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Effect of exogenous application of Methylamine on Osmolyte accumulation, yield, and ionic response of *Abelmoschus esculentus* L. under drought condition

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Abstract: Drought remains one of the foremost menaces to okra cultivation. Combating these menaces, various interventions such as the use of methylamine can be employed. However, the efficacy of methylamine in ameliorating the impact of water deficit in Okra remains limited. This study assessed the impact of exogenous intervention of methylamine on mineral ion composition, osmolyte content, and yield of three varieties of okra cultivated under drought conditions. Seedlings of Okra (NGBOO288, NGBOO289, and NGBOO290) were exposed to three levels of water treatment (Well-watered, mild, and severe drought) with (50 µM) and without methylamine (0). Drought triggered a variety-dependent reduction in Okra yield, mineral ions, and osmolyte parameters. Severe drought reduced leaf biomass and decreased Chl *a* of NGBOO288 and NGBOO290 compared to the well-watered treatments. Methylamine significantly improved the accrual of glycine betaine and proline content, irrespective of water treatment in NGBOO288 than in NGBOO289 and NGBOO290. Methylamine mitigates the harmful consequences of drought on photosynthetic pigments by substantially improving chlorophyll Chl *a* and *b* in NGBOO288, whereas in NGBOO289, methylamine increased Chl *a* under mild drought. Methylamine boosted Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} under mild drought in NGBOO288 than in NGBOO289 and NGBOO290. The findings highlighted the potential of methylamine intervention to improve drought tolerance of the Okra by enhancing yield parameters, glycine betaine, total soluble sugar, proline, and mineral ions. Nevertheless, methylamine application could be more beneficial to NGBOO288 than the other varieties under drought conditions.

Keywords: *Abelmoschus esculentus*, anions, cations, glycine betaine, methylamine, drought, total soluble sugar, yield

Introduction

Water is a requisite component for plants to grow and develop (VanHautegem et al., 2015; Okunlola et al., 2022). Plants require water for normal vegetative growth, flowering, and fruiting (Okunlola et al., 2023). However, water scarcity may damage the plant cells, and affect photosynthesis, hence, it remains a primary constraint for plant growth, and functional development (Rao et al., 2016; Fahad et al., 2017). Plants growing in an environment with inadequate water usually face the menace of physiological and morphological stress occasioned by substantially reduced water potential caused by the buildup of soluble salts and turgor pressure of the cells (Iqbal et al., 2018; Okunlola et al., 2023). Water shortage in plants could result in stunted growth, ionic imbalance, oxidative mutilation, and hormonal, and nutrient imbalance (Li et al., 2021). It can also cause several disruptions in plant activities leading to the deterioration of vital biomolecules such as chlorophyll, enzymes, and essential and non-essential nutrients (Shahbaz et al., 2011). Deficit irrigation at different levels negatively affects the rate of transpiration, and diminishes plant development, mineral intake, and yield (Sahin et al., 2018). Findings have reported short-term and mild drought to cause stomata impairment and reduce water exchange in the leaves (Ekinci et al., 2015) Plants suffer permanent damage from severe drought, which might even result in the death of the crop (Okunlola et al., 2023). Nowadays, several approaches are employed to intensify crop tolerance to drought and maximize growth and production. Exogenous applications of specific organic molecules which are known to be growth enhancers can surge a plant's ability to resist diverse environmental stresses (Okunlola et al., 2023; Olatunji et al., 2024). Among these potential enhancers and regulators are the compounds belonging to the polyamines group. Polyamines can facilitate several processes including cell division and enlargement, formation of roots, flowers, and fruit ripening (Zhao and Yang 2008). Findings have pointed out that polyamines could strengthen plant defense during stress events such as metal (Hasanuzzaman et al., 2019), drought, high and low temperatures (Chen et al., 2019). One of the important molecules belonging to the polyamines group is methylamine. Methylamine (CH_3NH_2) is an important precursor of interstellar amino acids (Prasad-Ramesh and Yuan-Pern, 2022). The applications of methylamine can be eco-friendly and economically viable to lessen the detrimental aftermath of drought and maintain the optimal mineral nutrient content of plants. According to Chen et al., (2019), this enhancer can interfere with the metabolic and photosynthetic mechanism, scavenge ROS, and increase the antioxidants, all of which can help plants grow and lessen the effects of drought. Methylamine may enhance biomass production, photosynthetic efficiency, biosynthesis of pigments, and regulate stomatal activities (Yildirim et al., 2021; Kumar et al., 2023).

Okra (*Abelmoschus esculentus* (L) Moench) is a nutritious vegetable produced in warm temperate, tropics, and subtropics regions (Durazzo et al., 2019; Ogunkunle et al., 2023). *A. esculentus* is a versatile crop treasured for its soft and delicious fruit. Okra leaves, buds, and flowers are also eaten in West Africa. Okra is a cost-effective means of getting minerals, dietary fiber, vitamins, protein, carbohydrates, and various phytonutrients (Petropoulos et al., 2018).

Prior research has indicated that drought decreases the rate of seed germination, causes abscission of flower buds, and yields losses (Pravisya and Jayaram, 2015; Rao et al., 2016). Okra is facing oxidative damage due to water shortage, which impaired leaf chlorophyll, lower ion accumulation, crop growth rate, relative growth rate, and nutritional contents in fruits and seeds (Eltigani et al., 2022). Varieties of the same plants could react to drought differently, as such the reaction to exogenous application of methylamine might vary depending on the cultivar. Despite the reported damaging influence of drought on okra plants, the prospect of developing a cost-effective and environmentally beneficial strategy to reduce and mitigate this impact has not received enough attention. The objectives of this study were twofold: (i) to evaluate how three different varieties of okra responded to extreme drought in terms of growth, pigment content, and ion accumulation, and (ii) to assess the efficacy of externally applied methylamine in alleviating drought stress and enhancing the growth, osmolyte accumulation, as well as anionic and cationic uptake of okra.

Materials and Methods

Study Site

The experiment was established at the Department of Plant Biology screen house, Osun State University, Nigeria. For this study, cultivars of okra (NGBOO288 (V1), NGBOO289 (V2), and NGBOO290 (V3)) were obtained from the National Centre for Genetic Resources and Biotechnology, Nigeria. The three cultivars were selected due to their significance in terms of nutrition and the paucity of data on their drought tolerance. The seeds were propagated (15-20 seeds) into six plastic pots and the emerging seedlings were later transplanted into fifty-four (10 L) plastic pots. Each pot contains 5 kg of homogenized topsoil.

Implementation of Drought and methylamine treatments

This experiment was implemented using a complete randomized block design, with three varieties of Okra, and three levels of moisture treatments with or without methylamine. Each Okra variety was exposed to three replicates of six treatments, that is, well-watered with methylamine (W_{ma}), well-watered without methylamine (W_{nma}), mild-drought stress with methylamine (MD_{ma}), mild-drought stress without methylamine (MD_{nma}), severe drought stress with methylamine (SD_{ma}) and severe drought stress without methylamine (SD_{nma}). Drought stress and exogenous application of methylamine on the three Okra varieties were introduced 21 days after transplanting. The soil relative water content (SRWC) was set as 80% for well-watered; 60% for mild drought and 40% for severe drought. Okra were kept at their designated moisture levels by adding the volume of water lost (Distilled water). Methylamine was administered exogenously at a concentration of 50 µM. Okra which was not designated for methylamine treatments received an equivalent amount of distilled water. To guarantee uniform dispersion on the leaves, an exogenous application of methylamine was carried out with a hand sprayer. Plant samples were carefully retrieved from each pot at the termination of the experiment for further analysis.

Plant growth and biomass estimation

A leaf area meter (CI 202, United States) was used to determine leaf area (cm^2). After being carefully retrieved from the pots, each seedling was detached into leaves, stems, and roots. The fresh weights were determined using an electronic weighing balance. The samples were dried for 24 hours at 80ºC to maintain steady mass and weighed to measure the biomass. Leaf area/total dry weight and root /shoot dry weight were employed to estimate leaf area ratio (LAR) and root shoot ratio (RSR), respectively.

Determination of Photosynthetic Pigments

Chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) of each okra variety were measured by extracting 0.2 g (fresh weight) of the leaf sample with acetone (80%, i.e, 80 ml of acetone). A spectrophotometer was used to measure the extracted supernatants at an absorbance of 663 nm, 646 nm, and 470 nm after the samples were left in a dark environment for the whole night. The following formula was used to calculate pigment concentrations: Chlorophyll $a = 12.21A663–2.81A646$, Chlorophyll $b = 20.13(A646) -5.03(A663)$, Lichtenthaler (1987).

Total Soluble Sugar (TSS), Proline, and Glycine Betaine Determination

The TSS was estimated by grinding and extracting 0.2 g of leaf samples (DW) with 80% ethanol (80 ml of pure ethanol). The extracts were subjected to a 10-minute centrifugation at 5000 g, and the filtrate was combined with 6 mL of anthrone. After 10 minutes of heating, ice cooling, and 20 minutes of incubation, the mixes were analyzed spectrophotometrically at 625 nm. Proline contents were evaluated using Bates technique (Bates et al., 1973). Five milliliters of 3% sulfosalicylic were used to extract proline from the plant's leaves. The filtrate (1 mL) was heated in a water bath for an hour at 100 °C after being mixed with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 g ninhydrin, 30 ml glacial acetic acid, and 20 ml H_3PO_4). Three milliliters (3 mL) of toluene were thoroughly incorporated into the mixes and the absorbances were measured spectrophotometrically at 520 nm.

Glycine betaine content in the leaf was determined by shaking 0.5 g of powdered leaf with 20 ml of purified water at 25°C for 24 hrs. Sample extracts were respectively blended with 0.5 and 0.2 ml of Aliquot and potassium iodide after being diluted in a ratio of 1:1 with 2N sulfuric acid. The extract was gently mixed, and centrifuged for 15 minutes at 10000 g and 8°C. The periodite crystals that developed after 2.5 hrs were thawed in 9 milliliters of 1,2-dichloro ethane. The absorbance at 365 nm was measured with a spectrophotometer.

Determination of inorganic ions

Each of the okra varieties (leaves) was pulverized into a dry 0.1 gram sample and decocted in 10 milliliters of deionized water at 80 degrees Celsius. The resulting extracts were then used to measure Cl^- and SO_4^{2-}. A chloride analyzer (Sherwood Scientific Ltd., Model 926) was used to measure the Cl^- concentration, and a modified protocol of Mussa et al., (2009) was employed to estimate the SO_4^{2-}.

To ascertain the quantities of cations (Ca^{2+}, Na^+, K^+, and Mg^{2+}), 100 mg of the powdered sample was digested using a digestion mixture that included 14 g $LiSO_4.2H_2O$, 0.42 g Se, 350 ml of hydrogen peroxide, and 420 mL of concentrated sulfuric acid. After heating the digested sample to a temperature of 200 °C, 500 µL of perchloric acid was added. The concentrations of Na^+, K^+, and Ca^{2+} in the filtrate were measured using a flame photometer (Model: PFP-7, Jenway Ltd. Felsted, Dunmow, Essex, UK) once the mixture had cooled. The Mg^{2+} was determined through an atomic absorption spectrophotometer.

Statistical Analysis

The effects of methylamine and drought treatment on the osmolyte, ion contents, and yield characteristics of the three-okra variety were examined using a generalized linear model (GLM). The impacts of water treatment and methylamine on the osmolyte, ion contents, and yield were evaluated using analysis of variance (ANOVA). Duncan's multiple range test was employed to assess for differences between treatment means at $p < 0.05$. Student's t-test was used to compare the means at two levels of a factor (methylamine-containing and non-methylamine) under each type of okra. SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) and Origin-Pro 8.5 (OriginLab) were used to perform the analyses and draw the figures, respectively. The relationship between the yield traits and Osmolytes and Ion contents was assessed by Pearson correlation for each of the varieties across the treatments using Past 4.03 (Past4Project).

Results

Effect of methylamine on Osmolyte accumulation, ion contents, and photosynthetic pigments of the three varieties of okra under drought.

Related to the well-watered treatment, drought significantly increased ($p< 0.05$) the proline contents and decreased the total soluble sugar but did not affect the glycine betaine content of NGBOO288 (Table 1). In NGBOO289, mild drought significantly decreased ($p< 0.05$) total soluble sugar and glycine betaine but enhanced proline content in comparison to the well-watered treatment. In NGBOO290, extreme drought enhanced glycine betaine and proline but decreased total soluble sugar as compared to the well-watered treatment. Whereas, mild drought decreased glycine betaine and did not affect the total soluble sugar.

Methylamine enhanced osmolyte accumulation contingent in the three varieties of Okra, irrespective of the level of drought (Table 1) In NGBOO288, methylamine significantly ($p< 0.05$) enhanced the quantity of glycine betaine and proline accumulated regardless of the water treatments but slightly promote the accrual of total soluble sugar under mild and severe drought. Whereas, in severe drought treatment, methylamine significantly ($p<0.05$) improved the buildup of proline and total soluble in NGBOO289. In NGBOO290, methylamine significantly ($p< 0.05$) promotes glycine betaine, total soluble sugar, and proline in mild drought treatment and total soluble sugar under severe drought (Table 1).

Table 1: Response of leaf osmolyte accumulation to exogenous application of Methylamine under drought condition

Varieties	**Treatments**	**Glycine Betaine ($\mu molg^{-1}$ DW)**	**Total soluble Sugar (mgg^{-1} DW)**	**Proline (µmol/mg FW)**
V1	W_{nMa}	12.41 ± 0.36^{a}	57.30 ± 1.06^{a}	0.02 ± 0.00^{c}
	W_{Ma}	$15.54 \pm 0.47^{A**}$	$61.81 \pm 1.00^{A**}$	$0.07 \pm 0.00^{C**}$
	MD_{nMa}	11.39 ± 0.09^{b}	50.61 ± 0.54^{b}	0.08 ± 0.00^{b}
	MD_{Ma}	$16.11\pm 0.09^{A**}$	52.10 ± 2.95^{B}	$0.14 \pm 0.00^{B**}$
	SD_{nMa}	12.64 ± 0.06^{a}	51.16 ± 0.51^{b}	0.11 ± 0.00^{a}
	SD_{Ma}	$16.05 \pm 0.07^{A**}$	53.13 ± 0.13^{B}	$0.21 \pm 0.00^{A**}$
V2	W_{nMa}	15.56 ± 0.40^{a}	51.00 ± 1.32^{a}	0.05 ± 0.00^{b}
	W_{Ma}	16.26 ± 0.22^{A}	51.16 ± 0.52^{B}	$0.10 \pm 0.00^{A**}$
	MD_{nMa}	11.37 ± 0.14^{b}	50.48 ± 0.41^{a}	0.12 ± 0.00^{a}
	MD_{Ma}	11.15 ± 0.13^{B}	51.35 ± 0.64^{B}	0.12 ± 0.00^{A}
	SD_{nMa}	15.55 ± 0.48^{a}	52.32 ± 0.58^{a}	0.03 ± 0.00^{c}
	SD_{Ma}	16.23 ± 0.11^{A}	$58.16 \pm 0.16^{A**}$	$0.08 \pm 0.00^{A**}$
V3	W_{nMa}	13.12 ± 0.24^{b}	51.12 ± 0.12^{a}	0.02 ± 0.00^{c}
	W_{Ma}	12.3 ± 0.27^{C}	52.55 ± 1.74^{B}	$0.08 \pm 0.00^{B**}$
	MD_{nMa}	11.37 ± 0.14^{c}	50.48 ± 0.41^{a}	0.10 ± 0.00^{a}
	MD_{Ma}	$14.5 \pm 0.5^{B**}$	$52.14 \pm 0.30^{B**}$	$0.20 \pm 0.00^{A**}$
	SD_{nMa}	15.4 ± 0.34^{a}	45.53 ± 2.45^{b}	0.07 ± 0.00^{b}
	SD_{Ma}	16.32 ± 0.28^{A}	$60.91 \pm 0.62^{A**}$	0.08 ± 0.00^{B}

Different lower-case letters represent significant differences among No- methylamine treatments @ $p< 0.05$. Different Upper-case letters represent significant differences among methylamine treatments @ $p< 0.05$. Asterisks (**) indicate significant differences between methylamine treatments (Ma) and No- methylamine treatments (nMa) treatments @ $p< 0.05$. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. W_{Ma}: Well-watered + methylamine, W_{nMa}: Well-watered – methylamine, MD_{ma}: Mild-drought+ methylamine, MD_{nma}: Mild-drought - methylamine, SD_{ma}: Severe-drought + methylamine, SD_{nma}: Severe-drought- methylamine

Accumulation of Na^+, Mg^{2+}, Cl^-, SO_4^{2-} K^+, and Ca^{2+} varied among the Okra varieties in drought treatments. In comparison to the well-watered, severe drought significantly ($p< 0.05$) reduced Mg^{2+}, Cl^-, and SO_4^{2-} in NGBOO288 while mild drought only decreased Ca^{2+} and SO_4^{2-} (Table 2). In NGBOO289 and NGBOO290, mild drought treatment significantly ($p< 0.05$) reduced Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} contents of the leaf when compared with the well-watered. Meanwhile, severe drought increased Na^+, Ca^{2+}, Cl^-, and SO_4^{2-} when compared with mild drought and well-watered treatments. In comparison to the non-methylamine treatments, methylamine increased cation and anion contents in the three Okra varieties (Table 2). Application of methylamine significantly enhances leaf Cl^-, SO_4^{2-} Na^+, Ca^{2+}, K^+, and Mg^{2+} in NGBOO288 and NGBOO290, regardless of the water treatments. In NGBOO289, methylamine has no significant effects on the leaf Ca^{2+} and Mg^{2+}, under severe drought conditions and SO_4^{2-} irrespective of the water treatments.

Table 2: Response ion contents to exogenous application of Methylamine under drought condition

Varieties	Treatments	Cation contents (mg/kg)				Anion contents (mg/kg)	
		Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	Cl^{-}	SO_4^{2-}
V1	W_{nMa}	6.48 ± 0.05^{a}	8.11 ± 0.09^{a}	12.62 ± 0.04^{a}	5.63 ± 0.10^{a}	10.05 ± 0.06^{a}	10.57 ± 0.11^{a}
	W_{Ma}	$13.42 \pm 0.15^{A**}$	$14.52 \pm 0.16^{A**}$	$17.26 \pm 0.07^{A**}$	$13.07 \pm 0.09^{A**}$	25.69 ± 0.24^{A}	$15.54 \pm 0.47^{C**}$
	MD_{nMa}	7.67 ± 0.11^{a}	8.37 ± 0.09^{a}	9.09 ± 0.07^{b}	6.13 ± 0.11^{a}	$10.40 \pm 0.18^{a**}$	8.16 ± 0.11^{b}
	MD_{Ma}	$13.80 \pm 0.21^{A**}$	$13.57 \pm 0.20^{A**}$	$16.62 \pm 0.30^{AB**}$	$12.98 \pm 0.05^{A**}$	$22.28 \pm 0.32^{B**}$	$22.33 \pm 0.42^{A**}$
	SD_{nMa}	6.34 ± 0.14^{a}	7.63 ± 0.46^{a}	12.42 ± 0.19^{a}	3.10 ± 0.09^{b}	6.21 ± 0.13^{b}	7.31 ± 0.12^{b}
	SD_{Ma}	$13.21 \pm 0.04^{A**}$	$13.78 \pm 0.20^{A**}$	$15.36 \pm 0.13^{B**}$	$12.23 \pm 0.13^{A**}$	$22.95 \pm 0.12^{B**}$	$20.60 \pm 0.22^{B**}$
V2	W_{nMa}	11.51 ± 0.20^{a}	12.5 ± 0.31^{a}	15.26 ± 0.31^{a}	12.38 ± 0.17^{a}	21.44 ± 0.21^{a}	19.91 ± 0.29^{a}
	W_{Ma}	$16.63 \pm 0.16^{A**}$	$15.17 \pm 0.36^{A**}$	$17.44 \pm 0.19^{A**}$	13.29 ± 0.10^{A}	$28.15 \pm 0.11^{A**}$	22.85 ± 0.18^{A}
	MD_{nMa}	5.97 ± 0.14^{b}	5.33 ± 0.33^{b}	3.99 ± 0.03^{b}	2.26 ± 0.14^{b}	3.38 ± 0.17^{b}	3.44 ± 0.21^{b}
	MD_{Ma}	$10.05 \pm 0.06^{B**}$	$10.05 \pm 0.06^{B**}$	$11.63 \pm 0.22^{B**}$	$10.12 \pm 0.08^{B**}$	$15.52 \pm 0.20^{C**}$	10.50 ± 0.19^{C}
	SD_{nMa}	11.39 ± 0.17^{a}	12.61 ± 0.21^{a}	15.51 ± 0.19^{a}	12.34 ± 0.16^{a}	20.93 ± 0.07^{a}	19.97 ± 0.06^{a}
	SD_{Ma}	$16.09 \pm 0.09^{A**}$	$14.26 \pm 0.14^{A**}$	16.64 ± 0.18^{A}	13.05 ± 0.06^{A}	$25.51 \pm 0.18^{B**}$	20.19 ± 0.12^{B}
V3	W_{nMa}	7.48 ± 0.15^{b}	11.22 ± 0.12^{a}	11.72 ± 0.24^{b}	10.11 ± 0.20^{a}	14.09 ± 0.15^{b}	10.14 ± 0.12^{b}
	W_{Ma}	$9.45 \pm 0.20^{B**}$	$11.91 \pm 0.28^{B**}$	12.45 ± 0.19^{B}	11.38 ± 0.19^{B}	$18.42 \pm 0.18^{B**}$	10.25 ± 0.15^{C}
	MD_{nMa}	5.97 ± 0.14^{c}	5.33 ± 0.33^{b}	3.99 ± 0.03^{c}	2.26 ± 0.14^{b}	3.38 ± 0.17^{c}	3.44 ± 0.21^{c}
	MD_{Ma}	$8.18 \pm 0.12^{C**}$	$10.99 \pm 0.03^{B**}$	$10.9 \pm 0.00^{C**}$	$9.26 \pm 0.07^{C**}$	$18.74 \pm 0.24^{B**}$	$19.52 \pm 0.19^{B**}$
	SD_{nMa}	11.42 ± 0.22^{a}	12.72 ± 0.22^{a}	15.40 ± 0.17^{a}	11.85 ± 0.26^{a}	20.01 ± 0.15^{a}	17.84 ± 0.16^{a}
	SD_{Ma}	$18.3 \pm 0.21^{A**}$	$15.17 \pm 0.13^{A**}$	$17.99 \pm 0.10^{A**}$	$13.66 \pm 0.16^{A**}$	$31.36 \pm 0.25^{A**}$	$23.98 \pm 0.29^{A**}$

Different lower-case letters represent significant differences among No- No-methylamine treatments @ *p< 0.05*. Different Upper-case letters represent significant differences among methylamine treatments @ *p< 0.05*. Asterisks (**) indicate significant differences between methylamine treatments (Ma) and No-methylamine treatments (nMa) @ p< 0.05. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. Na^{+}: Sodium cation, K^{+}: Potassium cation, Ca^{2+}: Calcium cation, Mg^{2+}: Magnesium cation, Cl^{-}: Chloride anion, SO_4^{2-}: anion.

Irrespective of varieties, drought stress significantly decreased Chl *a* when compared with the well-watered treatments. However, drought has no substantial effect on the Chl *b* in NGBOO289 and NGBOO290. Application of methylamine significantly (*p< 0.05*) increased Chl *a* and *b* in NGBOO288 under severe drought and well-watered conditions (Table 3). Whereas, in NGBOO289, methylamine increased Chl *a* under mild drought and well-watered conditions, and Chl *b*, irrespective of the water treatments. Methylamine significantly increased Chl *b* content in NGBOO290 under severe drought conditions but did not affect Chl *a* (Table 3).

Table 3: Effect of methylamine on the leaf chlorophyll content of the three okra varieties under drought conditions

Varieties	Treatments	**Chlorophyll *a*** (mg/g FW)	**Chlorophyll *b*** (mg/g FW)
V1	W_{nMa}	0.22 ± 0.01^{a}	0.98 ± 0.17^{a}
	W_{Ma}	$0.25 \pm 0.02^{A**}$	$2.13 \pm 0.27^{A**}$
	MD_{nMa}	0.09 ± 0.00^{b}	0.15 ± 0.00^{b}
	MD_{Ma}	0.09 ± 0.00^{B}	0.15 ± 0.00^{C}
	SD_{nMa}	0.09 ± 0.00^{b}	0.15 ± 0.00^{b}
	SD_{Ma}	$0.05 \pm 0.01^{C**}$	$0.41 \pm 0.11^{B**}$
V2	W_{nMa}	0.49 ± 0.05^{a}	0.15 ± 0.00^{a}
	W_{Ma}	$0.22 \pm 0.07^{B**}$	$0.28 \pm 0.02^{C**}$
	MD_{nMa}	0.09 ± 0.00^{b}	0.15 ± 0.00^{a}
	MD_{Ma}	$0.\ 47 \pm 0.09^{A**}$	$0.75 \pm 0.01^{A**}$
	SD_{nMa}	0.09 ± 0.00^{b}	0.15 ± 0.00^{a}
	SD_{Ma}	0.09 ± 0.00^{C}	$0.30 \pm 0.08^{B**}$
V3	W_{nMa}	0.31 ± 0.12^{a}	0.15 ± 0.00^{a}
	W_{Ma}	0.32 ± 0.00^{A}	0.15 ± 0.00^{B}
	MD_{nMa}	0.09 ± 0.00^{b}	0.15 ± 0.00^{a}
	MD_{Ma}	0.09 ± 0.00^{B}	0.15 ± 0.00^{B}
	SD_{nMa}	0.09 ± 0.00^{b}	0.15 ± 0.00^{a}
	SD_{Ma}	0.09 ± 0.00^{B}	$1.76 \pm 0.00^{A**}$

Different lower-case letters represent significant differences among No-methylamine treatments @ *p< 0.05*. Different Upper-case letters represent significant differences among methylamine treatments @ *p< 0.05*. Asterisks (**) indicate significant differences between methylamine treatments (Ma) and No-methylamine treatments (nMa) @ p< 0.05. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290.

Effect of application of methylamine on leaf area, biomass accumulation, root: shoot ratio, and leaf : area ratio of the three varieties of okra

Drought triggered a significant (*p< 0.05*) reduction in the leaf area, biomass, and root: shoot ratio of the Okra varieties when compared to the well-watered treatment (Fig. 1- 6). Compared to the severe drought, mild drought reduced the leaf area and root biomass in all the varieties

(Figs. 1 and 4). In NGBOO288 and NGBOO290, the leaf biomass decreased as drought severity increased (Fig. 2). Mild drought showed no significant effect on shoot biomass in NGBOO289 and NGBOO290, whereas mild and severe drought reduced shoot biomass in NGBOO288 (Fig. 3). Unlike NGBOO288 and NGBOO290, moderate and severe drought enhanced leaf: area ratio in NGBOO289 in comparison to the well-watered treatments (Fig. 5). Compared to NGBOO289 and NGBOO290, NGBOO288 has a higher root: shoot ratio under mild and severe drought (Fig. 6). However, methylamine significantly ($p< 0.05$) enhanced the leaf area of NGBOO288 under mild drought stress but increased that of NGBOO289 and NGBOO290 under mild and severe drought (Fig. 1). Irrespective of the water treatments, methylamine significantly ($p< 0.05$) enhanced the leaf area, shoot, and root biomass, leaf: area ratio in the three varieties (Fig 2-5). While methylamine had no significant effect on the root: shoot ratio of NGBOO289, it significantly increased the root: shoot ratio of NGBOO288 and NGBOO290 under mild and severe drought conditions (Fig. 6).

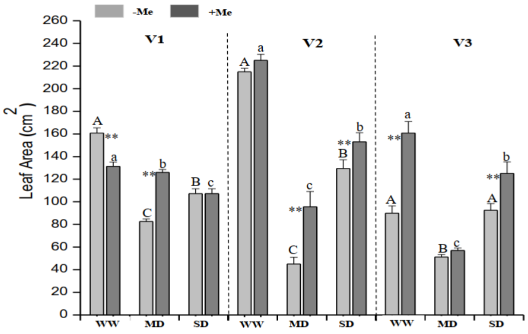

Fig. 1 Changes in Leaf Area of the three *Okra varieties* in response to drought and methylamine treatments. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. WW: Well-watered; MD: Mild-drought; SD: Severe-drought. Bar with different lower-case letters represents a significant difference among the drought treatments with the application of methylamine at $p < 0.05$. Bars with different Upper-case letters represent significant differences among the drought treatments without the application of methylamine at $p < 0.05$ using DMRT. Asterisks (**) indicate significant differences between methylamine and no methylamine treatments at $p< 0.05$

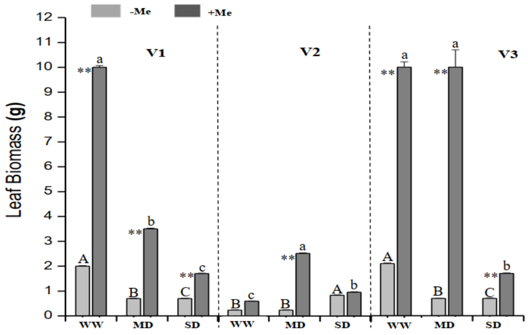

Fig. 2 Leaf biomass response of the three *Okra varieties* in response to drought and methylamine treatments. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. WW: Well-watered; MD: Mild-drought; SD: Severe-drought. Bar with different lower-case letters represents a significant difference among the drought treatments with the application of methylamine at $p < 0.05$. Bars with different Upper-case letters represent significant differences among the drought treatments without the application of methylamine at $p < 0.05$ using DMRT. Asterisks (**) indicate significant differences between methylamine and no methylamine treatments at $p< 0.05$

Relationship between yield parameters and Osmolyte accumulation, and ion contents, of the three varieties of okra

The relationship of yield traits with the osmolyte and ion content differed among the varieties (Fig 7A-C). In NGBOO288, RDW and SDW were positively correlated with total soluble sugar but negatively correlated with proline. A positive but weak correlation was observed between the SDW, RDW, LWR, and Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} (Fig 7A). In NGBOO289, a positive and significant correlation was observed between LA, LAR, and proline, while glycine betaine negatively correlated with the two traits (Fig 7B).

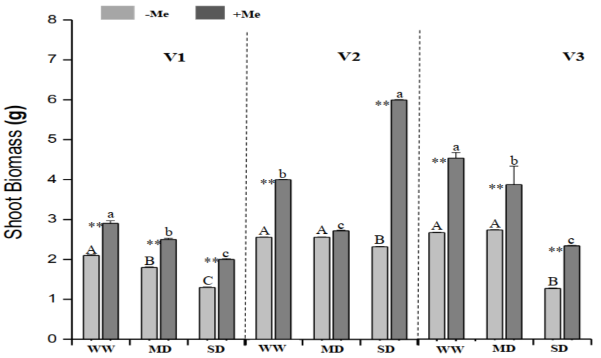

Fig. 3 Shoot biomass response of the three *Okra varieties* to drought and methylamine treatments. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. WW: Well-watered; MD: Mild-drought; SD: Severe-drought. Bar with different lower-case letters represents a significant difference among the drought treatments with the application of methylamine at $p < 0.05$. Bars with different Upper-case letters represent significant differences among the drought treatments without the application of methylamine at $p < 0.05$ using DMRT. Asterisks (**) indicate significant differences between methylamine and no methylamine treatments at $p< 0.05$

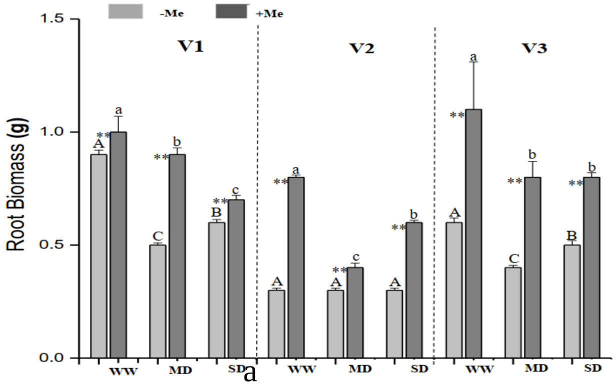

Fig 4. Root biomass response of the three *Okra varieties* in response to drought and methylamine treatments. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. WW: Well-watered; MD: Mild-drought; SD: Severe-drought. Bar with different lower-case letters represents a significant difference among the drought treatments with the application of methylamine at $p < 0.05$. Bars with different Upper-case letters represent significant differences among the drought treatments without the application of methylamine at $p < 0.05$ using DMRT. Asterisks (**) indicate significant differences between methylamine and no methylamine treatments at $p< 0.05$

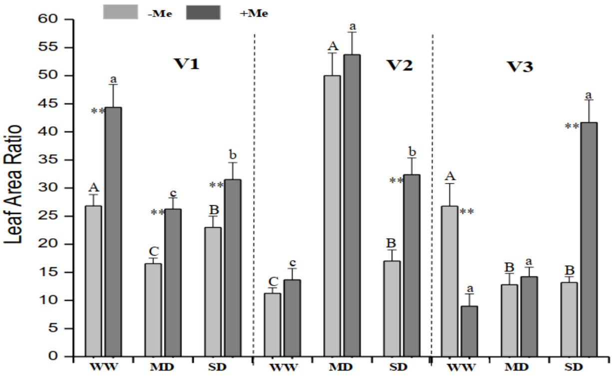

Fig 5 Response of the leaf area ratio of the three *Okra varieties* in response to drought and methylamine treatments. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. WW: Well-watered; MD: Mild-drought; SD: Severe-drought. Bar with different lower-case letters represents a significant difference among the drought treatments with the application of methylamine at $p < 0.05$. Bars with different Upper-case letters represent significant differences among the drought treatments without the application of methylamine at $p < 0.05$ using DMRT. Asterisks (**) indicate significant differences between methylamine and no methylamine treatments at $p< 0.05$

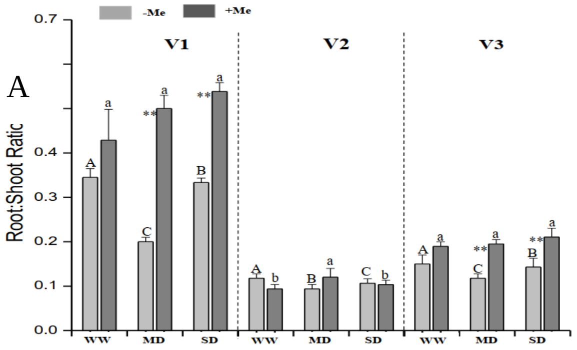

Fig. 6 Response of the root shoot ratio of the three *Okra varieties* in response to drought and methylamine treatments. V1: NGBOO288, V2: NGBOO289, V3: NGBOO290. WW: Well-watered; MD: Mild-drought; SD: Severe-drought. Bar with different lower-case letters represents a significant difference among the drought treatments with the application of methylamine at $p < 0.05$. Bars with different Upper-case letters represent significant differences among the drought treatments without the application of methylamine at $p < 0.05$ using DMRT. Asterisks (**) indicate significant differences between methylamine and no methylamine treatments at $p < 0.05$

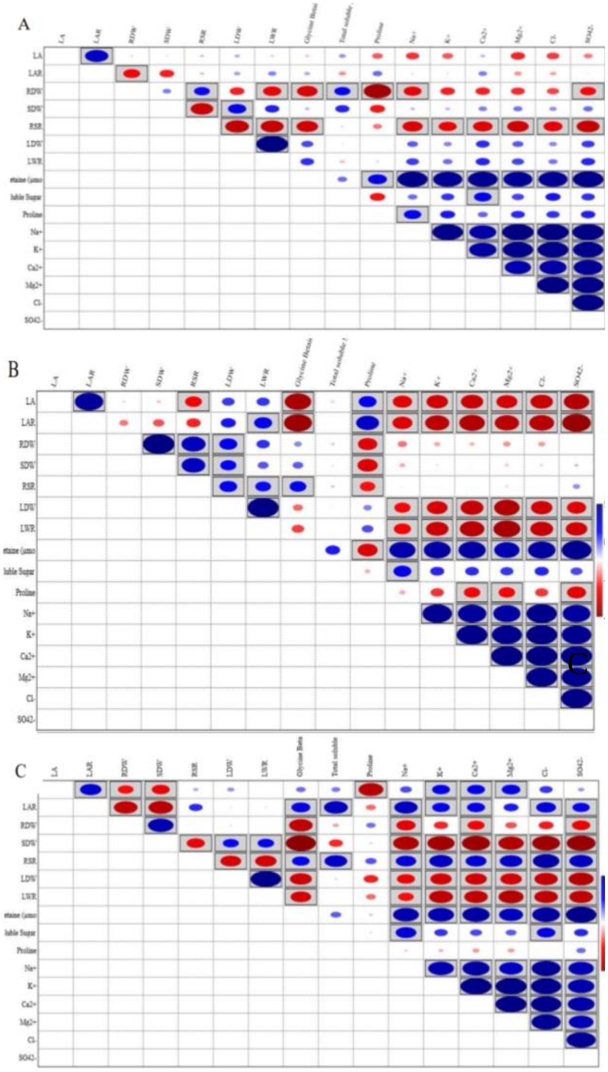

Fig. 7 Correlation analysis of the yield traits with osmolyte and ion contents of the three C varieties under stress. **A:** NGBOO288 (V1), B: NGBOO289 (V2), C: NGBOO290 (V3)

The SDW, RDW, and RSR significantly and negatively correlated with proline. The LA, LAR, LDW, and LWR significantly and negatively correlated with Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} (Fig 7B). The LA in NGBOO290 negatively correlated with proline but significantly and positively correlated with Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} (Fig 7C). RDW, SDW and LDW were significant and negatively correlated with glycine betaine, total soluble sugar, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} (Fig 7C).

Discussion

Okra is a significant horticultural crop, but susceptible to abiotic factors that have a damaging impact on its development, growth, and production (Rao et al., 2016; Eltigani et al., 2022). Aligned with the observation of Zhou et al., (2017) and Asadullah et al., (2024), growth indices such as root-to-shoot ratio, leaf area ratio, leaf area, and biomass decreased as drought severity increased. However, different okra varieties react differently to drought stress. For example, NGBOO289 showed an increase in leaf: area ratio under mild and severe drought, while NGBOO288 and NGBOO290 exhibited a reduction in leaf biomass as drought severity increased. This observation is consistent with typical responses of plants to water scarcity, where they invest fewer resources in above-ground and below-ground growth to survive the scarcity of water (Okunlola et al., 2023). The decrease in the root: shoot ratio in all three varieties indicates a shift in resource allocation, with a relatively smaller investment in root biomass compared to shoot biomass. This adaptation is common under drought conditions as plants allocate more resources to above-ground structures to capture available water and light (Olatunji et al., 2016). Findings have suggested that as an avoidance mechanism under drought plants increase root: shoot ratio which corresponds to an increase in the proportion of root biomass relative to aboveground (Zhou et al., 2017). Similarly, in this study, NGBOO288 has a higher root: shoot ratio which corresponds to higher root biomass than in the other varieties in mild and severe drought treatments. This suggests that NGBOO288 has a better avoidance mechanism to respond to drought than other varieties (Zhou et al., 2017).

However, the application of methylamine led to a substantial rise in the ratios of leaf area to shoot ratio, root, shoot, and leaf biomass. This is consistent with the report of Yildirim et al., (2021) and affirms the cell division potential and the overall contribution of methylamine plant growth and development, even under drought conditions. Studies have highlighted that exogenously applied methylamine might activate physiological processes and stimulate osmotic adjustment strategies of plants under stress (Zhang et al., 2009; Sanchez-Rodriguez et al., 2015). Previous findings have reported the accumulation of osmolytes as an essential mechanism for plants resistant to oxidative damage (Okunlola et al., 2022; Olatunji et al., 2024). Here, methylamine significantly upshot the accrual of osmolytes including glycine betaine, total soluble sugar, and proline. However, this was found to vary based on the Okra variety and the severity of drought. In NGBOO288, methylamine significantly improved the accrual of glycine betaine and proline content irrespective of water stress but only slightly promoted total soluble sugar under mild and severe drought. Whereas, in NGBOO289 and NGBOO290, methylamine significantly promoted total soluble sugar under severe drought conditions. These findings suggest that while methylamine enhances the capacity of NGBOO288 to prevent the accrual of ROS, protect the membrane, and reduce the impairment of the photosynthetic apparatus in chloroplasts, it has little impact on carbon storage and stabilization of protein structures of NGBOO288 under drought conditions. Whereas in NGBOO289 and NGBOO290, it plays a cogent role in maintaining turgor potential, carbon storage, and stabilization of protein structures in mild and severe drought conditions (Kavi-Kishor and Sreenivasulu 2013; Uttam et al., 2021). Nevertheless, it is imperative to note that while the application of methylamine enhanced the osmotic adjustment of the three varieties, NGBOO289 has a better capacity to tolerate drought conditions in the absence of methylamine than the other two varieties. This as the glycine betaine, total soluble sugar, anionic, and cationic compounds were found to be higher in NGBOO289, under severe drought conditions than in NGBOO288 and NGBOO290 when no methylamine was applied. Moreover, the increase in glycine betaine of NGBOO289 corresponds with the stability of Chl *b* content under drought.

Chlorophylls are vital biochemical building blocks that enable photosynthesis in plants, but they are also very susceptible to environmental stresses (Okunlola et al., 2022; Olatunji et al., 2024). A decrease in pigment concentrations is considered an indication of oxidative stress or chlorophyll degradation (Wang et al., 2018; Tariq et al., 2019). Consistent with the study of Tariq et al., (2019) and Okunlola et al., (2017), drought caused a substantial reduction in Chl *a* in all three okra varieties, suggesting the adverse consequences of drought on the primary photosynthetic pigment involved in capturing light for photosynthesis. Interestingly, Chl *b* content was not substantially affected by drought, especially in NGBOO289 and NGBOO290. This implies that these varieties might have a certain degree of resilience or adaptive mechanisms to maintain Chl *b* levels during hostile conditions (Okunlola et al., 2022). Although methylamine has significant helpful effects on Chl *a* and *b*, the effects differ among the cultivars. In NGBOO288, methylamine treatment mitigates the harmful consequences of drought on photosynthetic pigments by substantially improving chlorophyll Chl *a* and *b* whereas, in NGBOO289 methylamine increased Chl *a* under mild drought and well-watered conditions, and increased Chl *b* regardless of water treatment. This indicates that although the exogenous application of methylamine could enhance the photosynthetic activity of the three okra varieties, it is more beneficial to NGBOO288 under mild and severe drought conditions.

Previous studies reported polyamines to act as a vital regulator of ion homeostasis during stress (Moschou and Roubelakis-Angelakis, 2014; Okunlola et al., 2022). In this study, the protective upshot of methylamine is consistent with the reported upsurge in cation and anion contents due to the application of polyamines by previous studies (Zhang et al., 2013; Okunlola et al., 2022). Ion such as K^+, a well-known osmoticum, and Ca^{2+} plays a crucial part in water-use efficiency by boosting the rigidity of the cell wall and upsurging stomatal conductance (Okunlola et al., 2022). In this study, methylamine treatments increased cation and anion contents in okra exposed to well-watered, moderate, and severe drought treatments when compared to the non-methylamine treatments. However, the increments differed among the varieties such that in NGBOO288, methylamine boosted the accumulation of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} than in NGBOO289 and NGBOO290 in mild drought treatment. Contrary to a report that the upshot of Na^+ implies a decrease in K^+ (Jiang et al., 2016), the present finding showed that the application of methylamine enhances the accumulation of Na^+ and K^+, as well as Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} particularly in NGBOO288 than in NGBOO289 and NGBOO290 under mild drought condition. Although the role of methylamine in the accumulation of cations and anions by *Abelmoschus esculentus* under drought conditions has not been explored, the present observation is consistent with the report of Iqbal et al., (2018) and Okunlola et al., (2023). The observed increase in the cations and anions of NGBOO288 corresponds to the noticeable synthesis of glycine betaine and proline when methylamine was applied. This possibly improved its osmotic adjustment and eventually resulted in higher root biomass than in the other two varieties in mild drought treatment (Iqbal et al., 2018; Okunlola et al., 2023). This study highlighted that, unlike NGBOO289 and NGBOO290, methylamine is important for lessening the damaging consequences of water deficit on NGBOO288 by modulating the anion and cation concentrations.

Conclusion

Drought stress harmfully obstructed the growth, biomass accumulation, osmolyte, and ionic in all three varieties of Okra. However, there are variations in the aftermath of drought stress among okra varieties. The application of methylamine benefits okra plants subjected to mild and severe drought treatments by improving the leaf area, leaf area ratio, root-to-shoot ratio, biomass, osmolyte, and the buildup of anionic and cationic compounds. However, the application of methylamine was more beneficial to NGBOO288 as it significantly improved the accrual of glycine betaine and proline content irrespective of water stress, and substantially improved chlorophyll Chl *a* and *b* than in NGBOO289 and NGBOO290 under drought conditions. More so, methylamine boosted the accumulation of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} under mild drought in NGBOO288 than in NGBOO289 and NGBOO290. Nevertheless, further investigation considering the enzymatic antioxidant response and genetic diversity of the okra variety to methylamine application under environmental stressors should be carried out.

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