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Evaluation of different *Digera muricata* L. (Mart) biotypes for antioxidant activity

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Abstract
This study investigates the antioxidant enzymatic activity of different *Digera muricata* biotypes, a resilient plant species adapted to arid environments. The biochemical determination focused on malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) activities under stress conditions. The finding exhibited Biotype 2 had highest POD (1.737 ± 0.003) and APX (4.167 ± 0.024) activities, with higher mean SOD and CAT activity, indicating higher oxidative tolerance whereas, B5 lowest in APX (1.247 ± 0.030) and CAT (0.250 ± 0.006) activities, and showed a lower resistance to reactive oxygen species (ROS). Analysis of variance (ANOVA) confirmed significant differences ($p<0.01$) across tested biotypes and parameters. These findings suggested the critical role of antioxidant enzymes in mitigating oxidative damage and contributing to its ability to succeed under environmental stress conditions.

Keywords: Malondialdehyde, superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and antioxidant activity

Introduction

Digera muricata L. commonly known as false amaranth is a highly prostrate plant species associated with dry and semi-arid regions. This plant has throughout been recognized for the nutritional and therapeutic characteristics they possess; their ability to withstand unfavourable climate standards included. (Usmani, 2022) [25]. Oxidative stress is defined as a condition caused by increased production of ROS and the plant's inefficiency to control the amount produced through antioxidant defenses. Hydrogen peroxide (H_2O_2), superoxide radicals (O_2^-) and hydroxyl radicals (OH^-) are collected as ROS which may cause massive cellular damage due to their reaction with lipids, proteins, and nucleic acids. SOD converts super oxide radicals to hydrogen peroxide and the same is converted to water by the CAT and APX enzymes. In a study conducted by Ahanger *et al.* (2019) [2] they explained that proline level increases within the *D. muricata* plant during drought stress condition to support the structural stability of subcellular compartments besides preventing oxidative harm.

Influence of the proline on the antioxidant enzyme activity in *Digera muricata* as published in the article Verma *et al.* 2020 [26]. Rathore *et al.* (2018) [16, 17], previously revealed that the alterations in abundance of Digera sp. had lower MDA content as compared to low stress tolerable plants when exposed to salt stress implying more efficient antioxidant system in the plant. Perusing the work of Singh and Kumari (2022) [21], the researchers established that exogenous application of proline and other preventive chemicals significantly reduced MDA concentrations in *Digera muricata*, thereby minimising oxidative injury under drought stress. In the case of drought and salt stressed plants, the activity of *Digera muricata* was significantly enhanced to counteract the superoxide radicals thus lowering the levels of ROS in stressed plants (Ahmad *et al.* 2017; Kumar *et al.* 2020) [3, 11].

Catalase (CAT) performs a critical function in detoxifying hydrogen peroxide, turning it into water and oxygen, thereby limiting the buildup of this hazardous ROS. In research by Sharma and Meena (2019) [19, 20], CAT activity in *Digera muricata* rose dramatically in response to drought stress, which was directly connected to the plant's capacity to control oxidative stress effectively. CAT activity showed, lowering hydrogen peroxide activity and limiting cellular damage (Yadav *et al.*, 2021) [27]. Singh *et al.* (2017) [22] demonstrated that POD activity in *Digera muricata* assisted in maintaining cellular redox equilibrium. Peroxidase activity is not only connected with ROS detoxification but also plays a role in lignin production, which contributes to the strengthening of cell walls and boosts the plant's capacity to endure mechanical stress (Kaushik *et al.* (2018) [9]. Ascorbate peroxidase (APX) plays a vital function in maintaining the equilibrium between ROS generation and removal in stressed plants (Khan *et al.*, 2020) [10]. Tripathi and Sharma (2022) [24] found that APX, together with CAT and SOD, forms a coordinated antioxidant defense mechanism that protects from oxidative damage. The research is focused on the assessment of antioxidant enzymatic of *Digera muricata*. Among them, superoxide dismutase (SOD) is involved in the dismutation of superoxide radicals while catalase (CAT) plays an important role in the decomposition of hydrogen peroxide so as to check its accumulation which is a dangerous ROS.

According to the study done by Sharma and Meena (2019) [19, 20] exposed that under drought stress *Digera muricata* enhance CAT activity, which was related to its ability to regulate the oxidative stress. The activity of CAT in *Digera muricata* as an antioxidant enzyme that reduces hydrogen peroxide production and decreases the amount of cellular damage (Yadav *et al.*, 2021) [27]. Singh *et al.*, (2017) [22] proved that POD activity in *Digera muricata* helped in the achievement of oxidative homeostasis in the cells. Enzyme peroxidase is not only linked to the process of detoxifying ROS but also in the formation of lignin that strengthens cell walls which in turn boosts the plant's ability to withstand mechanical pressure (Kaushik *et al.* (2018) [9]. They reported that ascorbate peroxidase (APX) provides a significant role in the regulation of ROS production as well as scissoring in stressed plants. Tripathi and Sharma (2022) [24] observed that APX along with CAT and SOD is involved in the antioxidant defense system which protects *Digera muricata* from oxidative stress. This research work is aimed at evaluating the antioxidant enzymatic activity of *Digera muricata*.

Materials and method

Collection of plant materials: Plants were collected from the different agro-climatic regions of the Rohtak and stored in the deep freeze for biochemical analysis whereas morphological characteristics were studied at the collection site. Plants were collected in the triplicate and data were also collected in the triplicate.

Malondialdehyde (MDA) Measurement: Membrane lipid peroxidation was determined by the increase in the malondialdehyde (MDA), an index of lipid peroxidation, and the TBARS assay based on Heath and Packer (1968) [28]. Plant tissue was prepared in trichloroacetic acid (TCA) with which tissues were homogenized and then added to thiobarbituric acid (TBA). Following incubation, absorbance was then determined at 532 nm and non-specific turbidity determined at 600 nm was subtracted from the former.

Superoxide Dismutase (SOD) Activity: SOD activity was measured in terms of its capacity to suppress the photochemical reduction of nitro blue tetrazolium (NBT) as described elsewhere by Beauchamp and Fridovich (1971) [1]. The assay medium consisted of riboflavin, methionine and NBT and the absorbance was measured at 560 nm.

Catalase (CAT) Activity: Activity of CAT was determined by following the oxidation of hydrogen peroxide (H_2O_2) at 240 nm according to the method described by Aebi (1984) [1]. The reduction in absorbance signified the decomposition of H_2O_2 as the coloured complex formed disappeared.

Peroxidase (POD) Activity: Pod activity was determined using the procedure of Chance and Maehly (1955) [5] which involves the ability of POD to catalyse the oxidation of guaiacol in presence of H_2O_2. It was measured using spectrophotometer at 470 nm of increased absorbance.

Ascorbate Peroxidase (APX) Activity: In thus assessing the activity of APX, H_2O_2 was allowed to decompose through ascorbate as outlined by Nakano and Asada, 1981 [9]. Measurement of APX activity was done by the determination of the decrease in absorbance at 290 nm.

Results

The data presented in table (1) revealed mean values and standard errors for malondialdehyde, superoxide dismutase, and catalase activities among five different biotypes (B1 to B5). MDA values, which indicate lipid peroxidation, varied from 0.013 ± 0.001 in B1 to 0.043 ± 0.001 in B2, suggesting the maximum oxidative damage in B2 and the lowest in B1 and B5. SOD activity, responsible for converting superoxide radicals into hydrogen peroxide, was greatest in B2 (0.673 ± 0.009) and lowest in B5 (0.427 ± 0.009), suggesting a higher antioxidant response in B2.

Table 1: Antioxidant activity of different biotype of *Digera muricata*

Treatment	MDA		SOD		CAT	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
B1	0.013	±0.001	0.557	±0.003	0.320	±0.000
B2	0.043	±0.001	0.673	±0.009	0.393	±0.003
B3	0.024	±0.002	0.463	±0.009	0.270	±0.000
B4	0.031	±0.001	0.603	±0.012	0.353	±0.009
B5	0.015	±0.003	0.427	±0.009	0.250	±0.006
C.D.	0.011		0.028		0.016	
SE(m)	0.001		0.009		0.005	

MDA: Malondialdehyde, **SOD:** Superoxide Dismutase, **CAT:** Catalase

CAT activity, which breaks down hydrogen peroxide, followed a similar trend, with B2 having the greatest activity (0.393 ± 0.003) and B5 the lowest (0.250 ± 0.006). The crucial difference values for MDA, SOD, and CAT are 0.001, 0.028, and 0.016, respectively, demonstrating substantial variations between each biotype. A substantial variance was discovered in the chosen biotype with malondialdehyde, superoxide dismutase, and catalase activities at the 5% of CD level.

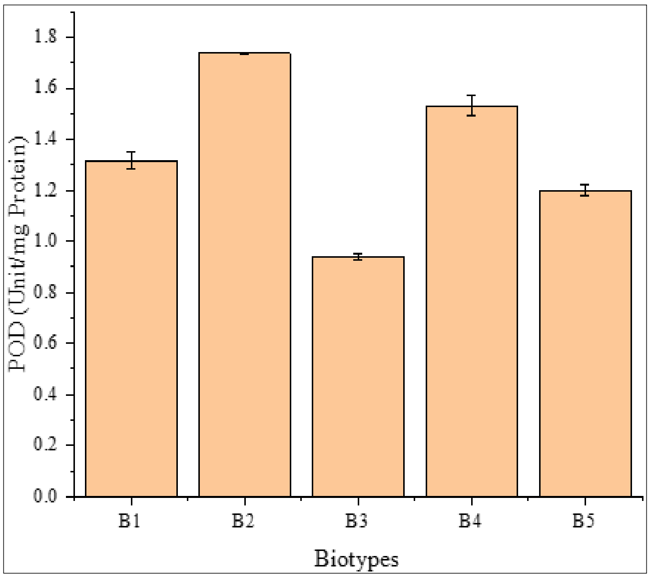

Fig 1: Peroxidase content (Unit/mg Protein) of different biotype of *Digera muricata*

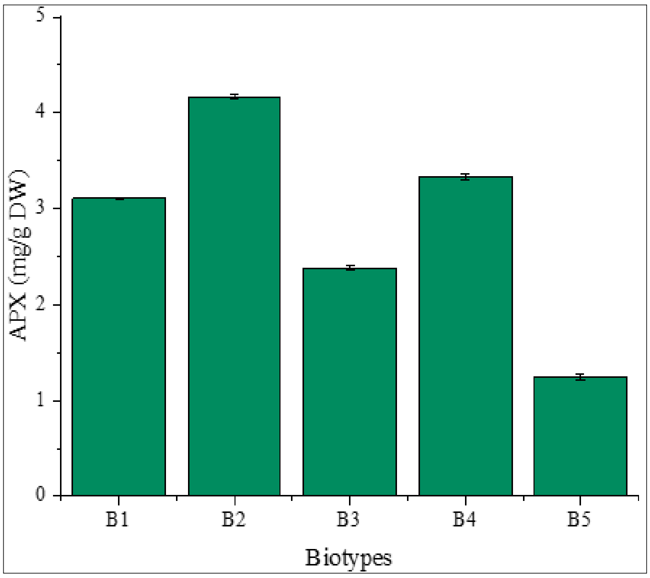

Fig 2: Ascorbate peroxidase (mg/g DW) of different biotype of *Digera muricata*

Biotype 2 shows the highest mean peroxidase activity (1.737±0.003), followed by Biotype 4 (1.533±0.038). Biotype 1 and Biotype 5 have moderate levels of POD (1.317±0.033 and 1.200±0.021, respectively) whereas biotype 3 shows the lowest POD activity (0.940±0.015) in all tested biotype. Highest APX activity (4.167±0.024) in B2, while B5 has the lowest (1.247±0.030) in APX activity. Biotypes 1, 3, and 4 show intermediate APX activities, with B1 having 3.107±0.003 and Biotype 4 having 3.337±0.033. POD and APX showed significant intraction with all tested biotypes.

Table 2: Mean sum of squares of the proline, malondialdehyde, superoxide dismutase, catalase, peroxidase, ascorbate peroxidase

Source of Variation	DF	MDA	SOD	CAT	POD	APX
Treatment	4	0.002**	0.122**	0.042**	1.124**	14.493**
Error	10	0.001**	0.002**	0.001**	0.019**	0.019**
Total	14	0.002	0.124	0.042	1.144	14.512

MDA: Malondialdehyde, **SOD:** Superoxide Dismutase, **CAT:** Catalase, **POD:** Peroxidase, **APX:** Ascorbate Peroxidase

Analysis of variance (ANOVA) for MDA, SOD, CAT, POD, and APX in Table-2 showed performance of different biotypes. Highly significant ANOVA ($p<0.01$) were observed for all parameters across treatments, indicated by the biotype sum of squares for MDA (0.002), SOD (0.122), CAT (0.042), POD (1.124), and APX (14.493). The mean sum of squares underlines the overall interaction in the data for each parameter indicates significant treatment effects on antioxidant activities.

Discussion

The relationship between the antioxidant activities of MDA, SOD, catalase, peroxidase, and ascorbate in plants is closely linked to the species' biochemical characteristics. Membrane lipid peroxidation was assessed by the rise in the malondialdehyde (MDA), an indicator of lipid peroxidation, and the TBARS test based on Heath and Packer (1968) [28]. Sharma and Meena (2019) [19, 20] found that CAT activity in *Digera muricata* significantly increased due to drought stress. The large rise in antioxidant enzyme activity in the context of drought and salt stress, boosted the SOD, CAT activities and a decrease in MDA levels (Singh and Kumari, 2022) [21]. Kaushik *et al.* (2018) [9] discovered which POD play in the synthesis of lignin; therefore, the improved capacity of D. muricata biotypes, notably Biotype 2, to withstand mechanical and environmental forces may be understood. The elevated APX activity in Biotype 2 enhances the plant's exceptional ability to regulate ROS levels, as asserted by Tripathi and Sharma (2022) [24], emphasizing the significance of APX in ROS management. Sharma *et al.* (2011) directly evaluated the plants from diverse places and the antioxidant activity of these plants had showed extremely obvious variances (Jafri *et al.*, 2022; Luo *et al.*, 2024) [30, 31].

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Conflict of Interest

The author(s) declares no conflict of interest.

Author Contribution

All authors contributed equally to establishing the topic of the research and design experiment.

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