*Mysore J. Agric. Sci., 57 (1) : 33-42 (2023)*

# Relevance of Stomatal Traits in Determining the Water Use and Water Use Efficiency in Rice Genotypes Adapted to Different Cultivation Systems

Abhishree Ramachandra, B. Mahadeva Prabhu and M. S. Sheshshayee
Department of Crop Physiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : abhishreeramachandra@gmail.com

**Authors Contribution**

Abhishree Ramachandra : Investigation, drafted the manuscript analysis;
B. Mahadeva Prabhu : Provided technical inputs;
M. S. Sheshshayee : Conceptualization, designing & final aproval

***Corresponding Author :***
Abhishree Ramachandra
Department of Crop Physiology, College of Agriculture, UAS, GKVK Bengaluru

*Received :* August 2022
*Accepted :* November 2022

## Abstract

Climate change has resulted in a highly unpredictable pattern of rainfall which is often insufficient for agriculture. Rice, being staple food and a water intensive crop, the water conservation strategy without much compromise in yield is always a challenge. The majority of water lost is because of the transpiration process, which is regulated by small opening on the leaf surface called stomata. Studying the stomatal arrangement between the genotypes paves the way to understand physiological responses of stomata to varying moisture regimes. In this study, the spatial variability of stomata existing in three well known rice varieties *viz.,* APO, Dhaksha and IR 64 was studied along with its effect on transpiration. Image J software was used to measure the stomatal size and phenomics facility was used to measure the transpiration. The data on stomatal frequency from both the lower and upper surface showed that APO and IR 64 had considerably higher stomatal frequency in the flag leaf with Dhaksha having higher stomatal frequency among all the three rice varieties. Total dry matter, cumulative water transpired and water use efficiency were significantly higher for Dhaksha followed by APO. The higher stomatal number in flag leaf and second leaf and lower stomatal number and higher size in third and fourth leaf of Dhaksha could be an adaptive strategy of the genotype to reduce the water loss from the leaves under water limited condition. This study indicates that higher stomatal numbers at top leaves and lower stomatal number at lower leaves is best suited for rice under aerobic condition to increase water productivity without decreasing yield.

*Keywords* : Stomatal traits, Water use efficiency, Rice genotypes

Climate change has a greater impact on agriculture. With the increasing demand for fresh water from civic and industrial sectors, water shortage would have a strong adverse effect on agricultural practices. Thus, there has been an increasing trend towards accessing ground water through tube wells. These anthropogenic interventions have exacerbated water crisis by depleting the water table.

Rice (*Oryza sativa* L.), the staple diet for more than half of the world population, is a water intensive crop. To sustain food security, under the scenario of a changing climate, it is imperative that the water productivity of rice has to be enhanced which can be achieved either by the adoption of specific water saving practices or by genetic enhancement to improve crop tolerance to water limitation stress (Vijayaraghavareddy *et al.,* 2020a). Semi-irrigated aerobic cultivation and upland rice is being practiced under both rainfed and irrigated conditions. However, concomitant reduction in yield is often noticed due to higher spikelet fertility. This led to the adoption of physiological breeding. For example, Dhaksha, has also been developed by introgressing specific physiological traits such as water use and water use efficiency (WUE) to grow under aerobic conditions (Sheshshayee *et al.,* 2019). Other approach was double haploids breeding technique for developing new varieties of crops (Chaitanya and Raju, 2022). It has become necessary to focus on water conservation strategy without much compromise in yield. There is a need to improve the yield of local rice varieties to combat the food demands of the increasing population (Karthika and Shanker, 2022).

Many studies have reported the improvement in water use efficiency (WUE) of the rice crop. The WUE is the function of the amount of dry matter produced per unit of water transpired. In plants, water can be used efficiently by regulating the transpiration process. This transpiration process is regulated by many external factors like light, temperature, humidity and an internal factor *i.e.* stomata. Stomata are extracellular openings present on the leaf surface. These provide access to the mesophyll cells (Zeiger *et al*., 1987; Hetherington and Woodward, 2003). Stomata are not uniform among the plants; their size, shape and number vary within and across different species. They exhibit differences in their development and patterning on the epidermis (Caine *et al.,* 2016).

The stomatal movements are the major driving force for both $CO_2$ fixation and transpiration. Due to varying response of rice crops to varied climatic conditions, there is differential arrangement of stomata (*i.e.* stomatal density and guard cell size) on leaf surface. These differences have led to the differential response of plants to stress resulting in differences in potential yield. Evolutionarily various stomatal traits have been altered enabling the plants to adopt to new environment (Taylor *et al*., 2012; Drake *et al*., 2013; Haworth *et al*., 2018). The plants with smaller size stomata show fast response to changing environmental conditions unlike the large sized stomata (Franks and Beerling, 2009 and Drake *et al*., 2013 and Lawson & Blatt, 2014). When the ratio of cell surface area to volume of smaller cells is higher, they facilitate faster ion fluxes, leading to faster guard cell turgor changes and a more rapid conductance response (Lawson and Chabrand, 2019).

An investigation was carried out to assess the role of stomatal factors such as number and size, in governing the variability in WUE. These influences were examined in rice cultivars representing the three distinct cultivation ecosystems, *viz.,* irrigated (IR64), rainfed upland (APO) and semi-irrigated aerobic (Dhaksha). Studying the variability of stomatal factors in these genotypes paves the way to understand physiological responses of stomata to varying moisture regimes.

## Material and Methods

### Plant Material and Environment

The experiment was conducted in the phenomics facility established in the Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bengaluru, during *Rabi* 2022 (Fig. 1A).


Fig. 1: (A) High throughput phenomics facility established at Department of Crop Physiology, University of Agricultural Sciences, Bangalore. (B) Automated software controlled system enables the measurement of hourly transpiration and cumulative water transpired.

Three well known Rice varieties *viz.,* Dhaksha, IR-64 and APO were used in studying the variability existing in the stomatal traits and water use.

**Growing Conditions**

The germinated seeds of Dhaksha, IR-64 and APO were directly sown into the pots of 30L capacity. Pots were filled with red soil and FYM in 3:1 ratio. Thinning was carried out at 21 days after sowing (DAS) to maintain two plants per pot. Recommended dose of fertilizers (100N: 50P: 50K) was added in three split doses during sowing, after thinning and at flowering. All the prophylactic measures were taken whenever necessary. The field capacity was calculated as described by Vijayaraghavareddy *et al.,* 2020a. The moisture conditions of 100 per cent Field Capacity (FC) (control) were maintained during the entire crop growth period by gravimetric method using the mini-lysimeter phenomics facility.

**Maintaining the Soil Moisture Status with Phenomics Facility**

The mini-lysimeter phenomics platform provides the cumulative water transpired by each genotype based on the gravimetric approach (Fig. 1B). The MLM drought simulator platform is capable of gravimetric determination of water loss due to the Evapo-Transpiration of container grown plants on a 'real-time' basis (Vijayaraghavareddy *et al.*, 2020b and Lekshmy *et al.*, 2021). Combined with a smart transpiration-interfaced automated irrigation facility, it ensures the precise maintenance of a specific water regime in the soil. The most prominent feature of this automated irrigation facility comes from its ability to ensure exactly the same level of soil moisture status for plants irrespective of differences in water used.

**Measurement of Stomatal Paramaters**

The leaf impressions for stomatal imprints were taken from flag leaf, second leaf, third leaf and fourth leaf from the top during the anthesis stage. The stomatal impressions were made using the nail enamel method. The nail enamel was smeared on the middle portion of leaf surface and allowed to dry for a minute and the smear was imprinted on the microscopic slides using the cello tape. Impressions were made for both the abaxial and adaxial sides of the same leaf for each variety, maintaining four replications for each variety (3 varieties x 2 surfaces x 4 replications x 3 images per slide) (Reddy *et al.*, 2020). The imaging was done using automated fluorescent microscope (EVOS M700) at magnification of 400X. The stomatal frequency was calculated by manually counting the number of stomata per microscopic area under 400X magnifaction and was further converted to number of stomata per $mm^2$ area (Franks and Beerling, 2009). The product of stomatal length and width as taken as stomatal size.

The stomatal length and stomatal width were measured using Image J software, an imaging tool, considering six stomata per microscopic view. From the tool bar the image to be analyzed was opened. Before starting the measurement of length or width of stomata using Image J, the standard scale was set using the scale mentioned in the image taken from the microscope (in the present experiment the scale was set to 75 µm). After setting the scale, the actual measurements were done using straight line tool. After drawing each line to the required length and / or width, the line drawn was measured by the software. The measured data was exported to excel sheets and calculated as stomatal size by multiplying stomatal length and stomatal width.

**Measurement of Morpho-Physiological Parameters and Transpiration**

When the plants reached physiological maturity (110 DAS), the panicles, leaves, stems and roots were separated from the plants and oven dried at 70°C for 3 days. The total leaf area (TLA) was calculated by multiplying the specific leaf area (SLA) ($cm^2/g$) with total dry weight of the leaves. The SLA is the ratio of leaf area to leaf dry weight (Garnier *et al.,* 2001). After threshing of panicles, the total weight of filled grains obtained was considered as yield per pot. The TDM was calculated by adding the dry weights of leaf, stem, root and yield.

Cumulative water transpired (CWT) was measured using the phenomics facility. Total water transpired from 20 Days after sowing (DAS) to 100 DAS was computed to arrive at total evapo-transpiration. The soil surface of the pots was covered with beads to avoid the evaporation loss of water. However, empty pots without plants were maintained to arrive at total evaporation loss of water for the crop period. Total evapo-transpiration was deducted with evaporation loss of water to arrive at CWT. Mean transpiration rate (MTR) was calculated as a ratio of CWT to the total leaf area. The WUE was calculated by taking the ratio of total biomass to the CWT by the plant between 20 DAS and 100 DAS.

## Results and Discussion

### Measurement of Stomata Using ImageJ

High-throughput phenotyping methods are becoming very crucial for developing genotypes for stress tolerance. Many software-based tools are highly used for measuring morpho-physiological traits (Zang and Zang 2018). However, traits such as stomatal size are not well studied due to lack of

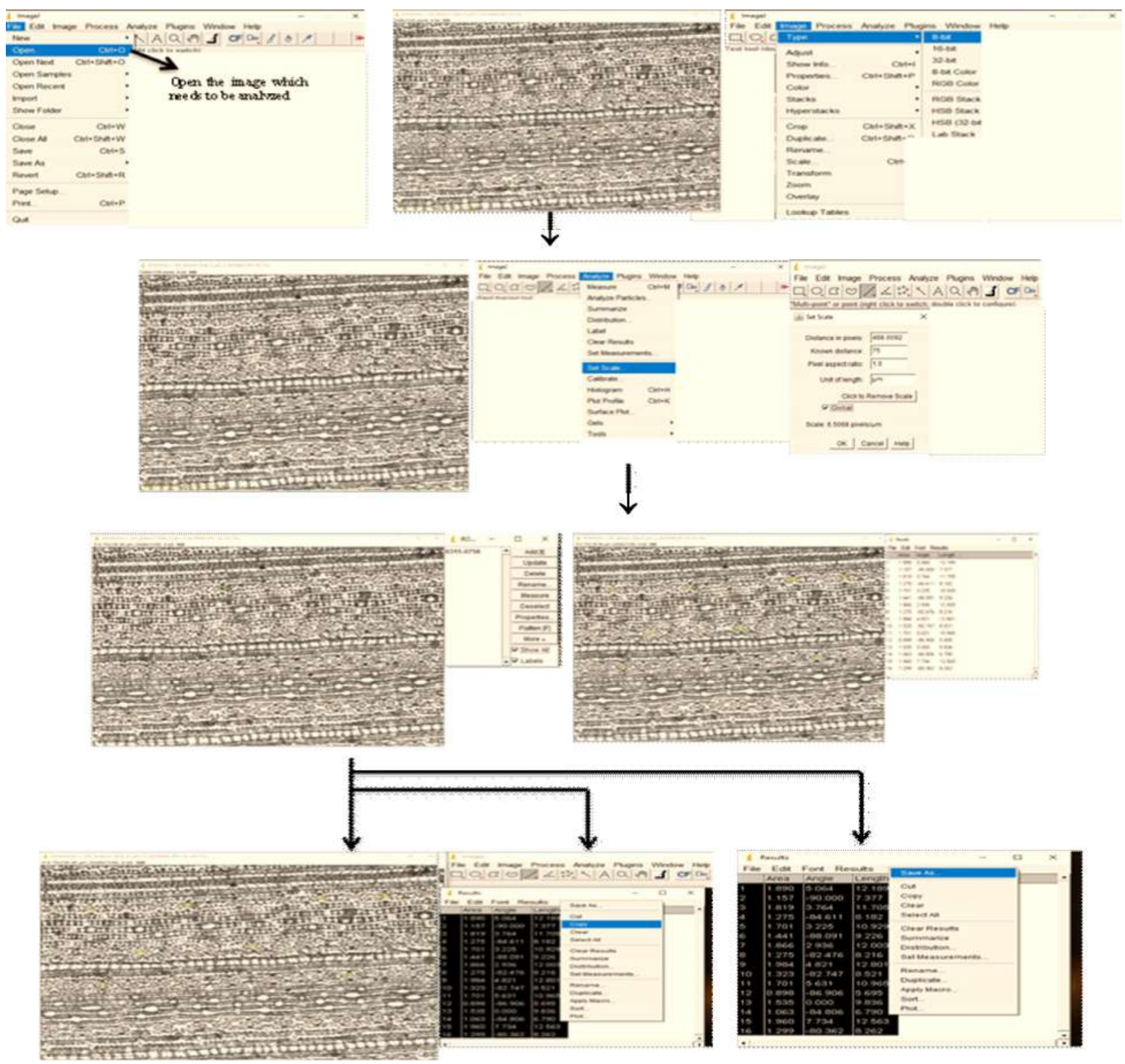



Fig. 2: Detailed illustration of measuring stomatal length and width using ImageJ software

phenotyping tools. Though scanning electron microscopy is highly used, it is not preferable to adopt for large set of genotypes or mapping population due to high cost of the technique. In this study, a quick method was standardized to measure the stomatal size in rice genotypes using ImageJ software. This system needs a clear image taken from the imprint method. In this study, fluorescent microscope was used for taking the images. ImageJ is an open source freeware mainly used for image analysis (Chatterjee *et al*., 2020). Images taken from the microscope were exported to the ImageJ software and the scale was set in µm (Fig. 2). Randomly five stomata were selected and measured for length and width. When the image to be analyzed is opened, in the tool bar, the 'Image' - 'Type' was set to 8 bit. Next, by selecting the straight line tool, the length was drawn on the scale bar in the image opened. By using 'Analyse' tool the scale was set to the same length as the scale bar (75 µm for 400x magnification). After setting the scale, using the same straight line tool, a line was drawn horizontally on the stomatal aperture which indicated the stomatal length and a line drawn vertically in the center of stomata indicated the stomatal width. The length and width of the measured stomata are taken from the results tab and exported to Microsoft excel for further analysis (Fig. 2).

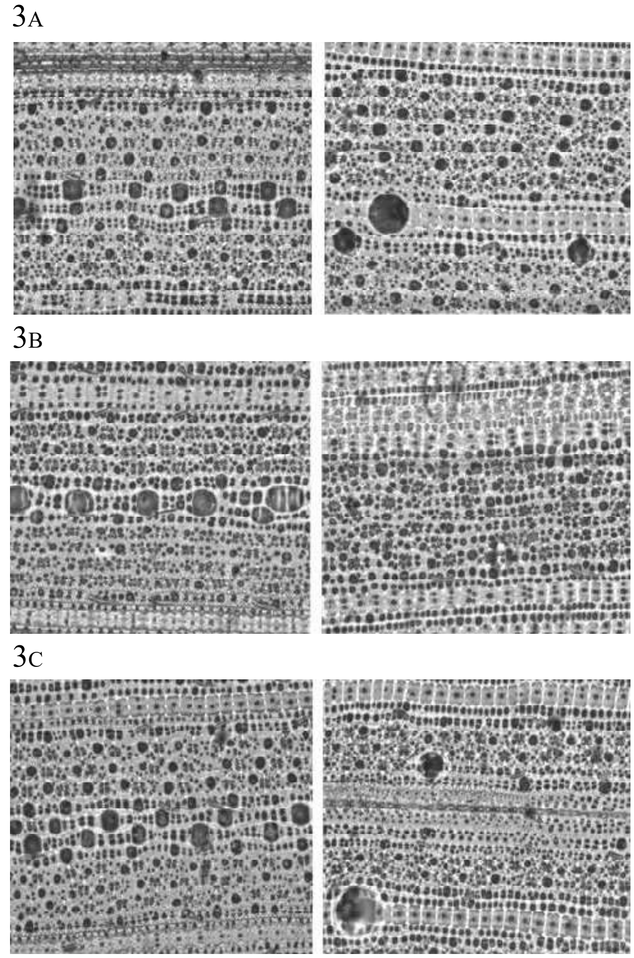



Fig. 3: Impressions of lower (left panel) and upper (right panel) leaf surface under magnification of 400 X. A) APO, B) Dhaksha, C) IR 64. Arrows indicate stomata.

**Variability in Stomatal Number and Size among the Rice Genotypes**

The stomata in rice are arranged alternatively in parallel rows on both the lower and upper leaf surface (Fig. 3). In APO, the flag leaf had higher stomatal frequency, whereas in Dhaksha both second leaf and flag leaf recorded higher stomatal frequency compared to other leaves (Fig. 4A). IR 64 did not show any difference in stomatal frequency between flag leaf, second and third leaf. In the fourth leaf, stomatal frequency was lower for IR 64 compared to APO and Dhaksha (Fig. 4A). Previous defoliation experiments have shown the importance of flag leaf during grain filling, where plants without flag leaf had a significant reduction in the yield (Acevedo *et al*., 2021). Flag leaf is the major source contributor during grain filling. Hence, higher stomatal frequency attributes to enhanced carbon fixation in the flag leaf (Acevedo *et al*., 2021). Under aerobic conditions, water use efficiency also plays a major role especially in semi aquatic crop plants like rice (Sheshshayee *et al*., 2003). Hence, it could be possible that in both aerobic type genotypes APO and Dhaksha, leaves which has greater light interception (flag leaf and second leaf) had the more number of stomata compared to lower leaves (Fig. 4A). There was no significant difference between the stomatal frequency of upper and lower surface except for fourth leaf of APO and IR 64. The lower surface of the fourth leaf of APO had lower stomatal frequency compared to upper surface. However, the lower surface of the fourth leaf of IR 64 had higher

TABLE 1

Variability in Stomatal frequency of lower and upper leaf surface among Apo, Dhaksha, IR64 through Flourescence microscope with 400X magnification

| | Stomatal frequency (per mm² area) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lower surface | | | | Upper surface | | | |
| | Flag Leaf | Second Leaf | Third Leaf | Fourth Leaf | Flag Leaf | Second Leaf | Third Leaf | Fourth Leaf |
| APO | 645.16 ± 28.71 | 476.03 ± 8.46 | 492.83 ± 8.46 | 380.82 ± 13.99 | 532.03 ± 13.58 | 498.43 ± 60.23 | 435.71 ± 30.49 | 505.15 ± 5.13 |
| Dhaksha | 650.76 ± 46.60 | 651.88 ± 62.77 | 493.95 ± 17.78 | 561.16 ± 26.24 | 621.64 ± 95.81 | 776.21 ± 10.08 | 562.28 ± 83.29 | 77.15 ± 45.21 |
| IR 64 | 599.24 ± 19.11 | 520.83 ± 34.92 | 539.87 ± 52.52 | 465.95 ± 102.27 | 484.99 ± 19.69 | 516.35 ± 38.95 | 570.12 ± 25.44 | 56.50 ± 20.25 |

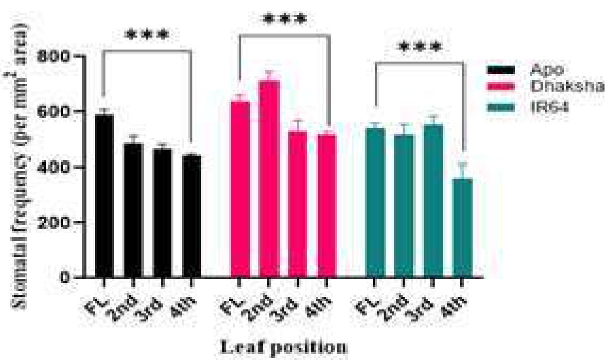
stomatal frequency (465.95 per mm$^2$ area) compared to upper surface (256.50 per mm$^2$ area). In Dhaksha, second and third leaf had high frequency of stomata in upper surface compared to lower surface (Table 1).

Stomatal size measured using ImageJ showed that, IR64 has the higher stomatal size in the fourth leaf compared to other top leaves. Whereas, Dhaksha had more stomatal size in both third and fourth leaf compared to flag leaf and second leaf (Fig. 4B). Genotype APO did not show any significant difference except for the flag leaf. Many studies have shown that increasing the stomatal size decreases stomatal density (Chatterjee *et al.,* 2020). Between upper and lower surface no significant difference in stomatal size was noticed in any genotype except for fourth leaf of APO (Table 2). Our data also showed a negative association between size and frequency of the stomata (Fig.5A). Size and frequency of the stomata between upper and lower surface did not show a strong association ($R^2$=0.42 and $R^2$=0.31 respectively) (Fig. 5B). The cause for variability in stomatal frequency and stomatal size existing between abaxial and adaxial surface and different leaves within same plant and the variability existing between the varieties needs to be further verified.

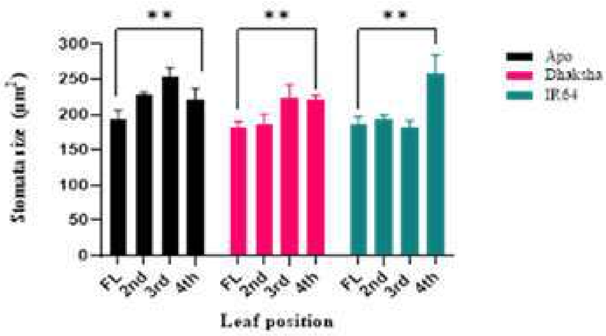
**Difference in the Cumulative Water Transpired Between the Genotypes**

The higher stomatal frequencies on all the four leaves in Dhaksha and APO contributed for higher transpiration during the entire growth period (Table 3). Because of higher stomatal frequency and size in APO and Dhaksha, the water transpired measured in term of cumulative water transpired (CWT) was significantly higher. IR64, a lowland cultivar showed significantly lower CWT because of lower stomatal size and frequency. It is well established that stomata is the major internal factor that drives transpiration. Higher stomatal number associates with higher carbon gain to more conductance. This study also confirms that higher stomatal number resulted in higher CWT in both APO and Dhaksha.

4A




4B




4C

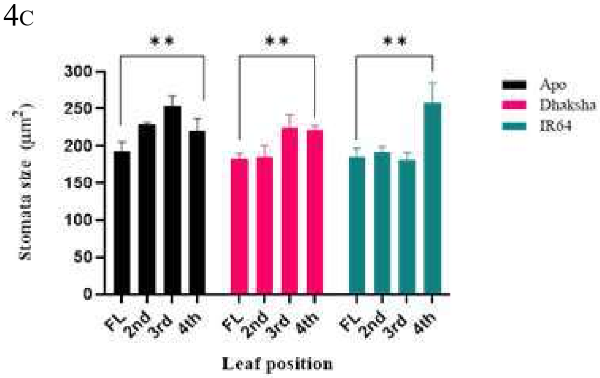



Fig. 4: A) Stomatal frequency and (B) stomatal size in flag leaf, second leaf, third leaf and fourth leaf from top of APO, Dhaksha and IR 64 (mean±SE). Significant difference at *** Pd" 0.01, **Pd" 0.05.

Further, results show that low stomatal frequency and small stomatal size types (IR 64) transpires less. These results would also be due to faster response of the stomata to varying vapor deficit conditions (Lawson and Chabrand, 2019). At the whole plant level, many factors regulate transpiration including the leaf area, canopy architecture and water availability (Caine *et al.*, 2019). It further needs to be studied which type of stomatal arrangement is physiologically important to conserve water without

Table 2

Variability in Stomatal size of lower and upper leaf surface among Apo, Dhaksha, IR 64 through ImageJ software

| | Stomatal size ($\mu m^2$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lower surface | | | | Upper surface | | | |
| | Flag Leaf | Second Leaf | Third Leaf | Fourth Leaf | Flag Leaf | Second Leaf | Third Leaf | Fourth Leaf |
| APO | 190.43 ± 18.01 | 218.75 ± 7.27 | 250.46 ± 18.55 | 245.90 ± 19.63 | 195.82 ± 13.06 | 238.61 ± 11.73 | 256.66 ± 9.78 | 194.36 ± 14.81 |
| Dhaksha | 198.65 ± 9.62 | 182.71 ± 8.91 | 233.94 ± 33.23 | 221.01 ± 14.14 | 166.98 ± 6.25 | 188.38 ± 22.83 | 215.38 ± 16.29 | 222.59 ± 4.38 |
| IR 64 | 189.15 ± 15.02 | 198.15 ± 17.81 | 177.91 ± 37.55 | 229.57 ± 45.11 | 181.92 ± 10.71 | 186.84 ± 19.57 | 183.85 ± 18.92 | 287.13 ± 38.41 |

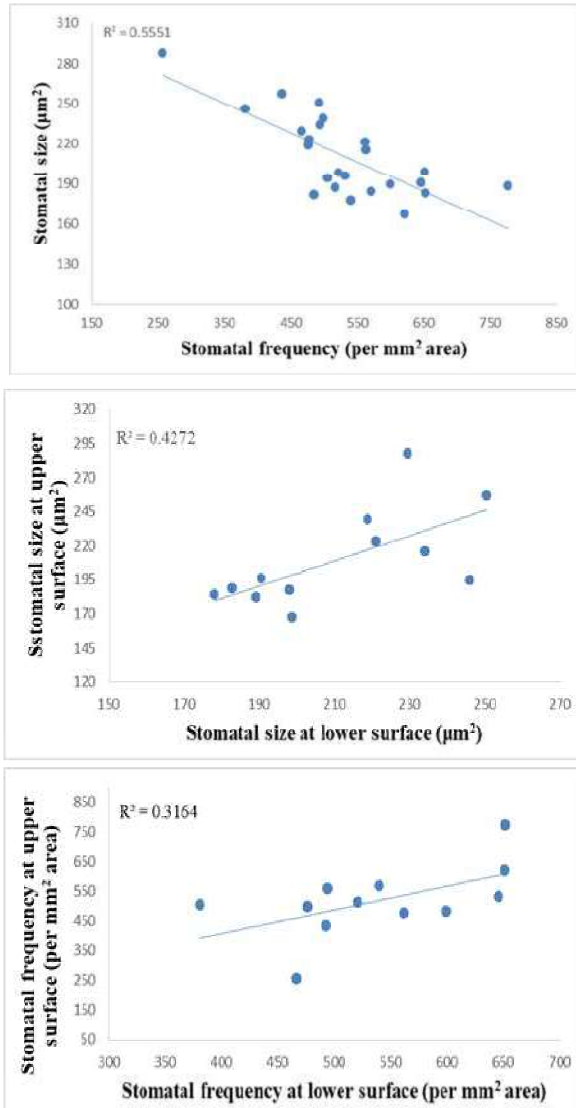



Fig. 5 : Regression analysis showing the relationship between the (A) stomatal frequency at upper and lower surface of the leaf, (B) stomatal size at upper and lower surface of the leaf and (C) stomatal frequency and stomatal size. (*P≤0.1, ns-non significant).

causing any yield penalties under both the well watered and water limited conditions (Caine *et al.*, 2019). It is most important to conserve water without greater compromise in yield to reduce the effects of climate change on future agriculture.

**Morpho-Physiological and Transpiration among the Genotypes**

Total dry matter (TDM) was significantly higher in Dhaksha and APO. However, yield per pot was significantly higher in Dhaksha compared to APO. The yield was significantly lower in IR 64. (Table 3). This could be due to the experience of mild drought stress under aerobic conditions. It has been well documented that, for lowland cultivars a smaller reduction in water availability causes significant yield loss (Vijayaraghavareddy *et al.,* 2020a). The reduction in yield also depends on the stage of stress occurrence. Since in the plants were maintained in aerobic conditions throughout the crop period, IR 64, the lowland cultivar might have experienced mild stress during all critical growth phases and hence resulted in lower yield. Among the genotypes, Dhaksha had the higher leaf area (5503.40 cm$^2$) (Table 3). CWT for entire crop period was also significantly higher for APO and Dhaksha. Difference in the CWT could be due to existing differences in the leaf area. Hence we calculated the MTR and it was significantly lower for Dhaksha indicating the lower transpiration per unit leaf area. APO recorded the higher MTR compared to IR 64. The lower MTR in Dhaksha could also be due to lower stomatal frequency in third and fourth leaf (Table 3). Since the lower leaves of the canopy

TABLE 3

Variability in transpiration and morpho-physiological traits among APO, Dhaksha, IR 64. Cumulative water transpired (CWT), Total dry matter (TDM), Total leaf Area (TLA), Water use efficiency (WUE) and Yield in Apo, Dhaksha and IR 64 respectively.

| | CWT (L) | TDM (g/pot) | TLA (cm2/pot) | WUE (g/L) | MTR (ml/cm2) | Yield (g/pot) |
|---|---|---|---|---|---|---|
| APO | 116.79 ± 0.70 | 368.80 ± 35.30 | 5068.45 ± 209.60 | 3.16 ± 0.31 | 23.1 ± 1.0 | 107.20 ± 8.71 |
| Dhaksha | 115.81 ± 0.75 | 374.37 ± 123.67 | 5503.40 ± 388.05 | 3.24 ± 1.09 | 20.4 ± 1.3 | 196.78 ± 27.37 |
| IR 64 | 82.41 ± 0.16 | 220.12 ± 7.34 | 3917.29 ± 27.70 | 2.67 ± 0.09 | 21.1 ± 1.5 | 65.60 ± 3.87 |

experience shade due to overlapping of leaves, carbon assimilation would be substantially low. Hence in Dhaksha, this could be a drought adaptive mechanism to reduce transpiration by minimizing the stomatal frequency in lower leaves. The water use efficiency (WUE) of both aerobic cultivars APO and Dhaksha was significantly higher compared to lowland cultivar IR 64. The genotype Dhaksha has been developed by physiological breeding by crossing high root and high WUE parents (Sheshshayee *et al.,* 2019). Hence despite the higher CWT, higher WUE indicates a better carbon assimilatory capacity.

This study depicts the importance of high through put techniques for measurement of drought adaptive mechanisms and the spatial variations in stomatal number and size indicates a strong G×E interaction. The importance of stomatal traits in determining the WUE explains the need of improving this trait for achieving drought tolerance.

*Acknowledgments* : AR would like to thank the Department of Science and Technology (DST), Government of India, for the DST-INSPIRE Fellowship. The research is supported by the National Agriculture Science Fund (NASF-2581). The authors also acknowledge the Pathogenomics Lab, Department of Plant Pathology, University of Agricultural Sciences, GKVK for providing microscope facility. The authors wish to thank Dr. Preethi Vijayaraghavareddy for useful suggestions during the experiment and manuscript preparation. The authors also thank the members of Molecular Marker Lab and Phenomics team, Department of Crop Physiology, for providing technical inputs during the experiment.

## References

Acevedo-Siaca, L. G., Coe, R., Quick, W. P. and Long, S. P., 2021, Variation between rice accessions in photosynthetic induction in flag leaves and underlying mechanisms. *J. Exp. Bot.*, **72** (4) : 1282 - 1294.

Caine, R. S., Chater, C. C., Kamisugi, Y., Cuming, A. C., Beerling, D. J., Gray, J. E. and Fleming, A. J., 2016, An ancestral stomatal patterning module revealed in the non-vascular land plant Physcomitrella patens. *Development*, **143** (18) : 3306 - 3314.

Caine, R. S., Yin, X., Sloan, J., Harrison, E. L., Mohammed, U., Fulton, T., Biswal, A. K., Dionora, J., Chater, C. C., Coe, R. A. and Bandyopadhyay, A., 2019, Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. *New Phytol.*, **221** (1) : 371 - 384.

Chaitanya, G. and Mohan Raju, B., 2022, Optimization of *in-vitro* androgenic protocol for development of haploids and double haploids in *indica* rice hybrid, KRH 4. *Mysore J. Agric. Sci.,* **56** (1) : 39 - 48.

Chatterjee, J., Thakur, V., Nepomuceno, R., Coe, R.A., Dionora, J., Elmido-Mabilangan, A., Llave, A. D., Reyes, A. M. D., Monroy, A. N., Canicosa, I. and Bandyopadhyay, A., 2020, Natural diversity in stomatal features of cultivated and wild *Oryza* species. *Rice*, **13** (1) : 1 - 20.

Dharmappa, P. M., Doddaraju, P., Malagondanahalli, M. V., Rangappa, R. B., Mallikarjuna, N. M., Rajendrareddy, S. H., Ramanjinappa, R., Mavinahalli, R. P., Prasad, T. G., Udayakumar, M. and Sheshshayee, S. M., 2019, Introgression of root and water use efficiency traits enhances water productivity : An evidence for physiological breeding in rice (*Oryza sativa* L.). *Rice*, **12** (1) : 1 - 14.

Drake, P. L., Froend, R. H. and Franks, P. J., 2013, Smaller, faster stomata : scaling of stomatal size, rate of response and stomatal conductance. *J. Exp. Bot.*, **64** (2) : 495 - 505.

Franks, P. J. and Beerling, D. J., 2009, Maximum leaf conductance driven by $CO_2$ effects on stomatal size and density over geologic time. *Proc. Natl. Acad. Sci.*, **106** (25) : 10343 - 10347.

Garnier, E., Shipley, B., Roumet, C. and Laurent, G., 2001, A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Funct. Ecol.,* 688 - 695.

Haworth, M., Marino, G., Brunetti, C., Killi, D., De Carlo, A. and Centritto, M., 2018, The impact of heat stress and water deficit on the photosynthetic and stomatal physiology of olive (*Olea europaea* L.) - A case study of the 2017 heat wave. *Plants*, **7** (4) : 76.

Hetherington, A. M. and Woodward, F. I., 2003, The role of stomata in sensing and driving environmental change. *Nature*, **424** (6951) : 901 - 908.

Karthika, V. and Shanker, A. G., 2022, Efficient callus induction and regeneration protocol for the rice *indica* genotype Sonali. *Mysore J. Agric. Sci.,* **56** (1) : 171 - 179.

Lawson, T. and Blatt, M. R., 2014, Stomatal size, speed and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiol.*, **164** (4) : 1556 - 1570.

Lawson, T. and Vialet Chabrand, S., 2019, Speedy stomata, photosynthesis and plant water use efficiency. *New Phytol.*, **221** (1) : 93 - 98.

Lekshmy, V. S., Vijayaraghavareddy, P., Nagashree, A. N., Ramu, V. S., Ramegowda, V., Makarla, U. and Sreeman, S., 2021, Induction of acquired tolerance through gradual progression of drought is the key for maintenance of spikelet fertility and yield in rice under semi-irrigated aerobic conditions. *Front. Plant Sci.*, pp. : 2275.

Reddy, S. H., Da Costa, M. V. J., Kambalimath, S. K., Rajanna Mavinahalli, P., Muthurajan, R., Chinnusamy, V., Sevanthi, A. M., Neelamraju, S., Gopala Krishnan, S., Singh, A. K. and Singh, N. K., 2020, Relative contribution of stomatal parameters in influencing WUE among rice mutants differing in leaf mass area. *Plant Physiol. Rep.*, **25** (3) : 483 - 495.

Sheshshayee, M. S., Bindumadhava, H., Shankar, A. G., Prasad, T. G. and Udayakumar, M., 2003, Breeding strategies to exploit water use efficiency for crop improvement. *J. Plant Biol.*, **30** (2) : 253 - 268.

Taylor, S. H., Franks, P. J., Hulme, S. P., Spriggs, E., Christin, P. A., Edwards, E. J., Woodward, F. I. and Osborne, C. P., 2012, Photosynthetic pathway and ecological adaptation explain stomatal trait diversity amongst grasses. *New Phytol.*, **193** (2) : 387 - 396.

Vijayaraghavareddy, P., Vemanna, R. S., Yin, X., Struik, P. C., Makarla, U. and Sreeman, S., 2020b, Acquired traits contribute more to drought tolerance in wheat than in rice. *Plant Phenomics*, pp. : 2020.

Vijayaraghavareddy, P., Xinyou, Y. I. N., Struik, P. C., Makarla, U. and Sreeman, S., 2020a, Responses of lowland, upland and aerobic rice genotypes to water limitation during different phases. *Rice Sci.*, **27** (4) : 345 - 354.

Zeiger, E., Farquhar, G. D. and Cowan, I. R., 1987, Stomatal function. *Stanford University Press*, Stanford : pp. : 115.

Zhang, Y. and Zhang, N., 2018, Imaging technologies for plant high-throughput phenotyping : A review. *Front. Agric. Sci. Eng.*, **5** (4) : 406 - 419.