of yuxiang oil decreased significantly (*Figure 9*). This suggests that fertilization of the Yuxiangyouzhanl during this period may help to increase the yield. However, as there is lack of evidence to support this; much work should be done in future studies.

Conclusion

In conclusion, the 2AP contents were substantially enhanced under drought stress in both rice cultivars whereas the 2AP contents were varied between grains and leaves. On the other hand, the drought stress decreased grain yield in fragrant rice cultivars as a consequence of the reduction related morphological traits i.e., plant height, the total dry weight, the relative water content, the leaf weight per unit area, the photosynthetic capacity, and the antioxidant enzyme activities. Therefore, in the future, we need research temporal transcription dynamics during plant drought needs to be further investigated to unravel the drought tolerant genes to develop drought - tolerant and high - yielding fragrant rice cultivars.

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