



## Morpho-physiological and biochemical attributes as tools to screen tolerance and susceptible rice cultivars for drought stress

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### ARTICLE INFO

Received : 08 June 2022

Revised : 27 October 2022

Accepted : 14 November 2022

Available online: 08 March 2023

#### Key Words:

Ascorbic acid

Drought

Glycine betaine

Proline

Rice

RWC

Total soluble sugars

### ABSTRACT

Present research work was aimed to observe possible changes in the metabolism of rice plants (*Oryza sativa* L.) through drought stress. Rice belongs to the family *Poaceae*. It is considered as a vital food crop across all the major countries worldwide. Rice is prone to be affected by drought stress. Therefore, developing the drought tolerant cultivars of cereal crops assumed considerable importance. This work was carried out with an objective to study the Screening of rice cultivars against water stress and compare biochemical characteristic among different drought tolerant and sensitive rice cultivars. A set of 25 cultivars of rice were screened against drought stress at vegetative stage through various morpho-physiological characters such as moisture, relative water content (RWC), membrane stability index (MSI), membrane injury (MI), seedling length and seedling weight. The RWC is a best criterion for plant water status. The osmotic adjustment is a influential mechanism of conserving cellular hydration under water stress and RWC expression also affects osmotic adjustment in this respect. Thus, it can be considered that the higher RWC having cv. GAR-13 and NWGR-16026 were tolerant and lower RWC having cv. NWGR-16009 and NWGR-16019 were susceptible. Hence, cv. GAR-13 & NWGR-16026 was used as tolerant and NWGR-16009 & NWGR-16019 were used as susceptible. On the basis of first experiment total four cultivars (Two tolerant NWGR-16026 & GAR-13, two susceptible NWGR-16009 & NWGR-16019) were selected for various biochemical analysis. The results indicated that total soluble sugars (TSS), glycine betaine and ascorbic acid content were found significantly higher in cultivar NWGR-16026. The proline content was found significantly higher in cultivar GAR-13. So, RWC and some biochemical parameters are best indicators for selection regarded as potentially useful for drought tolerant rice cultivars and targets for development through transgenic approaches.

### Introduction

Rice (*Oryza sativa* L.) belongs to the family *Poaceae*. The basic chromosome number of rice is  $n=12$ . Rice is considered as a main food crop across worldwide. As a food crop, it forms the staple food and which is more than three billion people accounting for about 50-80% of their daily calorie intake (Khush, 2005). In India rice production was 116.48 million tons in area of 43.80 million ha (United States Department of Agriculture, 2018-19).

It yields about one third of the total carbohydrates source. The usage of the crop diverges widely ranging from its use as food in cereals, snacks, brewed beverages, flour, rice bran oil to its use in religious occasions across India. It is the second most important crop in the world after wheat, covering almost 90% of area across Asia alone. Water deficiency is one of the major environmental constraints of plant growth and crop

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Doi: <https://doi.org/10.36953/ECJ.13212387>

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productivity (Chaves and Oliveira, 2004). The crop is subjected to soil moisture scarcities of varying degree and duration, which occasionally outcome in substantial loss of yield. Plant water status is disturbed due to water stress, resulting in disruption of important metabolic processes. A major effect of drought is reduction in photosynthesis, which arises by a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in food production (Farooq *et al.*, 2009). Drought stress leads to biochemical response such as transient decrease in photochemical efficiency, accumulation of stress metabolites (Reddy *et al.*, 2004). Drought increases the formation of reactive oxygen species (ROS) resulting in lipid peroxidation, protein denaturation (Hansen *et al.*, 2006). The effect of drought reduces in growth rates and nutritional quality of crops (Mahajan and Tuteja, 2005, Verslues *et al.*, 2006). Therefore, to develop the drought tolerant varieties of cereal crops assumed considerable importance. The present work aims to govern the effect of water stress on various morpho-physiological parameters like moisture, RWC, membrane injury, MSI, seedling length and seedling dry weight. Some biochemical parameters like proline, glycine betaine, TSS and ascorbic acid. The comparison of these responses will be beneficial in identifying the similarities and the differences associated to the relative ability of the rice seedlings to cope with diverse pattern of responses to drought stress and useful as potential targets for upgrading of rice cultivars by conventional and transgenic approaches.

### Material and Methods

There were total 25 cultivars of rice procured from Main Rice Research Station, Nawagam, Anand Agricultural University, Nawagam for investigation. All twenty-five rice cultivar's seed grown in pot. Then, after twenty-five days of germination, given treatment of water stress by five days holding of water in all rice cultivars. Then, taken seedlings of all cultivars and measured all morpho-physiological parameters. For biochemical attributes, from all twenty-five rice cultivar's we selected total four cultivars (Two drought tolerance and two drought sensitive) on the basis of morpho-physiological parameters total four cultivars seed grown in pot.

Then, after 25 days of germination taken seedlings of all cultivars and measured all biochemical attributes. The experiment was conducted under completely randomized block design

### Morpho-physiological parameters

#### Moisture

Moisture content of rice seedlings at vegetative stage were estimated as per procedure developed by A.O.A.C. (2000).

#### Relative water content (RWC)

The method described by Jayaweera *et al.* (2016) was used for the determination of relative water content of rice cultivars under control as well as water deficit stress conditions.

#### Membrane stability index (MSI)

Membrane stability index (MSI) was determined by using the method of Al-Ashkar *et al.* (2016).

#### Membrane injury (MI)

Membrane injury (MI) was determined by using the method of Atarzyna *et al.* (2010) with some modification in Caraway (*Carum Carvi* L.) Genotype in water deficit conditions.

#### Seedling length (cm)

Five randomly selected seedlings were taken from each growing treatment to calculate the length of seedling. It was measured with a measuring scale and stated in centimeters.

#### Seedling dry weight (mg)

Five randomly selected seedlings were occupied from each growing treatment to calculate the weight of shoot. Weight of seedling were measured by three-digit balance and expressed in milligram.

### Biochemical attributes

#### Osmolytes

Total soluble sugars were determined using the phenol-sulphuric acid method as described by Mostajeran and Rahimi-Eichi (2009) with some modification.

#### Proline

The proline content in the rice leaves samples were analyzed at vegetative stage by the method suggested by Mostajeran and Rahimi-Eichi (2009).

**Glycine betaine**

Glycine-betaine extraction and estimation was done in dried leaf powder of rice as per the method of Khattab *et al.* (2014).

**Ascorbic acid**

Ascorbic acid content was estimated from rice leaves by 2-4 dinitrophenylhydrazine (DNPH) method as described by Anthon and Barrett (2003).

**Statistical analysis**

The mean values were taken from measurements of total four replications and standard error (SE) of the means was calculated. Data obtained by biochemical constituents and enzymes determination were subjected to simple completely randomized design for study in the significance of various data using "F" test.

**Results and Discussion****Morpho-Physiological parameters****Moisture**

The moisture content is a supreme element in determining the distribution of species and the responses and adaptation of species to drought stress are severe for their success in any environmental niche and for their usage and productivity in agricultural systems. The moisture content of 25 cultivars of rice leaves were analyzed at vegetative stage, the data is presented in Table 1. The moisture content was found significantly varied among the rice cultivars and it was varied from 50.90 % to 77.86 % (Table 1). However, significantly the highest moisture content was recorded for NWGR-12015 (77.86%). The non-significant moisture difference was recorded among NWGR-16022 (72.27 %), NWGR-16026 (71.63 %), GAR-13 (68.87 %) and NWGR-14071 (68.78 %). Significantly lower moisture content was recorded for cultivar NWGR-12002 (50.90%) which was significantly at par with NWGR-16003 (54.62%), GR-6 (54.99 %) and GR-7 (55.21 %). Kumari *et al.* (2019) studied the physiological, biochemical and molecular screening of ten rice (*Oryza sativa* L.) genotypes. They observed that the moisture content between 43.7 to 49.9 among all the rice genotypes. With increasing the drought condition moisture content was also decreasing. Similar trend was also recorded by various researchers (Aly and Latif,

2011; Noorka and Silva, 2012) in wheat. The present experimental data of moisture content indicated that the cv. NWGR-12015, NWGR-16022, NWGR-16026, GAR-13 and NWGR-14071 are considered as tolerant and cv. NWGR-12002, NWGR-16003, GR-6 and GR-7 are considered as susceptible as compared to other cultivars.

**Relative water content(RWC)**

The osmotic adjustment is a commanding mechanism of preserving cellular hydration under water deficit stress. The RWC is a relevant tool for screening drought tolerance, it expresses the effect of osmotic adjustment in the drought condition.

The relative water content of 25 cultivars of rice leaves were analyzed at vegetative stage and the data are showed in Table 1. The relative water content was found knowingly differed among the rice cultivars and it was varied between 25.07 % to 58.06 %. However, significantly higher RWC was found in cultivar GAR-13 (58.06%), which was at par with cultivar NWGR-16026 (56.30%). Significantly lower RWC was recorded for cultivar NWGR-16019 (25.07%). The non-significant difference was recorded among NWGR-16009 (30.88 %), NWGR-16003 (32.24 %) and NWGR-15017 (32.46 %). The RWC is considered as the best integrated measurement of plant water status, and it represents the variations in water potential, turgor potential, and the osmotic adjustment (OA) of the plant (Bhushan *et al.*, 2007) though RWC vary due to differences in OA. Previously reported studies on different crop species like rice, wheat and foxtail millet have also shown wide genotypic variations in RWC under water stressed conditions (Garg *et al.* 2012, Jayaweera *et al.* 2016). This study showed highly tolerant cultivars have the ability to maintain high RWC. The susceptible cultivar may not maintain higher RWC. Thus it can be concluded that the cv. GAR-13 and NWGR-16026 are considered as tolerant and cultivar NWGR-16019 and NWGR-16009 are considered as susceptible as compared to all other cultivars in water deficit stress condition.

**Membrane injury and Membrane stability index**

Membrane stability index (MSI) is a physiological index widely used for the estimate of drought tolerance and it is the finest indicator for screening different varieties for water deficit stress. The higher

**Table 1: Effect of Water stress on morpho-physiological parameters**

Sr. No.	Cultivars	Moisture %	RWC %	MI %	MSI	Seedling length (cm)	Seedling dry wt. (gm)
1	NWGR-9081	60.60	34.54	19.74	0.80	17.50	0.020
2	NWGR-12002	50.90	37.07	9.26	0.91	17.40	0.017
3	NWGR-12015	77.86	35.03	9.76	0.90	15.20	0.021
4	NWGR-14071	68.78	47.17	64.35	0.36	20.53	0.062
5	NWGR-15017	62.57	32.46	47.73	0.52	17.43	0.018
6	NWGR-15024	66.36	39.66	54.31	0.46	17.93	0.035
7	NWGR-15038	64.97	49.60	72.83	0.27	22.93	0.041
8	NWGR-16003	54.62	32.24	14.69	0.85	16.93	0.018
9	NWGR-16009	62.47	30.88	26.23	0.74	12.30	0.010
10	NWGR-16019	56.50	25.07	72.93	0.27	17.10	0.025
11	NWGR-16022	72.27	44.85	41.77	0.58	14.20	0.027
12	NWGR-16026	71.63	56.30	3.66	0.96	17.77	0.043
13	NWGR-16031	56.75	34.63	52.05	0.48	20.03	0.039
14	NWGR-16033	64.45	38.58	62.55	0.37	18.57	0.052
15	Gurjari	62.52	34.66	77.78	0.22	20.40	0.042
16	Mahisagar	55.87	36.72	13.45	0.87	15.83	0.029
17	GAR-1	64.42	55.20	39.58	0.60	18.27	0.031
18	GAR-13	68.87	58.06	40.66	0.59	17.44	0.041
19	GR-4	58.82	40.44	27.92	0.72	14.47	0.015
20	GR-5	64.86	39.15	18.34	0.82	23.77	0.066
21	GR-6	54.99	51.25	15.27	0.85	19.63	0.059
22	GR-7	55.21	33.71	38.61	0.61	17.27	0.030
23	AAUDR-1	65.08	38.54	48.05	0.52	28.97	0.090
24	Dandi	56.38	54.93	35.78	0.64	16.57	0.032
25	SLR-51214	60.33	39.00	26.55	0.73	19.00	0.045
	S.Em. ±	1.69	0.91	1.56	0.02	0.68	0.001
	C.D.@ 5%	4.80	2.60	4.44	0.04	1.92	0.003
	C.V. %	4.70	3.88	7.24	4.31	6.39	5.503

and lower values of MI indicate that the susceptible and tolerant condition of cultivar. The membrane injury was analyzed for leaves of rice cultivars and the data are presented in Table 1. Significantly the highest and the lowest membrane injury were recorded for cultivars Gurjari (77.78 %) and NWGR-16026 (3.66 %), respectively. The minimum MI was recorded for NWGR 16026 (3.66 %) which, was followed by NWGR 12002 (9.26 %). NWGR 12015 (9.76 %) and Mahisagar (13.45 %). While, the non-significant difference was observed between cv. NWGR-16019 (72.93 %) & NWGR-15038 (72.83 %) and NWGR 12002 (9.26 %), NWGR 12015 (9.76 %). The result of membrane stability index indicated that MSI was varied from 0.22 to 0.96 of 25 rice cultivars (Table 1). Significantly maximum and minimum MSI was recorded for NWGR-16026 (0.96) and Gurjari (0.22), respectively. Whereas, the cv. GR-7(0.61), GAR-1 (0.60), GAR-13 (0.59) and

NWGR-16022 (0.58) were recorded significantly at par with each other. Our results are supported by Al-Ashkar *et al.* (2016). They observed that MSI reduced with increasing water stress and there were significant differences among the cultivars. The tolerant cultivars have higher membrane stability as compared to susceptible cultivars of wheat crop (Hasheminasab *et al.*, 2012). Thus from the above results it can be determined that the cv. NWGR-16026 and Gurjari are considered as a tolerant and susceptible respectively, as compared to all other cultivars in water deficit stress condition.

#### **Seedling length and seedling dry weight**

Seedling length was found significantly differed among the rice cultivars (Table 1). However, the seedling length of 25 cultivars of rice was recorded between 12.30 to 28.97 cm. significantly the highest seedling length was recorded for AAUDR-1 (28.97 cm). The lower seedling length was recorded for

cultivar NWGR-16009 (12.30 cm) which was at par with NWGR-16022 (14.20 cm). Seedling length was also affected by the water stress treatment and it is a one type of indicator which was used for the screening of various types of cultivars. Our results are in concurrence with Zain and his coworkers (2014). They have concluded that plant height reduced with increased duration of water stress cycle in rice. Sokoto and Muhammad (2014) have recorded the significant difference of plant height at 6, 9, 12 and 15 weeks after planting at tillering stage in rice. Seedling dry weight was found significantly different among the rice cultivars (Table 1). However, significantly the highest seedling dry weight was recorded in cultivar AAUDR-1 (0.09 gm). None of the cultivars were at par with AAUDR-1. Significantly lower seedling dry weight was recorded for cultivars NWGR-16009 (0.01 gm). Seedling dry weight is affected under the water deficit stress condition, which is a one type of indicator which is used for the screening of various types of cultivars. Our results are in accordance with Surapornpiboon *et al.* (2008). Among all morpho-physiological characters such as moisture, relative water content, membrane injury, membrane stability index, seedling length and seedling dry weight, the relative water content is a best criterion for plant water status. Then osmotic adjustment is a powerful mechanism of preserving cellular water under drought stress and relative water content expresses also the effect of osmotic adjustment in this respect. Hence, RWC is a suitable estimation of plant water status in terms of cellular hydration under the promising effect of both leaf water potential and osmotic adjustment. The GAR-13 and NWGR-16026 were registered with higher RWC, while NWGR-16009 and NWGR-16019 were registered with lower RWC. Hence, cv. GAR-13 and NWGR-16026 are used as tolerant and NWGR-16009 and NWGR-16019 are used as susceptible for further experiment.

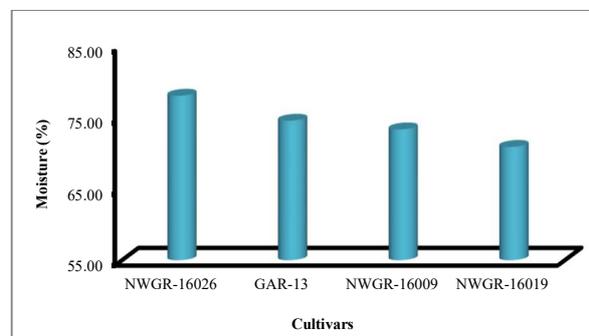
#### **Comparison of drought tolerant and drought susceptible cultivars in response to biochemical attributes at vegetative stage**

##### **Moisture**

Moisture is important to make photosynthesis possible. In the case of rice, higher moisture content around the plant is more important. If the plant has less amount of moisture the stomata will close with the result that photosynthesis process can be

inhibited. The maximum and minimum moisture content was recorded in NWGR-16026 (78.02%) and NWGR-16019 (70.88%), respectively. However, the non-significant difference was recorded between NWGR-16026 and GAR-13 as well as between NWGR-16019 and NWGR-16009 (Fig.1).

Our results are in agreement with the results of Kumari *et al.* (2019). They have analyzed ten rice cultivars and found that moisture content was varied between 43.70 to 49.90 %. Thus on the basis of moisture content it was concluded that the cv. NWGR-16026 is considered as a tolerant and cv. NWGR-16009 and NWGR-16019 both are considered susceptible.



**Figure 1: Effect of water stress on moisture content in rice cultivars**

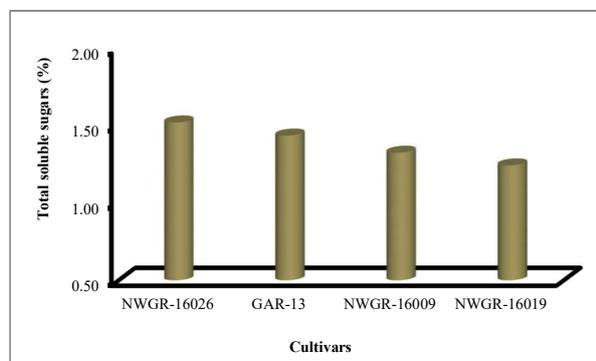
##### **Osmolytes**

The osmoregulation is one of the core mechanisms preserving turgor pressure in most plants against water loss from plant so, it causes plant to continue water absorption and preserve metabolic activities. Then osmotic adjustment is a powerful mechanism of conserving cellular hydration under drought stress.

##### **Total soluble sugars**

Mostly all soluble sugars play a dual role with respect to ROS, either promoting ROS production or contributing indirectly in ROS scavenging mechanisms. Under water stress, carbohydrate concentrations tended to rise as the level of stress increased, though the rise was greater in drought resistant than in the drought susceptible cultivars. The transformations in sugar concentrations between tolerant and susceptible genotypes were significant only for severe osmotic stress treatment. The total soluble sugars were significantly different among all rice cultivars. However, it was recorded

significantly higher and lower in cv. NWGR-16026 (1.52 %) and NWGR-16019 (1.24 %), respectively in Fig. 2(A).



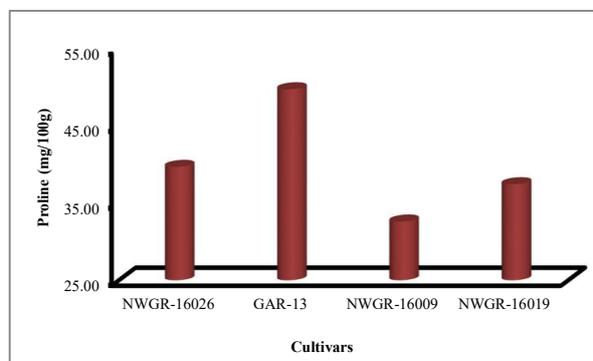
**Figure 2(A): Effect of water stress on total soluble sugars in rice cultivars**

Mostajeran and Rahimi-Eichi (2009) recorded that Zayandeh-Rood cultivar had significantly higher TSS content as compared to other cultivars in control condition. On the basis of their experiment they have concluded that tolerant cultivar has higher TSS content in control condition. Thus the present experimental data suggest that the cv. NWGR-16026 is considered as a tolerant and cv. NWGR-16019 is considered as a susceptible.

#### Proline

Proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures, scavenging free radicals and buffering cellular redox potential under stress conditions. Rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages. The proline content was varied between 32.59 to 49.69 mg/100g. However, the maximum and minimum proline accumulation was found in cv. GAR-13 (49.69 mg/100g) and NWGR-16009 (32.59 mg/100g), respectively in Fig. 2(B). Significantly NWGR-16026 & NWGR-16019 was found at par with each other. Various scientist has (Mostajeran and Rahimi-Eichi, 2009 and Shamsul *et al.*, 2012) suggested that proline plays three major roles during drought stress, i.e., as a metal chelator, an

antioxidative defense molecule and a signaling molecule. Al-Ashkar *et al.* (2016) studied six different rice genotypes and found that proline

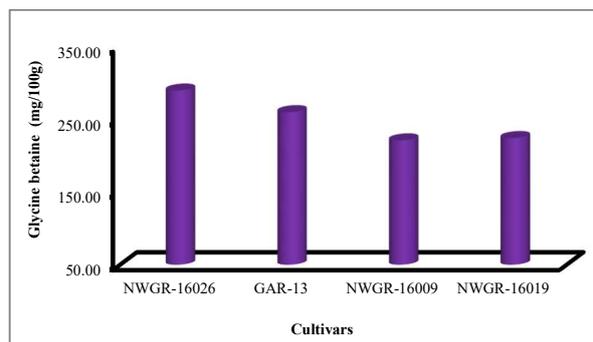


**Figure 2(B): Effect of water stress on proline content in rice cultivars**

content was significantly higher in IRAT 170 compared to other rice genotypes. Thus the result indicated that GAR-13 is considered as a tolerant and cultivar NWGR-16009 is considered as a susceptible.

#### Glycine betaine

Glycine betaine is rich mainly in chloroplast where it plays a vital role in adjustment and guard of thylakoid membrane, thereby conserving photosynthetic efficiency. In higher plants, glycine betaine is produced in chloroplast from serine via ethanolamine, choline and betaine aldehyde. The data presented in Fig. 2(C) recorded that significantly the highest glycine betaine content was found in cv. NWGR-16026 (289.54 mg/100g).



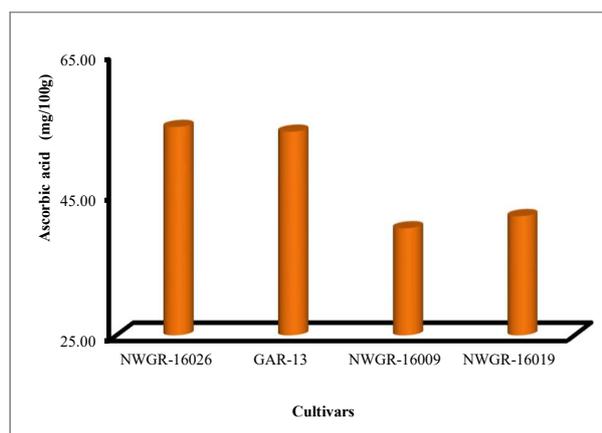
**Figure 2(C): Effect of water stress on glycine betaine content in rice cultivars**

Glycine betaine content was recorded lower in cv. NWGR-16009 (221.25 mg/100g) which was at par

with NWGR-16019 (224.41 mg/100g). Khattab and his co-workers (2014) observed that variety Giza 177 has higher glycine betaine content compared to variety IET 1444 in control condition. These results are also agreement with Kumari and Sairam (2013) in Foxtail millet. They observed that glycine betaine content was significantly higher in variety PBD-343 followed by HD-2987 and C-306 in control condition.

### Ascorbic acid

The ascorbic acid plays very crucial role as antioxidant in plant system. It influences many enzyme activities and reduces the damage caused by oxidative process. The ascorbic acid content was found higher in cultivar NWGR-16026 (54.64 mg/100g) which was at par with GAR-13 (53.97 mg/100g) in Fig. 2(D).



**Figure 2(D): Effect of water stress on ascorbic acid content in rice cultivars.**

Non-significant and minimum ascorbic acid content was recorded in cultivar NWGR-16009 (40.27 mg/100g) which was at par with NWGR-16019 (41.99 mg/100g). The results are in good concurrence with Hameed and his coworkers (2013). They have recorded that the tolerant variety FD-83 (275 µg/g) had meaningfully higher ascorbic acid as compared to sensitive variety Nesser (205 µg/g) in wheat under control condition. This higher accumulation of ascorbic acid by tolerant genotype may be in part responsible for its better performance under drought stress. Thus on the basis of ascorbic acid content it was concluded that the cultivars NWGR-16026 and GAR-13 both are considered as tolerant cultivar and NWGR-16009 and NWGR-16019 both are considered as susceptible cultivar.

### Conclusion

From the morpho-physiological characters the relative water content was found to be the best indicator to identify the tolerant or susceptible characteristics of crop. Cultivars NWGR-16026 and GAR-13 were found tolerant and NWGR-16009 and NWGR-16019 as susceptible. The NWGR-16026 having higher accumulation of all biochemical characters except proline. The maximum proline accumulation occurs in GAR-13. So, on the basis of all biochemical attributes we can also identify the tolerant or susceptible cultivars. These morpho-physiological and biochemical characters are very use full for the further studies to identify the tolerant or susceptible cultivars.

### Conflict of interest

The authors declare that they have no conflict of interest.

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