Genotypic variation in transpiration and grain yield in a japonica rice diversity set grown under aerobic conditions

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Abstract
Australian rice (*Oryza sativa* L.) is traditionally grown under flooded conditions in the Riverina, but with reduced water supply, non-flooded aerobic production has been suggested to improve water productivity. It is essential that donor varieties and traits adapted to aerobic conditions are identified, such that the breeding program can incorporate them with the aim of establishing a sustainable temperate aerobic production system. Hence, a field experiment was conducted to explore stomatal related traits in a japonica diversity set. It is hypothesised that genotypes that are able to maintain high transpiration will contribute to the maintenance of high grain yield. This experiment explored genotypic variation in canopy temperature differential (CTD) and stomatal conductance which may contribute to the maintenance of high transpiration under the cyclic nature of soil wetting and drying in aerobic production.
Genotypic variation existed among 241 genotypes for CTD which was negatively correlated with grain yield (r=-0.38**). Furthermore, the dissection of stomatal conductance in 31 genotypes provided strong evidence that genotypes with high stomatal conductance had low CTD at 2 to 4 days after irrigation (r=-0.39*~-0.88**), and tended to improve grain yield. The experiment quantified the existence of genetic variation in CTD which can potentially be exploited by the NSW-DPI rice breeding program to improve plant transpiration to improve yield of direct seeded aerobic rice. Donor genotypes with high stomatal conductance or low CTD have entered the pre-breeding program to improve the adaptation of germplasm for aerobic production in Australia.

Keywords
Stomatal conductance; canopy to air temperature differential

Introduction
Australian rice is traditionally grown under flooded conditions in the Riverina production environment. Drill seeding is now becoming common in Australia with many advantages such as reduced water supply, no wind or muddy water problems, changed weed spectrum and reduced duck and snail issues (Dunn and Ford 2018). However, reduced water supply has put increasing pressure to further improve water productivity. Aerobic rice that is grown in unsaturated, but well-watered conditions, has been suggested to improve water productivity as a result of reduced water losses (Lal et al. 2012). Rice crops in the Riverina district are among the highest yielding crops in the world. The target environment under aerobic conditions is for yields to be maintained close to that of flooded conditions while reducing water inputs. Traits that contribute to maintenance of transpiration have been explored under drought conditions but have not been explored under non-flooded, high water availability conditions. Stomatal conductance is the largest plant factor affecting transpiration. Thus, there is a need to explore genetic variation in stomatal conductance and CTD to identify genotypes able to maintain the influx of CO_2 for photosynthesis and thereby contribute to maintenance of high grain yield under reduced soil water availability. Under water deficit, stomatal conductance is generally reduced to conserve water and maintain leaf water potential (Agurla et al. 2018). According to Ohsumi et al. (2007), genetic variation for stomatal conductance has been identified in 67 rice varieties under flooded conditions. The method of measuring stomatal conductance is often time consuming and does not allow for the rapid estimation of a large number of genotypes, whereas CTD is relatively fast and a convenient measure that is indicative of evaporative cooling as a result of transpiration, and therefore indirect measurement of stomatal conductance (Urban et al. 2017). The genotypes that are able to have high conductance and low CTD would be advantageous for aerobic rice. In conclusion, under aerobic conditions where soil water content may decline temporarily maintenance of high stomatal conductance increases CO_2 uptake and promotes photosynthesis and ensures high biomass production. Therefore, a field experiment in Gatton was conducted to examine the genetic variation in stomatal conductance and CTD in relation to grain yield in a japonica rice diversity set grown under aerobic conditions.

Methods

Experiment

The experiment was conducted at Gatton (27.5636 ° S, 152.2800° E) in a vertisol soil in Queensland, Australia during the summer of 2019 - 2020. A japonica diversity set of 241 genotypes from a mix of lowland and upland varieties compiled by NSW DPI rice breeders was used. The experiment was arranged in a partially-replicated design due to low seed availability of some genotypes (44 genotypes had 1 replication while the remaining had 2). Canopy temperature was measured on 11 occasions with Everest 6210L Agri-Therm III, (Everest Interscience Inc, USA) and RS PRO thermohygrometer (Enrgtech, UK) between 10:00 and 14:00 during the reproductive stage and expressed as the canopy to air temperature differential (CTD). Stomatal conductance (g_s) was measured using AP4 porometer on a 31 genotype subset. This subset included the top 14 and bottom 14 genotypes (28 genotypes) based on CTD at 60 days after sowing (DAS) which was measured before irrigation and where two replications existed as well as standard check varieties Reiziq, Sherpa and Tachiminori. A 0.88m^2 area was harvested and panicles threshed at maturity to quantify grain yield.

Statistical Analysis

A multiplicative linear mixed model was used for analysis and was implemented in As-Reml-R (V4.3) in the R environment (V3.6.3) (Butler et al. 2009). The best spatial model was fitted for each trait (Gilmour et al. 1997). Genotype was treated as both a random effect to estimate heritability and a fixed effect to obtain best linear unbiased estimates (BLUEs). A repeated measures analysis was undertaken for CTD and stomatal conductance across measurement event, and an unstructured residual variance model was used.

Results and discussion

Stomatal Conductance and Canopy to Air Temperature Differential

There were highly significant (P<0.01) genotypic differences for CTD in the diversity set on all days recorded. The average CTD varied from -9.81 °C to -4.78 °C, with heritability of CTD in diversity set ranging from 0.43 to 0.57, and in the subset from 0.30 to 0.81. Genotype by CTD measurement date interaction was also highly significant (p<0.01) in the diversity set. Highly significant (P<0.01) genotypic differences existed for all g_s measurements. Heritability of g_s ranged from 0.66 to 0.85 which may be over inflated because of the selection of the tops and tails, while the means varied between 177.7 and 454.5 mmol m^{-2} s^{-1}. There was a highly significant (P<0.01) effect of genotype and genotype by measurement date interaction for g_s (Table 1). The average g_s dropped from 397.4 mmol m^{-2} s^{-1} at 2 days after irrigation on 76 DAS to 177.7 mmol m^{-2} s^{-1} at 4 days after irrigation on 78 DAS with the CTD increased from -9.78 ℃ to – 5.04 ℃ at the same time period. Similar results were found by Parent et al. (2010) who found that stomatal conductance of rice in greenhouse decreased from 500 to 100 mmol m^{-2} s^{-1} as the soil water potential decreased from flooded condition to -20kPa. Melandri et al. (2019) found that the average canopy temperature of 293 indica rice accessions was 2.27 °C lower under flooded conditions than under drought conditions. Significant negative correlations were found between g_s and CTD at 6 of the 7 days of measurements (r=-0.39*~-0.88**) in the present experiment, while similar results were also found by Takai et al. (2010) in a transplanted rice paddy experiment (r= -0.93**).

Table 1 BLUES of canopy air temperature differential (CTD ℃) in full diversity set and 31 genotype subset and stomatal conductance (g_s mmol m^{-2} s^{-1}) in subset, correlations between g_s and CTD in subset and days after irrigation for each measurement date, with repeated measures analysis on g_s and CTD in the subset of days of measurement (time) and genotype (*P<0.05, **P<0.01, ^{ns} not significant)

Date	CTD Diversity set	CTD Subset	g_s subset	Correlations between g_s & CTD	Days after irrigation
54 DAS	-5.60	-5.50	/	/	1
58 DAS	-7.15	-7.08	/	/	1
60 DAS	-4.69	-5.03	/	/	3
63 DAS	-4.78	-5.41	/	/	2
65 DAS	-9.27	-8.74	428.60	-0.50**	2
73 DAS	-5.89	-6.08	283.16	-0.49**	2
76 DAS	-9.81	-9.70	387.79	-0.25^{ns}	2
77 DAS	-6.10	-7.09	309.85	-0.67**	3
78 DAS	-5.46	-6.60	177.72	-0.88**	4
83 DAS	/	-6.35	454.46	-0.45*	2
85 DAS	/	-7.03	433.10	-0.39*	2

LSD5% G	0.79**	0.80**	51.41**
LSD5% G x D	2.65**	2.65**	142.36**

Grain Yield

Highly significant (P<0.01) genotypic differences existed for grain yield with high heritability (0.83). The average grain yield was 7.14 t ha^{-1}, with the highest grain yield produced by Calrose76 at 13.20 t ha^{-1} while IR34 produced the lowest grain yield at 1.84 t ha^{-1}. Australian standard varieties Sherpa and Reiziq had relatively high grain yield at 11.48 and 10.05 t ha^{-1}, respectively.

Relationship between Physiological Traits and Grain Yield

Grain yield was significantly correlated with both average CTD (r= -0.38**) and g_s (r=0.41*) (Figure 1). YRF210 and Gavina were found to have high stomatal conductance, low CTD and relatively high grain yield. According to Centritto et al. (2009), stomatal conductance was found to be associated with high yield and photosynthetic rate in rice. Melandri et al. (2019) found that CTD was negatively (r=-0.48**) related to grain yield under drought in rice. Fukuda et al. (2018) found a QTL, qCTd11, for CTD in a chromosomal segment substitution line population of Koshihikari and Takanari. In a near-isogenic line, the decreased CTD was also found to associated with increased photosynthesis.

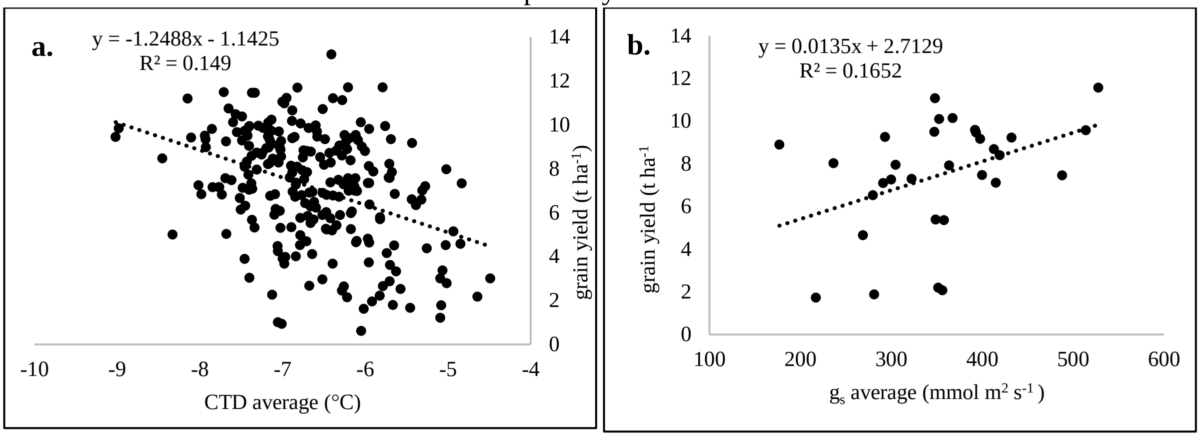

Figure 1 The relationship between grain yield (t ha^{-1}) and (a) canopy to air temperature differential (°C) in diversity set and (b) stomatal conductance (mmol m^{-2} s^{-1}) from 31 genotypes in subset

Conclusion

Genotypic variation existed among 241 genotypes in the diversity set for CTD, and among 31 genotypes in the subset for stomatal conductance. Both CTD and stomatal conductance were related to grain yield. Therefore, genotypes with greater stomatal conductance that would have maintained higher transpiration rate often achieved higher grain yield. These donors can be incorporated into the rice breeding program to improve the adaptation of germplasm for aerobic production in Australia. The genetic variation in stomatal conductance and CTD could be exploited to improve plant transpiration and photosynthesis thus improve yield under aerobic conditions. This has not been previously quantified for germplasm that is directly relevant to the Australian breeding program. Further research is currently underway to explore the genotypic variation under mild water stress conditions.

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References

Agurla, S., S. Gahir, S. Munemasa, Y. Murata and A. S. Raghavendra (2018). Mechanism of stomatal closure in plants exposed to drought and cold stress. Survival Strategies in Extreme Cold and Desiccation, Springer: 215-232.

Butler, D., B. R. Cullis, A. Gilmour and B. Gogel (2009). ASReml-R reference manual. Brisbane, The State of Queensland, Department of Primary Industries Fisheries.

Centritto, M., M. Lauteri, M. C. Monteverdi and R. Serraj (2009). "Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage." Journal of Experimental Botany 60(8): 2325-2339.
Dunn, B. and R. Ford (2018). Management of drill sown rice. Primefact 1253 3rd edn. O. NSW Department of Primary Industries, NSW.
Fukuda, A., K. Kondo, T. Ikka, T. Takai, T. Tanabata and T. Yamamoto (2018). "A novel QTL associated with rice canopy temperature difference affects stomatal conductance and leaf photosynthesis." Breeding science: 17129.
Gilmour, A. R., B. R. Cullis and A. P. Verbyla (1997). "Accounting for natural and extraneous variation in the analysis of field experiments." Journal of Agricultural, Biological, Environmental Statistics 2(3): 269-293.
Lal, B., A. Nayak, K, G. Priyanka, T. Rahul, S. Teekam and L. J (2012). "Aerobic Rice: A Water Saving Approach for Rice Production." International Journal of Research in BioSciences 1: 1-6.
Melandri, G., A. Prashar, S. R. McCouch, G. Van Der Linden, H. G. Jones, N. Kadam, K. Jagadish, H. Bouwmeester and C. Ruyter-Spira (2019). "Association mapping and genetic dissection of drought-induced canopy temperature differences in rice." Journal of experimental botany 71(4): 1614–1627.
Ohsumi, A., T. Kanemura, K. Homma, T. Horie and T. Shiraiwa (2007). "Genotypic variation of stomatal conductance in relation to stomatal density and length in rice (Oryza sativa L.)." Plant Production Science 10(3): 322-328.
Parent, B., B. Suard, R. Serraj and F. Tardieu (2010). "Rice leaf growth and water potential are resilient to evaporative demand and soil water deficit once the effects of root system are neutralized." Plant, Cell & Environment 33(8): 1256-1267.
Takai, T., M. Yano and T. Yamamoto (2010). "Canopy temperature on clear and cloudy days can be used to estimate varietal differences in stomatal conductance in rice." Field Crops Research 115(2): 165-170.
Urban, J., M. W. Ingwers, M. A. McGuire and R. O. Teskey (2017). "Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in Pinus taeda and Populus deltoides x nigra." Journal Of Experimental Botany 68(7): 1757-1767.