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Studies on association of physiological parameters contributing to yield in rice (*Oryza sativa* L.) under drought stress

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Abstract
The study on the association between eight physiological parameters with yield was conducted during summer 2019-20 at College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga in the field experiment plots of the department of Genetics and Plant Breeding under moisture stress condition. The experimental material consisted of 40 rice genotypes, including four checks Anagha, Jyothi, JGL-1798 and BPT 5204. It was observed that genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients indicating that the environmental influence reduces the relationship between yield and physiological parameters contributing towards yield under moisture stress condition in rice. Correlation coefficient analysis revealed a significant positive association of grain yield per plant with chlorophyll content, root length, root to shoot ratio, root volume, relative water content and proline content. A significant negative correlation was observed between leaf rolling and yield per plant. Path coefficient analysis revealed the highest positive direct effect of relative water content on yield per plant, while leaf rolling had the highest negative direct effect on yield. Correlation and path analysis indicated traits *viz.*, root length, root to shoot ratio, root volume, relative water content and proline content could be used as selection index for yield as they exhibited significant positive correlation and direct positive effect with yield per plant.

Keywords: rice, correlation, path coefficient analysis

Introduction

Rice is regarded as a key of life for Asian people. Rice is the major source of energy and also contains a good amount of carbohydrates, protein, phosphorous, magnesium, calcium, and folic acid. Rice protein contains balanced amino acid and biologically the richest by virtue of its high true digestibility (88%) among cereal proteins and also provides minerals and fibre. Calories from rice are particularly important for the poor, accounting for 50 to 80 per cent of the daily caloric intake (Gopalan *et al.*, 2007) [5]. The presence of essential amino acids and overall protein quality is comparatively higher in rice (0.55) than in the other important cereals such as wheat (0.40) (Bagchi *et al.*, 2015) [1].

Rice being the second most important crop of the world, occupies an area of about 162.06 million hectares and feeds more than one-third of the people worldwide. India, the second largest rice producer after china, occupies an area of 43 million hectares (22% crop land) with a production of 169.14 million tonnes. In Karnataka, rice occupies an area of 0.99 million hectares with a production of 4.53 million tonnes.

Global climate change influences the frequency and amount of precipitation, leading to fluctuations in the water table resulting in phenomena like drought and flood. Both high and low extremes in precipitation increasingly limit food production worldwide. Approximately 30% of the world's rice (*Oryza sativa*) farmlands are at a low elevation and are irrigated by rain (Bailey-Serres *et al.*, 2010) [2]. Such rain-fed farming is known to reduce groundwater depletion, water pollution, and soil salinization, which are most often associated with controlled irrigation systems. However, inadequate water supply under rainfed conditions leads to drought, which is more problematic in rice because it is considered as one of the most drought-susceptible plants due to its small root system, thin cuticular wax, and swift stomatal closure (Wang *et al.* 2012) [11].

Drought resistance may be defined as the mechanisms causing minimum loss of yield in a drought environment relative to the maximum yield in constraint-free or optimal for the crop. Drought tolerance mechanisms include cellular adjustments, physiological acclimation and morphological adaptations, which are controlled by genetic factors at different stages.

Cellular adjustments for drought tolerance involve the accumulation of organic osmolytes, most commonly proline, and increased chlorophyll content. Physiological acclimation comprises higher stomatal density and conductance, decreased transpiration rates, root thickness and length, waxy or/and thick leaf coverings, decreased leaf weight and size, smaller epithelial cells, delayed leaf senescence and increased green leaf area. (Oladasu *et al*., 2019).

Hence, it is necessary to study the degree of association between these physiological attributes contributing towards yield under drought condition. Correlation and path analysis thus helps to identify suitable selection criteria and pave the way for improving yield through indirect selection. In this context present study was conducted to determine correlation and path analysis of physiological parameters contributing to yield in rice (*Oryza sativa* L.) under drought stress.

Materials and Methods

The present study was conducted during summer 2019 and summer 2020 at College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, in the field experiment plots of the Genetics and Plant Breeding department. The experimental material consisted 40 rice genotypes including four checks Anagha, Jyothi, JGL-1798 and BPT 5204.

Experimental layout

The experiment was laid out in randomized completely block design (RCBD). PVC pipes of 100 cm length and 20 cm diameter were used. The pipes were filled with a mixture of red soil and FYM. Seeds were directly sown in the pipes. After 30 days, irrigation was withheld to create water stress.

Recording of observations

At the time of flowering, the pipes were carefully removed and immersed in water for few hours to loosen the soil, then washed with a jet of water. Then the plants were carefully removed and washed with water without damaging the roots; then plants were kept in a bucket containing water for recording observations.

Chlorophyll content

Chlorophyll content was measured using SPAD chlorophyll meter in random five leaves of each genotype at the flowering stage and readings were recorded.

Maximum root length (cm)

Root length was measured from the collar region to the tip of the longest root and recorded in centimetres.

Shoot length (cm)

Shoot length was measured from the base of the plant to the tip of the boot leaf and recorded in centimetres.

Root volume (cm)

Root volume was measured in ml by the water displacement method.

Root to shoot ratio

Root to shoot ratio was calculated by dividing root length by shoot length.

Relative water content (RWC)

The relative water content (RWC) was determined by the method described by Weatherley (1950) [12]. Relative water content was measured after thirty days of withholding irrigation. Leaf discs were cut from the collected leaf samples and fresh weight was recorded; then, leaf samples were immersed in distilled water in Petri dishes overnight. Next day turgid weight was recorded and the discs were surface dried, then dry weight was taken after keeping in an oven at 80 ºC for 20 hours dry. RWC was calculated by the following formula.

RWC (%) = (Fresh weight – dry weight)/(turgid weight – dry weight) x100

Proline content

Proline content was estimated as per Bates *et al.*, (1973) [3]. 0.5 g of leaf sample was collected and homogenized with 10ml 3 per cent aqueous Sulpho-salicylic acid in pestle and mortar, then filtered. Filtrate volume was made up to 25ml, then 2ml was pipetted out into test tubes. 2ml of the filtrate was reacted with 2ml acid ninhydrin and 2ml of glacial acetic acid for one hour at 100 ºC, and the reaction terminated in an ice bath. The reaction mixture was extracted with 4ml toluene, mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520nm in a spectrophotometer. The proline concentration was determined from a standard curve and calculated on a fresh weight basis.

$$\text{Proline } (\mu \text{ mol/g}) = \frac{\mu\text{g proline/ml x ml toluene)/ 115.5}\mu\text{g/mole}}{\text{g sample}}$$

Leaf rolling scores

The plants were scored for leaf rolling, 30 days after induction of water stress using 0-9 standard evaluation systems of rice which is given below. Leaf rolling score description (Gana. 2011) [4].

Scale	Description
0	Leaves healthy.
1	Leaves starts to fold
3	Leaves folding (V- shaped)
5	Leaves fully cupped (U- shaped)
7	Leaves margins touching (O-shaped)
9	Leaves tightly rolled

Result and Discussion

Phenotypic and genotypic correlation coefficients between yield per plant and eight physiological parameters are presented in Table 1 and 2, respectively. It was observed that genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients indicating that the environmental influence reduces the relationship between yield and physiological parameters contributing towards yield under moisture stress condition in rice. A similar result was obtained by Ratna *et al.,* (2015) [9]. Leaf chlorophyll content exhibited a significant positive association with root to shoot ratio, relative water content, proline content and grain yield per plant. There was a significant positive correlation between root length, root to shoot ratio, root volume, relative water content and proline content. Root volume and relative water content exhibited a significant negative correlation with leaf rolling. The result obtained is in accordance with Manickavelu *et al.*, (2006) [6], who also reported significant and positive correlation of yield with relative water content, root length and root to shoot ratio and a significant negative correlation of leaf rolling with yield. Leaf chlorophyll content, root length, root to shoot ratio, root volume, relative water content, proline content exhibited a significant positive correlation with yield per plant, thus indicating that indirect selection for these physiological parameters under drought condition could enhance yield. A significant negative correlation was observed between leaf rolling and grain yield per plant, indicating negative selection for this trait would increase yield under drought condition. Positive association of chlorophyll content, root length, root: shoot, root volume, relative water content, proline content with yield was reported by Navya *et al.*, (2019) [7].

Path coefficient analysis

Path coefficient analysis between yield per plant and eight physiological parameters are presented in table 3. Path coefficient analysis revealed the direct positive effect of five traits out of eight parameters on yield under moisture stress condition. The characters which exhibited positive direct effect with yield were root length (0.0919), root to shoot ratio (0.0539), root volume (0.0431), relative water content (0.7312), proline content (0.1187), while chlorophyll content (-0.1454), shoot length (-0.0381) and leaf rolling (-0.0952) exhibited negative direct effect with yield. Root length and root volume showed an indirect negative effect through shoot length. Path coefficient analysis revealed highest positive direct effect of relative water content (0.7312) on yield per plant but leaf rolling (-0.0952) had the highest negative direct effect on yield. The result obtained is in accordance with Navya *et al.*, 2019 [7], Uday *et al.*, 2013 [10].

Table 1: Estimation of phenotypic correlation between yield and physiological characters under drought condition

Traits	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
X_1	1	0.2953*	-0.247	0.4214**	0.2534*	0.5011**	0.3785*	-0.3009**	0.349*
X_2		1	0.0653	0.8397*	0.9302**	0.7516**	0.7929*	-0.5885**	0.811**
X_3			1	-0.4372	0.0843	-0.0098	-0.0341*	-0.1271	-0.004
X_4				1	0.7891*	0.7145*	0.7465*	-0.5217**	0.7546**
X_5					1	0.7135*	0.7739*	-0.6601**	0.7891**
X_6						1	0.8763*	-0.6807**	0.9274**
X_7							1	-0.6347**	0.8951**
X_8								1	-0.7121*
X_9									1

** Significance at 1% * Significance at 5%

X_1- Chlorophyll content (SPAD reading)	X_4 - Root to shoot ratio	X_7- Proline content (µmole/g FW)
X_2- Root length (cm)	X_5- Root volume (ml)	X_8- Leaf rolling
X_3- Shoot length (cm)	X_6- Relative water content (%)	X_9- Yield per plant (g)

Table 2: Estimation of genotypic correlation between yield and physiological characters under drought condition

Traits	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
X_1	1	0.3353	-0.254*	0.4636**	0.2735*	0.545**	0.4345**	-0.3334**	0.4138**
X_2		1	0.0693	0.8432**	0.9473**	0.7675**	0.8053**	-0.5931**	0.8401**
X_3			1	-0.4282**	0.0881	-0.0026	-0.0367	-0.1311	-0.0078
X_4				1	0.8095**	0.7322**	0.7646**	-0.5296**	0.7918**
X_5					1	0.7321**	0.7838**	-0.6668**	0.8226**
X_6						1	0.895**	-0.6967**	0.9663**
X_7							1	-0.6425**	0.9216**
X_8								1	-0.7393**
X_9									1

** Significance at 1% * Significance at 5%

X_1- Chlorophyll content (SPAD reading)	X_4 - Root to shoot ratio	X_7- Proline content (µmole/g FW)
X_2- Root length (cm)	X_5- Root volume (ml)	X_8- Leaf rolling
X_3- Shoot length (cm)	X_6- Relative water content (%)	X_9- Yield per plant (g)

Table 3: Estimation of direct and indirect effects of physiological parameters on yield under drought condition

Traits	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
X_1	-0.1454	-0.0487	0.0369	-0.0674	-0.0398	-0.0792	-0.0632	0.0485
X_2	0.0308	0.0919	0.0064	0.0775	0.0871	0.0706	0.074	-0.0545
X_3	0.0097	-0.0026	-0.0381	0.0163	-0.0034	0.0001	0.0014	0.005
X_4	0.025	0.0455	-0.0231	0.0539	0.0437	0.0395	0.0412	-0.0286
X_5	0.0118	0.0408	0.0038	0.0349	0.0431	0.0316	0.0338	-0.0288
X_6	0.3985	0.5612	-0.0019	0.5354	0.5353	0.7312	0.6544	-0.5094
X_7	0.0516	0.0956	-0.0044	0.0908	0.0931	0.1063	0.1187	-0.0763
X_8	0.0317	0.0564	0.0125	0.0504	0.0635	0.0663	0.0612	-0.0952
r value	0.4138	0.8401	-0.0078	0.7918	0.8226	0.9663	0.9216	-0.7393

Residual effect= 0.1344

Where

X_1- Chlorophyll content (SPAD reading)	X_4 - Root to shoot ratio	X_7- Proline content (µmole/g FW)
X_2- Root length (cm)	X_5- Root volume (ml)	X_8- Leaf rolling
X_3- Shoot length (cm)	X_6- Relative water content (%)	X_9- Yield per plant (g)

Conclusion

Yield is a complex trait that is highly influenced by environmental fluctuations. Adequate information about the magnitude and type of yield association with its attributing traits is of great importance in the selection programme. This investigation revealed the association of yield with leaf chlorophyll content, root length, root: shoot, root volume, relative water content, proline content under moisture stress condition. Hence these traits could be used for indirect selection for yield effectively under drought condition.

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