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ORIGINAL RESEARCH ARTICLE



## Evaluation of seedling growth of rice (*Oryza sativa* L.) genotypes under water stress and non-stress conditions

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### ABSTRACT

Rice (*Oryza sativa* L.) is a species of crops which is highly sensitive to drought. The sensitivity to water stress varies from genotypes to genotypes of rice. An experiment was performed to compare twenty-five different rice genotypes for drought tolerance in the seedling stage at Gokuleshwor Agriculture and Animal Science College, Baitadi Nepal from July to August 2018. The genotypes were tested under two conditions viz., water stress and non-water stress. The research work was carried out in a completely randomized design (CRD) with three replications. The results showed the height of seedling, length and breadth of leaf, number of leaves/plant decreased, and number of leaves shedding increased under water stress condition. Clustering was done by the method of average linkage, and genotypes were categorized into five clusters. The genotype, namely Radha 11 grouped in Cluster-V had higher plant height, bigger length and breadth of leaf, maximum number of leaves/plant and minimum number of leaf shedding. Thus, this genotype showed the best performance for morphological traits under water stress condition. Therefore, this genotype Radha 11 can be utilized further for developing rice variety with drought-tolerance.

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### INTRODUCTION

In the world, rice (*Oryza sativa* L.) is considered as the foremost cereals and ranks the second position after wheat. Rice is the principal nutritional source of energy for approximately 9 American countries, 17 Asia and the Pacific countries and 8 African countries in a global scenario. It offers 20 % of dietary supply of energy in the world (Adhikari, 2015). Rice is a chief energy source for over 60% of the world population, whereby about 90% of total cultivated rice is consumed in Asian countries (Kumar *et al.*, 2015). In Nepal, rice cultivation covered 1.47 million hectares (ha) during 2017-18, with the production of about 5.15 million tons (t) and an average yield of 3.5 t/ha (MoALC, 2018).

Tolerance of drought is considered as a complex feature in rice

and is considered by several component traits. Rice plant is sensitive to water stress, and when it prevails, it exhibits several undesired morphological changes at various growth stages (Henry, 2016). The growth of plants in nature is often visible to several abiotic factors like drought, decrease in temperature, floods, CO<sub>2</sub> concentration, oxidative stress, salt, heat, higher irradiation and heavy metal toxicity. Drought is a metrological term that refers to a duration lacking considerable rainfall. Usually, the condition of water stress occurs with the reduction of soil water content and gradual decrement of water due to the natural atmospheric process of transpiration or evaporation (Abdul Jaleel, 2009). When the supply of water doesn't meet the crop water requirement, this will undoubtedly affect the growth of crops and eventually, its yield (Venkatesan, 2005). The genotypes that respond better to drought during early developmental

(e.g., seedling) stages might produce greater yield (Dien, 2017). There might be a moderate reduction of yield due to drought during the vegetative stage (O'Toole, 1982; Zhang, 2009). Polyethylene glycol (PEG) can increase the stress of drought, which helps to decrease the moistness in tissues (El-Tayeb and Hassanein, 2000). This method is adopted as an effective way to select in the growth of rice at the initial stages (Jing and Chang, 2003). The recognition of varieties with greater drought tolerance in rice (Pirdashti et al., 2003) can be screened by using polyethylene glycol-6000 in aqueous solutions (Costa et al., 2004; Fanti and Perez, 2004). The development of different varieties of rice with the drought-tolerant feature is necessary to achieve the upcoming demand for food for the further increment of the population in the country. The principal purpose of applying cluster method in the trials of breeding of plant is for categorization of the several species into different standardized groups such that the species of a certain group comprises similar patterns of responses across the locations (Shrestha, 2016). This study focused on the water stress effects on growth stages of seedling of different genotypes of rice.

## MATERIALS AND METHODS

### Site of Experiment

The experimental work was carried out at the research laboratory of Gokuleshwor Agriculture and Animal Science College (GAASC) which is located at 29.6606°N, 80.5461°E, located in Gokuleshwor, Baitadi of province no. 7, Nepal.

### Agrometeorological features

The experimental site had a maximum temperature of 28°C, and the minimum temperature recorded was 24.6°C. The average relative humidity was 78% in the first week, and in the following weeks, relative humidity was increasing gradually. The maximum relative humidity of 91% was gained during the end of our research period. Temperature and relative humidity were noted daily using a hygrometer.

### Experimental setup

A laboratory experiment was conducted at Gokuleshwor Agriculture and Animal Science College, Baitadi Nepal from July to August 2018. The experiments were conducted in plastic pots. The twenty-five rice genotypes were tested under two conditions viz., water stress and non-water stress (normal condition) in completely randomized design (CRD) with three replications. The seeds of all the selected rice genotypes were presoaked in distilled water for 12 hrs. The surface water of presoaked seeds was eliminated from dry air. Then the dried seeds were kept on a blotting paper in the Petri plates and were permitted to germinate. The germinated seeds were raised in plastic pots adopting usual cultural practices with irrigating seedlings at weekly intervals under non-water stress condition. The condition of water deficit stress was artificially created by using polyethylene glycol 6000 (PEG6000; Sigma Chemicals) at the rate of 20 g per 100 ml distilled water. 5-6 drops of different

solutions of PEG-6000 were provided to the seeds placed in the Petri plates in every 12 hours of regular interval. After germination, seedlings were planted in plastic pot. After seedlings establishment in pots, irrigation was stopped for the experiment of water stress condition. The size of a plastic pot used in both experiments (water stress and non-stress conditions) had 16.5 cm of bottom diameter, 10 cm of top diameter and 13 cm of the height.

### Data collection

Data on plant height (10, 20 and 30 DAS), leaf length (10, 20 and 30 DAS), leaf breadth (10, 20 and 30 DAS), and number of leaves (10, 20 and 30 DAS) and leaves shedding number/plant (20 and 30 DAS) of rice were recorded under water stress and non-stress conditions.

### Statistical analysis

The statistical data collected during the research period were tabulated. Data entry and preparation of figures and graphs were done using Microsoft Excel 2016. These data were processed into R version 3.4.2 and R studio for analysis. The evaluation of genotypic effects was performed by carrying out analysis of variance (ANOVA) on the collected data, and estimation of mean comparisons among means of treatment was done by least significant difference test at 5% level of significance (Gomez and Gomez, 1984; Shrestha, 2019). The Euclidean average linkage method of clustering was done by Minitab ver.17.

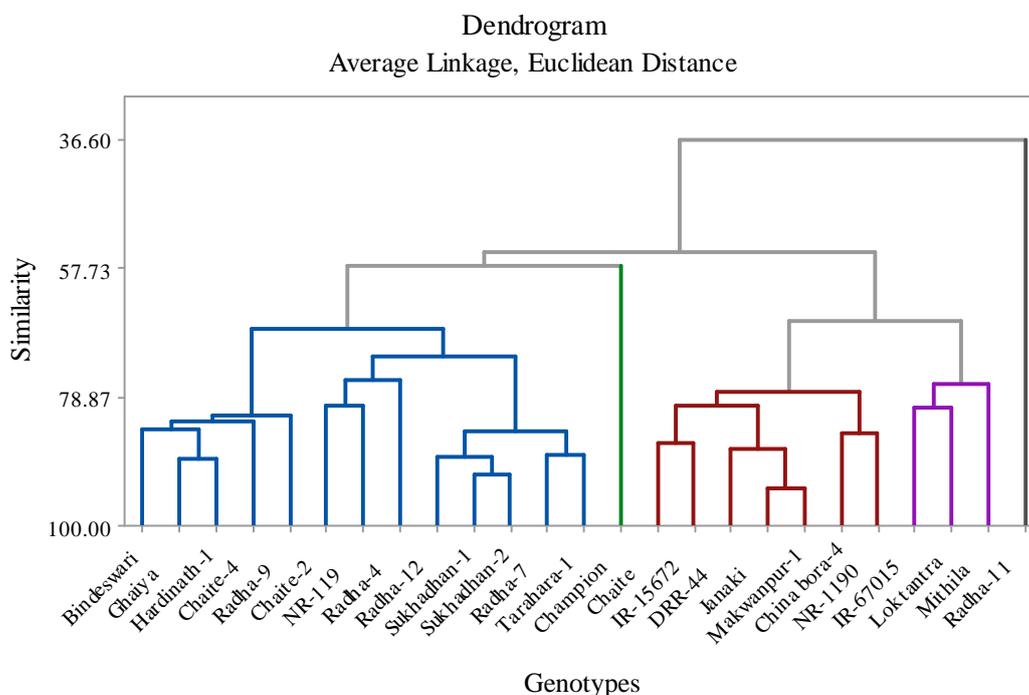
## RESULTS AND DISCUSSION

The results from analysis of variance (ANOVA) showed a significant difference for height of plant, leaf length, leaf number/plant and leaves shedding number/plant under water stress condition (Table 1). As per the results, the plant height, the length, and breadth of the leaf, number of leaves/plant were decreased under water stress condition (Table 1). The number of leaves shedding was higher under water stress condition. The growth and development of plant crucially depending on the drought stress directly affects the elongation, expansion, and enlargement of plants (Anjum et al., 2003; Kusaka et al., 2005; Shao et al., 2008). Islam et al. (2018) observed that the height of seedlings reduced under water stress conditions. The forbiddance of rise in radicle was visualized due to decreasing potential gradient of water between the seed and exterior surrounding. Therefore, it prevents the height of the seedling (Sokoto & Muhammad, 2014). The leaf area with the size of cell and intercellular volume was found to be reduced due to water stress (Kramer, 1969). The leaf area was found to be reduced at early on the senescence setting or by speeding up its rate due to water stress (Knapp et al., 1999). Smaller sized leaves were observed in stressed plants in comparison to that of controlled condition (Schurr et al., 2000). Also, the size and total area of the leaves in these species was observed to be decreased significantly due to water stress intensity (Cheng et al., 2003).

**Table 1.** Morphological traits (at 30 days after sowing) of rice genotypes under water stress and non-stress conditions at Gokulesh-wor, Baitadi, Nepal, 2018.

Genotypes	Plant height (cm)		Leaf length (cm)		Leaf breadth (cm)		No. of Leaves/plant		No. of leaves shedding/plant	
	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress
Bindeswari	16.59 <sup>b-e</sup>	18.23 <sup>b-e</sup>	7.93 <sup>a-h</sup>	7.23 <sup>a</sup>	0.21 <sup>a</sup>	0.19 <sup>ab</sup>	3.64 <sup>b-f</sup>	3.41 <sup>a</sup>	3.34 <sup>ab</sup>	2.63 <sup>a</sup>
Chaite	14.93 <sup>d-f</sup>	17.71 <sup>b-g</sup>	7.25 <sup>d-h</sup>	8.05 <sup>a</sup>	0.15 <sup>d</sup>	0.16 <sup>b</sup>	3.08 <sup>ef</sup>	3.63 <sup>a</sup>	2.07 <sup>ab</sup>	2.67 <sup>a</sup>
Chaite-2	16.89 <sup>b-e</sup>	16.03 <sup>e-g</sup>	9.68 <sup>ab</sup>	10.47 <sup>a</sup>	0.16 <sup>a-d</sup>	0.18 <sup>ab</sup>	2.80 <sup>f</sup>	3.58 <sup>a</sup>	1.93 <sup>ab</sup>	2.33 <sup>a</sup>
Chaite-4	17.38 <sup>b-e</sup>	21.05 <sup>abc</sup>	7.68 <sup>c-h</sup>	8.91 <sup>a</sup>	0.15 <sup>bcd</sup>	0.16 <sup>b</sup>	3.87 <sup>b-e</sup>	3.57 <sup>a</sup>	2.67 <sup>ab</sup>	1.61 <sup>a</sup>
Champion	20.61 <sup>a</sup>	17.58 <sup>b-g</sup>	7.83 <sup>b-h</sup>	7.82 <sup>a</sup>	0.16 <sup>a-d</sup>	0.18 <sup>ab</sup>	3.23 <sup>def</sup>	3.63 <sup>a</sup>	3.03 <sup>ab</sup>	1.78 <sup>a</sup>
China bora-4	13.24 <sup>f</sup>	15.34 <sup>g</sup>	6.64 <sup>fgh</sup>	6.77 <sup>a</sup>	0.14 <sup>d</sup>	0.18 <sup>ab</sup>	3.15 <sup>ef</sup>	3.67 <sup>a</sup>	2.47 <sup>ab</sup>	1.60 <sup>a</sup>
DRR-44	15.27 <sup>c-f</sup>	21.14 <sup>ab</sup>	6.13 <sup>gh</sup>	10.65 <sup>a</sup>	0.16 <sup>a-d</sup>	0.16 <sup>b</sup>	3.64 <sup>b-f</sup>	3.58 <sup>a</sup>	3.04 <sup>ab</sup>	2.22 <sup>a</sup>
Ghaiya	16.54 <sup>b-e</sup>	16.2 <sup>d-g</sup>	6.74 <sup>fgh</sup>	7.35 <sup>a</sup>	0.17 <sup>a-d</sup>	0.17 <sup>b</sup>	3.60 <sup>b-f</sup>	3.45 <sup>a</sup>	2.60 <sup>ab</sup>	1.79 <sup>a</sup>
Hardinath-1	16.07 <sup>c-f</sup>	15.49 <sup>g</sup>	6.71 <sup>fgh</sup>	6.72 <sup>a</sup>	0.16 <sup>a-d</sup>	0.20 <sup>ab</sup>	3.32 <sup>def</sup>	3.59 <sup>a</sup>	2.53 <sup>ab</sup>	1.93 <sup>a</sup>
Ir-15672	14.73 <sup>ef</sup>	15.95 <sup>fg</sup>	6.89 <sup>e-h</sup>	9.10 <sup>a</sup>	0.18 <sup>a-d</sup>	0.17 <sup>b</sup>	3.00 <sup>ef</sup>	3.69 <sup>a</sup>	2.53 <sup>ab</sup>	1.55 <sup>a</sup>
IR-67015	15.61 <sup>c-f</sup>	18.66 <sup>a-g</sup>	8.29 <sup>a-g</sup>	6.21 <sup>a</sup>	0.17 <sup>a-d</sup>	0.17 <sup>b</sup>	3.60 <sup>b-f</sup>	3.50 <sup>a</sup>	3.47 <sup>a</sup>	2.53 <sup>a</sup>
Janaki	14.66 <sup>ef</sup>	19.36 <sup>a-f</sup>	6.36 <sup>gh</sup>	9.81 <sup>a</sup>	0.15 <sup>cd</sup>	0.16 <sup>b</sup>	3.55 <sup>b-f</sup>	3.58 <sup>a</sup>	2.82 <sup>ab</sup>	1.96 <sup>a</sup>
Loktantra	15.95 <sup>c-f</sup>	20.03 <sup>a-e</sup>	8.76 <sup>a-f</sup>	9.14 <sup>a</sup>	0.20 <sup>ab</sup>	0.19 <sup>ab</sup>	3.40 <sup>c-f</sup>	3.73 <sup>a</sup>	3.00 <sup>ab</sup>	1.83 <sup>a</sup>
Makwanpur-1	14.51 <sup>ef</sup>	22.36 <sup>a</sup>	5.90 <sup>h</sup>	9.77 <sup>a</sup>	0.18 <sup>a-d</sup>	0.18 <sup>ab</sup>	3.55 <sup>b-f</sup>	3.70 <sup>a</sup>	2.68 <sup>ab</sup>	1.93 <sup>a</sup>
Mithila	15.65 <sup>c-f</sup>	16.98 <sup>c-g</sup>	9.42 <sup>abc</sup>	8.94 <sup>a</sup>	0.14 <sup>d</sup>	0.18 <sup>ab</sup>	3.31 <sup>def</sup>	3.78 <sup>a</sup>	2.60 <sup>ab</sup>	2.37 <sup>a</sup>
NR-119	17.4 <sup>a-e</sup>	15.06 <sup>g</sup>	8.98 <sup>a-f</sup>	7.58 <sup>a</sup>	0.15 <sup>ad</sup>	0.18 <sup>ab</sup>	3.56 <sup>b-f</sup>	3.85 <sup>a</sup>	2.47 <sup>ab</sup>	2.20 <sup>a</sup>
NR-1190	14.26 <sup>ef</sup>	18.33 <sup>b-g</sup>	7.29 <sup>d-h</sup>	8.24 <sup>a</sup>	0.19 <sup>a-d</sup>	0.19 <sup>ab</sup>	2.94 <sup>ef</sup>	3.63 <sup>a</sup>	2.22 <sup>ab</sup>	1.70 <sup>a</sup>
Radha-11	19.59 <sup>ab</sup>	16.04 <sup>efg</sup>	10.34 <sup>a</sup>	7.24 <sup>a</sup>	0.17 <sup>a-d</sup>	0.22 <sup>a</sup>	4.33 <sup>ab</sup>	3.41 <sup>a</sup>	1.47 <sup>b</sup>	2.30 <sup>a</sup>
Radha-12	18.21 <sup>a-d</sup>	17.18 <sup>b-g</sup>	8.38 <sup>a-g</sup>	8.90 <sup>a</sup>	0.17 <sup>a-d</sup>	0.18 <sup>b</sup>	3.83 <sup>b-e</sup>	3.67 <sup>a</sup>	2.13 <sup>ab</sup>	1.63 <sup>a</sup>
Radha-4	18.28 <sup>a-d</sup>	20.4 <sup>a-d</sup>	9.29 <sup>a-d</sup>	11.44 <sup>a</sup>	0.20 <sup>abc</sup>	0.19 <sup>ab</sup>	4.87 <sup>a</sup>	3.69 <sup>a</sup>	2.00 <sup>ab</sup>	2.13 <sup>a</sup>
Radha-7	17.49 <sup>a-e</sup>	17.97 <sup>b-g</sup>	8.80 <sup>a-f</sup>	8.26 <sup>a</sup>	0.17 <sup>a-d</sup>	0.16 <sup>b</sup>	3.73 <sup>b-f</sup>	3.61 <sup>a</sup>	2.13 <sup>ab</sup>	2.10 <sup>a</sup>
Radha-9	16.41 <sup>b-f</sup>	16.15 <sup>efg</sup>	7.58 <sup>c-h</sup>	6.78 <sup>a</sup>	0.17 <sup>a-d</sup>	0.17 <sup>b</sup>	4.13 <sup>a-d</sup>	3.80 <sup>a</sup>	2.2 <sup>ab</sup>	1.55 <sup>a</sup>
Sukhadhan-1	18.63 <sup>abc</sup>	17.07 <sup>b-g</sup>	8.41 <sup>a-g</sup>	7.13 <sup>a</sup>	0.19 <sup>abd</sup>	0.19 <sup>ab</sup>	4.17 <sup>a-d</sup>	3.77 <sup>a</sup>	1.6 <sup>ab</sup>	2.37 <sup>a</sup>
Sukhadhan-2	18.63 <sup>abc</sup>	15.07 <sup>g</sup>	8.09 <sup>a-h</sup>	7.39 <sup>a</sup>	0.18 <sup>a-d</sup>	0.18 <sup>ab</sup>	4.20 <sup>abc</sup>	3.43 <sup>a</sup>	1.86 <sup>ab</sup>	1.40 <sup>a</sup>
Tarhara-1	17.57 <sup>a-e</sup>	20.78 <sup>abc</sup>	9.20 <sup>a-e</sup>	8.67 <sup>a</sup>	0.15 <sup>d</sup>	0.18 <sup>ab</sup>	3.33 <sup>def</sup>	3.93 <sup>a</sup>	2.47 <sup>ab</sup>	2.088 <sup>a</sup>
Mean	16.6	17.85	7.94	8.34	0.17	0.18	3.59	3.64	3.59	2.01
CV%	10.62	12.08	15.3	38.8	16.86	15.85	13.5	9.09	13.5	32.7
LSD (0.05)	2.94	3.59	2.02	5.38	0.05	0.05	0.81	0.55	1.64	1.09
F test	**	**	**	Ns	Ns	Ns	**	ns	**	ns

LSD= Least Significant Difference, CV= Coefficient of Variation. \*\* = Significant at 1% level of significance, Ns= non-significant.



**Figure 1.** Cluster analysis of twenty five rice genotypes estimated for morphological attributes under water stress condition.

**Table 2.** Morphological characteristics of twenty five rice genotypes within and among five clusters under water stress condition.

Variable	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Centroid
Plant height (10 DAS)	11.9169	10.11	11.4	10.2333	14.55	11.2936
Plant height (20 DAS)	15.51	13.05	13.39	13.2333	17.63	14.548
Plant height (30 DAS)	17.3915	14.5143	20.61	15.7367	19.59	16.604
Leaf length (10 DAS)	6.0315	5.32	5.18	6.3367	6.92	5.8704
Leaf length (20 DAS)	7.1485	6.0143	6.07	7.7933	9.01	6.9396
Leaf length (30 DAS)	8.2669	6.6371	7.83	8.8233	10.34	7.9428
Leaf breadth (10 DAS)	0.1346	0.1214	0.13	0.1233	0.14	0.1296
Leaf breadth (20 DAS)	0.1523	0.1429	0.15	0.1333	0.15	0.1472
Leaf breadth (30 DAS)	0.1715	0.1643	0.16	0.17	0.17	0.1688
No. of leaves (10 DAS)	1.6354	1.42	1.27	1.42	1.4	1.5252
No. of leaves (20 DAS)	3.0777	2.7429	2.67	2.8633	3.47	2.9576
No. of leaves (30 DAS)	3.7731	3.2729	3.23	3.44	4.33	3.5936
No. of leaves shedding/plant (20 DAS)	1.4246	1.5429	1.6	1.69	1.53	1.5008
No. of leaves shedding/plant (30 DAS)	2.3023	2.5471	3.03	3.0233	1.47	2.4532

**Table 3.** Grouping of twenty-five rice genotypes into five clusters based on morphological traits under water stress condition.

Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Bindeswari, Ghaiya, Hardinath-1, Chiate-4, Radha-9, Chaite-2, NR-119, Radha-4, Radha-12, Sukhadhan-1, Sukhadhan-2, Radha-7, Tarahara-1	Chaite, IR-15672, DRR-44, Janaki, Makwanpur-1, China bora-4, NR-1190	Champion	IR67015, Loktantra, Mithila	Radha-11

### Cluster analysis

Twenty-five rice genotypes were categorized into 5 clusters on the basis of several morphological attributes (Table 2).

From the Cluster analysis, it was found that cluster I contained 13 genotypes, cluster II contained 7, cluster III contained 1, cluster IV contained 3 and cluster V contained 1 genotype (Figure 1). The cluster I consisted of the genotypes namely Bindeswari, Ghaiya, Hardinath-1, Chiate-4, Radha-9, Chaite-2, NR-119, Radha-4, Radha-12, Sukhadhan-1, Sukhadhan-2, Radha-7, Tarahara-1 which represent 53% of total genotypes. The genotypes categorized into cluster II were Chaite, IR-15672, DRR-44, Janaki, Makwanpur-1, China bora-4, NR-1190, that represent 28% of the total. The cluster III consisted of genotype, namely Champion. Cluster IV consisted of the genotypes IR67015, Loktantra, Mithila. Similarly, cluster V up one genotype Radha-11 (Table 3). The cluster V had a higher height of the plant, bigger length and breadth of the leaf, maximum leaves number per plant, and the minimum number of leaf shedding. Cluster II had a lower height of the plant, smaller length, and breadth of the leaf, minimum leaves number per plant.

### Conclusion

The variation was observed in the morphological traits viz., the height of plant, length and breadth of leaf, leaves number per plant and number of leaf shedding in the tested rice genotypes. The rice genotype grouped into cluster V was good due to their required morphological attributes, i.e. higher plant height, bigger leaf length and breadth, maximum leaves number per plant and a minimum number of leaf shedding. Thus, this genotype showed the best performance under water stress

condition. Therefore, the genotype Radha 11 can be utilized further to develop the variety which is tolerant towards drought. The high degree of heterogeneity present among the rice genotypes categorized into different clusters which showed the hybridization suitability, and different crosses can be developed among the varieties in breeding programs.

**Conflict of interest:** The authors declare that there are no conflicts of interest.

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