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BIOCHEMICAL ANALYSIS OF SORGHUM IN CONTEXT TO SALINITY TOLERANCE AT PRE-FLOWERING STAGE UNDER FIELD CONDITION

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is a major staple food and fodder crop of millions of poor and world's fifth most vital cereal after maize, rice, wheat and barley. It is cultivated in sub-tropical and semiarid regions. Estimates suggest that nearly 10% additional area is getting salinized every year, and by the year 2050, around 50% of the arable land could be salt-affected. Salt stress adversely effects plant growth and inhibits many physiological and biochemical processes in plants, alters the plant metabolism and decline the rate of photosynthesis. The present investigation entitled "Biochemical analysis of sorghum in context to salinity tolerance at pre-flowering stage under field condition" was conducted to evaluate the response of sorghum to salt stress during *Rabi* season of 2023-24 at field of Post Graduate Institute and Sorghum Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.). Seeds of five *Rabi* sorghum genotypes with five sorghum varieties were obtained from the All India Coordinated Research Project on Sorghum, MPKV, Rahuri and sown in saline and normal soil and evaluated for biochemical parameters *viz*: proline, superoxide dismutase, ascorbate peroxidase, lipid peroxidation and glutathione reductase, physiological attributes like relative leaf water content, chlorophyll content, membrane stability index at pre flowering stage of growth. Among the physiological attributes relative leaf water content, the highest relative leaf water content of 83.49 percent in normal soil and 71.43 percent in saline soil was observed in RSV 2481 and total chlorophyll content lowest decrease was recorded in RSV 2481 with 11.46% followed by RSLG 2419 with 12.18% at pre flowering stage of growth under normal to saline soil. Biochemical analysis of sorghum genotypes under saline stress conditions revealed that leaf proline content was significant in RSLG 1876, RSV 2481 and RSV 2482 with 63.40%, 53.73% and 53.10% respectively under saline soil as compared to normal soil.

Keywords: Sorghum, Salinity, Pre-flowering, Biochemical.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) (C_4 plant) is the fourth most cultivated cereal in the world, being produced most frequently in high temperature and low rainfall areas and in soil with salinity problems (Reddy *et al.*, 2010). It is a crop fairly tolerant to salinity. Salinity is a major environmental factor that influences crop productivity worldwide. Saline soils are predominantly found in arid and semi-arid regions where there is less rainfall and high evaporation rates; rarely found in humid regions. Estimates suggest that nearly 10% additional area is getting salinized every year, and by the year 2050, around 50% of the arable land could be salt-affected

(Mythili and Goedecke, 2016). Mandal *et al.*, (2018) reported that nearly 75% of salt-affected soils in the country exist in the states of Gujarat (2.23 million ha), Uttar Pradesh (1.37 million ha), Maharashtra (0.61 million ha) and West Bengal (0.44 million ha). Maharashtra (0.61 million ha) and West Bengal (0.44 million ha). The area under cultivation in India is 4.8 to 5 million hectares (Kumar & Jha 2023) and in Maharashtra is 1.5 to 1.7 million hectares (Jadhav & Patil, 2023). The production sorghum in Indian is 3.5 to 4 million metric tons (Sarker & Sinha 2023) and in Maharashtra is 1.2 to 1.5 million metric tons (Deshmukh & Pande 2023).

Sorghum, known for its drought tolerance, shows

moderate salt tolerance but is affected by salinity-induced ionic imbalances, osmotic stress, and oxidative stress from ROS like superoxide anions and H₂O₂ (Sharif *et al.*, 2019). Plants counter these effects through enzymatic (CAT, POD) and non-enzymatic (proline) antioxidant systems (Majeed *et al.*, 2019). Salinity causes ion toxicity, mineral deficiencies, and oxidative damage to cellular components, necessitating the development of salt-tolerant genotypes. Research on biochemical markers linked to salinity tolerance in sorghum is essential to identify resilient accessions. Salinity, drought, and high temperatures significantly reduce crop yields and threaten agricultural sustainability and food security (Munns and Tester, 2008). Around 7% of global agricultural land is affected by salinity, potentially rising to 20% (Kosova *et al.*, 2013). Salts in the root medium disrupt water balance, causing cellular dehydration and inhibiting enzyme activity (O'Donnell *et al.*, 2013). Poor-quality irrigation water and intensive farming exacerbate salinity stress, especially in arid regions. Salinity triggers chlorophyll degradation, reduced water content, and morphological changes. Plants respond by accumulating osmolytes like soluble carbohydrates, amino acids, and proline to maintain osmotic balance between vacuoles and cytoplasm.

Salinity affects plants by causing ion toxicity, osmotic stress, mineral deficiencies, and oxidative stress due to increased reactive oxygen species (ROS) like superoxide and hydrogen peroxide, leading to cellular damage (Munns, 2002; Apel and Hirt, 2004). Excess salt accumulation reduces plant growth and yield (Yun *et al.*, 2018) and is exacerbated by environmental factors and climate change (Hichem and Mounir, 2009). Salt stress negatively impacts sorghum growth by disrupting photosynthesis, ion balance, and antioxidant defenses (Younis *et al.*, 2010). Investigating physiological and biochemical traits linked to salt tolerance can help identify resilient genotypes for breeding programs aimed at enhancing sorghum yield under saline conditions.

Materials and Methods

Material

- **Seeds:** The seeds of following 10 genotypes and varieties of Rabi sorghum namely RSLG 1876, RSV 2481, RSV2482, RSV 2520, RSLG 2419, Selection 3, Phule Rohini, Phule Suchitra, Phule Vasudha, M-35-1 were obtained from AICRP on Sorghum, M.P.K.V, Rahuri.

Methods

- **Lipid Peroxidation:** Lipid peroxidation, the oxidative degradation of lipids by reactive oxygen

species (ROS), was measured as thiobarbituric acid reactive substances (TBARS) following Heath and Packer (1968). Leaf samples (0.25 g) were homogenized in 5 ml of 0.1% TCA, centrifuged at 15,000 ×g for 15 min, and the supernatant was used for TBARS estimation. A 1 ml aliquot was mixed with 4 ml of 0.5% TBA in 20% TCA, heated at 95°C for 30 min, cooled in an ice bath, and centrifuged at 10,000 ×g for 10 min. Absorbance was recorded at 532 nm, corrected for nonspecific absorption at 600 nm, and TBARS content was calculated using $\epsilon = 115 \text{ mM}^{-1} \text{ cm}^{-1}$, expressed as $\mu\text{moles MDA g}^{-1} \text{ F.W.}$

- **Total Chlorophyll Content:** The total chlorophyll content of sorghum leaves was estimated using Arnon's method (1949) with 80% acetone. Leaf samples (0.2 g) were macerated in 20 ml of 80% acetone, centrifuged at 5000 ×g for 10 min, and the supernatant's absorbance was measured at 645 nm and 663 nm against an 80% acetone blank.
- **Chlorophyll Stability Index (CSI%):** The chlorophyll stability index (CSI) was assessed using Kaloyereas' method (1958) to evaluate salinity tolerance based on chlorophyll thermal stability. Two 0.25 g leaf samples were immersed in 10 ml distilled water, with one as a control and the other heated in a water bath at 56°C for 30 min. The absorbance of the extracts was measured at 652 nm.
- **Relative Leaf Water Content:** Relative leaf water content (RRLWC) was measured following Molaei *et al.*, (2012). Fresh weights (FW) of five leaf samples per treatment were recorded, followed by turgid weights (TW) after 4 hours in distilled water at 25°C, and dry weights (DW) after oven-drying at 75°C for 24 hours.
- **Enzymes Extraction:** Antioxidative enzymes (SOD, GR, APX) were extracted from leaf tissue following Costa *et al.*, (2002). Leaf samples (200 mg) were homogenized in 2 ml ice-cold 0.1 mM potassium phosphate buffer (pH 7.5) with 1 mM PMSF and 5% PVPP, filtered through cheesecloth, and centrifuged at 15,000 ×g for 20 min at 4°C. The supernatant served as the crude enzyme extract.
- **Superoxide Dismutase:** Superoxide dismutase (SOD) activity was measured by its ability to inhibit the photochemical reduction of nitroblue

Table 1: Effect of salinity on relative leaf water content, Total chlorophyll and Chlorophyll stability index of sorghum genotypes at pre- flowering stage.

Sr. No.	Genotypes	Relative leaf water content (%)			Total chlorophyll (mg/g F.W)			Chlorophyll Stability Index (%)		
		Normal Soil	Saline Soil	% Dec-rease	Normal Soil	Saline Soil	% Dec-rease	Normal Soil	Saline Soil	% Dec-rease
1	RSLG 1876	68.70	56.51	17.69	2.69	2.24	12.35	90.75	74.92	15.10
2	RSV 2481	83.49	71.43	21.39	2.66	2.25	11.46	88.41	72.01	13.89
3	RSV 2482	77.55	58.63	24.39	2.62	2.02	17.89	89.52	66.95	19.06
4	RSV 2520	70.17	59.22	20.79	2.52	1.74	19.23	90.80	65.83	19.70
5	RSLG 2419	82.63	66.14	15.85	2.67	1.54	12.18	93.77	69.37	14.02
6	Selection 3	78.58	70.95	14.33	2.65	2.16	13.30	90.33	70.62	15.10
7	PhuleRohini	53.51	60.48	22.75	2.43	1.92	17.30	92.49	67.71	21.28
8	PhuleSuchitra	69.43	57.89	22.81	2.64	1.91	16.54	91.02	63.34	20.91
9	PhuleVasudha	68.12	57.48	18.60	2.73	2.14	14.57	91.72	74.35	12.66
10	M- 35-1	52.23	55.04	19.41	2.49	1.87	15.50	91.25	65.22	20.12
	Mean	70.44	61.38		1.98	2.61		69.02	91.00	
	Comparison	CD at 5%	SE		CD at 5%	SE		CD at 5%	SE	
	Variety	1.91	0.667		0.052	0.018		0.006	0.002	
	Treatment	2.96	1.03		0.081	0.028		0.010	0.003	
	Variety × Treatment	6.61	2.31		0.189	0.063		0.023	0.008	

tetrazolium (NBT) following Dhindsa *et al.*, (1981). The reaction mixture included methionine, NBT, EDTA, phosphate buffer, sodium carbonate, enzyme extract, distilled water, and riboflavin. After 15 min of light exposure, absorbance was measured at 560 nm. One SOD unit was defined as the amount of enzyme required to inhibit 50% NBT photoreduction.

- **Ascorbate peroxidase:** Superoxide dismutase (SOD) activity was measured by its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following Dhindsa *et al.*, (1981). The reaction mixture included methionine, NBT, EDTA, phosphate buffer, sodium carbonate, enzyme extract, distilled water, and riboflavin. After 15 min of light exposure, absorbance was measured at 560 nm. One SOD unit was defined as the amount of enzyme required to inhibit 50% NBT photoreduction.
- **Glutathione reductase:** Glutathione reductase activity was measured in fresh extracts following Smith *et al.*, (1988). The reaction mixture included phosphate buffer, EDTA, DTNB, NADPH, oxidized glutathione, enzyme extract, and distilled water. The reaction was initiated with 20 mM GSS, and the increase in absorbance at 412 nm was recorded. Enzyme activity was expressed as absorbance per min⁻¹ mg⁻¹ protein.

Results and Discussions

Relative Leaf Water Content

The relative leaf water content (RLWC) of sorghum genotypes under normal and saline conditions showed RSV 2481 had the highest RLWC (83.49% in normal soil, 71.43% in saline), while M-35-1 had the lowest (52.23% in normal, 55.04% in saline). Selection 3 exhibited the least RLWC decrease (14.33%), and RSV 2482 showed the greatest (24.39%). RLWC declined with increasing salinity, with RSLG 1876 and RSV 2481 showing the least reduction, indicating higher salt tolerance. These results align with Desai *et al.*, (2012), Mbinda and Kimtai (2019), and Zhang *et al.*, (2020), linking RLWC decline to reduced turgor pressure and inhibited growth under salt stress.

Total chlorophyll and Chlorophyll stability index

Total chlorophyll content in sorghum genotypes decreased under saline conditions, with RSV 2481 showing the least reduction (11.46%), followed by RSLG 2419 (12.18%). This decrease was linked to oxidative damage from ROS and salt accumulation, impairing chlorophyll biosynthesis, as reported by Turan *et al.*, (2009), Desai *et al.*, (2012), and Kumar *et al.*, (2017). Similar declines were observed in foxtail millet (Biswas *et al.*, 2019) and other crops (Kul *et al.*, 2021; Zahra *et al.*, 2020). RSV 1876 and RSV 2481 exhibited the least chlorophyll decrease, suggesting higher salt tolerance. These findings align with the idea that salinity reduces

Table 2: Effect of salinity on lipid peroxidation, Superoxide dismutase and Ascorbate peroxidase in sorghum genotypes at pre-flowering stage.

Sr. No.	Genotypes	Lipid peroxidation (μmoles of MDA formed g^{-1} F.W)			Superoxide dismutase (units mg^{-1} protein)			Ascorbate peroxidase (units mg^{-1} protein)		
		Normal Soil	Saline Soil	% Inc-rease	Normal Soil	Saline Soil	% Inc-rease	Normal Soil	Saline Soil	% Inc-rease
1	RSLG 1876	13.33	15.81	18.58	2.24	2.66	30.39	12.20	18.23	33.06
2	RSV 2481	11.62	14.10	21.31	2.17	2.72	29.29	14.22	17.63	19.35
3	RSV 2482	12.78	16.62	30.07	1.96	2.77	21.43	13.54	20.13	32.75
4	RSV 2520	10.98	15.04	36.95	1.73	2.53	15.50	13.31	20.49	35.04
5	RSLG 2419	10.60	14.23	22.25	2.07	2.64	30.65	16.23	18.73	13.35
6	Selection 3	13.72	16.50	20.29	2.16	2.65	28.90	15.22	17.67	13.87
7	PhuleRohini	11.92	16.84	41.24	1.91	2.51	17.28	13.54	19.04	28.89
8	PhuleSuchitra	10.73	15.04	40.22	1.95	2.77	18.15	12.64	18.54	31.83
9	PhuleVasudha	12.65	17.09	35.11	2.14	2.69	21.03	12.54	17.80	29.55
10	M- 35-1	12.78	16.84	31.80	1.83	2.50	18.56	15.19	20.87	27.22
	Mean	15.81	12.11		2.01	2.64		13.86	18.91	
	Comparison	SE	CD at 5%		SE	CD at 5%		SE	CD at 5%	
	Variety	0.048	0.138		0.002	0.008		0.004	0.011	
	Treatment	0.075	0.215		0.004	0.012		0.006	0.018	
	Variety \times Treatment	0.168	0.481		0.009	0.027		0.014	0.042	

photosynthetic efficiency, leading to chlorosis and early leaf senescence.

Chlorophyll stability index (CSI) in sorghum genotypes decreased under saline conditions, with Phule Vasudha and RSLG 2481 showing the smallest decreases (12.66% and 13.89%) compared to M35-1 (20.12%). Phule Rohini and Phule Suchitra had the largest decreases (21.28% and 20.91%). The decline in CSI across genotypes

reflects the negative impact of salinity on chlorophyll stability, aligning with previous research.

Lipid Peroxidation

Lipid peroxidation increased significantly under salinity, with Phule Rohini, Phule Suchitra, and Phule Vasudha showing the highest increases. Lower lipid peroxidation was linked to salt tolerance, highlighting membrane stability's role in coping with salinity stress.

Table 3: Effect of salinity on glutathione reductase, grain yield and fodder yield in sorghum genotypes at pre-flowering stage.

Sr. No.	Genotypes	Glutathione reductase (μmoles of NADPH oxidised mg^{-1} protein min^{-1})			Grain yield (qt/ha)			Fodder yield (qt/ha)		
		Normal Soil	Saline Soil	% Inc-rease	Normal Soil	Saline Soil	% Inc-rease	Normal Soil	Saline Soil	% Inc-rease
1	RSLG 1876	0.97	0.99	15.12	21.54	16.71	22.42	42.84	32.40	24.37
2	RSV 2481	1.06	1.11	16.49	24.68	19.60	20.58	58.20	46.12	20.76
3	RSV 2482	0.99	1.04	13.64	25.84	19.84	23.22	54.75	42.58	22.23
4	RSV 2520	1.17	1.25	6.66	19.54	13.12	32.85	45.82	31.44	31.38
5	RSLG 2419	1.15	1.22	11.00	20.50	13.50	34.15	50.18	32.54	35.15
6	Selection 3	1.31	1.39	13.20	14.10	9.08	35.60	28.30	18.55	34.45
7	PhuleRohini	1.44	1.54	7.12	17.88	11.65	34.87	48.42	31.47	35.01
8	PhuleSuchitra	0.99	1.03	4.18	23.46	17.54	25.23	56.20	44.22	21.32
9	PhuleVasudha	0.99	1.05	15.55	27.52	20.04	27.18	60.50	42.10	30.41
10	M- 35-1	1.45	1.56	8.64	15.52	12.08	22.17	44.22	32.46	26.59
	Mean	1.15	1.22		21.06	15.32		48.94	35.39	
	Comparison	SE	CD at 5%		SE	CD at 5%		SE	CD at 5%	
	Variety	0.004	0.011		0.35	1.245		0.036	0.103	
	Treatment	0.006	0.018		0.674	0.350		0.056	0.161	
	Variety \times Treatment	0.014	0.042		1.508	1.050		0.125	0.360	

Superoxide dismutase (SOD)

The superoxide dismutase (SOD) activity in sorghum genotypes increased significantly under saline soil conditions, with RSLG 1876, RSV 2481, and Selection 3 showing the highest percentage increases (30.39%, 29.29%, and 28.90%, respectively). In contrast, RSV 2520, Phule Rohini, and Phule Suchitra recorded minimum increases in SOD activity under saline conditions. The increase in SOD activity under salt stress is consistent with previous findings in sorghum, amaranth, and sugarcane. Higher SOD activity is associated with salt tolerance, as it enhances the detoxification of reactive oxygen species (ROS) and reduces oxidative damage. Genotypes RSLG 1876 and RSV 2481, which showed higher SOD activity, may be considered salt-tolerant.

Ascorbate peroxidase

The ascorbate peroxidase (APX) activity in sorghum genotypes increased significantly under saline soil conditions, with RSV 2520, RSLG 1876, and RSV 2482 showing the highest percentage increases (35.04%, 33.06%, and 32.75%, respectively). In contrast, RSV 2481, Selection 3, and RSLG 2419 recorded minimum increases in APX activity under saline conditions. These findings align with recent studies indicating that salt-tolerant sorghum varieties exhibit higher APX activity, suggesting APX as a useful marker for selecting and breeding salt-tolerant varieties.

Glutathione Reductase (GR)

The glutathione reductase (GR) activity in sorghum genotypes increased significantly under saline soil conditions, with RSV 2481, Phule Vasudha, and RSLG 1876 showing the highest percentage increases (16.49%, 15.55%, and 15.12%, respectively). In contrast, Phule Suchitra, RSV 2520, and Phule Rohini recorded minimum increases in GR activity under saline conditions. This increase in GR activity helps maintain reduced glutathione levels, mitigating oxidative damage caused by reactive oxygen species and contributing to better stress tolerance. GR works synergistically with other antioxidants to neutralize ROS and protect plant cells, and its overexpression has been shown to enhance stress tolerance in plants.

Grain and Fodder Yield

The mean grain yield of sorghum was 21.06 qt/ha in normal soil and 15.32 qt/ha in saline soil, with RSV 2481 showing the smallest yield decrease (20.58%) and Selection 3 and RSLG 2419 the highest. Kausar and Gull (2019) reported significant negative effects of salinity on sorghum yield, with reductions varying across genotypes.

Similarly, Ranjbar *et al.*, (2015) noted salinity significantly reduces grain yield. Fodder yield decreased from 48.94 qt/ha in normal soil to 35.39 qt/ha in saline soil, with RSV 2481 showing the lowest decrease (20.76%) and RSLG 2419 the highest (35.15%).

Plant biomass ranged from 0.32 ± 0.09 kg to 0.74 ± 0.16 kg/plant, with the highest biomass (0.91 kg) at 0.78 dS/m and the lowest (0.26 kg) at 14.04 dS/m. Biomass significantly decreased at 10.79 and 14.04 dS/m ($p=0.003$), with reductions of 27.03–56.76% as salinity increased (Joardar *et al.*, 2018). Increased salinity also significantly reduced fodder yield, with the highest losses at EC 3 (12.0 dS/m). Genotypes IS 3237 and SSG 9 were identified as salinity-tolerant based on green and dry fodder yield (Pummy Kumari *et al.*, 2017).

Conclusion

Five sorghum genotypes were screened for salinity stress tolerance, exhibiting significant variation in physiological and biochemical parameters. RSV 2481 and M 35-1 performed better in terms of relative leaf water content, chlorophyll content, and chlorophyll stability index under saline stress. RSV 2481 and RSV 2482 also showed increased antioxidative enzymes (superoxide dismutase and ascorbate peroxidase) under saline conditions. Additionally, RSV 2481 and RSV 2482 recorded maximum grain yield and fodder yield under salinity stress. Overall, RSV 2481, RSV 2482, M 35-1, and Phule Vasudha exhibited better performance under saline stress at the post-flowering stage.

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