

Original Research Article

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## Assessment of Physiological Traits for Adaptation to Flowering Stage Water Deficit in Rainfed Lowland Rice (*Oryza sativa* L.)

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### ABSTRACT

#### Keywords

Rice, Flowering stage, Moisture deficit, Physiological and biochemical traits

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Responses of 4 cultivars of rice viz. NDR-8002, TCA-48, IR-42 and BPT 5204 to varying levels of moisture deficit stress at flowering stage were assessed in the plastic tubs for their relative tolerance to drought. Moisture deficit adversely affected the apparent translocation rate, relative leaf water content, total chlorophyll content and total soluble carbohydrate content, while proline accumulation enhanced irrespective of varieties. Grain yield decreased conspicuously in all the varieties in response to water stress. Amongst the rice varieties, NDR-8002 and TCA-48 were noted to be relatively more drought tolerant than other 2 cultivars on the basis of their yield performance. The flowering stage in rice was found to be very critical to moisture deficit stress.

### Introduction

Drought and prolonged dry spells are world-wide problems that significantly influence the grain quality and quantity. The situation has

been worsening with the ever burgeoning global population and climate change (HongBo *et al.*, 2005). Rice, a semi aquatic field crop is very much susceptible to deficit water stress conditions (Tao *et al.*, 2006; Yang

*et al.*, 2008). Near about 50% of the world production of rice is affected more or less by drought (Bouman *et al.*, 2005). In rainfed areas such climatic aberrations are frequent having far off consequences with dwindling productivity. As stable and high yields of rainfed lowland rice are highly essential for food security in many of the subsistence farming system in Asia (Cooper, 1999), growing of rice in Eastern India in around 10 m ha under vulnerable rainfed lowland farming situation is the greatest challenge before us.

Drought at the flowering stage is highly detrimental to rice plants. It hampers anthesis and seed setting leading to higher spikelet sterility and lower yield in rice (Ram *et al.*, 1988). It also reduces effective leaf area and photosynthesis, thus plants have to depend on pre-anthesis reserves which may impart tolerance against internal water deficits (Austin *et al.*, 1980). Therefore, there is urgent need to develop flowering stage drought tolerant lines for rainfed lowlands. This necessitated the characterization of different physiological or morphological traits associated with flowering stage drought tolerance in rainfed lowland rice.

Though plants have naturally evolved several stress adoptive strategies, most of them relate to survivability under stress. However, from the agricultural point of view, any stress adoptive strategy, drought stress in particular, would be useful only if it is associated with superior crop growth rates under a given stressful environment (Kar *et al.*, 2005). Such adoptive types were traditionally selected based on empirical screening methods that did not always result in the expected increase in productivity under water limited conditions. To improve crop productivity, it is being suggested that a 'trait-based' approach to be adopted (Sheshshayee *et al.*, 2003), which would provide a strong impulse to the efforts

in assessing the genetic variability under moisture deficit stress condition. The present endeavour is an attempt to study the physiological and biochemical changes occurring due to drought during flowering stage in rainfed lowland rice and to identify the desirable physiological traits related to drought resistance.

## **Materials and Methods**

The current study was carried out in plastic tubs at the experimental site of the Department of Crop Physiology, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh, India during *Kharif* 2011 and 2012 as factorial completely randomized design with four rice varieties (viz. IR-42, NDR 8002, BPT 5204, TCA-48) in 3 replications and 3 drought treatments viz. 100% (saturated) available soil moisture regime (SMR), 50% available SMR, 25% available SMR), which was imposed at flowering stage. The native soil was sandy loam with pH of 6.8 and available N, Olsen's P and available K were of 124, 18 and 220 kg ha<sup>-1</sup>, respectively. Cold winter months with occasional frost and hot and dry summers have been the climatologic features in that area. Seeds were directly sown in the plastic tubs at equidistance. Thinning was done in each tub after seedling emergence and only 10 healthy plants were maintained. Plants under saturated condition were irrigated normally but 50% and 25% ASM regimes were maintained by withholding irrigation in tubs. After 4 days of water withholding in tubs, soil sampling was done periodically. After weighing, samples were oven dried and moisture content in soil was computed. When moisture content in soil reached to 14.5% and 10.75% in 50% and 25% available SMR treatments respectively, drought treatment was terminated by irrigating the tubs. The observations on different characters were taken as per the following procedures.

### **Grain /Economic yield**

Panicles of tagged plants were separated from plant then grains were collected and weighed with the help of electronic balance for finding out the grain yield in g plant<sup>-1</sup>.

### **Apparent translocation rate**

Apparent translocation rate (ATR) was computed by using the following formula (Reyniers *et al.*, 1982).

$$\text{ATR} = \frac{\text{WSF} - \text{WSM}}{\text{WPM} - \text{WPF}}$$

Where,

WSF is dry weight of stem at flowering,

WSM is dry weight of stem at maturity,

WPM is dry weight of panicle at maturity and

WPF is dry weight of panicle at flowering.

### **Relative leaf water content**

The relative leaf water content (RLWC) was calculated by using the following formula (Turner and Beg, 1981).

$$\text{RLWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{Dry weight}} \times 100$$

### **Total chlorophyll**

Total chlorophyll (Chl) content in leaf sample was estimated according to method of Arnon (1949) and expressed as mg g<sup>-1</sup> fresh weight of leaves. In this method, Chl was extracted in 80% acetone. 200 mg leaves in 5 mL of 80% aqueous acetone were centrifuged at 4,000 rpm for 20 minutes, and the absorbance of the

supernatant was measured at 645 and 663 nm on spectronic-20 using 80% acetone as blank.

The amount of chlorophyll was calculated as,

$$\text{Chl a} = 12.7 \times \text{OD}_{663} - 2.69 \times \text{OD}_{645} \text{ (mg L}^{-1}\text{)}$$

and

$$\text{Chl b} = 22.9 \times \text{OD}_{645} - 4.68 \times \text{OD}_{663} \text{ (mg L}^{-1}\text{)}.$$

Where,

OD = Optical density of the chlorophyll extract at a specific wavelength.

### **Total soluble carbohydrate**

The total soluble carbohydrate in plant extracts was estimated by the method of Yemmand Willis (1954). The fresh plant sample of 100 g was homogenized in 10 mL of 80% ethanol and centrifuged at 4,000 rpm for 20 minutes. The supernatant was collected and the residue was re-extracted twice with 10 mL of 80% ethanol and then again centrifuged at 4,000 rpm for 20 minutes.

All the supernatants were combined and the volume was made to 20 mL. A known volume (0.1 to 0.2 mL) of ethanol extract was evaporated to dryness in a test tube on water bath and cooled to room temperature. The distilled water of 1 mL was then added to each tube and mixed thoroughly. To each test tube, 4.0 mL of anthrone reagent was added and heated on a water bath at 100 °C for 10 minutes. Finally, it was cooled under running cold water and the absorbance was measured at 620 nm against reagent blank. A standard curve was prepared by using graded concentrations of glucose.

### **Proline content**

The Proline content was estimated spectrophotometrically according to the method of

Bates *et al.*, 1973. Fresh leaves were homogenized in 3 mL of 3% aqueous sulfosalicylic acid and centrifuged at 4,000 rpm for 20 minutes. The residue was re-extracted with 5 mL of 3% sulfosalicylic acid and volume was made to 10 ml. 2 mL of this aliquot was transferred into a test tube and 2 mL each of acid ninhydrin and acetic acid were added. The mixture was heated on a boiling water bath for one hour, after which reaction was terminated by placing the tube in icebox. Thereafter, the mixture was shaken vigorously with 4 mL toluene and kept for several hours at room temperature.

Chromatophor was thus extracted into toluene phase, which was separated and its absorbance was measured at 520 nm using toluene as blank. Standard curve prepared with graded concentration of DL-proline.

### Statistical analysis

The statistical analysis of experimental data was done by the method described by Panse and Sukhatme (1978) using factorial completely randomized design (CRD) experiment. The comparison of the treatment means made with the help of least significant differences calculated as here under.

$$SEm \pm \text{due to variety} = \sqrt{\frac{2 \times EMSS}{D \times r}}$$

$$SEm \pm \text{due to drought} = \sqrt{\frac{2 \times EMSS}{V \times r}}$$

$$SEm \pm \text{due to variety} \times \text{drought} = \sqrt{\frac{2 \times EMSS}{r}}$$

CD = SEm x t at 5% for error d.f.

Where,

V = number of varieties

r = number of replications

t = table value at 5% for error d.f.

D = Drought levels

EMSS = error means sum of square

d.f. = degrees of freedom

SEm = standard error of means

## Results and Discussion

### Grain yield

Moisture deficit stress at flowering stage caused a severe reduction in grain yield of all varieties (Table 1). The percent reduction in grain yield at 50% available SMR over saturated condition ranged from 17% to 31% which increased to the tune of 32.2% to 48.2% at 25% available SMR. Under both moisture deficit stresses, the minimum percent reduction in grain yield was observed in rice cv. NDR-8002 followed by TCA-48, while IR-42 had the highest reduction. The mean effect of varieties showed the highest grain yield in NDR-8002 followed by TCA-48, BPT-5204 and IR-42. The mean effect of stress exhibited 23.4% and 39.7% reduction in grain yield at 50% and 25% available SMR, respectively over saturated condition. The present result also corroborated the earlier findings of Sarvestani *et al.*, (2008) with reduction in total biomass, harvest index, filled grains, unfilled grains and 1000 grain weight under water stress in rice cultivars at flowering stage.

### Apparent translocation rate

The apparent translocation rate (ATR) is the relative decrease or increase in stem dry

weight compared to panicle dry weight. It is an indirect measurement of translocatory behaviour of stem reserve to panicle growth. The data presented in Table 1 clearly showed significant and progressive increase in ATR with increase in moisture deficit stress level. Significantly, the highest ATR value in all varieties was recorded at 25% available SMR and the saturated condition trailed behind in this line. At 50% and 25% available SMR, the increase in ATR over saturated condition ranged from 18.7% (IR-42) to 27.8% (TCA-48) and 35% (IR-42) to 50% (TCA-48), respectively. The mean effect of drought showed 26.7% and 40% increase in ATR at 50% and 25% available SMR, respectively over saturated condition. The present result also corroborated the findings of Reyniers *et al.*, (1982), Chaturvedi and Ingram (1991) and Arti (2006). Among the rice varieties, NDR-8002 and TCA-48 showed higher percent increase in ATR under moisture deficit stresses with respect to saturated condition. It means these varieties have better capacity to translocate reserve food materials from the stem to panicles under drought condition than other varieties.

### **Relative leaf water content**

The relative leaf water content (RLWC) was severely reduced under moisture deficit stress condition (Table 2). The reduction in RLWCs of all varieties were more at 25% available SMR as compared to 50% available SMR. At 50% available SMR, the maximum reduction in RLWC over saturated condition was recorded in IR-42 (24.3%), while TCA-48 showed the minimum reduction (13.1%). At 25% available SMR also the maximum and the minimum percent reduction in RLWC over saturated condition were found in IR-42 (31.8%) and TCA-48 (23.5%), respectively. The mean effect of varieties showed the highest RLWC in TCA-48 which was at par with NDR-8002 but significantly higher than

BPT-5204 and IR-42. The mean effect of drought showed 17.2% and 26.7% reduction in RLWC at 50% and 25% available SMR, respectively over saturated condition. Many researchers have reported that reduction in RLWC might be due to hydrolysis of reserve protein and starch or due to non-utilization of these products for growth, as a result concentration of soluble sugars, minerals, betaine, proline and other free amino acids increases in the cell sap, such accumulation might have resulted in the lowering of solute potential and consequently decrease the RLWC. It has also been observed in our experiment that proline and soluble carbohydrates were comparatively high in NDR-8002 and TCA-48 than other varieties of rice under moisture deficit stresses (Table 3). This might have resulted in maintaining better RLWC by above varieties than rest of the varieties. Our study also confirms the findings of Chaturvedi *et al.*, (2004) who observed water deficit at flowering stage declining the RLWC in rice significantly and the reduction was more in susceptible genotypes than tolerant ones. During recovery from moisture deficit stress, the reduction in RLWC with respect to saturated condition was also minimized.

### **Total chlorophyll**

At the end of drought, all the varieties showed decrease in total chlorophyll at both 50% and 25% available SMR relative to saturated condition, however extent of decrease was more at 25% available SMR (Table 2). At both 50% and 25% of the available SMR, the highest chlorophyll content was found in NDR-8002 followed by BPT-5204 and TCA-48 in descending order; IR-42 had the least. The mean effect of variety indicated the highest chlorophyll in NDR-8002 which was at par with BPT-5204, TCA-48 but significantly higher than IR-42. The mean effect of stress showed 14.7% and 19.7%

decrease in total chlorophyll content at 50% and 25% available SMR, respectively over saturated condition. At recovery, all the varieties exhibited decrease in total chlorophyll content at 50% and 25% available SMR with respect to saturated condition. Relatively higher chlorophyll content at both 50% and 25% available SMR was recorded in NDR-8002 and TCA-48, while BPT-5204 and IR-42 had lower chlorophyll content. The mean effect of drought showed that at 25% available SMR, chlorophyll content significantly declined below 50% available SMR and saturated condition. The decrease in chlorophyll content under drought was due to weakening of bonding between chlorophyll and protein complex (Samier and Mafrina, 1982). Sikuku *et al.*, (2010) had also observed decline in total chlorophyll content in rice with increasing water deficit. Similar to the present result, Pandey and Chaturvedi (1993) observed varietal variation in total chlorophyll content of rice under drought; the tolerant varieties had less decrease in total chlorophyll than susceptible ones.

### **Total soluble carbohydrate**

The total soluble carbohydrate (CHO) in culm decreased with increase in moisture deficit stress level and the extent of decrease was higher at 25% available SMR (Table 3). At 50% available SMR, the lowest reduction in total CHO with respect to saturated condition was found in NDR-8002 (9.7%) followed by TCA-48 (12.4%), BPT-5204 (14%) and IR-42 (19.9%). At 25% available SMR, above varieties followed similar order, however the reduction varied from 15.1% (NDR-8002) to 29.3% (IR-42). The mean effect of varieties showed the highest total CHO in NDR-8002 which was significantly higher than other varieties, while the lowest CHO was observed in IR-42. The mean effect of stress indicated 14% and 21.6% reduction in total CHO at

50% and 25% available SMR, respectively over saturated condition. The rice cv. NDR-8002 had the highest total CHO at both 50% and 25% available SMR, while IR-42 showed the lowest values. The mean effect of variety also showed the highest total CHO in NDR-8002 and the lowest in IR-42. As limited water supply inhibits the synthesis of current photosynthates which might be one of the reasons for reduced CHO level under drought. Secondly, when current photosynthates under drought are limiting, plant survives at the expense of energy obtained from breakdown of stored CHO pool, this also reduces the CHO status in culm. Similar to the present results, Weng *et al.*, (1986) found wide variability among rice genotypes for CHO content under drought and observed that the tolerant rice cultivars have higher CHO content than susceptible ones. According to Chaturvedi and Ingram (1989), high CHO and its remobilization are key components of drought recovery especially for drought tolerance at flowering stage. This indicated that higher CHO content in NDR-8002 and TCA-48 might be one of the tolerant characters for flowering stage drought. All the varieties showed lower CHO at both 50% and 25% available SMR as compared to saturated condition.

### **Free proline**

The proline accumulation increased with increase in moisture deficit stress level and the highest accumulation in all varieties was recorded at 25% available SMR (Table 3). At both 50% and 25% available SMR, the highest proline content was observed in NDR-8002 which was at par with TCA-48 but significantly higher than BPT-5204 and IR-42. At both 50% and 25% available SMR, the highest proline content was found in NDR-8002 followed by TCA-48, BPT-5204 and IR-42 in reducing manner.

**Table.1** Effect of flowering stage water deficit on grain yield and apparent translocation rate (ATR) of rainfed lowland rice

Varieties	Grain yield (g plant <sup>-1</sup> )				ATR			
	100% available SMR	50% available SMR	25% available SMR	Mean	100% available SMR	50% available SMR	25% available SMR	Mean
<b>IR-42</b>	16.41	11.32 (31.0)	8.50 (48.2)	12.07	0.13	0.16 (18.7)	0.20 (35.0)	<b>0.16</b>
<b>NDR-8002</b>	23.01	19.09 (17.0)	15.60 (32.2)	19.23	0.17	0.23 (26.0)	0.29 (41.4)	<b>0.23</b>
<b>BPT-5204</b>	20.01	14.80 (26.0)	11.40 (43.0)	15.40	0.15	0.19 (21.1)	0.24 (37.5)	<b>0.19</b>
<b>TCA-48</b>	19.36	15.10 (22.0)	12.00 (38.8)	15.48	0.13	0.18 (27.8)	0.26 (50.0)	<b>0.19</b>
<b>Mean</b>	19.70	15.08 (23.4)	11.87 (39.7)		0.15	0.19 (26.7)	0.25 (40.0)	
<b>S.Em (±)</b>	V = 0.38    D = 0.32    V x D = 0.65				V = 0.01    D = 0.01    V x D = 0.02			
<b>LSD at 5%</b>	V = 1.09    D = 0.94    V x D = NS				V = 0.02    D = 0.02    V x D = 0.04			

(SMR: Soil moisture regime; NS: Not significant)

(Values in parenthesis indicate percent decrease due to 50% and 25% available SMR calculated over 100% available SMR)

**Table.2** Effect of flowering stage water deficit on relative leaf water content (RWC) and total chlorophyll (mg g<sup>-1</sup> fresh weight) of rainfed lowland rice (recorded at end of the stress)

Varieties	RWC (%)				Total chlorophyll			
	100% available SMR	50% available SMR	25% available SMR	Mean	100% available SMR	50% available SMR	25% available SMR	Mean
<b>IR-42</b>	81.00	61.30 (24.3)	55.20 (31.8)	65.83	2.90	2.42 (16.5)	2.25 (22.4)	<b>2.52</b>
<b>NDR-8002</b>	82.10	70.30 (14.3)	62.00 (24.5)	71.47	3.01	2.70 (10.2)	2.52 (16.2)	<b>2.74</b>
<b>BPT-5204</b>	79.30	65.60 (17.3)	57.70 (27.2)	67.53	3.11	2.54 (18.3)	2.50 (19.6)	<b>2.72</b>
<b>TCA-48</b>	82.30	71.50 (13.12)	63.00 (23.5)	72.27	2.93	2.52 (14.0)	2.36 (19.4)	<b>2.60</b>
<b>Mean</b>	81.18	67.18 (17.2)	59.48 (26.7)		2.99	2.55 (14.7)	2.40 (19.7)	
<b>S.Em (±)</b>	V = 1.73    D = 1.49    V x D = 3.00				V = 0.06D = 0.05    V x D = 0.10			
<b>LSD at 5%</b>	V = 4.98    D = 4.32    V x D = NS				V = 0.17D = 0.15    V x D = NS			

(SMR: Soil moisture regime; NS: Not significant)

(Values in parenthesis indicate percent decrease due to 50% and 25% available SMR calculated over 100% available SMR)

**Table.3** Effect of flowering stage water deficit on total soluble carbohydrate (mg g<sup>-1</sup> dry weight) and proline (µg g<sup>-1</sup> fresh weight) in leaves of rainfed lowland rice (recorded at the end of stress).

Varieties	Total soluble carbohydrate				Proline			
	100% available SMR	50% available SMR	25% available SMR	Mean	100% available SMR	50% available SMR	25% available SMR	Mean
<b>IR-42</b>	223.43	178.98 (19.9)	157.96 (29.3)	186.79	69.63	79.16 (12.0)	87.96 (20.8)	<b>78.92</b>
<b>NDR-8002</b>	237.63	214.57 (9.7)	201.80 (15.1)	218.00	74.53	99.14 (24.8)	117.45 (36.5)	<b>97.04</b>
<b>BPT-5204</b>	225.57	194.00 (14.00)	173.41 (23.12)	197.66	71.90	86.45 (16.8)	93.13 (22.8)	<b>83.83</b>
<b>TCA-48</b>	229.47	201.10 (12.4)	185.18 (19.3)	205.25	66.98	96.34 (30.5)	111.78 (40.0)	<b>91.70</b>
<b>Mean</b>	229.23	197.16 (14.0)	179.59 (21.6)		70.76	90.27 (21.6)	102.58 (31.0)	
<b>S.Em (±)</b>	V = 4.73	D = 4.09	V x D = 8.19		<b>V = 2.07</b>	<b>D = 1.79</b>	<b>V x D = 3.58</b>	
<b>LSD at 5%</b>	<b>V = 13.67</b>	<b>D = 11.83</b>	<b>V x D = NS</b>		<b>V = 5.97</b>	<b>D = 5.17</b>	<b>V x D = 10.34</b>	

(SMR: Soil moisture regime; NS: Not significant)

(Values in parenthesis indicate percent decrease due to 50% and 25% available SMR calculated over 100% available SMR)

The percent increase in the proline content at 50% and 25% available SMR with respect to saturated condition varied from 12% (IR-42) to 30.5% (TCA-48) and 20.8% (IR-42) to 40% (TCA-48), respectively. At recovery, all 4 varieties showed higher proline at 50% and 25% available SMR with respect to saturated condition, however values were less than that found at end of the drought. The mean effect of stress exhibited 21.6% and 31.0% increase in proline content at 50% and 25% available SMR, respectively over saturated condition.

The increase in free proline content under water deficit condition could occur due to *de novo* synthesis of proline or breakdown of proline rich proteins or shift in metabolism. According to Dubey (1997), increase in proline level helps the cell in osmoprotection as well as in regulating their redox potential, scavenging hydroxyl radicals and protection

against denaturation of various macro molecules. Higher proline accumulation under drought has been also observed by a number of researchers (Blum, 1998; Mostajenan and Rahimi-Eichi, 2009). High proline content of NDR-8002 and TCA-48 might have contributed to increasing water absorption capacity of plant through osmotic adjustment and provided a readily available pool of nitrogen at recovery. Hence, proline is considered as a measure of drought resistance in various crops. High proline accumulation under stress might be a tolerant trait for drought.

The grain and biochemical processes of rice were adversely affected due to moisture deficit stress at flowering stage. Rice varieties NDR-8002 and TCA-48 have the potential to produce better yield under moisture deficit stress. Initial high carbohydrate coupled with

less reduction under stress seems to be useful adaptive trait for flowering stage drought tolerance. High proline accumulation under moisture deficit stress can also be taken as a measure of flowering stage drought tolerance. High apparent translocation rate value is an indicator of high stem reserve mobilization to sink during moisture. This parameter can be used to screen large number of genotypes for flowering stage drought tolerance. Maintenance of good relative leaf water content during moisture deficit stress can be considered adaptive trait for drought tolerance. Rice varieties NDR-8002 and TCA-48 have adaptive features for flowering stage drought tolerance and may be used as tolerant lines under breeding programme. Further studies are required to validate present results with larger population and search other tolerant traits.

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### **Conflict of interest**

There is no conflict of interest among the authors.

### **Ethical approval**

This article does not contain any studies with human participants or animals performed by any of the authors.

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