

Original Research Article

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Behavior of Sorghum Cultivars under Decreasing Levels of Soil Moisture Condition

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is one among the five major cereals of the world, being grown extensively in tropical and sub-tropical climate. In the present investigation six existing and recently released cultivars of Sorghum were taken to test their water stress tolerance. These cultivars are presently used extensively in the commercial production in Indian farmers. The pot trial was laid out in Factorial Completely Randomized Design with two replications involving with six genotypes viz., Phule Yashoda, Phule Revati, Phule Chitra, Phule Vasudha, Phule Anuradha and Phule Maulee and four moisture regimes (25%, 50%, 75% and > 90 % of field capacity). The effect of moisture stress was assessed using various physiological parameters. Among the six genotypes, the Phule Yashoda and Phule Revati showed significantly maximum mean chlorophyll a, b and total, RLWC, rate of photosynthesis, rate of transpiration, A-PAR, stomatal conductance and resistance at 25 % of F.C. It could be inferred that the genotype Phule Chitra and Phule Maulee are more suited under limited soil moisture condition (which moisture regime). While, the genotype RSV-1006 and Phule Yashoda found well suited for medium soil for stress as well as non-stress condition. Irrespective of moisture regime Phule Yashoda and RSV-1006 found to better than rest of genotypes based on physiological parameters. However, Phule Chitra and Phule Maulee had some physiological parameters which suited under water stress condition (M₁). The resilience to drought shown by recent varieties is a good premise for their use in areas subjected to dry spells.

Keywords

Sorghum,
Physiological
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Introduction

Sorghum (*Sorghum bicolor* L.) is grown for food, feed and industrial purposes and cultivation has been the heart of dry land agriculture from years together. It is considered more tolerant to many stresses, including heat, drought, salinity and flooding

as compared to other cereal crops (Ejeta and Knoll, 2007). Sorghum is drought resistant because of its ability to minimize tissue water loss (Rao and Sinha, 1990). These and other specialized physiological features, make it drought resistant species (Arnon, 1972). In Maharashtra *rabi* sorghum is predominantly grown in the rainfed condition, on residual soil

moisture as drought is major problem in *rabi* sorghum. Though sorghum possess excellent drought resistance, as compared to the most other field crops generally it suffers from severe moisture stress during the stages of growth and development (Kebede *et al.*, 2001). Thus situation totally disturbs *rabi* production levels especially on light and medium soils where grain and fodder yield get drastically reduced.

Water stress has emerged as one of the most severe stresses faced by the sustainable crop productivity all over the world (Tahir and Mehdi, 2001; Sher *et al.*, 2013). Drought adversely affects some of the important physiological, biophysical and biochemical processes of the plants, like chlorophyll distribution, enzymatic activities and protein synthesis. In view of this, it is necessary to identify the plant factors which extract more moisture and render a genotype more drought tolerance with maximum productivity.

Screening varieties for relative drought tolerance has been attempted by various workers using different physiological and biochemical parameters. Giles *et al.*, (1976) reported that bundle sheath chloroplasts in sorghum leaves were much affected during increased moisture stress.

It has been documented that root growth, leaf area development, synthesis of epicuticular wax and osmotic adjustment under stress are some of the guidelines in characterizing the genotypes for stress tolerance in sorghum. Barrs and Weatherly (1962) developed the concept of relative water content and the reduction in relative water content under stress has been used as a measure of drought tolerance by several workers. Similarly, stomatal resistance is also one of the important adaptive mechanisms under drought conditions. The response of stomata to moisture stress by way of increase in stomatal

resistance on imposition of stress and decrease upon rewatering varies from genotype to genotype. Yoshida *et al.*, (1972) used this as a parameter to screen the genotypes for drought tolerance in the rice. In the present investigation, some of the existing and recently released cultivars of sorghum were studied to test their water stress tolerance.

Materials and Methods

Experimental design

The experiment was carried out at Sorghum Improvement Project, M.P.K.V., Rahuri, Dist. Ahmednagar during *rabi* 2009-10. Experiment was laid out in Factorial Randomized Block Design (FRBD). In which mainly 24 treatment combinations (6 cultivars x 4 moisture levels) were involved, of which first factor was moisture regimes i.e. M₁ (25 % of F.C.), M₂ (50 % of F.C.) and M₃ (75 % of F.C.) and M₄ (> 90 % of F.C.) and second factor was genotypes i.e. V₁ (Phule Yashoda), V₂ (RSV-1006), V₃ (Phule Chitra), V₄ (Phule Vasudha), V₅ (Phule Anuradha) and V₆ (Phule Maulee).

Chlorophyll stability index

The Chlorophyll stability index (CSI) was computed by using the methodology proposed by Arnon (1949). Two glass vials of 0.5 g sample in respective tubes, with 50 ml distilled water was taken. One subjected to heat in water bath at 56 + 1^oC for 30 min while other was kept as control. Then leaves were ground in mortar for 5 min. with 50 ml, 80 % acetone. It was filtered through whatman No. 1 filter paper and examined immediately for light absorption at 652 nm wavelength. Other leaf samples were then estimated for chlorophyll content without heating, simultaneously and the light absorption was measured 652 nm. The difference between two readings (Readings without heating – Reading after heating at 56^oC) is CSI.

Chlorophyll content a, b and total

Total chlorophyll, Chl a and Chl b contents were determined following the method of Arnon (1949) in field condition. Third fully opened leaf from the top was used for chlorophyll estimation. The leaf sample of 0.2 g from each plot was homogenized by adding sufficient pure acetone in porcelain mortar. The homogenized material was filtered through a Whatman No. 1 filter paper into 25 ml volumetric flask.

The extraction was repeated twice by using 80 per cent acetone and the final volume was made upto 25 ml using 80 % acetone. The absorbance of the leaf extract was measured at 645 and 663 nm in spectrophotometer. The Chl a, Chl b and total chlorophyll contents were calculated by using the following formula and expressed in mg g⁻¹ fresh weight.

Chl. a = $12.7 (A_{663}) - 2.69 (A_{645}) \times 25 / (1000 \times w)$,

Chl. b = $22.9 (A_{645}) - 4.68 (A_{663}) \times 25 / (1000 \times w)$,

Total chlorophyll (mg/g fresh weight) = $[(O.D. 652 \times 1000) / 34.5] [25 \times 1000 \times W]$

Relative leaf water content

Relative water content (RLWC) was estimated following the procedure of Barrs and Weatherly (1962) at 30, 60 and 90 DAS. Twenty leaf discs 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-disc 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 80⁰C for 48 hours and dry weight was recorded. The

RWC (%) was calculated by using the formula,

$RWC (\%) = \frac{Fresh\ weight\ (g) - Dry\ weight\ (g)}{Turgid\ weight\ (g) - Dry\ weight\ (g)} \times 100$.

Stomatal frequency (Abaxial and Adaxial)

Stomatal frequency was measured at 10 days after 50 per cent flowering stages of the crop for this purpose sufficient colourless thermocol and xylene solution was applied on both the adaxial and abaxial surface of the freshly harvested second leaf from the top of selected plants during bright sunshine hours.

The thermocol, xylene was applied at four different spots, rubbed gently to form a thin and uniform layer on the leaf surface and there after dried for about 1-2 hours. The peelings were taken out after drying which as stomatal impression and these peelings were observed under microscope. Stomatal frequency was calculated by counting the number of stomata per microscopic field under high power (40 x) of the microscope and it was expressed as number of stomata per cm² using the formula, Stomatal frequency = $(1 \times A) / 0.0068$. Where, 'A' is the number of stomata per microscopic field 0.068 is the area of microscopic field under 40 X magnification.

Canopy temperature depression

Calculating difference of a canopy temperature from air true temperature. Infrared thermometer was used to measure canopy temperature. If value is negative then canopy temperature was lower than air temperature which indicates sufficient water in plant. Resistance genotypes had low leaf temperature. If value is positive then canopy temperature is higher than air temperature which indicates moderate to severe water stress and stomata have began to close or are

closed. When the canopy temperature equals to air temperature irrigation needed for optimum yield and water use efficiency.

Drought Susceptibility Index (DSI)

The drought susceptibility index was calculated by using formula suggested by Fischer (1968) as below. $S = (1 - Y/YP) / DI$, where, S = Drought susceptibility index, DI = Drought index, Y = Yield in water stress condition, YP = Yield in irrigated condition. Drought index is calculated as $1 - (Xs/ Xp)$, Where, Xs = Mean yield of all genotypes in water stress condition and Xp = Mean yield of all genotypes in irrigated condition.

Portable Infra-Red Gas Analyser (IRGA)

For measuring the photosynthesis rates in the pot conditions a portable IRGA has been developed in the recent years this can be used for measuring the rate of photosynthesis (CO₂ fixation) of crop plants in the field condition. The rate of transpiration (\square moles m⁻²s⁻¹), Rate of photosynthesis (μ moles m⁻² s⁻¹), Leaf temperature (°C) Photosynthetically active radiation (μ moles m⁻² s⁻¹), Stomatal conductance (mmoles H₂O m⁻²s⁻¹) Stomatal resistance (mmoles H₂O m⁻²s⁻¹) were recorded at 50% flowering. For measurement an intact leaf of crop plant is clamped into the chamber and from 2 to 10 observations of the measurable parameters was logged. The time between observations can be fixed to 20 seconds (2 seconds on observation upto total 10 observation). During the measurements of photosynthetic rate the data about leaf temperature, chamber RH and CO₂ fixation rate (CFR/PE) PAR and the stomatal resistance, is computed and stored in the memory. The data on the stomatal resistance and apparent photosynthesis rate for each part of observation were logged. Also summary statistics were computed on all the variables. Finally transpiration rate and initial value of CO₂ (internal to the leaf) were optionally

calculated. After examining the data on the system conserve the data stored in the internal memory and next observation was taken following the same procedure.

Statistical analysis

Fisher's method of analysis of analysis of the data and interpretation of the results as suggested by Panse and Sukatme (1967). The level of significance used in 'F' and 't' test was P = a0.05. Critical difference (CD) values were calculated at 5 per cent probability level, wherever 'F' test was significant. Correlation analysis was carried out to study the nature and degree of relationship between growth parameters, as well as morphophysical, biochemical parameters and yield and yield components, following the method of Panse and Sukhatme (1985).

Results and Discussion

Sorghum cultivation has been the heart of dry land agriculture from years together. Sorghum has twice the number of secondary roots than maize and also only half the leaf area exposed for evaporation than maize. Being a C₄ plant, it can utilize sunlight and water very efficiently. The genotype V₃ (Phule Chitra) was recorded significantly lowest mean chlorophyll stability index (0.24). Chlorophyll degradation is one of the consequences of water stress that may result from photo inhibition and photobleaching. These finding confirmed the earlier report of Lim *et al.*, (2007). The genotype V₂ (RSV-1006) was recorded significantly superior genotype with respect of total chlorophyll (2.97 mg g⁻¹ fr.wt.). Absorbed photosynthetic active radiation under moisture stress conditions. Since chlorophyll - a is essential for the conservation of light ending into chemical emerging (carbohydrates) and consists major portion of both the pigment PS-I and PS-II (Table 1).

Table.1 Physiological parameters as influenced by genotypes, moisture regimes and their interactions of flowering stage in sorghum at pot condition

Genotypes	CSI	Chlo-a	Chlo-b	Total chlo.	RLW	Stomatal frequency		Canopy Temp.	Photosynthetic rate	Transpiration rate	A-PAR	Stomatal conductance	Stomatal resistance	Leaf Temp.
						abaxial surface	adaxial surface							
M₁	0.34	1.84	0.68	2.52	35.37	157.40	136.74	-3.32	20.86	1.30	344.11	16.17	0.062	-4.78
M₂	0.38	1.84	0.69	2.53	37.44	160.73	140.27	-1.21	21.71	1.32	377.09	17.63	0.057	-4.31
M₃	0.42	2.01	0.70	2.71	41.45	164.76	142.91	-0.71	23.67	1.42	392.94	21.34	0.048	-3.50
M₄	0.46	2.02	0.71	2.73	42.70	169.70	146.53	-0.41	25.23	1.50	406.21	23.49	0.043	-1.26
S.E. ±	0.001	0.001	0.001	0.001	0.394	0.399	0.804	0.112	0.358	0.003	2.309	0.258	0.003	0.171
CD at 5 %	0.002	0.002	0.002	0.003	1.153	1.171	2.353	0.327	1.047	0.009	6.760	0.757	NS	0.501
Genotypes														
V₁	0.46	2.05	0.78	2.83	36.78	182.73	163.10	-1.29	25.45	1.39	419.11	21.03	0.049	-3.18
V₂	0.59	2.16	0.81	2.97	33.02	179.32	159.75	-1.05	26.82	1.56	405.15	21.85	0.047	-2.85
V₃	0.24	2.00	0.68	2.68	43.54	154.14	135.46	-1.51	21.60	1.45	373.27	17.88	0.057	-3.77
V₄	0.45	1.97	0.65	2.61	39.17	167.11	142.16	-1.46	25.34	1.31	377.80	20.49	0.049	-3.59
V₅	0.26	1.88	0.63	2.49	41.01	149.44	126.36	-1.43	18.27	1.34	338.14	19.72	0.052	-3.31
V₆	0.39	1.53	0.62	2.15	41.91	146.13	122.83	-1.73	19.74	1.28	367.06	16.98	0.060	-4.08
S.E. ±	0.002	0.001	0.001	0.001	0.482	0.489	0.984	0.137	0.438	0.004	2.828	0.316	0.0009	0.209
CD at 5 %	0.002	0.003	0.002	0.004	1.412	1.433	2.882	0.401	1.282	0.012	8.280	0.927	0.0027	0.614
Interaction														
S.E. ±	0.002	0.002	0.002	0.002	0.965	0.979	1.969	0.274	0.876	0.008	5.656	0.633	0.002	0.419
CD at 5 %	0.007	0.008	0.006	0.11	3.685	3.740	7.520	N.S	3.344	0.031	21.60	2.420	0.006	NS

Candido *et al.*, (2009) reported decrease in total chlorophyll at vegetative were 38, 28 and 60 percent, reproductive and maturation stages, respectively. The chlorophyll - a, b and total chlorophyll and carotenoids significantly decreased in all stages after the water restriction.

The genotype V₃ (Phule Chitra) was found significantly maximum relative leaf water content (43.54 %). A dramatic decline in relative water content (RWC) and leaf water potential has been reported in various plants which were imposed to water deficit conditions. Similar results were also reported by Ahmadi and Siosemardeh (2005), Pirdashti *et al.*, (2009).

The reduction in leaf RWC has been induced by the water deficiency in soil as a consequence of water loss via the stomata and water stress reported by Pirdashti *et al.*, (2009). Studies have disclosed that the decline in RWC lead to a reduction in leaf photosynthetic activity under water stress. Similar results were also reported Siddique *et al.*, (2000) and Ahmadi and Siosemarideh (2005).

Furthermore, positive relation between grain yields with RWC has been reported under various levels of water stress by Azizie-Chakerchaman *et al.*, (2009). The genotype V₁ (Phule Yashoda) was recorded significantly highest stomatal frequency of abaxial of leaves (182.73 mm²) as compared to rest of genotypes, while the genotype V₆ (Phule Maulee) was observed significantly minimum stomatal frequency of abaxial of leaves (146.13 mm²) at pot condition than among rest of genotypes.

Similar result was found in case of mean stomatal frequency of adaxial of leaf surface. The total stomata number per plant were positively correlated with leaf area, stomatal

frequency seemed to be the desirable character for higher productivity and did not affect growth rate of plant in sorghum. Similar results were also reported by Surwenshi *et al.*, (2007), Shawesh *et al.*, (1985). The significantly highest canopy temperature depression (-1.05 °C) was recorded in genotype V₂ (RSV-1006) as compared with rest of genotypes while the genotype V₆ (Phule Maulee) was observed significantly lowest canopy temperature depression (1.73°C). Singh *et al.*, (1990) reported a negative correlation between dry matter and transpiration cooling (canopy temperature minimum air temperature).

The genotype V₂ (RSV-1006) was noticed significantly maximum in mean drought susceptible index (0.75) than rest of genotypes. However, the genotype V₁ (Phule Yashoda) was found significantly minimum in mean drought susceptible index (0.22). Birari *et al.*, (1995) reported drought index, DSI and drought tolerance index were used to determine degree of resistance of genotypes to drought. Lower drought tolerance index was found to be more susceptible variety to drought.

Lower drought susceptibility index higher in the drought tolerance. These findings are in conformity with Golabadi *et al.*, (2006) opined that larger value of tolerance index and stress susceptibility index show relatively more sensitiveness to stress and Guttieri *et al.*, (2001) noticed stress susceptibility index criterion and suggested that stress susceptibility more than one indicates above average susceptibility to drought stress. The genotype V₂ (RSV-1006) had the maximum photosynthetic rate (26.82 μ mol m⁻²s⁻¹) with compared rest of treatments.

The present findings are in agreement with Massacci *et al.*, (1996) observed progressive reduction of photosynthesis proportionately

with decreasing water potential. Both stomatal and non stomatal limitations cause reductions in photosynthesis during drought reported by Zhou *et al.*, (2007). The maximum transpiration rate ($1.56 \text{ m mole m}^{-2} \text{ s}^{-1}$) was recorded in genotype V₂ (RSV-1006). However the genotype V₆ (Phule Maulee) was found significantly lowest transpiration rate ($1.28 \text{ m mole m}^{-2} \text{ s}^{-1}$) with the rest of genotypes. The present investigations are supported by Yadav *et al.*, (1991) observed that higher rate of stomatal resistance and lower rate of transpiration can be used for screening genotypes for drought clearance. The genotype V₁ (Phule Yashoda) was recorded significantly highest absorbed photosynthetic active radiation ($419.11 \mu\text{mole m}^{-2} \text{ s}^{-1}$).

The genotypes V₂ (RSV-1006) was recorded significantly highest mean stomatal conductance ($21.85 \text{ m mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). The genotype V₆ (Phule Maulee) had significantly superior in mean stomatal resistance ($0.060 \text{ m mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than rest of genotypes. Treatment V₆ (Phule Maulee) was found significantly lowest in mean leaf temperature depression (-4.08°C). While, significantly highest mean leaf temperature depression (-2.85°C) was found in genotype V₂ (RSV-1006). These results coincided with Singh and Kanemasu (1983) who reported that average leaf temperature on non-irrigated pearl millet genotypes was higher (34.1 to 36.8°C) than irrigated plants of the same genotypes was higher (34.1 to 36.8°C) than irrigated plants of the same genotypes. This was also supported by Verma and Easton (1986) in sorghum and Khera and Sandu (1986) in sugarcane.

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