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Research

Effects of *Sub1* and *qDTY* on The Agro-Morpho-Physiological Traits of Rice (*Oryza sativa* L.) Under Reproductive Stage Drought Stress

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ABSTRACT

Drought is one of the major abiotic stresses that affects plants at various levels and stages as it also reduces crop productivity and weakens global food security. Breeding and screening of drought tolerant varieties are crucial to ensure the continuity of food supply. Two breeding lines, UKM-112 and UKM-114 with QTL(s) for submergence tolerance (*Submergence 1* [*Sub1*]) and yield under drought stress (*qDTY*) and together with their recurrent parental line, UKM-5, and a drought-sensitive cultivar, MR219 were screened for their agro-morpho-physiological responses under reproductive stage drought stress (RS) and non-stress (NS) to determine how the *Sub1* interacts with *qDTY* in enhancing drought tolerance. Under NS, UKM-112 (with $qDTY_{3.1}$ and *Sub1*) recorded the highest values while MR219 (no QTL) recorded the lowest values for most parameters. Furthermore, lines with QTL (either single or two QTLs) outperformed MR219 in all agronomic parameters including grain yield (GY) under RS. UKM-114, which carries the *Sub1*, demonstrated higher values for important agronomic traits such as the number of filled spikelets (FS), thousand-grain weight (TGW), and GY when exposed to drought stress. The enhanced values in UKM-114 are a good indication that the presence of *Sub1* minimized the negative impacts of drought on yield-related traits. The genotype with *qDTY* or a combination of *qDTY* and *Sub1* has a lower photosynthetic rate under RS compared to the genotype with only *Sub1* but higher than MR219 suggesting that *qDTY* improved the photosynthetic rate, though lesser compared to *Sub1*. These results indicate that *Sub1+qDTY* does not effectively improve drought stress survivability and yield. The presence of *qDTY* appears to mask the beneficial effect of *Sub1* in improving photosynthetic rate and yield. While the present study did not report a positive outcome for *Sub1+qDTY*, the combination of these traits has the potential to provide beneficial effects without any negative interactions.

Key words: Agronomy, drought, photosynthesis, physiology, QTL, transpiration

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INTRODUCTION

Rice is the primary source of nutrition for more than half of the world's population and makes up a significant portion of the food consumed daily in countries located in Southeast Asia including Malaysia. Asia is the region that produces and consumes about 90% of the world's rice and is the key to global food security in rice production (Bandumula, 2018). In the previous half century, rice output in Asia has expanded by nearly three times due to the rise in yields brought on by the so-called "Green Revolution" in agricultural technology (Nahar *et al.,* 2016; Horie, 2019).

The rice sector is a crucial sector in Malaysia that provides food and jobs for the community and generates income for the country. Due to factors including growing demand and population expansion, the amount of rice consumed in this nation has increased (Harun *et al.,* 2021). As the average Malaysian consumes 82.3 kg of rice annually and an average of 3.7 metric tonnes (MT) of rice is produced per hectare of paddy field, rice cultivation serves as the country's main source of food (Panchakaran, 2015).

Drought is one of the major abiotic stresses that affects plants at various levels and stages as it also reduces crop productivity and weakens global food security (Mohd Ikmal *et al.,* 2023). Of all the abiotic stresses that commonly severely reduce crop productivity, drought is the most threatening. Over 50% of the world's rice land is thought to be affected by drought, which is a severe constraint on rice production (Bouman *et al.,* 2005; Fukao *et al.,* 2011; Selvaraj *et al.,* 2020). Every year, drought stress in Asia affects 8 million hectares of highland rice and 34 million hectares of lowland rice and the intensity of the stress varies (Singh *et al.,* 2016). The effect of drought on Malaysian farmers caused around RM1 million in losses (Wan Mansor, 2020).

This abiotic stress also affects stomatal closure, restricts gas exchange, lowers transpiration, and interferes with photosynthesis in addition to reducing water content, turgor, and total water in plants (Daszkowska-Golec and Szarejko 2013; Razak *et al.,* 2013). Drought stress significantly decreased rice genotype yield, plant height, tiller count, and total chlorophyll content (Singh *et al.,* 2018).

In rice mapping populations that were subjected to drought conditions, a total of 262 quantitative trait loci (QTL) traits were found for the root according to Li *et al.,* (2017). Under non-stress and reproductive-stage drought stress circumstances, Catolos *et al.,* (2017) identified three QTL significant consistent-impact QTLs for grain yield ($qDTY_{1.1}$, $qDTY_{1.3}$, and $qDTY_{8.1}$). These QTLs were found to have an effect on grain yield. Sabar *et al.,* (2019) identified three quantitative trait loci (*qDRL3*, *qDRL9*, and *qDRL11*) for deep root length (below 60 cm), as well as two quantitative trait loci (*qHt6*, *qHt1*) for plant height. Apart from that, several meta-QTLs linked to various traits for drought response were also identified by Selamat and Nadarajah (2021). These features have the potential to be exploited to improve rice's resistance to drought and to raise overall rice yield.

In reaction to submergence stress, *Submergence 1* (*Sub1*) QTL has been introduced in many rice cultivars to improve tolerance in flash flooding fields (Fukao *et al.,* 2006; Sarkar & Bhattacharjee, 2011; Kumar *et al.,* 2020). Although many exposures of rice plants to prolonged submergence, *Sub1* is also able to respond to drought stress. According to Saha *et al.,* (2018), *Sub1* minimizes oxidative damage as well as protection through the chelation of reactive oxygen species.

Sub1 also helps to reduce the amount of growth that occurred while water was being withheld, and there was a steady decline in the amount of chlorophyll, lycopene, and carotenoids present throughout the length of the water stress. The findings of Fukao *et al.,* (2011) reveal that *Sub1* has a role in preventing the loss of leaf water during periods of drought. As a result of reoxygenation, the role of *Sub1* is to keep the leaf water content stable, which may be seen when there is a drought. According to the findings of the research, *Sub1* QTL does not only respond favourably toward submergence in rice, but they can also react favourably when subjected to the stress of drought. However, very limited studies reported on the response of genotypes with *Sub1* under drought stress. In the present study, we aimed to evaluate the performance of advanced breeding lines possessing the combination of *Sub1* and a QTL for yield under drought stress (*qDTY*) to obtain preliminary pictures of how *Sub1* and its combination with *qDTY* confer drought tolerance. This study focuses on the screening of the lines under drought stress and non-stress conditions for morphological, agronomical, and physiological traits.

MATERIALS AND METHODS

Study site and plant materials

The study was conducted at Pusat Kecemerlangan Padi, Department of Agriculture in Titi Serong, Perak (2°55'21"N 101°46'57"E) during the 2019 main season. Two advanced breeding lines, namely UKM-112 and UKM-114 were evaluated together with their recurrent parent, UKM-5, and one of the most popular cultivars in Malaysia, MR219. MR219 is a high-yielding cultivar but performed poorly under water stress (Mohd Ikmal *et al.,* 2019; Mohd Ikmal *et al.,* 2021). Therefore, it was included as the susceptible check in this study. UKM-112 and UKM-114 were developed through marker-assisted breeding from the population of UKM5*/IR64-Sub1. Table 1 shows the list of genotypes used in this study and their information.

Drought stress and non-stress screening procedures

The genotypes were arranged in a split-plot completely randomized design with three replications. The main plot is the field which was divided into drought and non-stress conditions, while the subplot is the genotypes. The seedlings were transplanted into the puddled field at the age of 21 days after seeding. For each genotype, 189 seedlings were transplanted in each replication. Planting space was 0.25 m between rows and 0.25 m between hills. Drought stress (RS) was imposed 30 days after transplanting, before the starting of the reproductive phase by draining the irrigation water out from the field. The field was maintained unflooded until maturity and harvest for the drought stress treatment while for the non-stress (NS) treatment, the field was maintained flooded with 5 cm standing water until a week before harvest. The gravimetric method was used for measuring the moisture content of the soil in the drought stress plot throughout the growing period. The moisture content of the soil in the RS trial was kept between 5-15% to ensure uniform stress throughout the experiment period.

Table 1. Information of genotypes used in this study

Genotype	Source	QTL
UKM-5	Universiti Kebangsaan Malaysia	$qDTY_{3.1}+qDTY_{12.1}$
MR219	Malaysian Agricultural Research and Development Institute	-
UKM-112	Universiti Kebangsaan Malaysia	$Sub1+qDTY_{3.1}$
UKM-114	Universiti Kebangsaan Malaysia	*Sub1*

Phenotypic data collection

The genotypes were measured for morphological traits, viz. days to 50% flowering (DTF) and plant height (PH) in cm, agronomical traits, viz. number of panicles per plant (NP), number of spikelets per panicle (SPP), number of filled spikelet per panicle (FS), thousand-grain weight (TGW) in g, grain yield (GY) in kg ha^{-1} at 14% moisture content. At the maturity stage, in each replication, four representative hills were sampled for PH, and NP. FS was hand-calculated from three panicles from each representative hill. Five panicles were sampled from each of the four representative hills to count for SPP and to measure the TGW. All plants were harvested for determination of GY.

Photosynthetic parameters measured were photosynthesis rate (P_n, μmol m^{-2} s^{-1}), transpiration rate (E, $mmol^{-2}$ s^{-1}), and stomatal conductance (g_{sw}, mol m^{-2} s^{-1}). The measurements were conducted using a portable photosynthesis instrument, Li-6400XT (Li-Cor Inc., Lincoln, NE, USA) by referring to Wang and Kinoshita (2017) and Siti Nurfaeiza *et al*. (2022) with some modifications. The air temperature in the leaf chamber was maintained at 30 °C, CO_2 concentration at 400 μmol mol^{-1}, relative humidity between 55%-65% and photosynthetic photon flux density of 1300 μmol mol^{-1}. The measurements were conducted after 70 days of treatment between 0800-1100 hours. A young and mature leaf was selected for each measurement. For each replication, three plants were sampled and three measurements were taken from each plant.

Statistical analysis

Analysis of variance (ANOVA) was conducted for all the measured traits using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 developed by the International Rice Research Institute (2014) followed by Fisher's LSD test for traits recording the significant difference. The Pearson's correlation coefficient values among traits recorded in this study were computed in RStudio version 1.2.5003 by using the *Stat* package (R Core Team, 2020) and were visualized into a graphical correlation matrix using the *Corrplot* package (Wei and Simko, 2017)

RESULTS

The results of a two-way ANOVA in Table 2 revealed that there was not a statistically significant interaction between the effects of genotypes and treatment for plant height (PH), number of panicles per plant (NP), and thousand-grain weight (TGW). A statistically significant interaction between the effects of genotypes and treatment was found for days to 50% flowering (DTF), number of spikelet per panicle (SPP), number of filled spikelet per panicle (FS), and grain yield (GY). Simple main effects analysis showed that treatment has a statistically significant effect on DTF and GY. Simple main effects analysis also showed that genotypes did have a statistically significant effect on all morpho-agronomic traits except FS. The lack of a significant interaction effect suggests that the relationship between genotype and PH, as well as between genotype and NP, is consistent across the treatment levels.

Under NS, MR219 was the earliest to flower (Table 3). The two advanced breeding lines, UKM-112 and UKM-114, both harbouring the submergence tolerance QTL (*Sub1*) flowered later than MR219. However, while being the earliest to flower under NS, MR219 recorded the longest DTF under RS. UKM-5, UKM-112 and UKM-114 flowered slightly earlier by 21 to 24 days. All genotypes recorded a reduced height under RS compared to under NS. The highest reduction was recorded for UKM-114 (reduced by 15.89 cm, 14.15%), followed by MR219 (reduced by 13.11 cm, 11.17%). UKM-5 recorded the lowest reduction of PH by a 7.44 cm difference (7.26%).

Table 2. Analysis of variance for morpho-agronomical traits (mean square values)

Source of variation	df	DTF	PH	NP	SPP	FS	TGW	GY
Treatment (T)	1	1162.04***	791.43	9.13	486.88	313.61	0.02	248909868.68***
Rep(T)	4	0.21	122.32	24.20	404.62	178.95	3.84	575213.97
Genotypes (G)	3	147.49***	203.99*	44.53*	2048.55*	739.76	12.37*	13310466.42**
G × T	3	251.60***	21.17	7.50	4256.31**	1189.16*	4.82	11902635.86**
Pooled error	12	0.38	36.89	9.61	407.13	297.83	2.31	1249931.53

*, **, *** indicate significant at $p < .05$, $p < .01$ and $p < .001$ respectively

Days to 50% flowering (DTF), plant height (PH), number of panicles per plant (NP), number of spikelet per panicle (SPP), number of filled spikelet per panicle (FS), thousand-grain weight (TGW) and grain yield (GY)

Table 3. Mean values comparison of genotypes in each treatment condition (G × T) for days to flowering (DTF), plant height (PH) and number of panicles per plant (NP)

Genotype	DTF (days)		PH (cm)		NP	
	NS	RS	NS	RS	NS	RS
MR219	75.67	109.00	119.33	106.22	20.67	17.47
UKM-5	77.33	84.67	102.44	95.00	24.00	20.87
UKM-112	79.67	87.67	111.66	102.17	25.00	25.87
UKM-114	80.00	87.00	112.33	96.44	23.67	24.20
F-value	670.93		0.57		0.78	
p-value	<0.001		>0.05		>0.05	
$\text{LSD}_{0.05}$	1.09		-		-	
d.f.	12		12		12	

Table 4 shows the mean values for all genotypes in both NS and RS conditions for the SPP, FS, TGW and GY. In the NS, UKM-112 had the highest SPP (191.57) followed by MR219 (157.75) and UKM-5 (151.11). UKM-114 recorded the lowest SPP in NS. Meanwhile, the highest SPP in RS was recorded for MR219 (194.83), and the lowest was recorded for UKM-112. Although MR219 had the highest SPP in RS, the recorded FS was the lowest. UKM-114 recorded the highest FS in RS followed by UKM-5.

Table 4. Mean values comparison of genotypes in each treatment condition (G × T) for number of spikelet per panicle (SPP), number of filled spikelet per panicle (FS), thousand-grain weight (TGW) and grain yield (GY)

Genotype	SPP		FS		TGW (g)		GY (kg ha^{-1})		%GYR
	NS	RS	NS	RS	NS	RS	NS	RS	
MR219	157.75	194.83	94.61	82.40	25.30	23.50	10674.13	3295.00	69.13
UKM-5	151.11	135.87	108.69	101.77	27.43	27.30	13328.00	5632.67	57.74
UKM-112	191.57	109.93	131.28	92.17	28.17	25.70	14105.60	5683.13	59.71
UKM-114	121.00	144.77	97.67	127.00	24.77	26.33	8414.40	6147.80	26.94
F-value	10.45		3.99		2.09		9.52		-
p-value	<0.01		<0.05		>0.05		<0.01		-
$\text{LSD}_{0.05}$	35.90		30.70		-		1988.92		-
d.f.	12		12		12		12		-

The significant main effect of genotype on TGW indicates that the different genotypes have varying effects on this trait, regardless of the treatment applied. TGW recorded for all genotypes were between 23.50 g to 27.30 g in NS and 25.33 g to 27.30 g in RS. UKM-112 recorded the highest TGW in NS, while the lowest TGW was recorded for UKM-114. In RS, UKM-5 recorded the highest TGW, followed by UKM-114. The lowest TGW in RS was recorded for MR219.

All five genotypes had GY of more than 8000 kg ha^{-1} in the NS and more than 3000 kg ha^{-1} in RS. All genotypes also had reduced GY in the RS compared to the NS. UKM-112 recorded the highest GY in NS (14105.60 kg ha^{-1}) while the lowest was recorded for UKM-114 (8414.40 kg ha^{-1}). In the RS, the highest GY was recorded for UKM-114 (6147.80 kg ha^{-1}) followed by UKM-112 (5683.13 kg ha^{-1}) and UKM-5 (5632.67 kg ha^{-1}). Meanwhile, MR219 (3295.00 kg ha^{-1}) recorded the lowest GY in RS.

Table 5 shows the results of ANOVA for physiological traits. The results indicated that the effects of G × T were significant for transpiration rate (E). Meanwhile, the effects of treatment were significant for net photosynthesis (P_n), and stomatal conductance (g_{sw}) but not for transpiration rate (E).

Figure 1(A) shows the P_n for all five genotypes. In NS, UKM-112 had the highest P_n while UKM-114 recorded the lowest P_n. However, UKM-114 recorded the highest P_n in RS, while MR219 recorded the lowest. UKM-114 recorded higher P_n in RS compared to UKM-5 (no *Sub1*) and MR219 (susceptible to drought). In Figure 1(B) UKM-112 shows the highest g_{sw} in NS followed by UKM-114. MR219 recorded the lowest g_{sw} in NS. UKM114 also recorded higher g_{sw} in RS compared to UKM-5 and MR219. Figure 1(C) shows UKM-112 with the highest E in NS compared to other genotypes while MR219 recorded the highest E in RS. The lowest E in NS was recorded for MR219. UKM-114 recorded higher E compared to UKM-5 in RS.

Figures 2 and 3 show the graphical correlation matrix for the traits evaluated in the NS and RS trials respectively. In the NS trial, the agronomic traits, namely NP, TGW and SPP were found to respond positively with GY, shown by the positive correlation, although not significant. GY was also positively correlated with P_n, g_{sw}, and E but the correlation was not significant. All the physiological traits measured were positively correlated with each other in the NS trial. On the other hand, GY was significantly negatively correlated with PH. Delayed DTF in the NS trial was found to affect the GY based on the negative correlation recorded for both traits, but the correlation was very low and not significant.

In the RS trial, delayed DTF was also found to negatively affect the GY as recorded in the NS trial but the correlation was higher and significant. The same result as in the NS trial was recorded for GY and NP in the RS trial, but SPP was found to have a negative correlation with GY. The physiological traits were positively correlated with GY in the RS trial, except for E, which was negative and significant.

Table 5. Analysis of variance for physiological traits (mean square values)

Source of variation	df	P_n	g_{sw}	E
Treatment (T)	1	220.48*	0.13*	2.50
Rep(T)	4	14.27	0.01	0.92***
Genotypes (G)	3	30.38	1.08***	1.58***
G × T	3	37.05	0.08	6.92***
Error	12	11.93	0.03	0.14

, * indicate significant at $p < 0.01$ and $p < 0.001$ respectively

net photosynthesis (P_n), stomatal conductance (g_{sw}) and transpiration rate (E)

DISCUSSION

In general, the presence of *Sub1* has been found to confer advantageous effects on rice plants under conditions of drought stress. Among the five genotypes studied, UKM-114, which carries the *Sub1* gene, demonstrated higher values for important agronomic traits such as FS, TGW, and GY when exposed to drought stress. These traits are directly linked to rice yield, and therefore the enhanced values in UKM-114 are a good indication that the presence of *Sub1* minimized the negative impacts of drought on yield-related traits. The significant effect of genetic variation (G), treatment effects (T), and their interaction (G × T) on GY underscores the importance of genotypic factors in determining the response of rice to drought stress and ultimately affecting its yield.

In terms of morphological traits, the *Sub1*-bearing genotype exhibited a reduced growth relative to other genotypes, consistent with previous findings that the presence of *Sub1* reduces growth in conditions of water scarcity (Fukao *et al.,* 2011; Saha *et al.,* 2018). The ANOVA analysis showed that variation in G had a significant effect on three morphological traits, namely DTF, PH, and NP, while the effects of T and their interaction were only significant for DTF. This indicates that G is the primary source of variation affecting the morphological traits in this study. Therefore, the observed differences in response to normal and reduced watering conditions among the five genotypes can be contributed primarily to their respective genotypes.

The measurements of photosynthetic parameters provide further evidence that genotypes harboring the *Sub1* QTL are better equipped to withstand drought stress. Photosynthesis is known to have a significant impact on crop yield (Parry *et* al., 2011; Long *et al.,* 2015) and the present study likewise found a significant correlation between all three photosynthetic parameters with GY (Figure 3). Furthermore, both variations of G and G × T exerted significant effects on the response of all three photosynthetic parameters to drought stress, indicating that the genotypes themselves play a critical role in photosynthesis, and its relationship to crop yield. Specifically, *Sub1*-bearing genotypes (UKM-114 and 112) displayed lower transpiration rates during RS, indicating reduced water loss relative to genotypes that do not carry *Sub1* QTL. Additionally, the tolerant genotypes exhibited stable stomatal conductance levels under both normal and reduced watering conditions, demonstrating the ability of *Sub1* to regulate water loss through the regulation of stomatal conductance under drought stress. Lastly, the photosynthetic rate of *Sub1*-bearing genotypes was found to be consistently higher than that of genotypes that do not bear *Sub1* genotypes, such as MR219 and UKM-5.

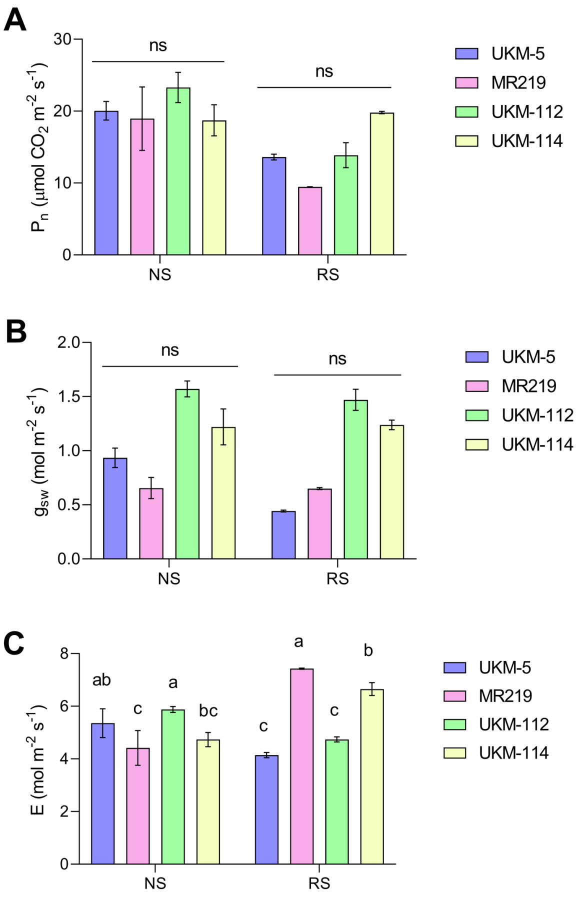

Fig. 1. Mean values comparison of genotypes in each treatment condition (G × T) for net (A) photosynthesis (P_n), (B) stomatal conductance (g_{sw}) and (C) transpiration rate (E). Mean values with different alphabets are significantly different by Fisher's LSD ($p < 0.05$)

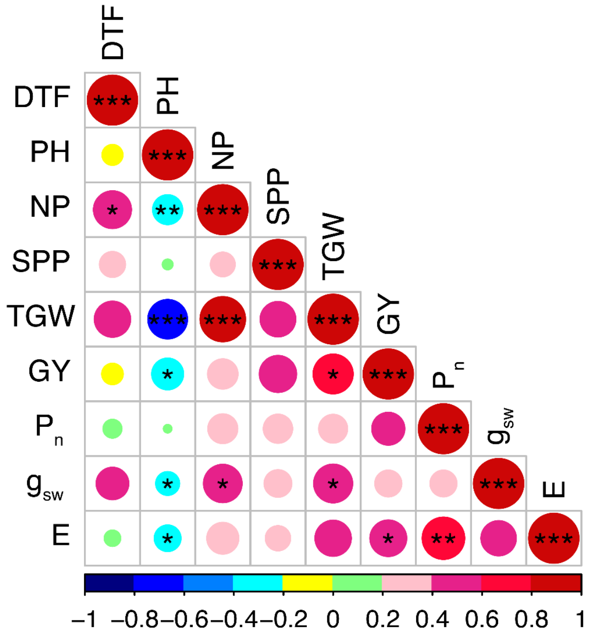

Fig. 2. Matrix showing the correlation among traits evaluated in the NS trial.

*, **, *** indicate significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively
Days to 50% flowering (DTF), plant height (PH), number of panicles per plant (NP), number of spikelet per panicle (SPP), number of filled spikelet per panicle (FS), thousand grain weight (TGW) and grain yield (GY), net photosynthesis (P_n), stomatal conductance (g_{sw}) and transpiration rate (E)

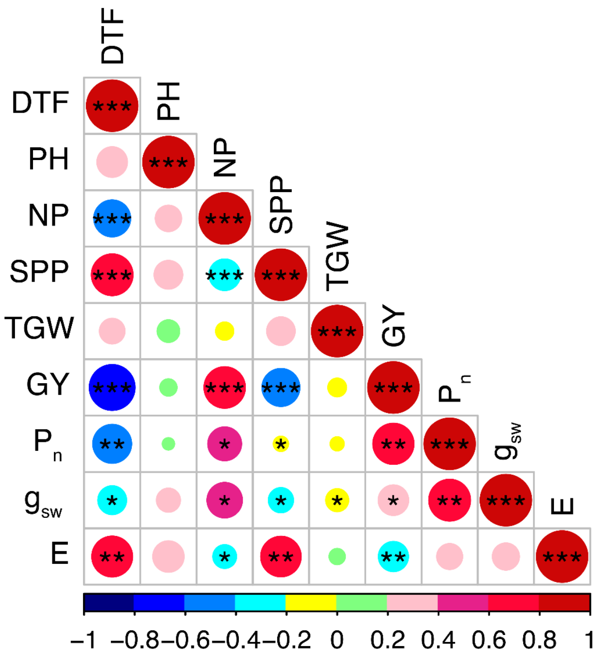

Fig. 3. Matrix showing the correlation among traits evaluated in the RS trial.

*, **, *** indicate significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively
Days to 50% flowering (DTF), plant height (PH), number of panicles per plant (NP), number of spikelet per panicle (SPP), number of filled spikelet per panicle (FS), thousand grain weight (TGW) and grain yield (GY), net photosynthesis (P_n), stomatal conductance (g_{sw}) and transpiration rate (E)

The submergence gene *Sub1* is a well-known major QTL that helps rice cope with submergence stress. However, this study suggests that *Sub1* also enhances the performance of rice under drought stress. The role of Sub1 in improving the survival under drought stress was also reported by Fukao *et al*. (2011). *Sub1* has been suggested to be involved in chlorophyll protection regardless of the submergence condition (Vijayalakshmi *et al.,* 2020). *Sub1* introgression lines have been shown to maintain high levels of chlorophyll content and fluorescence during submergence and the recovery phase after submergence. This indicates that the ability of *Sub1* QTL to maintain high chlorophyll content and fluorescence is not limited to submergence conditions only. As chlorophyll fluorescence is positively correlated with photosynthesis rate, which impacts yield (Elanchezhian *et al.,* 2013; Vijayalakshmi *et al.,* 2020), the ability of *Sub1* introgression lines maintain high levels of chlorophyll content and fluorescence suggests their ability to maintain photosystem II (PSII) efficiency, which affects photosynthetic activity. Given that photosynthetic activity plays a critical role in determining the yield of rice, the high photosynthetic rate observed in this study may be associated with the ability of *Sub1* to retain high chlorophyll content and fluorescence. Although most studies have focused on the role of *Sub1* under submergence conditions, the possibility of *Sub1* retaining high chlorophyll content and fluorescence under drought conditions and improving its survivability and yield should not be ignored. As also shown in this study, the effects of *Sub1* under non-stress condition is not profound, which suggests that *Sub1* only works under stress condition. Even though no yield penalty was reported in the current study, the yield of the *Sub1*-only genotype under non-stress was lower than other genotypes. In the absence of stress, *Sub1* showed no effects on yield and growth (Mackill et al. 2012), which is in line with the results obtained in the non-stress condition in the current study. This is because *Sub1* is an ethylene response factor (ERF) and its effects are only induced in the presence of stress (Fukao *et al*. 2006). Therefore, we suggest that a deeper exploration of the role of *Sub1* in the absence of stress on the physiological traits can be carried out to dissect the mechanism of its action.

The interactive effect of *Sub1* and *qDTY* under submergence stress has been studied, and the combination seemed to improve the submergence tolerance in rice (Mohd Ikmal *et al.,* 2021). It is noteworthy that the same combination may not necessarily confer benefits to rice under drought stress. In fact, the present study revealed that the *Sub1*+*qDTY* bearing genotype, UKM-112, exhibited lower yield-related traits such as SPP, FS and, TGW than the *Sub1*-only and *qDTY*-only bearing genotypes, such as UKM-5 and UKM-114. Furthermore, despite having the highest photosynthetic rate under normal condition, the photosynthetic rate of UKM-112 was relatively low compared to UKM-114 (*Sub1*-only genotype). Under RS, the photosynthetic rate of UKM-114 was higher compared to the susceptible genotype MR219. The genotype with *qDTY* or a combination of *qDTY* and *Sub1* has a lower photosynthetic rate under RS compared to the genotype with only *Sub1* but higher than MR219 suggesting that *qDTY* improved the photosynthetic rate, though lesser compared to *Sub1*. Taken together, these results suggest that *Sub1*+*qDTY* is not an effective combination for improving survivability and yield under drought stress. Instead, the presence of *qDTY* appears to mask the beneficial effect of *Sub1* in improving photosynthetic rate and yield.

In previous studies, epistatic interaction between *Sub1* and *qDTY* has been reported. Yadav *et al.* (2019) reported a significant epistatic interaction between introgressed QTL $qDTY_{6.1}$ and background loci of TDK1-Sub1. Five significant epistatic interactions between $qDTY_{6.1}$ and background loci located across chromosomes 2, 3, 5, 6, and 11 have been identified, resulting in those NILs of TDK1-Sub1 background being classified as low yielding. In another study conducted by Majumder *et al.*, (2021), negative interaction between $qDTY_{2.2}$, $qDTY_{3.2}$, $qDTY_{4.1}$, and $qDTY_{12.1}$ with background loci of IR64-Sub1 pyramided lines (PLs) resulted in 58% reduction in GY compared to the high yielding IR64-Sub1 PLs. Nonetheless, both studies also identified Sub1 NILs and PLs that do not exhibit negative interaction between *qDTY* and background loci, while demonstrating high-yielding capability. Therefore, the negative effect observed with UKM-112 is most likely a result of the negative interaction between the *Sub1* and *qDTY* loci in UKM-112. If another *Sub1*+*qDTY* genotype was included in this study, it might not have the same outcome as UKM-112.

CONCLUSION

In summary, the presence of *Sub1* QTL has demonstrated a significant enhancement in drought tolerance in rice. While the present study did not report a positive outcome for *Sub1*+*qDTY*, the combination of these traits has the potential to provide beneficial effects without any negative interactions. A further investigation incorporating another *Sub1*+*qDTY* genotype with a different recurrent *Sub1* or *qDTY* parent from UKM-112 in this study could produce different outcomes. While chlorophyll content and fluorescence measurements were not undertaken in this study, it would be worthwhile to examine these parameters in future research to verify the ability of *Sub1* to retain a high amount of chlorophyll under drought stress, thereby contributing to the observed high photosynthetic rate. Nonetheless, the high-yielding ability and high photosynthetic rate of UKM-114 still demonstrated the ability of *Sub1*-only genotype to endure drought stress, even in the absence of *qDTY*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL STATEMENT

Not applicable.

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