

Character association analysis on morpho-physiological and yield parameters of rice (*Oryza sativa* L.) under water stress

ABSTRACT

The present character association study aims to understand the correlation between morphological, physiological and yield traits of rice genotypes under water stress and non-stress conditions, so as to identify the differentially expressing traits under both the conditions. Such differentially expressing traits with positive influence on yield can serve as selection criteria for selection of stress tolerant plants. The experiment was carried out using randomized complete block design with three replications at department of plant breeding and genetics, college of agriculture, Kerala Agricultural University between December 2021 and March 2022. Sixteen characters pertaining to morphological, physiological and yield of three rice genotypes were recorded under reproductive stage water stress and non-stress conditions. The intensity of trait correlations varied significantly under drought and non-drought conditions. The results of correlation studies indicate positive and significant correlation of grain yield with number of tillers, 1000 grain weight, photosynthetic rate, inter cellular CO₂ content and water use efficiency under well-watered condition and of these, 1000 grain weight, and inter cellular CO₂ content had significant positive influence on grain yield under drought stress. Principal component analysis results indicated two principal components *i.e.*, PC1 and PC2 accounted 70.5% and 29.5% of the total variation in grain yield under drought and 89% and 11% respectively under non drought condition. Correlation and principal component analysis concluded that plants with higher grain weight along with improved high internal carbon dioxide content in leaves can be selected for attaining high yield potential under drought and non-drought conditions and will serve as climate adaptive crops under varying soil moisture levels.

Keywords: *Biplot; Correlation; Drought; Principal component analysis; Rice; Water stress; Yield.*

INTRODUCTION

Rice (*Oryza sativa* L.) is the major staple food that supplements the dietary requirements of more than half of world population. The crop is cultivated under rainfed, irrigated conditions in lowland and uplands and even at elevated altitudes. Being a crop which is cultivated in diverse agro-climatic regimes, maintaining stable yield have turned out to be a major hurdle due to shift in climatic pattern in the recent decades. In the world scenario where attaining food security for the growing population itself is a distant goal, crop yield loss due to water scarcity further exacerbate the situation [1].

Drought stress results in maximum yield loss compared to any other abiotic or biotic stress. Meta-data analysis on drought stress in rice revealed that mild, moderate, and severe water stress causes 17.0%, 27.8%, and 32.0% reduction in rice yield [2]. Sensitivity of rice crop towards water stress differs even with the stage of growth. Rice is most sensitive to water scarcity during its reproductive stage, even under moderate stress levels [3]. In some cases, yield loss due to reproductive stage stress have led to 65-85% reduction in rice grain yield [4]. A better insight into morpho-physiological dynamics and their relationships with grain yield attributes under water stress can contribute towards selection and development of varieties with better performance under drought stress [5].

Current scenario of climate change demands rice varieties with high yield potential in combination with drought tolerance so as to improve the productivity with available resources [6]. Correlations studies of data from contrasting rice growth conditions can help in deciphering such relationships between traits. Principal component analysis helps to partition this data based on contribution of the traits to the variance, providing a reliable basis for selecting characters contributing to grain yield under diverse soil water regimes.

The present study was taken up based on the above reports to identify the correlations and contribution levels of major morphological, physiological and grain characters of rice under well-watered and water limiting conditions which can be rewarding for efficient selection of high yielding genotypes in future crop improvement programmes.

MATERIAL AND METHODS

The present research experiment was carried out under the department of plant breeding and genetics, college of agriculture, Vellayani, Thiruvananthapuram, Kerala Agricultural University between December 2021 and March 2022. Three rice genotypes i.e., Kinandang patong, Manu Ratna and Jyothi with contrasting drought stress responses were considered for the study. Kinandang patong is a tropical japonica tolerant to water stress while Manu Ratna and Jyothi are short and medium duration high yielding indica cultivars whose water stress tolerance levels needs to be investigated. The statistical design used in the study was Randomized Complete Block Design (RCBD) with three replications. Breeder seeds of the three genotypes were collected from National Bureau of Plant Genetic Resources, New Delhi; Agricultural Research Station, Mannuthy and Rice Research Station, Moncompu respectively. Two sets of each genotypes were maintained for the study; one under water stressed and another under irrigated conditions. Water stress treatment was initiated for one of the above set of genotypes during flowering stage by withholding irrigation for a period of 10 days. After the stress treatment period, the genotypes were re-watered and maintained until harvest.

Morphological and grain yield parameters included in the study are plant height (cm), number of tillers, days to 50% flowering, panicle length (cm), 1000 grain weight (g), shoot fresh weight (g), shoot dry weight (g), specific leaf area ($\text{cm}^2 \text{g}^{-1}$), root volume (cm^3), root length (cm), root dry weight (g) and grain yield per plant (g). All morphological and grain yield parameters were recorded at crop maturity. Shoot and root samples were oven dried at 80°C for 3 days for recording shoot dry weight and root dry weight.

Physiological traits such as photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), transpiration rate ($\text{mmol m}^{-2} \text{ s}^{-1}$), stomatal conductance ($\text{mmol m}^{-2} \text{ s}^{-1}$), inter cellular CO_2 (mmol mol^{-1}) and water use efficiency were recorded on the final day of drought stress imposition. Here, photosynthetic traits were estimated using CIRAS-3 portable photosynthetic system (PP systems USA) between 10 AM and 12 PM.

Karl Pearson's coefficient of correlation and principal component analysis on morpho-physiological and yield traits of the genotypes under water stress and non-stress conditions were carried out using R software version 4.2.2 package.

RESULTS AND DISCUSSION

Grain yield is a complex character influenced by the dynamic changes in plant system with a complex genetic control mechanism. The modulations of source- sink of assimilates widely according to stage of plant growth and with environmental influences. Rice being a mandate crop with high production requirement around the globe, a fall in soil water levels during

reproductive phase creates remarkable variations in plant morphology, biomass accumulation, photosynthetic efficiency, root system dynamics and osmotic balance causing severe impact on rice grain yield. Correlation coefficients can be worked out to estimate the direction and degree of association between a pair of traits and selection of such rewarding traits help in simultaneous improvement of multiple correlated traits [7].

The results of correlation studies indicate positive and significant correlation of grain yield (GY) with number of tillers (NTL), 1000 grain weight (TGW), photosynthetic rate (PR), inter cellular CO₂ content (ICCC) and water use efficiency (WUE) under well-watered condition and of these, 1000 grain weight, and inter cellular CO₂ content had significant positive influence on grain yield under drought stress (Fig. 1). Similar reports of significant positive correlation of grain yield and grain weight under both water stressed and non-stressed conditions are reported in rice [8,9,10].

During water stress, days to 50% flowering (DFPF) showed significantly negative relation with grain yield. Reduction of crop duration by accelerating flowering is considered to be a drought escape mechanism where crop yield is sacrificed to a greater extent. Similar reports are available in correlation studies by Abarshahr et al. [11]. Other than days to 50% flowering, characters such as plant height (PH), panicle length (PL), shoot fresh weight (SFW), specific leaf area (SLA) and stomatal conductance (SC) had significant negative influence on grain yield under well-irrigated condition.

Considering grain yield under normal soil water levels, the relation of number of tillers per plant with respect plant height and panicle length towards yield was mutually exclusive while the relations were insignificant under water stress. A negative correlation between plant height and grain yield was also reported by Al-Salim [12]. Usually, traditional tall rice genotypes tend to have long panicles with reduced primary and secondary branching on the panicle which is the major reason for grain yield reduction. Negative correlations of panicle length and plant height towards grain yield had been reported in rice in studies by Pant et al. [10]. Genotypes with higher tillering is considered beneficial in terms of grain yield due to an increase in number of panicles per plant with moderate panicle length. Plants with short stature also has the added advantage of lodging tolerance and easy intercultural operations. Such contrasting relationships between plant height and number of tillers were reported by Kondhia et al. [13]. Positive contribution towards grain yield due to increased tillering is reported in water limited environment in various studies [14].

Shoot fresh weight showed significant relationship with grain yield only under well-watered condition. An increase in vegetative plant parts under reproductive stage results in reduced translocation of assimilates to the grain resulting in yield reduction. Under drought, no significant increase in shoot fresh weight was observed due to reduced osmotic levels in the plant system due imposition of drought stress.

All the observations on root and shoot biomass such as root length (RL), root volume (RV), root dry weight (RDW) and shoot dry weight (SDW) could not influence the grain yield of rice under both the water regimes except for shoot fresh weight (SFW), showing a negative relationship under well-watered treatments. Such insignificant effects of major root traits such as root length, root volume, root surface area and root diameter in water stressed and non-stressed conditions were reported aerobic rice cultivars [15]. The main reason for the above observation is that plants under both treatments completed the vegetative phase of growth under sufficient water levels and the stress was imparted only at flowering stage. Some genotypes that tend to increase drought tolerance with more developments in root

system. The present study indicates only a marginal influence on grain yield due to such adaptive root modifications under drought stress.

Photosynthetic rate had significant contribution towards grain yield under well irrigated condition while the relation with grain yield was insignificant and positive under drought stress. Observations on positive correlation of photosynthetic rate with grain yield was in line with the findings of Yang et al. [16]. In the aforementioned study stomatal conductance was reported to have positive influence on grain yield, but we could not observe such significant effect for stomatal conductance under both soil water regimes. Efficient photosynthesis requires water and CO₂, while in stressed conditions water becomes a limiting factor resulting in reduction in photosynthetic rate during drought. The condition is supported with the observations on insignificant positive relationship of water use efficiency and highly positive correlation of inter cellular CO₂ content towards grain yield under water stress. Under well irrigated condition both water use efficiency and inter cellular CO₂ content made significant contribution towards grain yield. Similar positive correlations use of water use efficiency as a criteria for selection of high yielding and combatant rice genotypes have been reported previously in rice [17]. Notable increase in intercellular CO₂ levels was observed both under water stress and non-stress conditions which have direct influence on photosynthetic efficiency and grain yield. An increased intercellular CO₂ concentration in the plant system also results in reduced stomatal opening, thereby acting as a drought adaptive mechanism which reduces transpiration rate (TR) and prevents photoinhibition of photosystem I [18,19]. Under drought stress, water use efficiency could not make a significant positive impact on grain yield compared to well irrigated control despite of high inter cellular CO₂ concentration. Inter cellular CO₂ concentration was observed to be positively correlated with water use efficiency and similar observation was reported by Liu et al. [20].

Transpiration rate and stomatal conductance were found to be highly correlated with each other, showing similar relationship trends with all the other traits considered in the study. Stomatal conductance had significant negative influence on grain yield under irrigated condition due to high rate of transpiration and water availability in the leaf tissue while the relation was insignificant under water scarce environment. Influence of short term dynamic changes in water levels in the soil can result in loss of synchrony between photosynthesis and stomatal conductance. Stomatal responses are slower compared to dynamics in photosynthetic rate, resulting in the aforementioned imbalance, resulting in limited photosynthetic carbon assimilation and poor plant water use efficiency under drought stress [21, 22].

Table 1. Estimation of correlation between morph physiological and yield characters under water stress (lower quadrant) and non-stress (upper quadrant) condition

	PH	NTL	DFPF	PL	TGW	SFW	SDY	SLA	RV	RL	RDW	PR	TR	SC	ICCC	WUE	GY
PH	1	-0.79*	0.825**	0.976***	-0.938***	0.884**	0.505	0.899***	0.526	0.607	0.821**	-0.794*	0.82**	0.95***	-0.994***	-0.929***	-0.936***
NTL	-0.212	1	-0.532	-0.774*	0.695*	-0.496	0.037	-0.681*	-0.125	-0.586	-0.479	0.751*	-0.477	-0.742*	0.792*	0.641	0.76*
DFPF	0.367	-0.629	1	0.866**	-0.937***	0.694*	0.401	0.582	0.233	0.097	0.613	-0.679*	0.724*	0.712*	-0.816**	-0.815**	-0.811**
PL	0.979***	-0.243	0.292	1	-0.915***	0.841**	0.45	0.84**	0.457	0.566	0.762*	-0.798*	0.774*	0.899***	-0.964***	-0.899***	-0.955***
TGW	-0.76*	0.5	-0.865**	-0.674*	1	-0.808**	-0.459	-0.764*	-0.381	-0.301	-0.755*	0.754*	-0.818**	-0.87**	0.939***	0.908***	0.866**
SFW	0.867**	0.233	-0.109	0.86**	-0.377	1	0.826**	0.932***	0.815**	0.62	0.972***	-0.616	0.905***	0.917***	-0.9***	-0.927***	-0.76*
SDY	0.857**	0.24	0.217	0.804**	-0.597	0.872**	1	0.633	0.829**	0.401	0.796*	-0.339	0.685*	0.555	-0.526	-0.669*	-0.415
SLA	0.944***	-0.01	0.122	0.924***	-0.581	0.945***	0.865**	1	0.788*	0.746*	0.943***	-0.593	0.895**	0.976***	-0.922***	-0.902***	-0.739*
RV	0.987***	-0.114	0.275	0.951***	-0.709*	0.91***	0.869**	0.97***	1	0.604	0.855**	-0.138	0.736*	0.681*	-0.552	-0.61	-0.344
RL	0.971***	-0.121	0.156	0.978***	-0.591	0.932***	0.824**	0.962***	0.969***	1	0.579	-0.491	0.399	0.639	-0.598	-0.499	-0.574
RDW	0.987***	-0.177	0.293	0.958***	-0.718*	0.889**	0.851**	0.974***	0.993***	0.969***	1	-0.507	0.942***	0.919***	-0.859**	-0.904***	-0.635
PR	0.63	-0.133	-0.24	0.727*	-0.096	0.703*	0.413	0.652	0.605	0.775*	0.623	1	-0.455	-0.632	0.785*	0.756*	0.897**
TR	0.974***	-0.384	0.458	0.962***	-0.79*	0.768*	0.774*	0.89**	0.936***	0.935***	0.957***	0.641	1	0.919***	-0.86**	-0.925***	-0.612
SC	0.971***	-0.113	0.149	0.961***	-0.603	0.937***	0.827**	0.977***	0.983***	0.992***	0.984***	0.732*	0.928***	1	-0.969***	-0.931***	-0.79*
ICCC	0.1	0.342	-0.788*	0.224	0.527	0.441	0.099	0.24	0.127	0.317	0.127	0.742*	0.043	0.286	1	0.954***	0.906***
WUE	-0.885**	0.436	-0.727*	-0.83**	0.941***	-0.573	-0.74*	-0.739*	-0.837**	-0.761*	-0.849**	-0.301	-0.919***	-0.762*	0.328	1	0.828**
GY	-0.269	0.357	-0.906***	-0.145	0.798**	0.108	-0.252	-0.101	-0.233	-0.04	-0.229	0.509	-0.295	-0.066	0.917***	0.632	1

*** Correlation is significant at 0.001 level (two tailed)

** Correlation is significant at 0.01 level (two tailed)

* Correlation is significant at 0.05 level (two tailed)

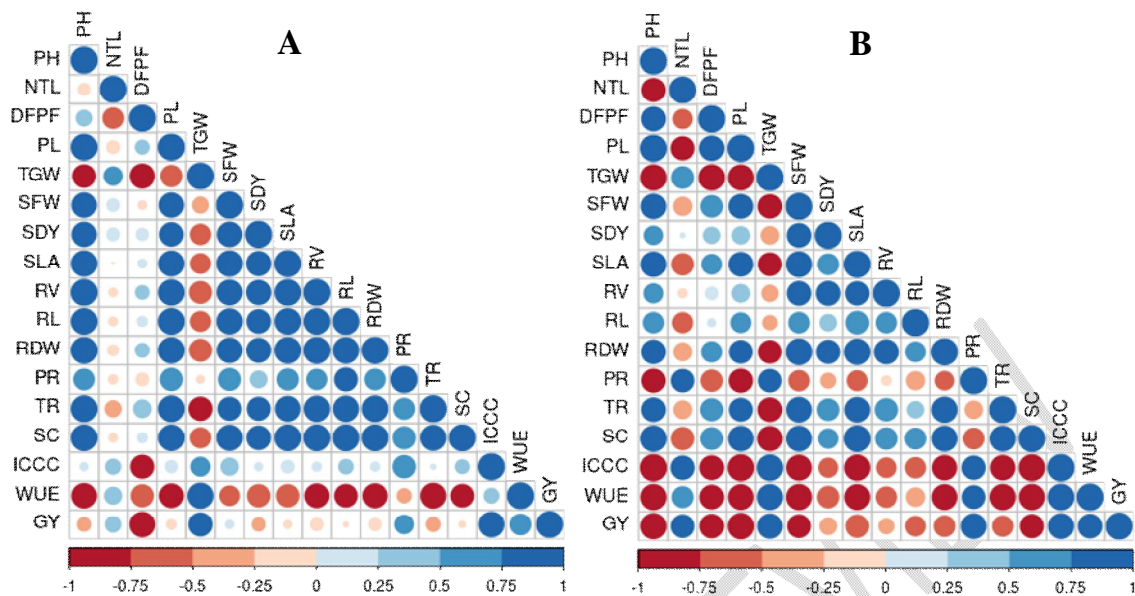


Fig.1 Intensity of correlation and correlation coefficients between grain yield per plant under water stress (A) and non-stress (B) condition.

The principal component analysis (PCA) on the morpho-physiological and yield parameters was used to divide the variance of projections into multiple principle component dimensions. Biplots developed through PCA analysis from the contrasting environments provides 2D graphical representation of associations between studied traits and the genotypes. The results of PCA indicated two principal components *i.e.*, PC1 and PC2 accounted 70.5% and 29.5% of the total variation in grain yield under drought and 89% and 11% respectively under non drought condition (Fig. 2,3). Under drought stress, characters such as number of tillers, days to 50% flowering, 1000 grain weight, photosynthetic rate, intercellular CO₂ concentration and grain yield contributed mainly to PC2 under drought while all the other traits were contributing to variation in PC1. Under non-drought condition, PC1 had the maximum share of variation and major contribution form most of the traits except days to 50% flowering, root length, root volume, 1000 grain weight and photosynthetic rate. Similar results were reported in rice by Bhattarai and Subudhi [23] and also by Tejaswini et al. [24]. Individual contributions of the traits towards each principal components under drought and non-drought condition are given in table 2. The relative position of genotypes in the biplot also shows that Manu Ratna can provide a higher yield than other genotypes under water stress and non-stress conditions. The close association of traits such as number of tillers, 1000 grain weight, water use efficiency, intercellular CO₂ concentration and photosynthetic rate with high yield of rice is evident from the biplot clusters.

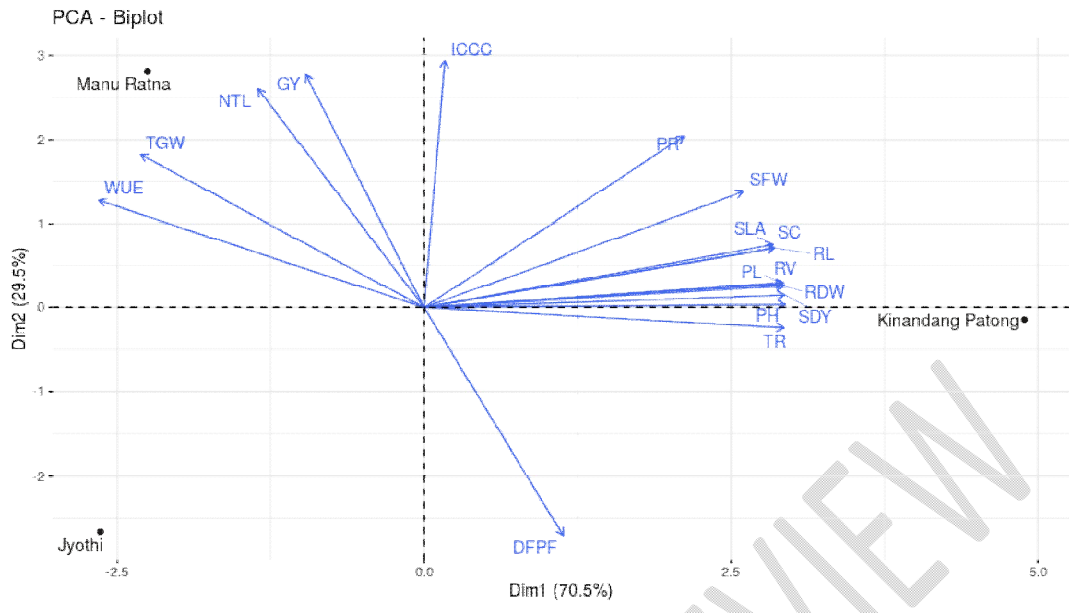


Fig. 2. Graphical representation of biplot from principal component analysis of water stress condition

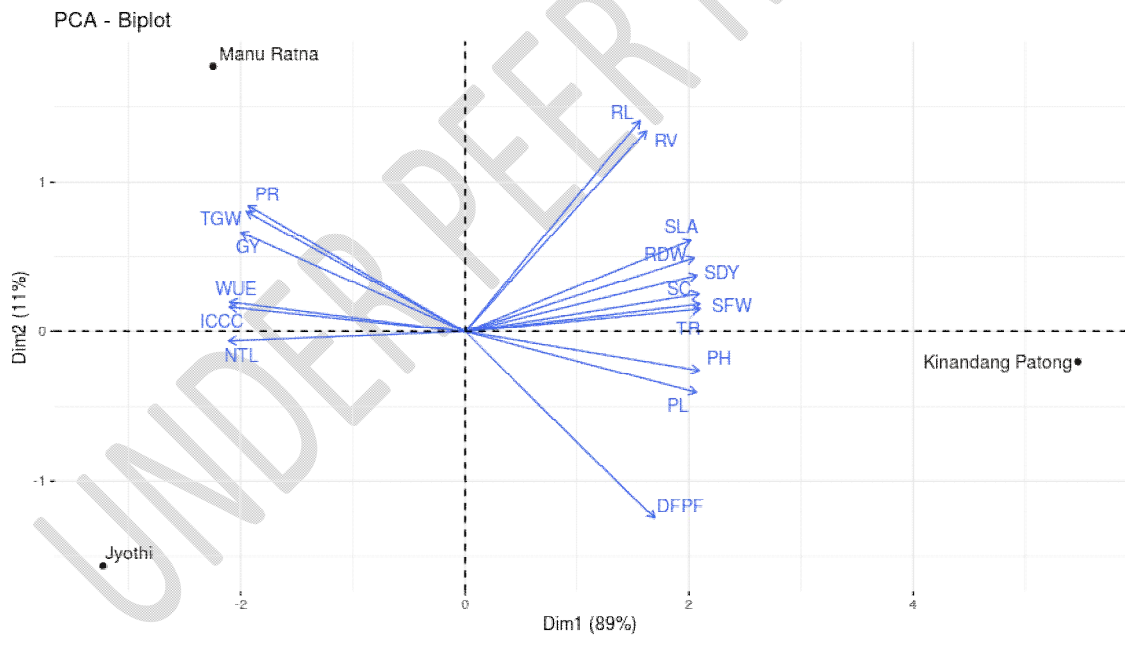


Fig. 3. Graphical representation of biplot from principal component analysis of non-stress condition

Table 2: Percentage contribution of variables on PCs

Variables	Water stress		Non-stress	
	PC1	PC2	PC1	PC2
PH	8.346	0.003	6.513	0.802
NTL	1.768	15.698	6.606	0.047
DFPF	1.242	16.954	4.287	18.742
PL	8.272	0.182	6.366	1.98
TGW	5.145	7.642	5.654	7.724
SFW	6.519	4.363	6.561	0.415
SDY	8.327	0.049	6.4	1.706
SLA	7.801	1.305	6.059	4.456
RV	8.261	0.206	3.924	21.671
RL	7.861	1.161	3.65	23.878
RDW	8.285	0.151	6.249	2.926
PR	4.325	9.598	5.557	8.504
TR	8.293	0.132	6.577	0.283
SC	7.859	1.166	6.517	0.764
ICCC	0.028	19.852	6.571	0.331
WUE	6.774	3.755	6.553	0.475
GY	0.895	17.782	5.955	5.297

CONCLUSION

The results from correlation and PCA analysis under diverse soil water levels suggest that the grain yield in rice is showing similar relationship trends under water stressed and non-stressed conditions, but high grain weight and intercellular CO₂ concentration are the two most significant and reliable parameters that can be used for selection of high yielding genotypes under conditions of falling soil water levels. Under conditions of sufficient water availability, genotypes with maximum tillering, higher grain weight, photosynthetic rate, intercellular CO₂ concentration and high water use efficiency can be selected for yield improvement. The present investigation that unravels the character contributions under diverse soil moisture regimes can support future research and development towards adaptability to climate change for attaining food security for the upsurging world population.

REFERENCES

1. Lesk C, Rowhani P, Ramankutty N. Influence of extreme weather disasters on global crop production. *Nature*. 2016 Jan 7;529(7584):84-7.

2. Zhang J, Zhang S, Cheng M, Jiang H, Zhang X, Peng C, Lu X, Zhang M, Jin J. Effect of drought on agronomic traits of rice and wheat: A meta-analysis. *International journal of environmental research and public health*. 2018 May;15(5):839.
3. Hsiao TC. Soil-plant-atmosphere continuum in relation to drought and crop production. Drought resistance in crops with emphasis on rice. 1982.
4. Kumar A, Bernier J, Verulkar S, Lafitte HR, Atlin GN. Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. *Field Crops Research*. 2008 June;107(3):221-31.
5. Kumar A, Bernier J, Verulkar S, Lafitte HR, Atlin GN. Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. *Field Crops Research*. 2008 June;107(3):221-31.
6. Tripathi BP, Bhandari HN, and Ladha JK. Rice Strategy for Nepal. *Acta Scientific Agriculture*. 2018, 3 (2): 171-180.
7. Panigrahi AK, Bharathi M, Kumaravadeivel N. Genetic variability and character association studies in advanced backcross generation of rice (*Oryza sativa* L.). *Journal of pharmacognosy and phytochemistry*. 2018;7(1):2397-400.
8. Ghiasy M, Farahbakhsh H, Sabouri H, Mohammadinejad G. Effect of drought stress on yield and yield components in rice landraces and improved cultivars under Gonbad Kavous environmental condition. *Cereal Research*. 2012 Dec 27;2(3):165-79
9. Haider Z, Khan AS, Zia S. Correlation and path coefficient analysis of yield components in rice (*Oryza sativa* L.) under simulated drought stress condition. *American-Eurasian Journal of Agricultural & Environmental Sciences*. 2012;12(1):100-4.
10. Pant D, Acharya SS, Poudel A. Correlation coefficient and path analysis of rice (*Oryza sativa*) genotypes under reproductive drought stress in mid hill of Nepal. *Crop Research*. 2019;54(5and6):152-7.
11. Abarshahr M, Rabiei B, Lahigi HS. Genetic variability, correlation and path analysis in rice under optimum and stress irrigation regimes. *Notulae Scientia Biologicae*. 2011 Nov 17;3(4):134-42.
12. Al-Salim SH. Evaluation of the performance of some rice (*Oryza sativa* L.) varieties in two different environments. *Open Access Library Journal*. 2016;3(01):1.
13. Kondhia A, Tabien RE, Ibrahim A. Evaluation and selection of high biomass rice (*Oryza sativa* L.) for drought tolerance. *American Journal of Plant Sciences*. 2015;6(12):1962.
14. Manjappa GU, Hittalmani S. Association analysis of drought and yield related traits in F2 population of Moroberekan/IR64 rice cross under aerobic condition. *International Journal of Agricultural Science and Research*. 2014 Apr;4(2):79-88.
15. Terra TG, Leal TD, Rangel PH, Borém A. Phenotypic correlation and path analysis in cultivars and strains of upland rice for drought tolerance.
16. Yang X, Wang B, Chen L, Li P, Cao C. The different influences of drought stress at the flowering stage on rice physiological traits, grain yield, and quality. *Scientific reports*. 2019 Mar 6;9(1):3742.
17. Hussain T, Hussain N, Tahir M, Raina A, Ikram S, Maqbool S, Fraz Ali M, Duangpan S. Impacts of Drought Stress on Water Use Efficiency and Grain Productivity of Rice and Utilization of Genotypic Variability to Combat Climate Change. *Agronomy*. 2022 Oct 15;12(10):2518.
18. Thompson M, Gamage D, Hirotsu N, Martin A, Seneweera S. Effects of elevated carbon dioxide on photosynthesis and carbon partitioning: a perspective on root sugar sensing and hormonal crosstalk. *Frontiers in Physiology*. 2017:578.

19. Wada S, Miyake C, Makino A, Suzuki Y. Photorespiration coupled with CO₂ assimilation protects photosystem I from photoinhibition under moderate poly (ethylene glycol)-induced osmotic stress in rice. *Frontiers in Plant Science*. 2020 Jul 24;11:1121.
20. Liu X, Zhang H, Wang J, Wu X, Ma S, Xu Z, Zhou T, Xu N, Tang X, An B. Increased CO₂ concentrations increasing water use efficiency and improvement PSII function of mulberry seedling leaves under drought stress. *Journal of Plant Interactions*. 2019 Jan 1;14(1):213-23.
21. Lawson T, Simkin AJ, Kelly G, Granot D. Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour. *New Phytologist*. 2014 Sep;203(4):1064-81.
22. McAusland L, Vialet-Chabrand S, Davey P, Baker NR, Brendel O, Lawson T. Effects of kinetics of light-induced stomatal responses on photosynthesis and water use efficiency. *New Phytologist*. 2016 Sep;211(4):1209-20.
23. Bhattarai U, Subudhi PK. Genetic analysis of yield and agronomic traits under reproductive-stage drought stress in rice using a high-resolution linkage map. *Gene*. 2018 Aug 30;669:69-76.
24. Tejaswini KL, Manukonda S, Kumar BR, Rao PR, Raju SK. Application of principal component analysis for rice F5 families characterization and evaluation. *Emergent Life Sciences Research*. 2018 Jun;4:72-84.

UNDER PEER REVIEW