Characterizing drought response strategies in diverse Asian rice varieties

by

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Abstract

Drought events are being exacerbated by climate change and result in the reduced growth, yield, and survival of crops. Rice, a staple crop in the diets of nearly half of the global population, is a semi-aquatic plant that is extremely sensitive to drought. However, the natural and artificial selection of rice across varying ecological regions and climates worldwide has resulted in the adaptation of different cultivated varieties (cultivars) of rice to diverse environmental conditions, leading to variable drought resistance capabilities and differential investments into distinct drought response strategies, such as increased root growth or early maturation, across cultivars. These response strategies can be largely grouped into three categories: drought tolerance, drought avoidance, and drought escape. Rice drought response, however, is a complex, multigenic trait involving many converging molecular pathways that regulate plant structure and function. As a result, much about the coordinated internal networks controlling rice drought responses remains unknown. In this study, growth and gas exchange parameters were compared across 17 regionally distinct Asian rice (*Oryza sativa*) cultivars grown in either well-watered (3 g H_2O/g dry soil) or growth-limiting drought (1 g H_2O/g dry soil) soil conditions to screen for drought response. The chlorophyll content and relative transcript abundance of target genes (*OsABA2*, *OsADF3*, *OsIAA6*) in the leaf tissue were additionally assessed in two identified drought-resistant cultivars (Bala, Krasnodarskij 424) relative to a drought-sensitive common reference cultivar for rice (Nipponbare). Altogether, these data were used to investigate whether geographically diverse rice cultivars invested in reducing the effects of drought stress on the plant through distinct pathways, where differences were expected to indicate specific drought response strategies characteristic of that cultivar.

Bala and Krasnodarskij 424 had diverse morphological and functional drought responses, with differences in water use, leaf developmental timing, biomass, and transcript abundance for genes *OsADF3* and *OsIAA6*. These differences suggest that Krasnodarskij 424 may use drought avoidance strategies, while Bala may use both drought avoidance and drought escape strategies. This study aims to supplement ongoing research into the identification of agronomically beneficial drought response strategies, assisting in the targeted development of climate change-resilient crops.

Acknowledgements

I would like to sincerely thank Dr. Olivia Wilkins for her incredible guidance, support, and encouragement throughout her supervision of this Honours project. As well, I am grateful to my committee members, Dr. Kevin Campbell and Dr. Colin Garroway, for their insightful feedback and advice. I would also like to thank the members of Wilkins Lab, including Obaid Maqsood, Elisa Gan, and Lukas Lam, for their eager assistance, Dharti Sevak and Chi Dang, for their expert mentorship throughout my molecular work, and Sean Robertson, for the time and effort he dedicated to this project, which included the identification and curation of stress-resistant rice cultivars from existing studies, his provision of valuable knowledge and advice, and help with plant care, experimental execution, data collection, and computational analysis. Additionally, I would like to thank the staff of the Dale Bumpers National Rice Research Center of the United States Department of Agriculture, namely Trevis Huggins and Jonathan Moser, for their curation and provision of the Rice Diversity Panel germplasm, from which the cultivars used in this project were selected. As well, special thanks are owed to the farmers and researchers who have contributed to germplasm collections worldwide. Finally, I am deeply grateful to my family – Wayne, Andrea, Lauryn, my grandparents, and my wonderful cats, Sparkles and Ginger – for their unceasing love and support throughout this process. And, ultimately, I give the glory of these accomplishments to God, who has set me on this path and to whom I attribute both my persistence and success in the completion of this project.

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Introduction

Global climate change is synonymous with trends of annually rising temperatures in many agriculturally important regions worldwide (Arnell et al. 2019; Guo et al. 2022). Climate shifts resulting from atmospheric and surface heating, including increased evaporation rates, unpredictable rainfall, heavier reliance on irrigation, and reduced snowfall, are leading to measured depletions in soil moisture content and exacerbating the frequency, severity, duration, and scale of drought events (Cook et al. 2018; Li et al. 2024; Thackeray et al. 2019). Drought is a major stressor limiting global crop production and has been historically linked to wide-scale famines and large economic losses, disproportionately affecting vulnerable populations where economically disadvantaged communities depend on agriculture for subsistence, and where the most severe droughts occur (Hertel and Lobell 2014; Pandey et al. 2007). Drought is also widespread, causing marked yield reductions in crops like maize, soy, and rice, and affecting an estimated three-fourths of arable land worldwide (Kim et al. 2019; Pandey et al. 2007).

Asian rice (*Oryza sativa* L.) is a dietary staple for nearly half of the global population, comprising roughly 20% of all human-consumed calories (Stein et al. 2018). Rice is also a semi-aquatic plant that requires an abundance of water to grow and reproduce, making it particularly vulnerable to drought (Molina et al. 2011; Serraj et al. 2009). Drought conditions confer measured reductions in height, biomass, and yield, as well as alterations in development compared to well-watered rice (Haefele et al. 2009; Zhang et al. 2018). Additionally, there is extensive genetic diversity within rice, resulting from both natural and artificial selective pressures, with six major subpopulations present within cultivated Asian rice (*indica*, *aus*, *aromatic*, *rayada*, *temperate japonica*, and

tropical japonica) (Wang et al. 2014) and over 130,000 unique rice accessions, cultivated and wild, stored in germplasm banks today (Jamora and Ramaiah 2022). After the first domestication of rice in China, as far back as 10,000 years ago, Asian rice traits have been undergoing artificial selection by farmers (Fornasiero et al. 2022; Garris et al. 2005). This selection has strong ties to the geographic region in which the rice is grown, as the selection of plants with enhanced growth, survival, and reproduction within a specific ecological region adapted them to diverse climates with distinct and varied temperature regimes, soil conditions, and moisture availability (Vaughan et al. 2008). Farmers also selected for agronomically favourable traits within their region (Huang et al. 2010; Yonemaru et al. 2012), which were further informed by differences in consumer preference between cultures for different culinary traits, such as palatability, pasting temperature, and breakdown viscosity, all of which are controlled by genetic factors (Hsu et al. 2014; Tiozon et al. 2025). This divergent selection on geographically isolated rice populations has resulted in the extensive genetic diversity seen within rice populations today (Sun et al. 2002), leading to rice cultivars with markedly different phenotypic responses to environmental stressors, such as drought, even within a subpopulation (Kakar et al. 2022; Lenka et al. 2010; Ramya et al. 2021). This diversity provides useful resources for researchers, as individual cultivars may possess distinct strategies of varying agricultural benefit to tolerate environmental stressors such as drought (Mickelbart et al. 2015) that have allowed for the development of rice varieties with increased resistance to drought (Chengqi et al. 2024; Datta et al. 2012; Tang et al. 2019).

Genotype, environment, and their interactions have been established as primary sources of variation in trait expression in rice (Habib et al. 2024; Justo et al. 2024;

Kunjaroenruk et al. 2025). For example, many of these traits are influenced by water availability in the environment, including shoot and root dry weight, water use, plant height, and yield, which are limited under drought conditions, and developmental and reproductive timing, which tend to be delayed in drought-stressed plants (Adzigbe et al. 2025; Degenkolbe et al. 2013; Robertson et al. 2025; Wening et al. 2020). Functional traits are also under both genetic and environmental influence, including carbon assimilation rate, stomatal conductance, transpiration rate, and chlorophyll content, which typically decrease under drought conditions (Tabassum et al. 2016; Wang et al. 2015), and water use efficiency, which is expected to increase in drought-stressed plants due to stomatal closure (Pitaloka et al. 2022; This et al. 2010). The genetic component of these responses has been assessed by examining differential gene expression across cultivars, with the specific genes being enriched indicating distinct stress response pathways in the plant (Kaur et al. 2023; Lenka et al. 2010). During rice drought response, low soil moisture is first detected by the roots, causing a variety of signals to be passed through the vasculature and towards the shoots (Ma et al. 2024). These signals include abscisic acid (ABA), which signals a wide variety of downstream drought responses in leaf and root tissue and is regulated by genes such as the ABA biosynthesis pathway genes *OsNCED1* and *OsABA2* (Xiang et al. 2026; Zhang et al. 2022). When soil moisture is low, there are three main categories of response strategies plants employ to resist drought: drought escape, drought avoidance, and drought tolerance (Panda et al. 2021).

Drought escape is the rapid acceleration of a plant through its life cycle, allowing it to mature and reproduce before the development of severe moisture deficits can cause further harm to the plant (Ahmad et al. 2020; Manavalan et al. 2009). Genes regulating

this drought response pathway include those controlling flowering time (Du et al. 2018; Fang et al., 2019), as well as genes that increase photosynthetic capacity, remobilize various assimilates (e.g., sucrose transporters) (Mathan et al. 2021), and inhibit tillering (e.g., *OsIAA6*) (Du et al. 2018; Jung et al. 2015). These plastic responses to environmental change allow rice plants to complete their lifecycle before drought can further affect the plant (Guan et al. 2010; Tsuji et al. 2011).

Drought avoidance strategies in rice plants involve adaptations to maintain a high tissue water content, preventing dehydration and allowing for sustained plant function under moderate drought conditions (Cardoso et al. 2025; Panda et al. 2021). These strategies include the formation of deep and branching root systems, involving genes related to root growth and cytoskeletal rearrangement (e.g., *OsADF3*), to increase water uptake into the plant (Huang et al. 2012). As well, limiting moisture loss via transpiration helps maintain a high internal water content, and involves genes related to stomatal closure, reduced stomatal density, increased cuticular wax deposition, and leaf rolling (Caine et al. 2019; Jian et al. 2022; Li et al. 2017; Tao et al. 2024; Zhou et al. 2016), thus allowing the plant to avoid drought (Zhang et al. 2021).

Drought tolerance is the ability of plants to respond to a low internal water content (Kumar et al., 2017). Rice plants are known to have a variety of strategies they employ to respond to this kind of stress. For example, osmotic adjustment is the accumulation of osmolytes like proline, soluble sugars, and starches within plant tissues to lower their osmotic potential and draw in moisture by osmosis (Girma and Krieg 1992). The production of antioxidant compounds is another important element of drought tolerance, as they neutralize the reactive oxygen species that can accumulate under drought stress

and damage cellular components and membranes (Hayat et al. 2012; Long et al. 2023; Wang et al. 2021; Xiong et al. 2018). In general, all drought response strategies that are induced by an internal moisture deficit can be characterized as drought tolerance strategies.

Extensive research over the last few decades has greatly improved the mechanistic understanding of drought response strategies in rice (Panda et al. 2021). However, rice drought response traits are under the control of thousands of genes, with their expression being regulated by multiple converging molecular pathways that ultimately alter structural and functional features of the plant in response to drought stress signals (Ma et al. 2024; Zhang et al. 2012). Even small changes in the soil moisture environment are associated with marked changes in gene expression patterns (Ong et al. 2023). This complexity suggests that, despite the large quantity of research already conducted into rice drought responses, the intricacies of these molecular pathways still remain largely unknown (Li et al. 2019).

Ultimately, there is a wide variety of genetically controlled, environmentally responsive mechanisms of drought response in Asian rice. This Honours project aimed to test whether geographically distinct Asian rice cultivars responded differently to environmental drought. Diverse Asian rice cultivars were hypothesized to invest in reducing the effects of drought stress through different pathways, which were measured as differences in growth, gas exchange, chlorophyll content, and gene expression under drought stress. These differences were indicative of specific drought response strategies characteristic of a certain cultivar. This study addresses the wide knowledge gaps surrounding the intricate, mechanistic responses of rice plants to drought stress and

contributes to the global effort to generate crops with increased resilience to the drought events being enhanced by climate change.

Methods

Drought response screening

A panel of 17 geographically distinct Asian rice cultivars with known or suspected drought resistance was selected from the Rice Diversity Panel shared by the United States Department of Agriculture (USDA) based on high scoring for various stress metrics identified from published genome-wide association studies (GWAS) (Guo et al. 2018; Woldegiorgis et al. 2022). These cultivars were then screened for drought response. Seeds were sourced from the USDA and germinated in moist, dark conditions at 30 °C for 72 hours. Soil (Sungro Sunshine Mix 1) was oven dried at 60 °C for 48 h, then combined with tap water to create well-watered (WW, 3 g water/g soil, control) and mild growth-limiting drought (mild GLD, 1.5 g water/g soil) soil moisture conditions. Six germinated seedlings for each cultivar were potted in large, round pots (15 cm diameter, depth = 11.4 cm), in both well-watered and growth-limiting drought conditions (~216 total plants) (Fig. A1), with soil added up to a predetermined weight that varied according to the soil's moisture content (Table A1). Soil moisture content was maintained by watering all pots daily at ZT6 (midpoint of the photoperiod) to their initial weight. Plants were grown in a controlled growth chamber (Conviron® PGR15 Plant Growth Chamber) with a 12-hour photoperiod, 30 °C day/25 °C night thermal cycle, 80% relative humidity, and an 800 $\mu mol/m^2/s$ light intensity at 80% lamp light output. The pot and tray positions within the growth chamber were rotated daily (Fig. A1, Fig. A2). After all shoots had emerged > 2.5 cm above the soil, the mild GLD soil treatment was allowed to dry down to a moderate

growth-limiting drought condition (GLD, 1 g water/g soil) by temporarily ceasing watering of these pots, as measured by pot weight. Two weeks after planting, all pots were treated with 20 g of fertilizer (1 g 13.2% Fe chelate, 4 g 202020 MiracleGro, 1 L tap water).

For each plant, leaf emergence dates, daily pot weight, total leaf and tiller numbers, and leaf length were measured during the three-week experimental period. Evaporative moisture loss was assessed by comparing daily water loss in pots containing plants to pots containing only 3 g/g or 1 g/g soil, but no plants. These measurements were used to calculate daily and cumulative water use (Table A2). GLD:WW tiller and leaf ratios were calculated from the total tiller and leaf numbers (mean count in GLD/mean count in WW) and represent the proportion of tillers or leaves a cultivar was able to maintain under drought relative to the well-watered count (1 = the same between soil moisture conditions). After three weeks, shoots were collected at the stem base using a razor blade and immediately weighed to the nearest 0.001 g on a precision balance (PB303-S DeltaRange) to determine fresh weight. Shoot and root dry weight were measured in the same way following 72 hours in a 70 °C drying oven. Measurements of gas exchange parameters (carbon assimilation rate, stomatal conductance, transpiration rate, and water use efficiency) were taken between ZT5 and ZT9 from the fully elongated fourth leaf of each plant 2.5-3 weeks after planting using a portable photosynthesis system (LI-COR LI-6800).

Measured parameters were compared between cultivars, and two cultivars (Bala, Krasnodarskij 424) that performed in an agronomically favourable way under drought compared to the other cultivars, and that had diverse physiological and morphological

drought-response parameters, were selected for further investigation. *Oryza sativa* cultivar Nipponbare was included to standardize results and facilitate comparisons with previous work, as Nipponbare is a common Japonica reference cultivar in rice genomics due to it being the first cereal crop genome to be fully sequenced (Eckardt 2000).

Growth and collection of sample leaf tissue

Seedlings of three cultivars (Bala, Krasnodarskij 424, and Nipponbare) were again germinated and grown as above. Eight seedlings of each cultivar type were individually potted in 10.66 cm square pots (soil surface = 10.66 x 10.66 cm, depth = 9.19 cm) and grown under both WW and GLD conditions (48 pots total) (Fig. A2). Soil moisture content maintenance and plant growth conditions were performed as in the drought response screening experiments. Leaf emergence dates and daily pot weight were recorded.

Twenty-four hours after the fifth leaf had emerged in all biological replicates for a specific sample (cultivar and soil moisture condition), the fourth leaf was removed at the base with a razor blade, and the middle third of it was dissected out. The entire third leaf was removed at the leaf base using a razor blade. Both leaf cuttings were flash-frozen in liquid nitrogen and stored in a -80 °C freezer.

RNA and chlorophyll extraction

Total RNA was extracted from the fourth leaf tissue of three plants of each cultivar in each condition (WW or GLD) using the Qiagen RNeasy Plant Mini kit. DNase digestion was performed during the extraction of RNA from each sample (RNase-Free DNase Set, Qiagen). The quality of the extracted RNA was verified using a Nanodrop One

Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific) by ensuring $A_{260/280}$ and $A_{260/230}$ ratios greater than 1.8 (Deepa et al. 2014) (Table A3) and confirming the presence of four clear bands on an agarose gel (Fig. A3).

Chlorophyll content within the third leaf of three plants collected from each cultivar and soil water condition was extracted and quantified using a protocol adapted from Pocock et al. (2004). Arnon's equations (Arnon 1949) were used to calculate chlorophyll a, chlorophyll b, and total chlorophyll content (a + b).

Primer design and validation

Commonly upregulated genes under osmotic or drought stress were identified from datasets available through previous comparative transcriptomic studies of rice (Baghyalakshmi et al. 2024; Baldoni et al. 2016; L. Huang et al. 2014) and a list of common, drought-responsive rice genes from the literature was also compiled (Dien et al. 2020; El-Esawi and Alayafi 2019; Jung et al. 2015; Miao et al. 2020; Mohammed et al. 2019; Zhang et al. 2022) (altogether, $n = 88$). To verify that transcripts of these genes should be present in a high enough abundance within samples for complementary DNA (cDNA) to amplify within the limit of detection during qPCR (quantitative polymerase chain reaction), which is an absolute abundance of three or greater (Forootan et al. 2017), Rice eFP Browser (Winter et al. 2007), a tool that displays rice gene expression data from published studies, was used to examine select genes within an existing whole-transcriptome *O. sativa* dataset that used these same soil conditions (WW and GLD) (Robertson et al. 2025). If the absolute abundance of transcripts for a gene was lower than three in this dataset, that gene was excluded. Sequences of the remaining, select genes (*n*

= 15) (Ouyang et al. 2007) were retrieved from Phytozome (Goodstein et al. 2012), and PrimerQuest (Integrated DNA Technologies, 2025) was used to create two qPCR primers per target gene. Primer-BLAST (Ye et al. 2012) was then used to assess primer binding specificity. Primer pairs for genes that met the above criteria (n = 9) were ordered from Integrated DNA Technologies (IDT) (Table A4).

Each primer pair was validated using a standard polymerase chain reaction (PCR), using genomic DNA (*O. sativa* cultivar Nipponbare) as a template and the DreamTaq Green PCR Master Mix reagent (Thermo Fisher Scientific) as per the manufacturer's instructions. Cycling conditions were 95 °C for one minute, then 30 cycles of 95 °C for 30 seconds (denaturation), the chosen annealing temperature for 30 seconds (annealing), and 72 °C for one minute (extension), followed by a one-cycle final extension at 72 °C for five minutes. Reactions for each primer pair were run at two different annealing temperatures (57°C, 58°C) to assess the optimal annealing temperature for downstream qPCR. PCR products were assessed using agarose gel electrophoresis, where a single clear, bright band at the expected size indicated a high quantity of product, as well as the absence of primer-dimer formation and non-specific amplification in the reaction sufficient for use of primer pairs for downstream qPCR (Fig. A4).

Complementary DNA (cDNA) synthesis and assessment of primer binding efficiency

Complementary DNA was synthesized from RNA extracted from leaf tissue samples using the SuperScript™ IV First-Strand Synthesis System (Thermo Fisher Scientific) as per the manufacturer's instructions. Briefly, 2 ng of sample RNA was combined with 50 μM oligo $(dT)_{20}$ primer and 10 mM dNTP mix in a total volume of 13 μL. After template

RNA denaturing and primer annealing, a master mix containing the reverse transcriptase (SuperScript™ IV) was added to each reaction, and reverse transcription and enzyme inactivation were performed according to manufacturer specifications.

The amplification efficiency of select validated primer pairs (n = 3) was determined using a standard curve generated from a 1:3 serial dilution (81 – 1 ng total DNA) of a genomic DNA (*O. sativa* cultivar Nipponbare) template to ensure sufficient efficiency for qPCR (Table A5). Primer pairs with efficiencies near 100% are ideal for downstream use in qPCR reactions (Rodríguez et al. 2015). To increase primer binding specificity, the higher annealing temperature (58°C) was used if both tested temperatures were deemed optimal in the validation step.

Quantitative real-time PCR (qRT-PCR) analysis

Primer pairs for select target genes (n = 3) were used to analyze relative transcript abundance in the leaf tissue of *O. sativa* samples using qRT-PCR (quantitative real-time PCR). Reactions were prepared using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific) in a total volume of 10 µL, with 5 µL of master mix, 0.25 µL of each forward and reverse primer, and 4.5 µL of 100 µM sample cDNA. Three technical replicates of each reaction were analyzed using a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories). Standard cycling mode (primer Tm <60°C) conditions were followed according to the manufacturer's instructions, followed immediately by a melt curve analysis to verify the presence of a single peak, indicating amplification of a single, specific product. No-template controls were included to confirm the absence of amplification of non-sample components (contaminants) in the reaction mixture.

Relative transcript abundance levels were calculated using the ΔΔCT method. Briefly, cycle threshold values of the target gene were normalized to the internal reference (housekeeping) gene *eIF-4a* (eukaryotic translation initiation factor), which is involved in ribosome attachment, as expression of this gene is stable under drought stress (L. Huang et al. 2014; Jain et al. 2006). These calculations were performed using the pcr package (version 1.2.2) (Ahmed and Kim 2018) in R/R Studio version 4.5.0 (Posit team 2026).

Statistical analyses

All analyses were conducted in R/R Studio version 4.5.0 (Posit team 2026). A factorial ANOVA accompanied by a Tukey's Honestly Significant Difference post-hoc test was used to examine the effects of cultivar, soil water content, and their interaction on all measured parameters. Mean ± standard error is reported for all measurements taken of the second set of plants, consisting of the three selected cultivars. As well, reported values are mean ± within-pot standard deviation for all measurements of the 17 cultivars in the drought response screening panel, with no statistical analyses being performed due to within-pot replicates.

Results

Diverse cultivars respond to drought by altering their growth habit

Various growth parameters were measured in a panel of 17 geographically diverse Asian rice cultivars (Fig. 1), grown in both a well-watered control condition (WW, 3 g water/g soil) and a moderate growth-limiting drought condition (GLD, 1 g water/g soil) (Table B1). These measurements guided the selection of three cultivars (Bala, Krasnodarskij 424, Nipponbare) to undergo further experimental investigation (Fig. 2).

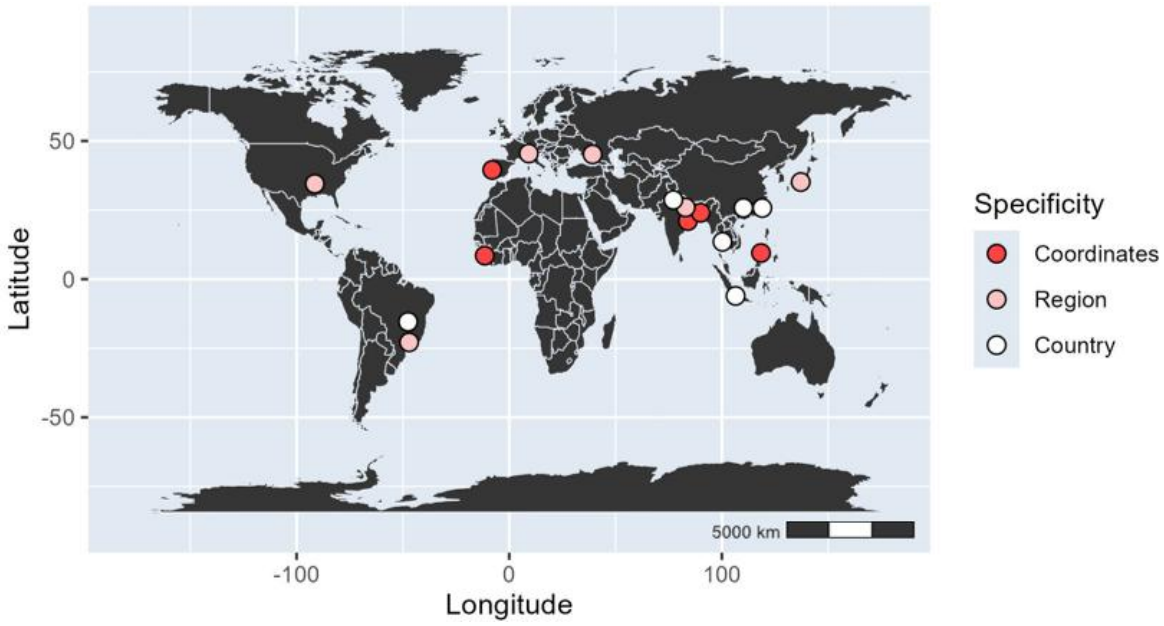


Fig. 1. Map of the geographic origins of 17 Asian rice (*Oryza sativa*) cultivars examined in this study. The colour of each point indicates the degree of specificity with which the geographic origin of each cultivar could be determined.

Fig. 2. Three Asian rice (*Oryza sativa*) cultivars selected from a drought response panel to undergo further experimentation. Plants were grown in either a well-watered (WW, 3 g H_2O/g dry soil) control soil condition or a growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil condition.

The cumulative amount of water used by rice plants over 20 days differed among cultivars and was reduced under drought conditions compared to watered conditions (12 – 59 g and 69 – 198 g, respectively) (Fig. 3A). Under drought conditions, Krasnodarskij 424 had the highest cumulative water use of all cultivars (59 g) after 20 days. Bala also had a high cumulative water use relative to other cultivars under drought conditions (44 g), while Nipponbare used a similar amount of water to many other cultivars under drought (19 g, median for all GLD cultivars = 24 g). Also, Krasnodarskij 424 exhibited a more similar water use under drought and watered conditions (59 vs. 94 g, $\Delta = 35$ g) than either Bala (44 vs. 139 g, $\Delta = 95$ g) or Nipponbare (85 vs. 94 g, $\Delta = 66$ g). These measurements were repeated on a second set of plants, consisting of only the three selected cultivars ($n = 4$) (Fig. 3B). The order of highest to lowest cumulative water use in watered control plants was consistent in this trial compared to the panel (Bala, Krasnodarskij 424, then Nipponbare, from high to low) (Fig. 1A, B).

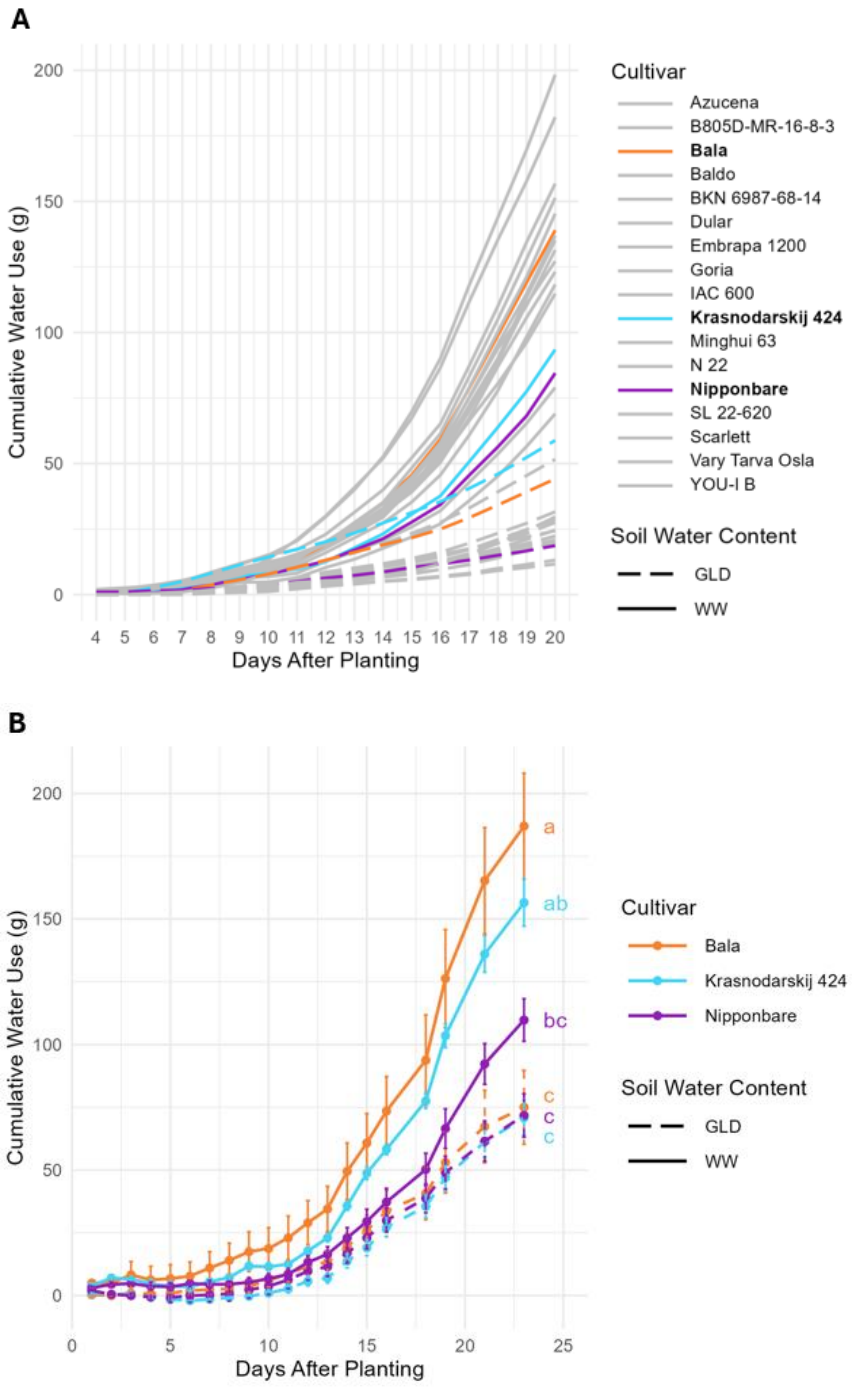
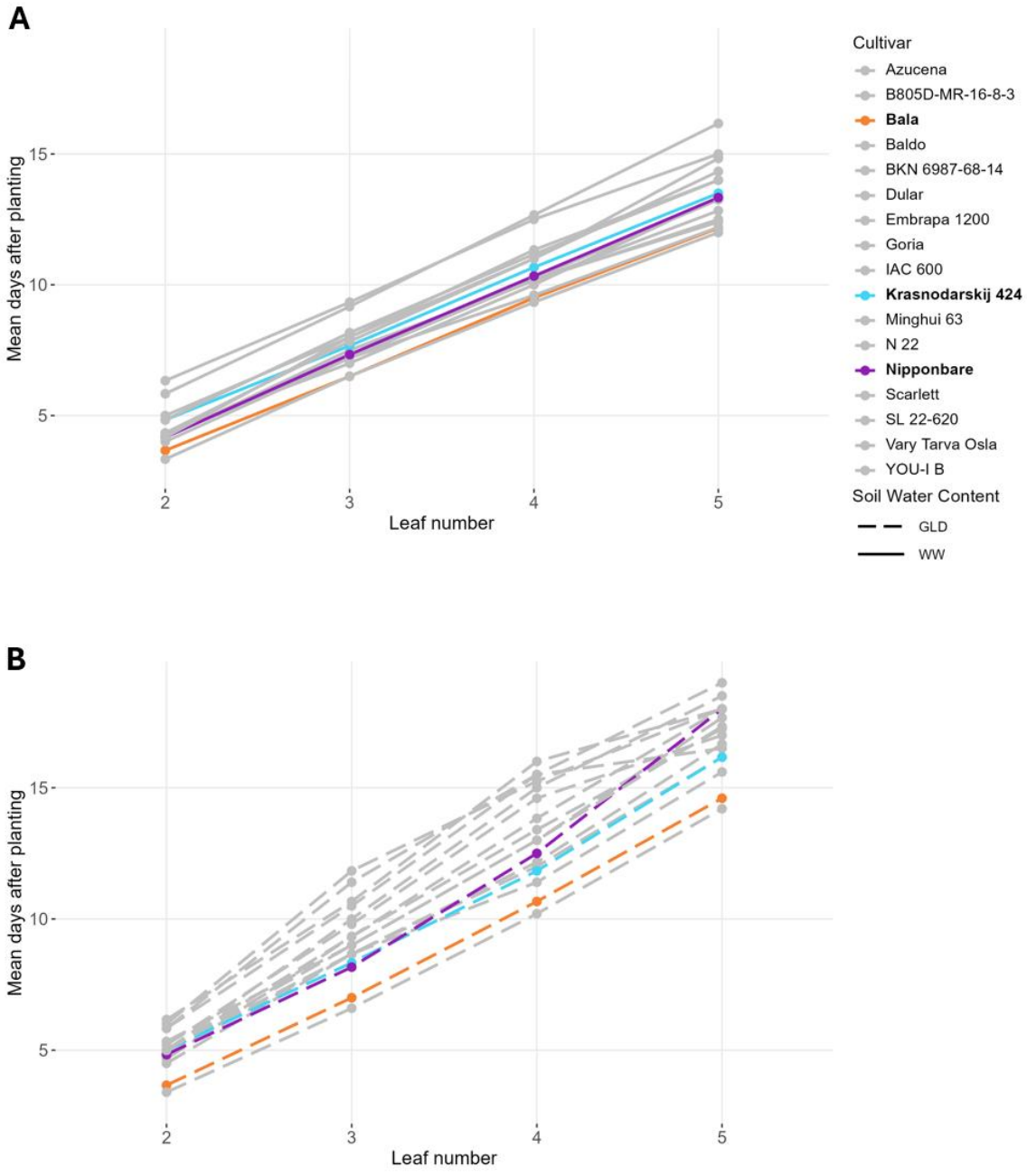


Fig. 3. Mean cumulative water use of Asian rice (*Oryza sativa*) cultivars over three weeks. Plants were grown in either well-watered (WW, 3 g H_2O/g dry soil) or growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil conditions. (A) Cumulative water use for a panel of 17 cultivars ($n = 1$), with the three cultivars selected for further experimentation highlighted. (B) Cumulative water use of these three cultivars in a second set of plants ($n = 4$). Letters denote statistically significant differences (factorial ANOVA, Tukey's Honestly Significant Difference test, $P < 0.05$), and error bars represent standard error.

As well, in this second trial, well-watered plants exhibited much greater variability in cumulative water use among the three cultivars (range: 110 ± 17 – 187 ± 42 g) compared to plants grown under drought (range: 71 ± 17 – 75 ± 26 g), and well-watered Krasnodarskij 424 and Bala plants had a significantly higher cumulative water use than both well-watered Nipponbare and all cultivars grown under drought (Tukey's HSD, $P < 0.05$) (Fig. 3B). The overall interaction effect between soil water content and cultivar was significant (factorial ANOVA, $F(2, 17) = 4.495$, $P = 0.0271$).

Leaf emergence dates for leaves 2 through 5 of each plant were measured to compare developmental timing between 17 cultivars under drought. In general, leaf emergence date was later in drought-limited conditions across cultivars (leaf 5 range: 14.2 ± 1.3 – 19.0 ± 0 days after planting) compared to well-watered conditions (leaf 5 range: 12.0 ± 0.6 – 16.2 ± 0.4 days after planting) for leaves 3 through 5 (Fig. 4A, B). When comparing leaf development among cultivars for leaves 2 through 5, leaf emergence in Bala was earlier in both drought (GLD mean: 3.7 ± 0.5, 7.0 ± 0.6, 10.7 ± 0.8, 14.6 ± 1.3, respective of leaf order) and watered (WW mean: 3.7 ± 0.5, 6.5 ± 0.5, 9.5 ± 0.5, 12.2 ± 0.4, respectively) conditions relative to Krasnodarskij 424 (GLD mean: 5.0 ± 0, 8.3 ± 0.5, 11.8 ± 1.2, 16.2 ± 1.6; WW mean: 4.8 ± 0.8, 7.7 ± 0.8, 10.7 ± 0.8, 13.5 ± 1.0) and Nipponbare (GLD mean: 4.8 ± 0.8, 8.2 ± 0.8, 12.5 ± 1.2, 18.0 ± 1.0; WW mean: 4.2 ± 0.4, 7.3 ± 0.5, 10.3 ± 0.5, 13.3 ± 0.5), both of which had relatively similar developmental timing for leaves 2 through 4, and were visibly similar in amplitude to most other cultivars in the panel. These measurements were repeated on a secondary set of plants, consisting of only the three selected cultivars ($n = 8$ plants per cultivar per condition) (Fig. 4C). General trends between cultivars appeared to follow a similar pattern as above, with

Bala grown in well-watered conditions having the earliest emergence dates of all cultivars and conditions (mean: 3.5 ± 0.3, 6.9 ± 0.2, 10.1 ± 0.4, and 13.0 ± 0.3 days after planting, respectively) for all leaves (Fig. 4C).


A
Mean days after planting
15
10
5
2
3
4
5
Leaf number
Cultivar
Azucena
B805D-MR-16-8-3
Bala
Baldo
BKN 6987-68-14
Dular
Embrapa 1200
Goria
IAC 600
Krasnodarskij 424
Minghui 63
N 22
Nipponbare
Scarlett
SL 22-620
Vary Tarva Osla
YOU-I B
Soil Water Content
GLD
WW
B
Mean days after planting
15
10
5
2
3
4
5
Leaf number

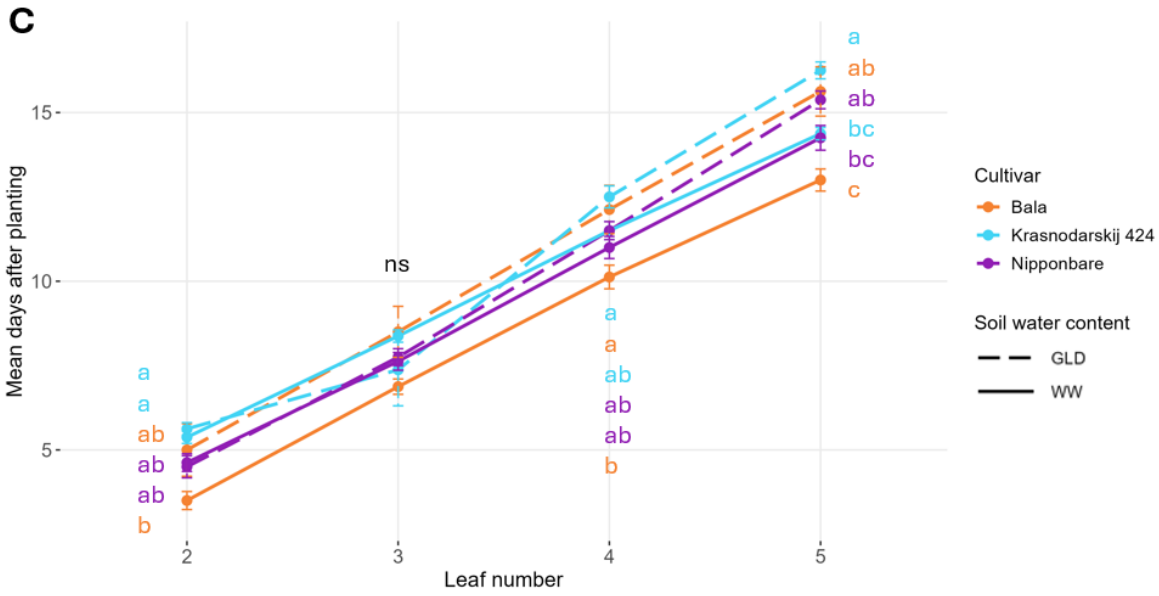


Fig. 4. Mean emergence dates of leaves 2 – 5 for 17 (A, B) and three (C) Asian rice (*Oryza sativa*) cultivars over 20 days of growth. Plants were grown in either a well-watered (WW, 3 g H_2O/g dry soil) control soil condition or a growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil condition. Leaf emergence dates were compared among a panel of 17 cultivars (n = 1) in WW (A, solid line) and GLD (B, dashed line) conditions, with the three cultivars selected for further experimentation highlighted. (C) Leaf emergence dates of the three selected cultivars (n = 4). Letters denote statistically significant differences (factorial ANOVA, Tukey's Honestly Significant Difference test, $P < 0.05$), and error bars represent standard error. No significance = ns.

Leaves 4 and 5 emerged at significantly different times between drought and watered conditions (factorial ANOVA: $F(1, 24) = 12.305$, $P = 0.0012$; $F(1, 24) = 33.670$, $P =$ 7.64e-07), with Bala and Krasnodarskij 424 being significantly different at the level of soil water content (Tukey's HSD, $P < 0.05$) (Fig. 4C).

Additionally, leaf and tiller numbers on each plant were counted at the end of the growing period for all 17 cultivars grown in the panel, to assess development between drought-stressed cultivars. In general, Bala had high tiller counts in both watered (mean: 3.3 ± 0.5) and drought (mean: 1.7 ± 0.8) conditions relative to other cultivars (WW range:

1.7 ± 0.5 – 4.0 ± 1.7; GLD range: 0.0 ± 0 – 1.7 ± 0.5) and had the highest GLD:WW tiller ratio of all 17 cultivars (0.52) (Table B2). Bala also had high leaf counts (WW mean: 14.8 ± 1.3, cultivar range: 9.0 ± 2.0 – 18.2 ± 5.8; GLD mean = 9.5 ± 2.1, cultivar range: 4.0 ± 0.9 – 9.5 ± 2.1) and the second-highest GLD:WW leaf ratio (0.64) to Krasnodarskij 424 (0.68). Leaf and tiller numbers universally decreased under drought conditions (Table B2).

Additional measurements of biomass accumulation on this panel of 17 cultivars included root and shoot biomass, as well as leaf length, to assess how successfully cultivars were able to convert carbon into biomass under drought, and what structures they invested more into. Reductions in all measured parameters were seen under drought in all cultivars (Table B3, Table B4). Krasnodarskij 424 ranked among the top cultivars for shoot fresh weight (0.912 g), shoot dry weight (0.220 g), and root dry weight (0.042 g) under drought relative to other cultivars (means across cultivars: 0.590 ± 0.228, 0.141 ± 0.058, and 0.025 ± 0.009 g, respectively) (Table B3). Krasnodarskij 424 also had more similar root and shoot biomass measurements at both soil water contents relative to other cultivars (Table B3). Additionally, Bala had a high shoot dry weight under drought conditions (0.218 g), while Nipponbare had a very low shoot fresh and dry weight, as well as root dry weight, under drought conditions compared to other cultivars (0.312, 0.083, and 0.016, respectively), with large changes in biomass between soil water conditions. When examining leaf length, Krasnodarskij 424 had long third, fourth, and fifth leaves under drought (163.2 ± 14.4, 225.2 ± 18.6, and 246.2 ± 77.7 mm, respectively) compared to other cultivars (ranges: 108.0 ± 6.2 – 164.5 ± 13.5, 119.0 ± 51.0 – 232.0 ± 8.5, and 53.0

± 38.5 – 242.7 ± 47.9 mm, respectively), with more similar leaf lengths between soil moisture conditions (Table B4).

Bala and Krasnodarskij 424 were selected for further experimental investigation based on these notable performances in the drought response panel, while Nipponbare, a model organism among rice species, was included as a more drought-susceptible standard, allowing for these results to be more easily compared to other work.

Diverse cultivars respond to drought by altering their gas exchange rates

Gas exchange rates in the leaves were measured using a portable photosynthesis system for each of the 17 cultivars in the drought response screening panel. Carbon assimilation rate, or photosynthetic rate, universally decreased under drought in all cultivars (range: 5.9 ± 5.0 – 15.2 ± 3.5 μmol m^{-2} s^{-1}) compared to the well-watered samples (range: 18.1 ± 4.9 – 26.5 ± 2.9 μmol m^{-2} s^{-1}) (Fig. 5A). Krasnodarskij 424 had a high carbon assimilation rate under drought (mean: 14.5 ± 3.7 μmol m^{-2} s^{-1}) compared to other cultivars (range: 5.9 ± 5.0 – 15.2 ± 3.5 μmol m^{-2} s^{-1}) and had a similar assimilation rate between soil moisture treatments (WW mean: 20.5 ± 5.1 μmol m^{-2} s^{-1}). In comparison, Nipponbare had the lowest carbon assimilation rate of all cultivars under drought (mean: 5.9 ± 5.0 μmol m^{-2} s^{-1}), which was much lower than its carbon assimilation rate under well-watered conditions (mean: 24.6 ± 4.1 μmol m^{-2} s^{-1}) when compared to Krasnodarskij 424. Water use efficiency, or how much carbon is gained per unit of water lost via transpiration, increased in all cultivars under drought stress (Fig. 5B). Bala had a high water use efficiency under drought (mean: 3.5 ± 1.2 μmol CO_2/mmol H_2O) compared to most other cultivars (range: 1.8 ± 1.1 – 4.2 ± 1.2 μmol CO_2/mmol H_2O) and increased its water use efficiency greatly

relative to well-watered conditions (mean: 1.3 ± 0.5 μmol CO_2/mmol H_2O). Krasnodarskij also had a moderately high water use efficiency under drought conditions (mean: 3.1 ± 0.8 μmol CO_2/mmol H_2O).

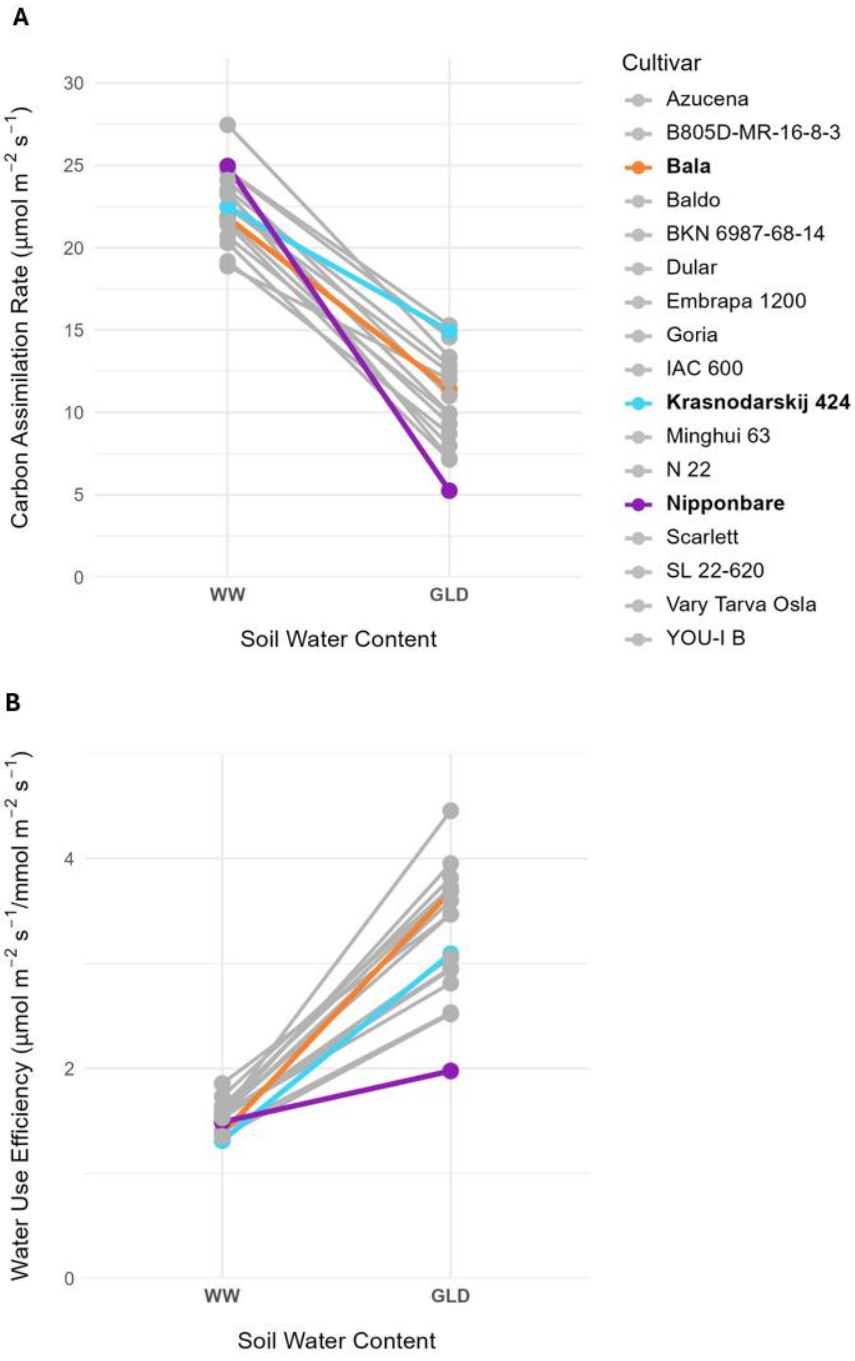


Fig. 5. Gas exchange parameters for 17 Asian rice (*Oryza sativa*) cultivars grown in well-watered (WW, 3 g H_2O/g dry soil) or growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil conditions. Carbon assimilation rate (A) and water use efficiency (B) were compared between a panel of cultivars (n = 1), with the three cultivars selected for further experimentation highlighted. Measurements were taken between ZT5 and ZT9 from the fully elongated fourth leaf of each plant three weeks after planting, using a portable photosynthesis system (LI-COR LI-6800).

In comparison, Nipponbare only minimally increased its water use efficiency under drought compared to watered conditions (WW mean: 1.5 ± 0.3 μmol CO_2/mmol H_2O; GLD mean: 1.8 ± 1.1 μmol CO_2/mmol H_2O) and had the lowest water use efficiency of all cultivars under drought (Fig. 5B). Stomatal conductance (g_{sw}) and transpiration rate (E) were also measured, and in all cultivars, both parameters were lower in drought (E mean across cultivars: 3.3 ± 0.6 mmol m^{-2} s^{-1}; g_{sw} mean across cultivars: 0.2 ± 0.0 mol m^{-2} s^{-1}) compared to well-watered conditions (E mean across cultivars: 14.7 ± 1.9 mmol m^{-2} s^{-1}; g_{sw} mean across cultivars: 1.4 ± 0.2 mol m^{-2} s^{-1}) (Fig. B1). Stomatal conductance and transpiration rate in both soil moisture conditions were similar across all cultivars.

In addition to the growth parameter measurements, gas exchange parameters were also considered when selecting Bala, Krasnodarskij 424, and Nipponbare to move forward with for this project. Overall, the drought response screening identified drought resistance and diverse drought responses in Krasnodarskij 424 and Bala, which led to their selection.

Chlorophyll a and b concentration does not significantly differ across diverse cultivars

Chlorophyll was extracted from the leaves of the three selected cultivars (n = 3) in the second set of plants to assess potential differences under drought. No significant differences between soil water conditions were found for chlorophyll a and b concentration in Bala (WW: 5.754 ± 0.333; GLD: 5.56 ± 0.090), Krasnodarskij 424 (WW: 5.295 ± 0.245; GLD:5.062 ± 0.236), or Nipponbare (WW: 4.728 ± 0.604; GLD: 5.204 ± 0.293), nor were cultivars significantly different from each other (factorial ANOVA, P < 0.05 for all factors) (Fig. B2).

High-quality RNA was extracted from samples, and primer validity and efficiency were confirmed for drought-responsive genes

To examine differentially expressed genes in well-watered versus drought conditions across all three selected cultivars, RNA was extracted from the middle third of the fourth leaf for three replicates of each cultivar in both soil water conditions. When examining all samples using a Nanodrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific), all extracted RNA samples contained a high concentration of RNA (all > 200 ng/μL) (Table A3). As well, there was minimal protein and buffer contamination in the RNA samples, demonstrated by an A_{260}/A_{280} ratio that remained above 2.0 for all samples, and an A_{260}/A_{230} ratio that remained above 1.8 for all samples, respectively. RNA quality was further assessed via agarose gel electrophoresis, where the presence of four clear bands, representing the two ribosomal RNA subunits from both the nucleus and the organelles, confirmed that RNA samples had not been heavily degraded and were of sufficient quality to use in a qPCR reaction (Fig. A3).

Of all tested primer pairs for select drought-responsive genes (n = 9), three genes resulting in a PCR product forming a single, bright band at the expected size were selected for qPCR (Fig. A4). A standard curve using a genomic DNA template (Nipponbare) was used to confirm sufficient efficiency of all primers to amplify their target sequence for qPCR (101.9 – 115.1 % efficiency; values > 100% indicate minor technical variation), and melt curve analyses showed a single, sharp peak indicative of a specific product (Table A5).

Drought alters transcript abundance of OsABA2*,* OsADF3*, and* OsIAA6

Normalized transcript abundance of *OsABA2*, a gene in the abscisic acid (ABA) biosynthesis pathway, showed similar amplitude increases under drought compared to well-watered samples for all three cultivars (Fig. 6A).

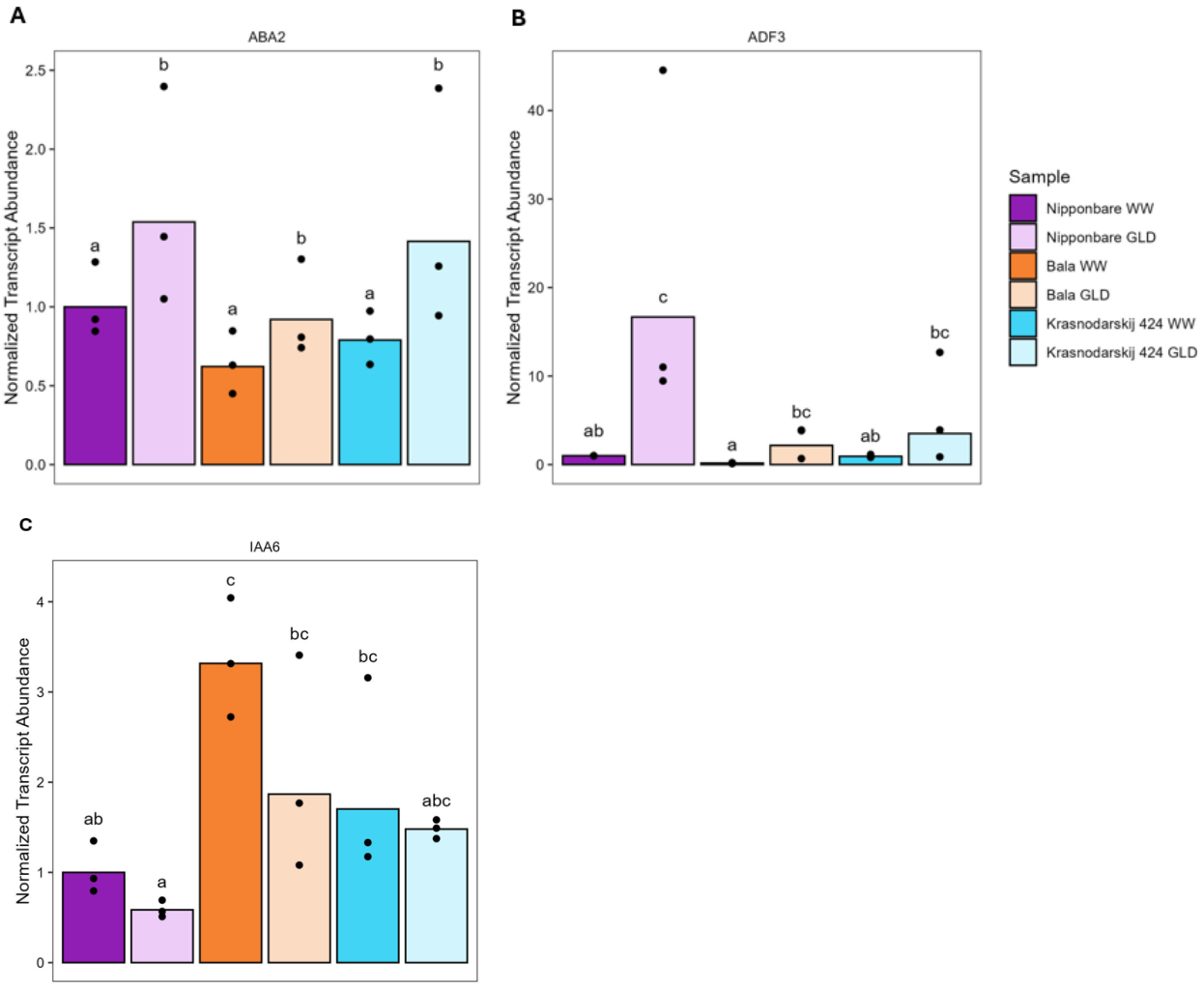


Fig. 6. Quantitative real-time PCR (qRT-PCR) analysis of transcript abundance for three target genes (*OsABA2*, *OsADF3*, and *OsIAA6*, labelled as ABA2, ADF3, and IAA6, respectively) (A–C) in the leaf tissue of three Asian rice (*Oryza sativa*) cultivars (Nipponbare, Bala, and Krasnodarskij 424) grown in well-watered (WW, 3 g H_2O/g dry soil) or growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil conditions. Transcript abundance is represented as normalized relative abundance, calculated using the ΔΔCt method using eukaryotic initiation factor 4A (*eIF-4a*) as a reference gene. Dots represent individual data points for each replicate of each sample ($n = 3$), with the bar indicating the mean. Letters denote statistically significant differences (factorial ANOVA, Tukey's Honestly Significant Difference test, $P < 0.05$). Samples were taken from the middle third of the fourth leaf, collected 24 hours after the fifth leaf emerged. Analyses were run on the CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories) with PowerUp™ SYBR™ Green qPCR Master Mix reagent (Thermo Fisher Scientific).

OsABA2 transcripts were significantly more abundant in drought samples (mean: Nipponbare = 1.54, Bala = 0.92, Krasnodarskij 424 = 1.42) compared to watered samples (mean: Nipponbare = 1.0, Bala = 0.62, Krasnodarskij 424 = 0.79) (factorial ANOVA, $F(1, 12) = 8.659$, $P = 0.0123$). As well, there was increased variation in transcript abundance for biological replicates of each cultivar grown in drought (Nipponbare range: 1.05 – 2.40, $\Delta = 1.35$; Bala range: 0.74 – 1.30, $\Delta = 0.56$; Krasnodarskij 424 range: 0.94 – 2.39, $\Delta = 1.44$) compared to the same cultivar grown under well-watered conditions (Nipponbare range: 0.85 – 1.28, $\Delta = 0.44$; Bala range: 0.45 – 0.85, $\Delta = 0.40$; Krasnodarskij 424 range: 0.63 – 0.97, $\Delta = 0.35$).

Normalized transcript abundance of *OsADF3* (actin depolymerizing factor 3) differed significantly between cultivars (factorial ANOVA, $F(2, 11) = 9.717$, $P = 0.0037$) and between drought and well-watered conditions (factorial ANOVA, $F(1, 11) = 29.761$, $P = 0.0002$) (Fig. 6B). Transcript abundance was higher in drought samples (mean: Nipponbare = 16.68, Bala = 2.17, Krasnodarskij 424 = 3.51) compared to well-watered samples (mean: Nipponbare = 1.00, Bala = 0.17, Krasnodarskij 424 = 0.93) for all cultivars, and there was a substantial increase in variation of transcript abundance for biological replicates of each cultivar grown under drought conditions (Nipponbare range: 9.45 – 44.55, $\Delta = 35.10$; Bala range: 0.68 – 3.90, $\Delta = 3.22$; Krasnodarskij 424 range: 0.87 – 12.68, $\Delta = 11.81$) compared to that same cultivar grown under watered conditions (Nipponbare range: 0.98 – 1.02, $\Delta = 0.03$; Bala range: 0.11 – 0.22, $\Delta = 0.11$; Krasnodarskij 424 range: 1.14 – 0.82, $\Delta = 0.33$). Additionally, amplitudes differ across cultivars under drought, with Nipponbare having a much higher *OsADF3* transcript

abundance compared to Krasnodarskij 424 under drought, and Krasnodarskij 424 having a higher transcript abundance relative to Bala under drought.

The normalized transcript abundance of *OsIAA6* (common auxin-class phytohormone, indole-3-acetic acid) was significantly different between both cultivar and soil water condition (Fig. 5C) (cultivar: factorial ANOVA, $F(2, 12) = 16.717$, $P = 0.0003$; soil water condition: factorial ANOVA, $F(1, 12) = 6.146$, $P = 0.0290$). Bala contained the highest abundance of *OsIAA6* transcripts among all cultivars in both soil water conditions and showed a reduction in *OsIAA6* transcript abundance in drought-limited plants compared to well-watered plants (WW mean = 3.32, GLD mean = 1.87). This pattern of reduction in drought was also seen, though to a lesser extent, in Nipponbare (WW mean = 1.00, GLD mean = 0.59), which had the lowest *OsIAA6* transcript abundance of all three cultivars in both conditions. Unlike the other two cultivars, Krasnodarskij 424 plant leaves had a much more similar abundance of *OsIAA6* transcripts between watered and drought conditions (WW mean = 1.70, GLD mean = 1.48).

Discussion

Growth and gas exchange parameters differ across diverse cultivars under drought

When examining the various measurements from the drought response screening panel and the subsequent measurements of the three selected cultivars (Bala, Krasnodarskij 424, Nipponbare), potential drought tolerance mechanisms can begin to be revealed. This study found that Krasnodarskij 424 had a high cumulative water use in drought compared to other cultivars, with a comparatively minimal reduction from its well-watered control. Krasnodarskij 424 also maintained a high shoot and root biomass under drought, had long

leaves, and was able to maintain the highest leaf count in drought relative to well-watered plants of all 17 tested cultivars. In addition, this cultivar was able to maintain a high carbon assimilation rate under drought conditions. These behaviors are associated with a drought avoidance strategy, where rice plants have adapted to maintain a high tissue water content (Panda et al. 2021). Previous studies have shown that *O. sativa* cultivars capable of deeper root growth can better avoid drought, showing greater drought resistance and a higher yield (Henry et al. 2011). Studies into other rice species have linked root-related traits to the maintenance of shoot biomass under drought (Thathapalli Prakash et al. 2023), and it has been shown that increased carbon assimilation rates correspond to increases in biomass (Simkin et al. 2015). These various measured parameters may suggest that Krasnodarskij 424 plants invest in increased root growth, allowing for the maintenance of a higher water content in the plant. The detection of drought causes reduced stomatal conductance and a subsequent decrease in transpiration rate, but biological functions independent of stomatal aperture size, such as the ease with which carbon can enter the chloroplast and the photosynthetic capacity of the leaf's biochemical machinery (Huang and Zeng 2023; Ouyang et al. 2022), may create a reduced level of internal carbon dioxide that increases carbon uptake, and thus carbon assimilation rate. High leaf water content can also increase carbon assimilation rate, as water is a reactant of the photosynthesis reaction (Wang et al. 2022). An increase in carbon assimilation rate compared to transpiration rate would also lead to the observed increase in water use efficiency under drought, which was higher in Krasnodarskij 424 than in most other cultivars. In comparison, Bala showed rapid development relative to the other cultivars within the panel in both drought and watered conditions, along with a high dry weight of the shoots, likely in relation to the high tiller number maintained in drought conditions

and the early emergence of leaves compared to other cultivars in both watered and drought-stressed conditions. Previous agronomic studies have suggested that the correlation between high leaf and tiller counts, along with shoot biomass, is due to tillers developing their own leaves as the plant progresses, increasing the overall weight of the shoot structures (Barnaby et al. 2022). Rice plants are also known to develop a somewhat fixed number of leaves before flowering (Joshi et al. 2002), meaning that earlier, more rapid emergence of leaves could be pushing the plant to reproduce faster, which would be indicative of a drought escape strategy. Additionally, this study showed that Bala plants had a high cumulative water use under drought stress, as well as a high water use efficiency under drought compared to cultivars Krasnodarskij 424 and Nipponbare, indicating that Bala may maintain a high internal water content, which would suggest drought avoidance as a response strategy. In contrast, Nipponbare rice plants, well-characterized as being drought susceptible (Degenkolbe et al. 2009; Wu et al. 2016), had reduced biomass, development, carbon assimilation, water use, and water use efficiency under drought compared to both Krasnodarskij 424 and Bala, confirming its usefulness as a comparative, less drought-tolerant cultivar in this analysis.

One apparent inconsistency of note was the lack of any significant difference in cumulative water use among drought-stressed Bala, Krasnodarskij 424, and Nipponbare plants in the second growth trial. This may be an artifact of growing the individual plants in slightly smaller, shorter pots when compared to the trial pots, as root volume is known to change in response to pot size in plants (Poorter et al. 2012). However, the reduction in many growth and functional metrics in plants as a result of drought is well characterized

(Lamlom et al. 2026) and, in general, this study also demonstrated that variability in growth decreases under drought stress.

Chlorophyll concentration does not differ across cultivars or soil moisture conditions

While expected decreases in amplitude for chlorophyll concentration were observed in Bala and Krasnodarskij 424 plants under drought conditions, as well as an increase in chlorophyll concentration for cultivar Nipponbare, significant differences between cultivars and soil moisture treatments were not found. However, previous studies comparing rice cultivars have consistently demonstrated significant reductions in chlorophyll content under drought stress, with a less pronounced decrease in more drought-resistant cultivars (Khan et al. 2013; Saha et al. 2020). As well, existing data collected from Nipponbare plants grown under drought conditions show significant increases in chlorophyll concentration compared to a watered control, in both mild and moderate drought conditions (Robertson et al. 2025, unpublished data). This context may suggest that the non-significance of the results in this study could be an artifact of a small sample size ($n = 3$), and further investigation with an increased number of plants may uncover significant changes in chlorophyll content between soil moisture conditions or cultivars across these three cultivars.

Diverse cultivars differentially alter their transcriptome under drought

Abscisic acid (ABA) is a phytohormone known for being involved in the response of plants to abiotic stressors, such as drought. The accumulation of this hormone in various plant tissues under moisture-limited conditions has long been established (Audran et al. 1998; Zeevaart 1980; Zhang and Davies 1990), and ABA is known to alter stomatal

conductance under drought conditions to reduce water loss by transpiration (Zhang et al. 2023). The process of ABA biosynthesis from xanthophyll carotenoids involves numerous enzymes, including xanthoxin dehydrogenase, which is encoded by the gene *OsABA2* (Liao et al. 2025). I found that the normalized transcript abundance of *OsABA2* increased in drought-stressed leaf tissue compared to plants grown in well-watered soil conditions, which is indicative of increased ABA synthesis in the leaves under drought. While drought-stressed root tissue, as in dry soil conditions, has historically been established to affect stomatal conductance through root-synthesized ABA being transported to the shoots via the xylem, a process presumably signalled by the moisture deficits in the soil (Jiang and Hartung 2008; Zhang and Davies 1990), leaf tissues can synthesize ABA locally as well. In fact, studies on tomato and Arabidopsis plants show that leaf-localized ABA synthesis is required for stomatal closure under drought (Christmann et al. 2007; Holbrook et al. 2002), and ABA from the leaf has been shown to more successfully induce stomatal closure compared to translocated root-synthesized ABA under root drought stress in peanut plants (Hu et al. 2016). Thus, the presence of *OsABA2* transcripts in the leaf tissue of plants grown in moisture-limited soil conditions was anticipated. Expression of this gene, as represented by transcript abundance, was also found to be similarly heightened in drought among all tested cultivars (Nipponbare, Bala, Krasnodarskij 424), likely contributing to the unanimous and visibly similar decrease in stomatal conductance under drought seen across all 17 cultivars examined in the drought response panel. Additionally, this suggested increase in ABA synthesis may correspond to the increased expression of additional genes known to be involved in rice drought response. ABA is known to be involved in signalling pathways that modulate the expression of drought-responsive genes, such as those that encode enzymatic antioxidants or are involved in

osmolyte biosynthesis (e.g., *CAT*, *OsP5CS1*) (Jin et al. 2018; Xiang et al. 2026; Zhang et al. 2023). Other studies have found that increased expression of *OsABA2* is associated with increased antioxidant activity and reduced levels of reactive oxygen species (ROS) and lipid peroxidation products (Zhang et al. 2022). A recent study of abiotically stressed Nipponbare rice also found that increased expression of another ABA biosynthesis pathway enzyme (encoded by gene *OsNCED1*) in leaf tissue resulted in increased antioxidant enzyme activity and reduced levels of hydrogen peroxide (a ROS) under osmotic stress (Xiang et al. 2026). These findings suggest that the cultivars examined in this study may have additionally responded to drought stress with increased expression of other drought-responsive genes, perhaps to deal with ROS accumulation in leaf tissue and mitigate oxidative damage, the examination of which could make for a good follow-up study to this research. As well, I found that *OsABA2* transcripts in the examined rice cultivars showed more variability between plants under drought compared to the well-watered treatments. Much previous research has demonstrated that there is increased transcriptional variability in plants undergoing stress, a feature thought to be useful in helping these organisms adapt to more unpredictable environmental conditions (Cortijo et al. 2019; Rurek and Smolibowski 2024). Ultimately, these three diverse cultivar types showed an overall uniform response in *OsABA2* transcript abundance in drought conditions, suggesting that the ABA-dependent pathway of rice drought response does not differ among these diverse cultivars.

ADF (actin depolymerizing factor) proteins function to deconstruct actin filaments, allowing for the reorganization of intracellular structures in a process essential for plant growth and development (Inada 2017). The *OsADF3* gene has been implicated in

increased root growth under drought stress in Arabidopsis (Huang et al. 2012), but comparative transcriptome studies examining drought or osmotic stress in rice identified *OsADF3* upregulation in leaf tissue within cultivars previously characterized as being resistant to drought (Baghyalakshmi et al. 2024; Baldoni et al. 2016), which may indicate the presence of cytoskeletal rearrangements occurring in leaf tissue under drought stress. The presence of this transcript in Nipponbare, Bala, and Krasnodarskij 424 rice leaves was thus examined, and this study found significantly higher transcript abundance in drought-limited plant samples compared to well-watered samples, along with greater transcript variability between drought-stressed individuals, as was expected. It has been amply shown that under dry conditions, osmotic movement of water from the plant cell causes the protoplast to shrink, causing it to become detached from the cell wall, which can be harmful or fatal to the cell (Lang et al. 2014). Previous work has also shown that actin filaments are involved in mitigating the volume and shape of stressed plant cells (Komis et al. 2002), suggesting that the breakdown and reconstruction of the cell's cytoskeleton may assist in preventing the collapse of dehydrated plant cells and reduce the negative effects of low turgor pressure in drought-stressed plants. Actin filaments have also been implicated in chloroplast movement and restructuring under drought stress, to assist in homeostasis of the photosynthesis reaction (Gayen et al. 2019; Śniegowska-Świerk et al. 2015; Yamashita et al. 2011). As well, *OsADF3* is most highly expressed in the vasculature in Arabidopsis plants (Huang et al. 2012). Actin filaments have been shown to contribute to secondary cell wall development in xylem vessels (Kijima et al. 2025), which may increase functional water transport in Arabidopsis (Lefebvre et al. 2011). An increased area of xylem and phloem tissue, which is contributed to by secondary cell wall deposition, is also known to correlate with hydraulic conductance in

rice (Huang et al. 2022). It is difficult to speculate on what specific purpose the increased abundance of *OsADF3* transcripts serves in these studied cultivars without further investigation, but some level of intracellular structural change under drought is suggested. This study also found that *OsADF3* transcript abundance was significantly different among cultivars, and that these cultivars showed differential expression of this gene under drought. Nipponbare, which has not been classified as a drought-resistant cultivar, showed the highest *OsADF3* expression under drought, suggesting that it may be undergoing structural modifications to a greater degree to cope with drought. Meanwhile, Bala and Krasnodarskij 424 had comparatively lower *OsADF3* expression, suggesting fewer intracellular modifications necessary to express a more drought-resistant phenotype. This may indicate the maintenance of a higher internal water content in these cultivars, which would characterize them as drought avoidant.

OsIAA6 is an auxin biosynthesis gene and a known regulator of rice tillering, shown to have increased expression in rice under drought (Jung et al. 2015). Increased *OsIAA6* expression reduces basal tillering in rice plants, indicating a greater investment in apical dominance for taller growth, perhaps for the purpose of increasing light access (Aarssen 1995; Barbier et al. 2017; Jung et al. 2015). Each rice tiller has the potential to develop into a panicle (grain-producing structure), meaning an increase in tiller number, especially for the more productive, earlier-developing tillers, will result in a higher grain yield (Kalaitzidis et al. 2025). I found that *OsIAA6* transcripts were significantly more abundant in well-watered plants compared to those grown under drought, and that transcript abundance differed between cultivars. Bala plants showed a decrease in transcript abundance for this tillering regulatory gene under drought conditions, which

may indicate an increased investment in reproduction over taller growth and correspond to a drought escape strategy. In contrast, there was no distinct difference in tillering-regulation transcript abundance between moisture conditions in Krasnodarskij 424, indicating that tillering is less regulated in this cultivar under drought. Nipponbare plants showed a mild, visible decrease in tiller regulation under drought, but transcript abundance was low in both moisture conditions in Nipponbare compared to the other cultivars, suggesting that Nipponbare may not invest in tillering regulation to respond to drought. This assessment is supported by differences in tiller counts between watered and drought conditions from the 17-cultivar drought response panel. Although drought reduces plant growth and, ultimately, tiller number across all cultivars, Bala plants were able to retain the highest proportion of tillers under drought compared to the well-watered treatment of all 17 cultivars, further supporting the suggestion that Bala plants may have a greater investment into reproduction compared to the other varieties and demonstrating a notable difference between geographically diverse cultivars.

Conclusion: diverse rice cultivars use distinct drought response strategies

Oryza sativa (Asian rice) is highly genetically diverse, even containing distinct subspecies within it (Wang et al. 2014), and has long been under selection for agronomically favourable traits in geographically separated populations across the globe. This has led to strong genetic divergence among rice varieties (Reig-Valiente et al. 2016), with different rice cultivars being known to have varied responses to drought stress. These diverse responses, at varying levels of observation, can be used to discern distinct drought response strategies in rice cultivars (Lamlom et al. 2026; Roy et al. 2021). However, rice drought tolerance responses involve thousands of genes governed by multiple complex

regulatory networks, meaning that the characterization of rice drought response strategies has vast potential for continued agricultural research (Guo et al. 2022; Wilkins et al. 2016). I found that rice cultivars originating from diverse regions showed differences in their drought response strategy. Krasnodarskij 424, a cultivar from the Krasnodar region of Russia, retains a high water uptake, carbon assimilation rate, and root and shoot biomass under drought stress. As well, only a small increase in *OsADF3* transcript abundance under drought suggests that the plant is not modifying its intracellular structure in response to dehydration. These combined measurements suggest that Krasnodarskij 424 avoids drought as a response strategy, which confers drought resistance. Bala, on the other hand, takes in high amounts of water under drought, uses water efficiently compared to other cultivars, and does not appear to have much increased abundance of *OsADF3* under drought compared to Nipponbare or Krasnodarskij 424, which may indicate that this cultivar is able to maintain a high internal water content, which is indicative of drought escape. As well, Bala showed rapid leaf development and high tiller and leaf numbers compared to other cultivars under drought and had highly reduced abundance of transcripts involved in tillering regulation, all suggesting Bala may also use a drought escape strategy. There were no cultivar-level differences in ABA biosynthesis gene expression, which may correspond to the lack of variation in stomatal closure and transpiration rate measurements across cultivars. This suggests that even diverse cultivars respond to ABA signalling and close their stomata to reduce water loss under drought conditions.

Future studies should examine differences in drought response to flowering time to further support the use of drought escape strategies in diverse rice cultivars. As well,

further experimental examination of various drought stress response indicators, such as proline, malondialdehyde, or antioxidant compounds, which are well-known components involved in rice drought response (Ma et al. 2024), may better clarify what signalling is occurring in these plants to stimulate the observed drought responses and better characterize drought tolerance strategies across diverse cultivars. Ultimately, an increased understanding of how plants respond to drought stress at all levels will aid in the generation of drought-resistant rice crops through genetic engineering and breeding, allowing rice to better grow, survive, and maintain yield under climate change-induced drought.

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Appendix A – Experimental set-up and sample validation information

Figures

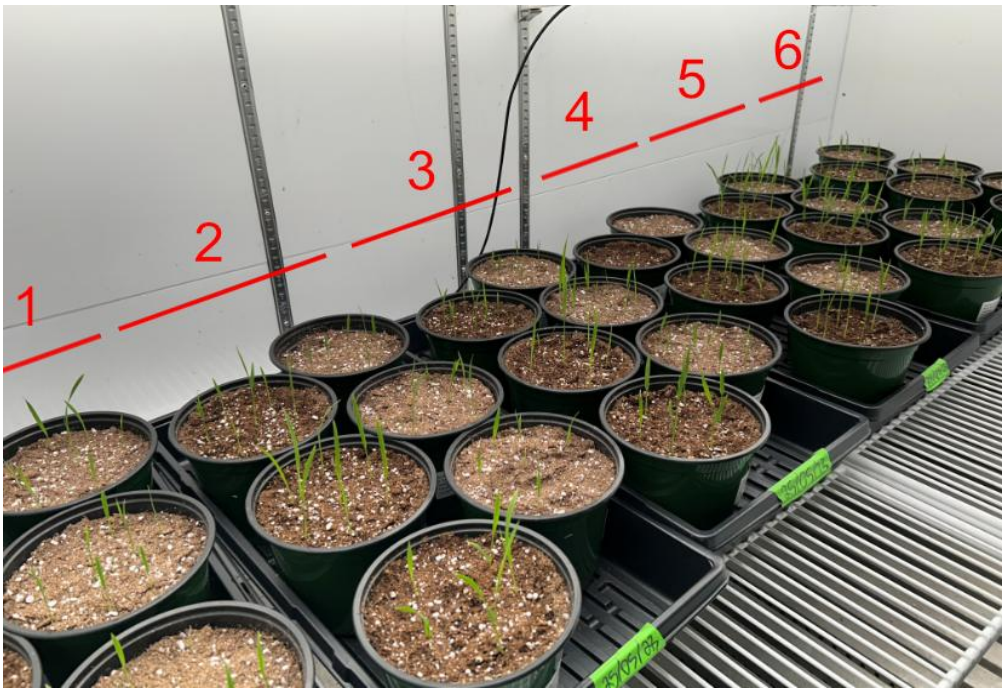


Fig. A1. Growth chamber set-up with 15 cm round pots in trays. Individual pots within each tray and each tray within the growth chamber were rotated daily (one position to the left for trays, one position forward for pots).

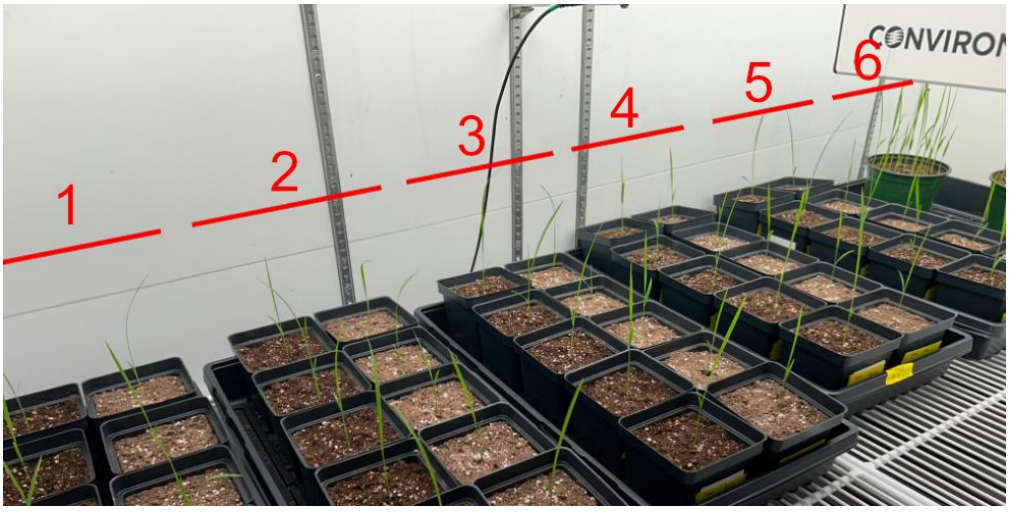


Fig. A2. Growth chamber set-up with 11.4 cm square pots in trays. Individual pots within each tray and each tray within the growth chamber were rotated daily (one position to the left for trays, one position forward for pots).

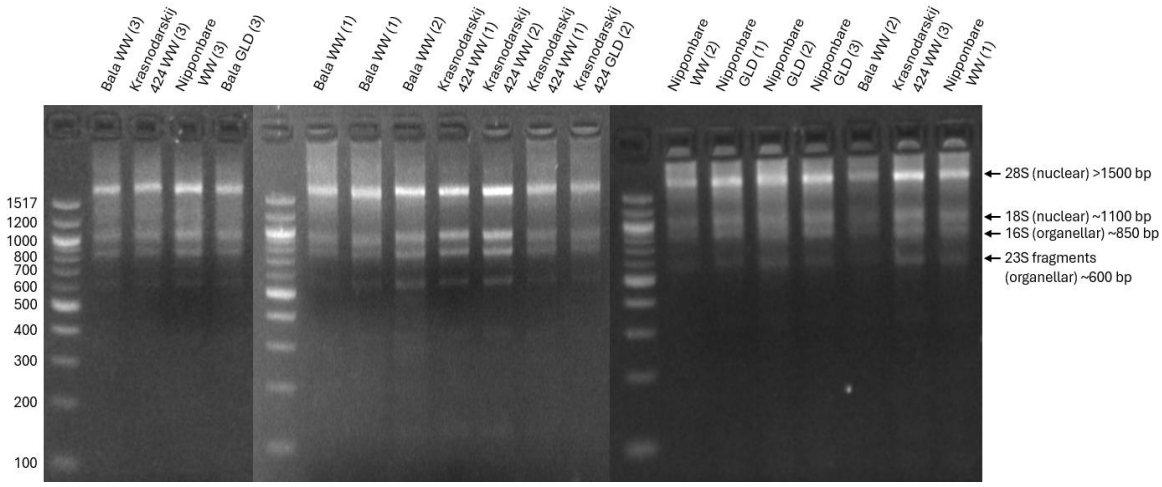
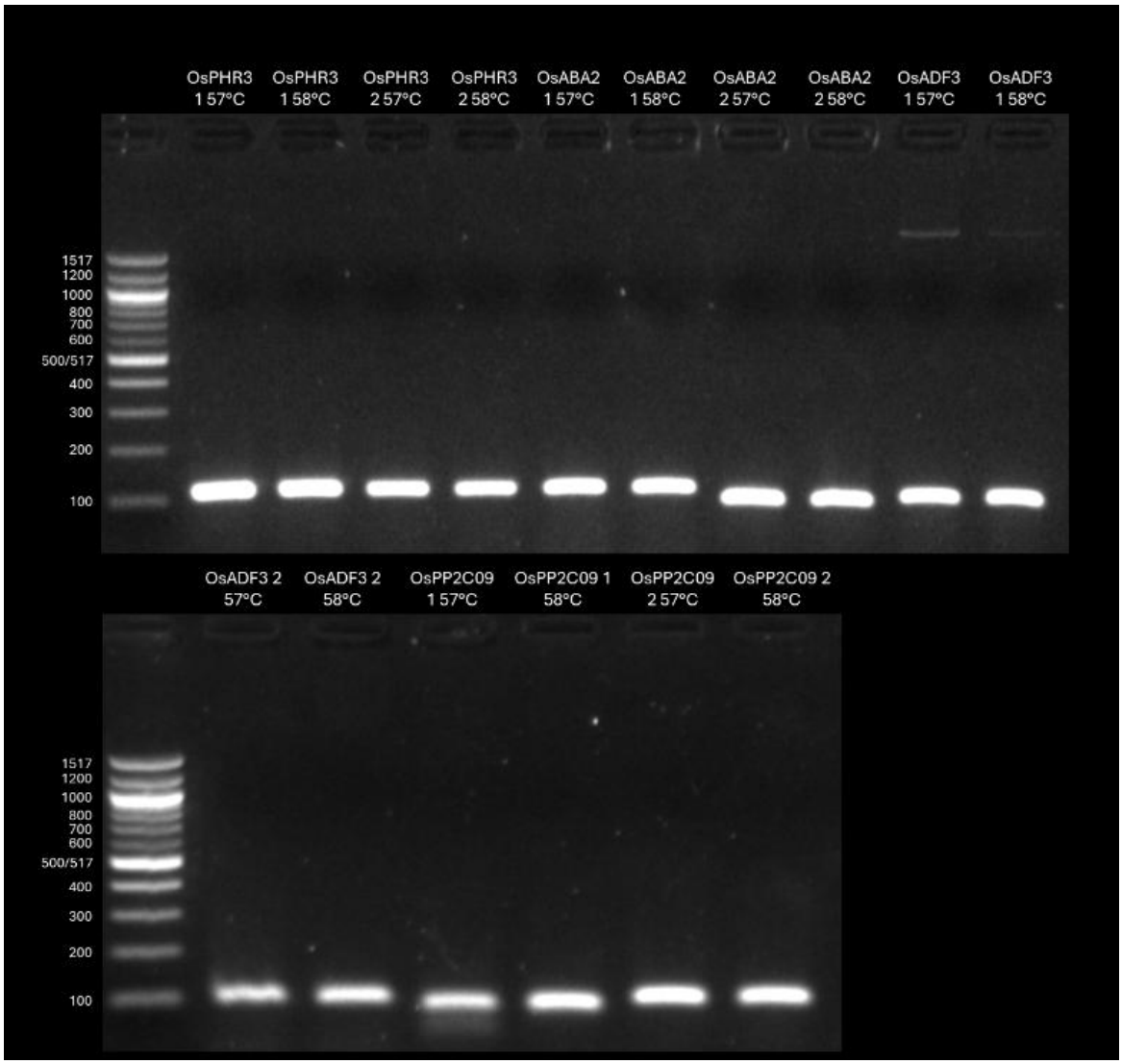


Fig. A3. Agarose gel electrophoresis (2% agarose) of extracted RNA from three replicates (R) of three rice cultivars (Bala, Krasnodarskij 424, Nipponbare) in two soil moisture conditions (WW = 3 g H_2O/g dry soil, GLD = 1 g H_2O/g dry soil). Gels were stained with SYBR Green dye and run at 50 V for 75 minutes. Unmarked lanes contain 3 µL of Quick-Load 100 bp DNA Ladder (New England Biolabs), and marked lanes contain 10 µL of 0.75 µg sample RNA. Same exposure settings used for left and central gels (black = 0, white = 12 000, gamma = 1.00). Exposure settings of the rightmost gel are similar. Fragment size estimations (base pairs, bp) for each ribosomal (rRNA) component correspond to the DNA ladder.


OsPHR3 1 57°C
OsPHR3 1 58°C
OsPHR3 2 57°C
OsPHR3 2 58°C
OsABA2 1 57°C
OsABA2 1 58°C
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OsADF3 1 57°C
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1517
1200
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200
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OsADF3 2 57°C
OsADF3 2 58°C
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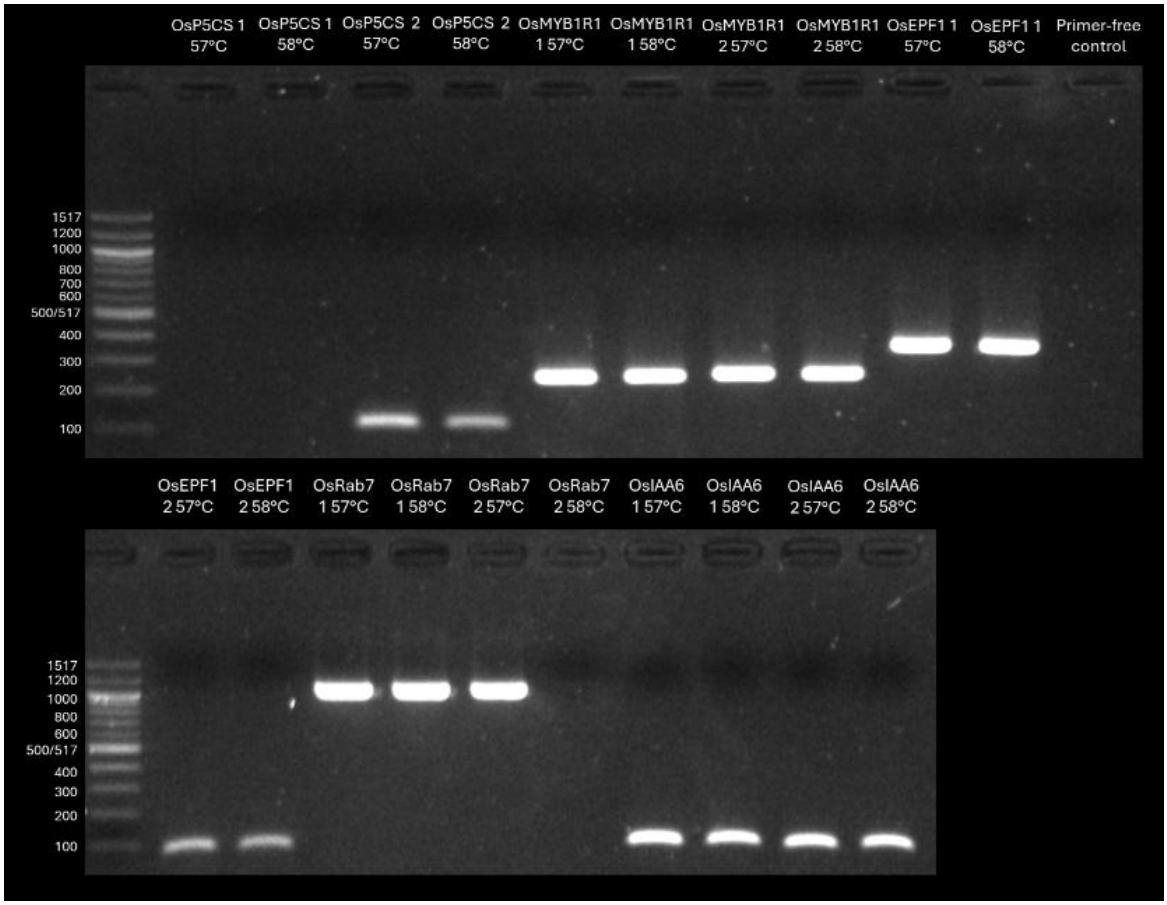

Fig. A4. Agarose gel electrophoresis (2% agarose) of polymerase chain reaction (PCR) product for validation of PCR primers for 9 candidate Asian rice (*Oryza sativa*) target genes. Unmarked lanes contain 3 µL of Quick-Load 100 bp DNA Ladder (New England Biolabs), while marked lanes contain 10 µL of PCR product. Gels were stained with SYBR Green dye. Two primer pairs were tested for each gene (1, 2), and each primer pair was assessed at two annealing temperatures (57°C, 58°C). The presence of a single, bright, clean band of the expected size indicates the absence of primer-dimer formation and non-specific amplification sufficient for downstream use. The following primer pairs were selected for downstream experimentation: *OsABA2* 1 58°C, *OsADF3* 2 58°C, *OsIAA6* 1 58°C.

Tables

Table A1. Predetermined pot weights for each soil moisture condition.

Soil condition	Soil water content	Pot weight (4.5" square)	Pot weight (6" round)
Well-watered	3 g tap water/g oven-dried soil	280 g	500 g
Mild growth-limiting drought	1.5 g tap water/g oven-dried soil	180 g	320 g
Moderate growth-limiting drought	1 g tap water/g oven-dried soil	150 g	260 g

Table A2. Formulas used for daily evaporative water loss, daily water use, and cumulative water use calculations.

Product	Formula
Daily evaporative water loss	(Predetermined empty pot weight) - (measured empty pot weight, day x) = water loss due to evaporation, day x
Daily water use	(Predetermined pot weight) - (measured pot weight, day x) - (evaporation, day x) = water use, day x
Cumulative water use	(Daily water use 1) + (daily water use 2) + ... + (daily water use x) = total water use, up until day x

Table A3. RNA quality measurements for RNA extractions on the leaf tissue of three Asian rice (*Oryza sativa*) cultivars. Plants were grown in either a well-watered (WW, 3 g H_2O/g dry soil) control soil condition or a growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil condition.

Cultivar	Soil water content	Replicate	ng/uL	A_{260}/A_{280}	A_{260}/A_{230}
Bala	WW	1	279.6	2.17	2.25
Bala	WW	2	863.0	2.17	2.36
Bala	WW	3	1043.1	2.16	2.07
Bala	GLD	1	309.5	2.16	1.89
Bala	GLD	2	816.0	2.18	2.37
Bala	GLD	3	497.8	2.17	2.13
Krasnodarskij 424	WW	1	1015.7	2.19	2.41
Krasnodarskij 424	WW	2	1374.3	2.19	2.39
Krasnodarskij 424	WW	3	616.9	2.15	2.20
Krasnodarskij 424	GLD	1	542.5	2.20	2.37
Krasnodarskij 424	GLD	2	460.4	2.18	2.33
Krasnodarskij 424	GLD	3	1100.3	2.19	2.39
Nipponbare	WW	1	1066.4	2.18	2.34
Nipponbare	WW	2	327.7	2.17	2.27
Nipponbare	WW	3	566.3	2.14	2.14
Nipponbare	GLD	1	890.3	2.17	2.34
Nipponbare	GLD	2	647.2	2.18	2.33
Nipponbare	GLD	3	852.7	2.18	2.16

Table A4. Drought-responsive Asian rice (*Oryza sativa*) target genes for which primers were designed and assessed.

Gene symbol	Function in drought response	Drought response indicated	Source of gene identification
OsADF3	Rearranges cytoskeletal elements (YHuang et al. 2012)	Drought avoidance	Baghyalakshmi et al. 2024; Baldoni et al. 2016
OsMYB1R1	Regulates protective solute production (Yin et al. 2017)	Drought tolerance	Baghyalakshmi et al. 2024; Baldoni et al. 2016
OsPHR3	Nitrogen and phosphate homeostasis (Shintani and Bono 2025; Sun et al. 2018)	Drought tolerance	Baghyalakshmi et al. 2024; Baldoni et al. 2016
OsABA2	Synthesizes stress response hormone ABA	Drought avoidance, drought tolerance	Zhang et al. 2022
OsPP2C09	Regulates stress response hormone ABA	Drought avoidance, drought tolerance	Miao et al. 2020
OsIAA6	Regulates tillering	Drought escape	Jung et al. 2015
OsP5CS	Proline metabolism	Drought tolerance	Dien et al. 2020
OsRab7	Regulates protective solute production	Drought tolerance	El-Esawi and Alayafi 2019
OsEPF1	Stomatal regulation	Drought avoidance	Mohammed et al. 2019

Table A5. Standard curve parameters for primer pairs of select drought-responsive genes. T_m = primer melting point (annealing temperature), E = amplification efficiency, y-int = y-intercept, MCA = melt curve analysis.

Gene symbol	T_m (°C)	E	R^2	Slope	y-int	MCA
ABA2	58°C	109.30%	0.991	-3.117	21.704	Single, sharp peak
ADF3	58°C	115.10%	0.99	-3.007	21.077	Single, sharp peak
IAA6	58°C	101.90%	0.929	-3.278	20.72	Single, sharp peak

Appendix B – Additional results and data

Figures

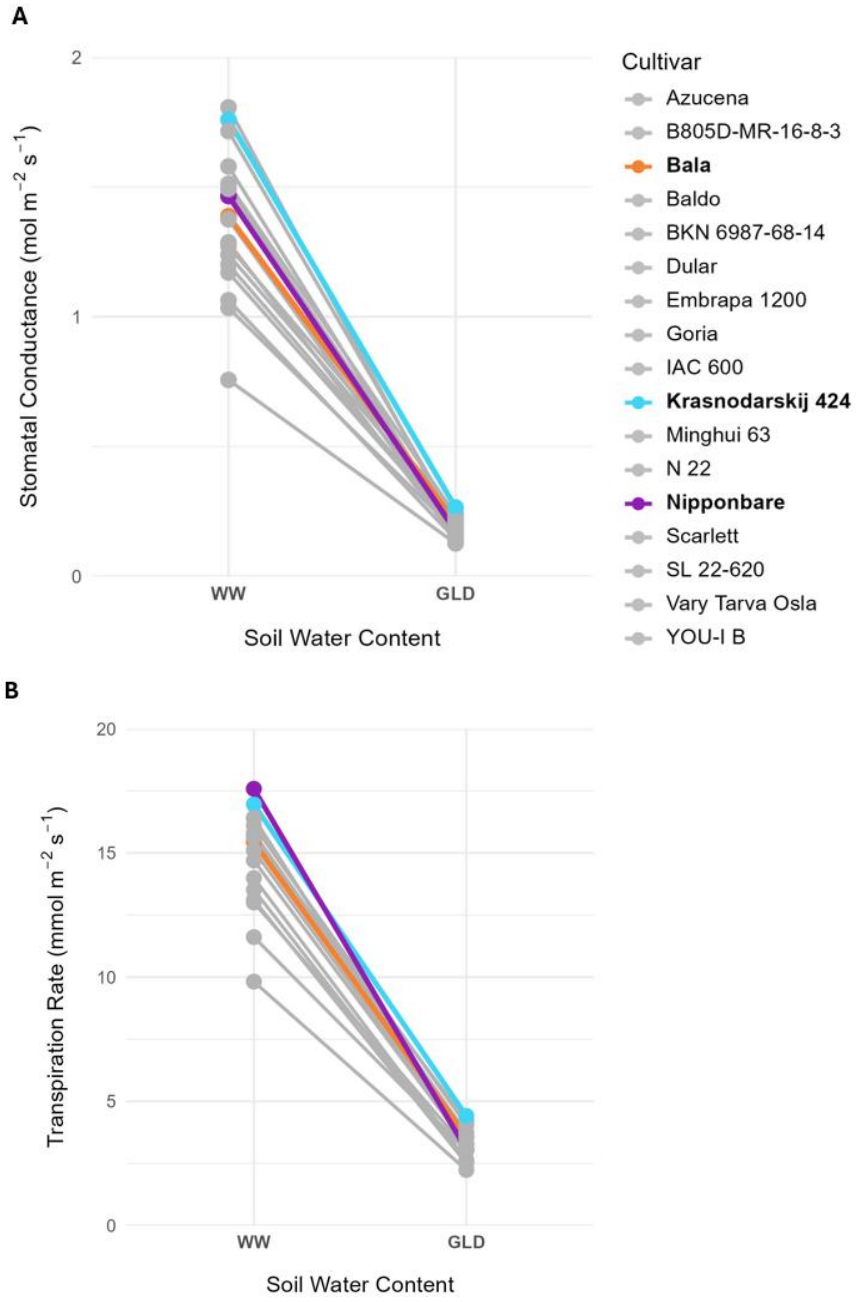

Fig. B1. Gas exchange parameters for 17 Asian rice (*Oryza sativa*) cultivars grown in well-watered (WW, 3 g H_2O/g dry soil) or growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil conditions. Stomatal conductance (A) and transpiration rate (B) were compared between a panel of cultivars (n = 1), with the three cultivars selected for further experimentation highlighted. Measurements were taken between ZT5 and ZT9 from the fully elongated fourth leaf of each plant three weeks after planting, using a portable photosynthesis system (LI-COR LI-6800).

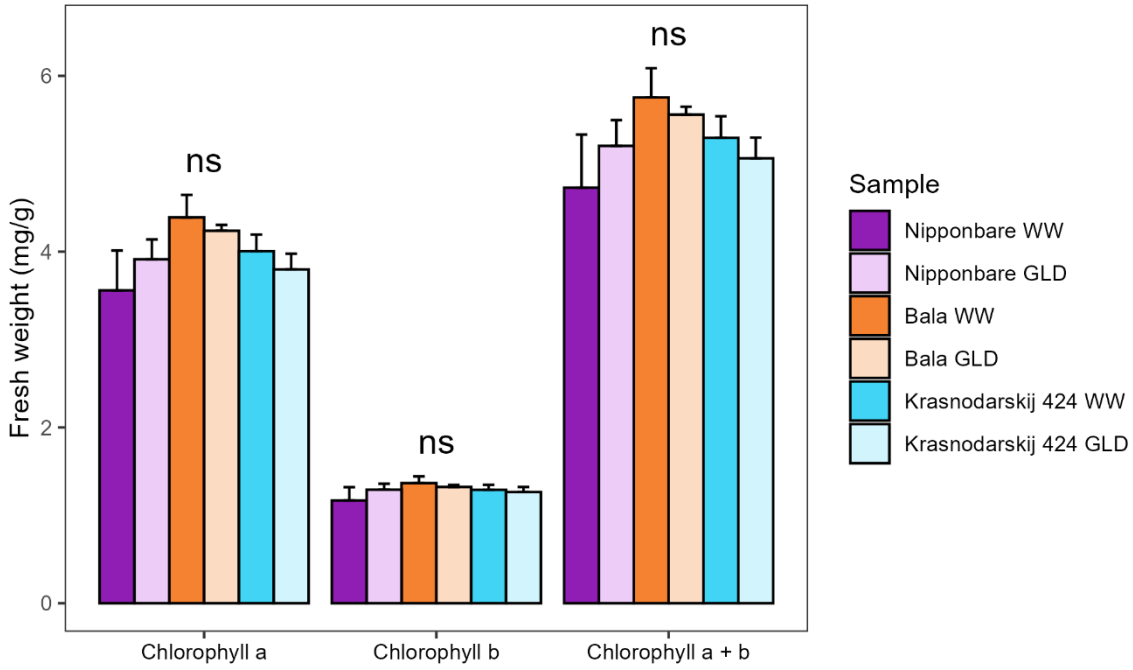

Fig. B2. Mean concentration of chlorophyll (mg chlorophyll/g fresh leaf tissue) in three Asian rice (*Oryza sativa*) cultivars (Nipponbare, Bala, Krasnodarskij 424) grown under varying moisture conditions. Plants were grown in either a well-watered (WW, 3 g H_2O/g dry soil) control soil condition or a growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil condition. Values were averaged across three biological replicates and three analytical replicates for each sample, and error bars represent standard error. No statistically significant differences (ns = non-significant) were found between samples (factorial ANOVA, Tukey's Honestly Significant Difference test, $P < 0.05$).

Tables

Table B1. Summary of information regarding 17 Asian rice (*Oryza sativa*) cultivars grown in a drought response screening panel. Plants were grown in either a well-watered (3 g H_2O/g dry soil) control soil condition or a growth-limiting drought (1 g H_2O/g dry soil) soil condition. Measured parameters that were distinct compared to the other cultivars in the panel ("standout traits") under drought conditions are noted. All reported values are within-pot means. DAP = days after planting.

Cultivar	Subpopulation	Geographic origin	Standout traits under drought
Azucena	Tropical Japonica	Philippines	High shoot (1.020 g fresh, 0.240 g dry) and root (0.036 g dry) weight, long fifth leaf (231.0 mm), early leaf emergence (leaves 2, 4-5: 4.5, 10.5, 14.8 DAP)
B805D-MR-16-8-3	Indica	Indonesia	High carbon assimilation rate (14.512 µmol m^{-2} s^{-1})
Bala	Indica Intermediate	India	High shoot dry weight (0.218 g), high leaf (9.5) and tiller (1.7) counts, early leaf emergence (leaves 2-5: 3.7, 7.0, 10.7, 14.6 DAP)
Baldo	Admixed Japonica	Italy	N/A
BKN 6987-68-14	Indica II	Thailand	High leaf (9.2) and tiller (1.7) counts, high carbon assimilation rate (15.230 µmol m^{-2} s^{-1})
Dular	Aus	India	N/A
Embrapa 1200	Tropical Japonica	Brazil	High carbon assimilation rate (13.542 µmol m^{-2} s^{-1}), high water use efficiency (4.169 µmol m^{-2} s^{-1}/mmol m^{-2} s^{-1})
Goria	Aus	Bangladesh	High root dry weight (0.042 g)
IAC 600	Temperate Japonica	Brazil	N/A
Krasnodarskij 424	Temperate Japonica	Russia	High total water use (59 g), high shoot (0.912 g fresh, 0.220 g dry) and root (0.042 g dry) weight, long leaves (leaves 3-5: 163.2, 225.2, 246.2 mm), high carbon assimilation rate (14.452 µmol m^{-2} s^{-1})
Minghui 63	Indica II	China	N/A
N 22	Aus	India	N/A
Nipponbare	Temperate Japonica	Japan	N/A
Scarlett	Admixed Japonica	United States	N/A
SL 22-620	Aus	Sierra Leone	N/A
Vary Tarva Osla	Japonica Intermediate	Portugal	Long leaves (leaf 4-5: 232.0, 242.7 mm)
YOU-I B	Indica I	China	Early leaf emergence (Leaves 2-5: 3.4, 6.6, 10.2, 14.2 DAP), high leaf (8.4) and tiller (1.4) numbers

Table B2. Total leaf and tiller numbers for 17 Asian rice (*Oryza sativa*) cultivars (n = 1) grown in well-watered (WW, 3 g H_2O/g dry soil) or growth-limiting drought (GLD, 1 g H_2O/g dry soil) conditions three weeks after planting. Values represent mean ± standard deviation for within-pot replicates, so no statistical interpretations were performed.

Cultivar	Soil water content	Tillers		Leaves	
		Count	GLD:WW	Count	GLD:WW
Azucena	WW	2.7 ± 0.8	0.44	13.5 ± 3.9	0.54
	GLD	1.2 ± 1.0		7.3 ± 2.9	
B805D-MR-16-8-3	WW	2.5 ± 0.5	0.48	13.2 ± 2.6	0.62
	GLD	1.2 ± 1.0		8.2 ± 3.1	
BALA	WW	3.3 ± 0.5	0.52	14.8 ± 1.3	0.64
	GLD	1.7 ± 0.8		9.5 ± 2.1	
Baldo	WW	2.0 ± 0.9	0.00	10.8 ± 3.4	0.37
	GLD	0.0 ± 0.0		4.0 ± 0.9	
BKN 6987-68-14	WW	4.0 ± 1.7	0.43	18.2 ± 5.8	0.51
	GLD	1.7 ± 0.5		9.2 ± 2.9	
Dular	WW	1.8 ± 1.5	0.22	11.0 ± 5.2	0.49
	GLD	0.4 ± 0.9		5.4 ± 3.4	
EMBRAPA 1200	WW	1.7 ± 0.5	0.00	9.0 ± 2.0	0.44
	GLD	0.0 ± 0.0		4.0 ± 0.9	
Goria	WW	2.7 ± 0.8	0.26	14.2 ± 2.6	0.39
	GLD	0.7 ± 1.0		5.5 ± 2.3	
IAC 600	WW	2.5 ± 0.5	0.28	11.8 ± 1.0	0.51
	GLD	0.7 ± 0.5		6.0 ± 1.3	
Krasnodarskij 424	WW	2.0 ± 0.0	0.15	9.3 ± 1.8	0.68
	GLD	0.3 ± 0.5		6.3 ± 1.4	
Minghui 63	WW	4.0 ± 1.2	0.30	16.6 ± 3.2	0.48
	GLD	1.2 ± 0.8		8.0 ± 2.5	
N 22	WW	2.7 ± 0.5	0.26	13.7 ± 1.2	0.42
	GLD	0.7 ± 0.8		5.8 ± 2.0	
Nipponbare	WW	2.7 ± 0.5	0.07	13.2 ± 2.6	0.40
	GLD	0.2 ± 0.4		5.3 ± 1.0	
Scarlett	WW	2.2 ± 0.4	0.14	12.7 ± 1.0	0.34
	GLD	0.3 ± 0.5		4.3 ± 1.5	
SL 22-620	WW	3.2 ± 1.6	0.22	14.7 ± 6.7	0.36
	GLD	0.7 ± 0.5		5.3 ± 1.2	
Vary Tarva Osla	WW	2.8 ± 0.4	0.46	11.3 ± 2.0	0.62
	GLD	1.3 ± 0.6		7.0 ± 1.7	
YOU-I B	WW	2.3 ± 0.5	0.61	13.7 ± 2.1	0.61
	GLD	1.4 ± 0.5		8.4 ± 2.6	

Table B3. Biomass measurements of root and shoot tissue for 17 Asian rice (*Oryza sativa*) cultivars (n = 1) grown under drought and watered conditions. Plants were grown in either well-watered (WW, 3 g H_2O/g dry soil) or growth-limiting drought (GLD, 1 g H_2O/g dry soil) soil conditions and collected for measurement three weeks after planting.

Cultivar	Shoot fresh weight (g)		Shoot dry weight (g)		Root dry weight (g)	
	WW	GLD	WW	GLD	WW	GLD
Azucena	3.667	1.020	0.882	0.240	0.196	0.036
B805D-MR-16-8-3	2.943	0.715	0.608	0.153	0.147	0.018
Bala	2.935	0.808	0.743	0.218	0.158	0.025
Baldo	3.247	0.270	0.627	0.063	0.128	0.010
BKN 6986-68-14	3.172	0.653	0.658	0.138	0.129	0.022
Dular	4.263	0.570	0.978	0.135	0.264	0.031
Embrapa 1200	2.105	0.413	0.352	0.097	0.058	0.023
Goria	3.333	0.508	0.865	0.125	0.223	0.042
IAC 600	1.915	0.392	0.375	0.100	0.056	0.017
Krasnodarskij 424	2.150	0.912	0.428	0.220	0.083	0.042
Minghui 63	3.430	0.385	0.800	0.090	0.185	0.018
N 22	3.102	0.654	0.710	0.160	0.235	0.024
Nipponbare	1.883	0.312	0.392	0.083	0.096	0.016
Scarlett	2.930	0.372	0.555	0.088	0.165	0.016
SL 22-620	2.708	0.328	0.585	0.087	0.148	0.022
Vary Tarva Osla	3.293	1.043	0.643	0.240	0.179	0.029
YOU-I B	2.733	0.674	0.623	0.158	0.157	0.026

Table B4. Leaf lengths for 17 Asian rice (*Oryza sativa*) cultivars (n = 1) grown in well-watered (WW, 3 g H_2O/g dry soil) or growth-limiting drought (GLD, 1 g H_2O/g dry soil) conditions and measured three weeks after planting. Values represent mean ± standard deviation for within-pot replicates, so no statistical interpretations were performed.

Cultivar	Soil water content	Leaf length (mm)				
		Leaf 1	Leaf 2	Leaf 3	Leaf 4	Leaf 5
Azucena	WW	58.7 ± 7.5	161.2 ± 13.2	251.8 ± 14.9	327.5 ± 27.4	370.0 ± 43.4
	GLD	52.8 ± 9.6	122.5 ± 6.8	164.5 ± 13.5	181.0 ± 65.4	231.0 ± 97.3
B805D-MR-16-8-3	WW	49.7 ± 4.1	117.2 ± 17.7	167.5 ± 12.0	217.2 ± 9.6	259.7 ± 10.2
	GLD	38.7 ± 3.4	86.7 ± 7.6	115.0 ± 8.6	137.5 ± 9.0	160.8 ± 29.0
Bala	WW	38.0 ± 5.0	120.5 ± 9.6	169.5 ± 9.3	216.2 ± 5.9	279.8 ± 9.7
	GLD	31.7 ± 4.5	91.0 ± 9.1	127.0 ± 6.3	157.3 ± 12.2	170.2 ± 57.3
Baldo	WW	37.8 ± 5.4	112.2 ± 17.3	193.5 ± 26.4	273.0 ± 17.6	332.8 ± 15.6
	GLD	30.5 ± 6.0	86.7 ± 10.7	117.8 ± 53.7	138.3 ± 52.2	93.0 ± 41.0
BKN 6987-68-14	WW	42.0 ± 6.4	124.4 ± 16.5	171.8 ± 9.3	221.0 ± 10.0	264.8 ± 17.5
	GLD	35.0 ± 10.1	103.0 ± 14.1	119.5 ± 9.8	135.8 ± 8.2	166.7 ± 30.3
Dular	WW	54.8 ± 4.6	151.0 ± 8.1	226.3 ± 6.7	288.5 ± 6.2	358.3 ± 13.1
	GLD	35.2 ± 6.9	85.8 ± 25.5	139.8 ± 41.9	156.5 ± 87.0	189.0 ± 73.7
Embrapa 1200	WW	47.7 ± 10.6	118.8 ± 19.4	199.0 ± 12.3	241.8 ± 8.3	282.5 ± 25.1
	GLD	43.0 ± 7.1	112.0 ± 16.4	144.7 ± 33.0	138.2 ± 72.8	80.0 ± 91.9
Goria	WW	62.2 ± 6.0	142.5 ± 11.6	226.7 ± 14.3	299.2 ± 10.5	367.3 ± 19.5
	GLD	54.7 ± 14.0	114.7 ± 23.2	161.3 ± 28.3	165.5 ± 53.9	227.5 ± 19.1
IAC 600	WW	27.5 ± 3.7	100.5 ± 9.8	171.0 ± 11.6	212.5 ± 11.0	266.0 ± 3.2
	GLD	26.2 ± 2.7	98.7 ± 14.0	134.5 ± 9.1	156.2 ± 5.8	53.0 ± 38.5
Krasnodarskij 424	WW	27.5 ± 4.7	103.5 ± 18.1	184.7 ± 31.3	255.8 ± 14.5	299.3 ± 22.7
	GLD	25.5 ± 4.8	99.0 ± 7.2	163.2 ± 14.4	225.2 ± 18.6	246.2 ± 77.7
Minghui 63	WW	52.6 ± 14.0	133.4 ± 19.6	214.2 ± 23.1	265.6 ± 14.4	331.0 ± 17.7
	GLD	38.8 ± 15.6	88.6 ± 23.9	122.0 ± 13.7	145.6 ± 15.4	106.0 ± 64.4
N 22	WW	52.8 ± 1.9	148.0 ± 5.4	214.5 ± 6.4	269.3 ± 3.6	346.5 ± 7.7
	GLD	39.5 ± 6.4	105.3 ± 10.6	146.0 ± 26.8	202.6 ± 17.8	156.4 ± 92.2
Nipponbare	WW	19.0 ± 4.9	79.2 ± 13.3	149.8 ± 17.0	190.5 ± 22.3	234.8 ± 20.7
	GLD	23.0 ± 4.3	77.3 ± 5.3	116.3 ± 9.8	129.2 ± 24.1	85.0 ± 40.8
Scarlett	WW	34.8 ± 3.7	123.8 ± 13.9	188.3 ± 7.2	223.5 ± 10.9	292.8 ± 11.3
	GLD	26.8 ± 3.90	85.2 ± 14.9	108.0 ± 6.2	127.8 ± 23.7	73.0 ± 50.9
SL 22-620	WW	58.0 ± 12.4	131.7 ± 20.4	209.0 ± 31.4	254.0 ± 35.5	269.5 ± 109.1
	GLD	58.5 ± 13.4	106.8 ± 16.6	143.8 ± 11.9	119.0 ± 51.0	94.5 ± 79.9
Vary Tarva Osla	WW	31.2 ± 7.4	163.3 ± 14.1	246.0 ± 8.6	289.7 ± 42.2	380.7 ± 9.5
	GLD	29.3 ± 6.7	129.0 ± 11.5	184.0 ± 18.5	232.0 ± 8.5	242.7 ± 47.9
YOU-I B	WW	26.0 ± 6.0	100.5 ± 10.4	154.8 ± 9.3	213.3 ± 8.2	249.7 ± 17.1
	GLD	20.6 ± 4.7	76.8 ± 13.8	110.0 ± 8.3	127.4 ± 11.4	153.4 ± 12.0