



# Evaluation of Physiological Efficiency in Sorghum (*Sorghum bicolor* (L.) Moench) Genotypes under Drought Stress

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

## Article Information

DOI: <https://doi.org/10.9734/ijpss/2024/v36i74735>

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here:

<https://www.sdiarticle5.com/review-history/117784>

Original Research Article

Received: 29/03/2024

Accepted: 02/06/2024

Published: 08/06/2024

## ABSTRACT

In an experiment conducted during the rabi season at Sorghum Improvement Project, M.P.K.V., Rahuri, Maharashtra during the period of 2016 to 2017. Present study to observed the physiological parameters under non stress and moisture stress condition with cross two genotypes RSV 1237 x RSV 1703. In this study of mean performance of the basic generation P1, P2, F1, F2, B1 and B2 of the cross RSV 1237 x RSV 1703 was observed various physiological parameters viz. photosynthetic rate, rate of transpiration, stomatal conductance, relative leaf water content, SPAD reading, total chlorophyll content and chlorophyll stability index. The significantly highest

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Cite as: A. Tambe, Snehal, Jondhale, A. S, and B.D. Wagh. 2024. "Evaluation of Physiological Efficiency in Sorghum (*Sorghum Bicolor* (L.) Moench) Genotypes under Drought Stress". *International Journal of Plant & Soil Science* 36 (7):309-16. <https://doi.org/10.9734/ijpss/2024/v36i74735>.

photosynthesis rate recorded for B2 generation 24.39  $\mu$  mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> under non stress condition and 20.01  $\mu$  mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> moisture stress condition. The highest transpiration rate (2.94 and 1.98 mmole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>) recorded for parent P<sub>1</sub> (RSV 1237) was under non stress and moisture stress condition, respectively. The F<sub>2</sub> generation was recorded the lowest transpiration rate (2.51 and 1.77 mmole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>) under non stress and moisture stress condition, respectively. In the F<sub>1</sub> and B<sub>2</sub> generation recorded significantly the highest mean of stomatal conductance (0.24 and 0.18 mole m<sup>-2</sup> s<sup>-1</sup>) under non stress condition and moisture stress condition, respectively. The segregating generation B<sub>2</sub> recorded significantly the highest SPAD reading (55.43) under non stress condition and B<sub>2</sub> generation was recorded highest SPAD reading (44.41) in moisture stress condition. The B<sub>2</sub> generation recorded the highest total chlorophyll content (3.31 and 2.07 %) under non stress and moisture stress condition, respectively. Overall experimental study to observed physiological parameters of sorghum cross two genotype (RSV 1237 x RSV 1703) directly affected in growth and grain yield under non-stress and moisture stress condition.

**Keywords:** Chlorophyll content; non-Stress; RSV 1237; SPAD; physiological parameters.

## 1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is essential food crop and it is originated in Africa. In India, Sorghum is a major staple cereals crop and vernacular is called as Jowar belong to the grass family Poaceae. During rabi season (October to February) is a mainly cultivated period in India. Globally, India is a second largest production with 4.81million tonnes during 2021-22 in sorghum crop. In India, Sorghum production in 2022-23 was 13.37lakh hectares (33.04 lakh acres) and Maharashtra 1.45 lakh ha (3.58 lakh acres). Jangid and Dwivedi [1] reported that drought is a major problem in India, which directly affects agriculture production. Overall, two to third parts rainfall less than 1000mm as compared a total geographical area of India. In India has only 40% water use efficiency of total existing irrigation as per the statistical review. Sorghum crop is efficiently grow in minimum adverse climatic condition with very low amount of water condition. However, the biotic and abiotic stresses is indirectly or directly effects on sorghum yield potential in arid and semi-arid regions mentioned by Kachare [2].

Drought tolerant is a complex trait, expression of which depends on action and interaction of different morphological, physiological and biochemical characters. The management practices and breeding efforts is important aspect for better understanding of effects of drought on sorghum crop. Drought tolerance mechanism can be studied based on the interpretation of relationship between leaf structure function and stress tolerance reported by Tambe et al.,[3] and [4]. The drought and high temperature stresses are the major abiotic stresses to reduce sorghum

yield potential. Water stress is one of the significant environmental factors affecting crop growth and productivity worldwide observed by Eggen et al., [5]. In tropical regions drought stress has become as a serious challenge for crop growth and development and the reproductive stages are very sensitive and serious condition in sorghum crop mentioned by Xu et al.,[6] and Prasad et al.,[7]. Drought stress decreases in physiological parameters like stomatal conductance, carbon assimilation and cell turgor and moisture stress also effects of leaves and reduction in leaf area and overall reducing crop normal growth and yield reported by Earl, et al.,[8] and Djanaguiraman et al.,[9]. High temperature stress beyond the physiological optimum that disturbs the normal growth and development was suggested by IPCC [10].

Kadam et al.,[11] reported that soil moisture content decreased gradually from sowing to harvesting and it was negatively correlated with grain yield. However, some other physiological parameters as like photosynthesis rate, rate of transpiration, stomatal conductance, relative leaf water content, SPAD reading and total chlorophyll content or index are also effects on crops growth and yields. Therefore, above mentioned facts indicate that the irregular water condition, water temperature and physiological properties are associated with effects on sorghum yield. For that reason, present study to understanding for mechanism that are directly and indirectly affected by physiological characters in sorghum. Moreover, it will also find out that physiological characters impressive parameter to improve and utilization in the sorghum variety or hybrids of breeding programs.

## 2. MATERIALS AND METHODS

The present investigation was conducted at Sorghum Improvement Project, M.P.K.V., Rahuri, Maharashtra during the year rabi 2016-17 under non stress and moisture stress condition. Present study of mean performance of the basic generations P1, P2, F1, F2, B1 and B2 of the two genotypes cross RSV 1237 x RSV 1703 showed physiological parameters viz. photosynthetic rate, rate of transpiration, stomatal conductance, relative leaf water content, SPAD reading, total chlorophyll content and chlorophyll stability index was conducted at the time of 50% flowering and harvesting. Following are the methods used for physiological parameters investigation.

### 2.1 Photosynthetic Rate, Transpiration Rate and Stomatal Conductance

The net photosynthetic rate ( $\mu$  mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>), transpiration rate (mmole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>) and stomatal conductance (mole m<sup>-2</sup> s<sup>-1</sup>) were measured using Infra-Red Gas Analyzer (IRGA; Model Portable Photosynthesis System LI6400, LI-COR® Inc, Lincoln, Nebraska, USA). The transpiration rate and stomatal conductance were measured continuously monitoring H<sub>2</sub>O of the air entering and existing in the IRGA head space chamber and the measurements were made at midday, between 11:30 and 12:00 eastern day time (1400–1800 mmole m<sup>-2</sup> s<sup>-1</sup> PPF), on top fully expanded leaf blades. The flow-rate of air in the sample line was adjusted to 500  $\mu$ mol s<sup>-1</sup>.

### 2.2 Relative Leaf Water Content (%)

Relative leaf water content was estimated as per Barrs and Weatherly [12] at 50 percent flowering. Twenty leaf discs of third fully expanded leaf from the top were collected and weighted on an electronic balance, and fresh weight was determined. The weighted leaf discs were floated in a Petri-dish containing distilled water for four hours and subsequently blotted gently and weighted again, which was referred to as the turgid weight. After taking turgid weight, the leaf discs were oven dried at 80°C for 48 hours and dry weight was recorded. The relative leaf water content (RLWC) was calculated in percentage by using the following formula.

$$\text{Relative Leaf water content (\%)} = \frac{\text{Fresh weight (g)} - \text{dry weight (g)}}{\text{Turgid weight (g)} - \text{dry weight (g)}} \times 100$$

### 2.3 SPAD Reading

SPAD (Soil Plant Analytical Development) chlorophyll meter reading (SPAD 502; Minolta Company Ltd) measures the greenness or relative chlorophyll content of the leaves. Measurements were taken at three points of each leaf (upper, middle and lower parts). Average of these three readings was considered as SPAD reading of the leaf. SPAD reading was carried out at 50% flowering in the third leaf to the top of the plant. The readings were taken between 10.00 to 12.00 hours of the day.

### 2.4 Chlorophyll Content (mg/g)

Total chlorophyll content was determined by following DMSO (dimethyl sulfoxide) method of Hiscox and Israeltam [13] at 50 percent flowering. Third fully expanded leaf from the top was brought from the field in polyethylene bags kept in an ice box and was cut in to small pieces, known weight of fresh leaf sample was macerated in a mortar and pestle and extracted with 7.0 ml of DMSO. The test tube incubated at 65°C for 30 minutes, leaf residue was removed by decanting the solution and final volume was made in 10 ml with DMSO. The absorbance of the extract was measured at 645 and 663 nm in a UV-vis spectrophotometer (Elico, SL-159) and a blank was run using DMSO. The total chlorophyll content was calculated by using the following formula and expressed in mg g<sup>-1</sup> fresh weight.

$$\text{Total chlorophyll} = 20.2 (A_{645}) + 8.02 (A_{663}) \times \frac{V}{1000 \times W}$$

Where,

A<sub>645</sub> = Absorbance of the extract at 645 nm

A<sub>663</sub> = Absorbance of the extract at 663 nm

W = Fresh weight of the sample (g)

V = Final volume of the chlorophyll extract (ml).

### 2.5 Chlorophyll Stability Index (CSI)

The chlorophyll stability index was computed by using the methodology proposed by Arnon [13].

$$\Delta R = \text{Reading without heating} - \text{Reading after heating at } 56^{\circ}\text{C.}$$

Where,  $\Delta R$  = CSI

### 3. RESULTS AND DISCUSSION

The present study of mean performance of the basic generations P1, P2, F1, F2, B1 and B2 of the cross RSV 1237 x RSV 1703 showed physiological parameters viz. photosynthetic rate, rate of transpiration, stomatal conductance, relative leaf water content, SPAD reading, total chlorophyll content, chlorophyll stability index are presented in following Tables 1 to 4.

#### 3.1 Photosynthesis Rate ( $\mu$ mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>)

In the present investigation for cross RSV 1237 x RSV 1703 the photosynthesis rate ranged from 19.1 to 24.39 with the mean of 21.35  $\mu$  mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> and 15.97 to 20.01 with the mean of 18.49  $\mu$  mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> under non stress and moisture stress condition, respectively. The B2 generation recorded significantly highest photosynthesis rate (24.39 and 20.01  $\mu$  mole CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>) while under non stress and moisture stress condition, respectively. Amongst the different generations, the percent reduction in photosynthesis rate varied from as low as 9.32 per cent in B1 generation to as high as 17.95 per cent in B2 generation. The rate of photosynthesis is an important physiological parameter and consequently the yield. The high yielding generation possessed higher rate of photosynthesis indicating the importance in determining the productivity. Similar findings were also reported by Narkhede *et al.*, [15] and Gadakh *et al.* [16].

#### 3.2 Rate of Transpiration (mole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>)

The stressed leaves showed decline in transpiration rate over non stressed leaves of different rabi sorghum generations. The mean transpiration rate under non stress condition was 2.85 m mole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup> and was reduced to 1.92 mmole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup> under moisture stress condition. The parent P1 (RSV 1237) recorded highest transpiration rate (2.94 and 1.98 mmole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>) under non stress and moisture stress condition, respectively. The F2 generation was recorded the lowest transpiration rate (2.51 and 1.77 mmole H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>) under moisture stress condition, respectively. Amongst the different generations, the per cent reduction in transpiration rate varied from as low as 28.46 per cent in P2 (RSV 1703) to as high as 36.07 per cent in B2. Transpiration rate is an important bio-physical trait which helped to gas exchange. It

showed that the stressed leaves showed decline in transpiration rate over non stressed leaves of different rabi sorghum generations (Table 1). The variations in transpiration rate were also reported by Narkhede *et al.* [15] and Shinde *et al.* [17].

#### 3.3 Stomatal Conductance (mole m<sup>-2</sup>s<sup>-1</sup>)

The stomatal conductance ranged from 0.20 to 0.24 and 0.15 to 0.18 mole m<sup>-2</sup> s<sup>-1</sup> under non stress and moisture stress condition, respectively. The F1 and B2 generation recorded significantly the highest mean of stomatal conductance (0.24 and 0.18 mole m<sup>-2</sup> s<sup>-1</sup>) under non stress condition and moisture stress condition, respectively. Among the segregating generation, the per cent reduction in stomatal conductance ranged from low 21.74 % in B1 to high as 33.33 % in B2 generation. The rate of stomatal conductance (Table 2).was observed more in non stress than that in moisture stress condition. The data also indicated that the generations differ significantly in respect of stomatal conductance rate under non-stress stress and moisture stress condition. The high yielding generations possessed higher rate of stomatal conductance indicating the importance in determining the productivity. A similar result was reported by Gadakh *et al.* [16].

#### 3.4 Relative Leaf Water Content (RLWC) (%)

The mean relative leaf water content of the parents and different generations of sorghum under non stress was 84.19 % and it reduced to 74.42 % under moisture stress condition. The parent P2 (RSV 1703) recorded significantly the highest relative leaf water content (81.74 and 71.74 %) and in different generations highest relative leaf water content was recorded in F1 and B2 generations (84.19 and 74.42 and 74.22 %) under non stress and moisture stress condition, respectively. Among the segregating generation, the per cent reduction in RLWC varied from as low as 9.90 % in B1 generation to as high as 12.75 % in F2 generation.

The control of stomatal aperture is one of the major mechanism by which plant regulates water status. Water status of a plant is a measure of drought tolerance and it could be conveniently studied in term of relative leaf water content (RLWC). Relative leaf water potential is very important phenomenon as retention of water in the leaf under moisture stress condition is a major indication of drought tolerance. The



**Table 4. Chlorophyll Stability Index in the leaves of different generations of rabi sorghum cross RSV 1237 x RSV 1703 under non stress and moisture stress condition**

Sr. No.	Generations	Chlorophyll Stability Index (%)		
		Non stress	Moisture stress	Per cent decrease
1	P <sub>1</sub>	26.92	23.89	11.26
2	P <sub>2</sub>	23.50	20.43	13.06
3	F <sub>1</sub>	34.34	31.67	7.78
4	F <sub>2</sub>	30.74	27.77	9.66
5	B <sub>1</sub>	34.49	32.08	6.99
6	B <sub>2</sub>	30.92	30.34	1.88
<b>Mean</b>		<b>30.15</b>	<b>27.70</b>	
<b>SE±</b>		<b>0.74</b>	<b>0.77</b>	
<b>CD at 5%</b>		<b>2.32</b>	<b>2.41</b>	

CD= critical difference

SE= standard error

### 3.5 SPAD Reading

The mean SPAD reading at 50% flowering was recorded 53.80 and 42.47 under non stress and moisture stress condition, respectively. The segregating generation B2 recorded significantly the highest SPAD reading (55.43) under non stress condition and B2 generation was recorded highest SPAD reading (44.41) in moisture stress condition. Amongst the segregating generations, the per cent reduction in SPAD reading varied from as low as 13.41 per cent in B1 generation to as high as 28.76 per cent in B1 generation (Table.3). The variation in SPAD chlorophyll reading is depends on greenness of leaves. The stay green genotypes recorded higher reading than non stay green. Similar result was also reported by Subudhi *et al.* [20].

### 3.6 Total Chlorophyll Content (mg g-1FW)

The mean total chlorophyll content at 50 per cent flowering was estimated 3.03 mg g-1FW and 1.87 mg g-1FW under non stress and moisture stress condition, respectively. Amongst the parents and different generations, the percent reduction in total chlorophyll content varied from as low as 37.05 per cent in B1 generation to as high as 39.46 per cent in P1 ( RSV 1237). The B2 generation recorded the highest total chlorophyll content (3.31 and 2.07 %) under non stress and moisture stress condition, respectively. Mean total chlorophyll content was less in water stress condition than non stress condition which may be due to adverse effect of water stress on plant metabolic processes (Table 3). According to Vinita *et al.*, [21] and Deshmukh *et al.*, [22] water stress leads to decline in total chlorophyll content. Their findings were in agreement with the observations recorded in the present investigation.

### 3.7 Chlorophyll Stability Index (%)

The mean chlorophyll stability index at 50 % flowering was 30.15 and 30.34 % under non stress and moisture stress condition, respectively. The parent B2 generation recorded the highest chlorophyll stability index (34.49 and 32.08%) under non stress and moisture stress condition, respectively. The parent P2 (RSV 1703) recorded significantly the lowest chlorophyll stability index (23.50 and 20.43%) under non stress and moisture stress condition respectively (Table 4). Amongst the different generations, the percent reduction in chlorophyll stability index varied from as low as 1.88 per cent in B2 generation to as high as 13.06 per cent in P2. Chlorophyll stability is a function of temperature and found to correlate with drought tolerance. Chlorophyll stability index is a measure of integrity of membrane or heat stability of pigments under stress condition reported by Koleyoreas [23]. The chlorophyll destruction commences rapid at control temperature of 55 to 56°C. Chlorophyll stability is an important trait which indicate an ability of genotype to survive under stress condition [24-26]. The lower chlorophyll stability index is desirable parameter for drought tolerance in *rabi* sorghum genotypes.

## 4. CONCLUSION

In the present study, the B<sub>2</sub> generation performed well under moisture stress condition by maintaining high photosynthetic rate, relative leaf water content, SPAD reading, and stomatal conductance, total chlorophyll content. Similarly, chlorophyll stability index and transpiration rate were also higher. Therefore, the B<sub>2</sub> generation could be effectively utilized in breeding program for the development of drought tolerant genotypes.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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