Eco. Env. & Cons. 30 (January Suppl. Issue) : 2024; pp. (S385-S388)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2024.v30i01s.077

Comparative study of enzyme Oxalate oxidase in *Portulacaria afra* (L.) Jacq. subjected to abiotic stress

Ruchira V. Javkar[1]* and Anil E. Avhad[2]

Department of Biological Sciences, Ramniranjan Jhunjhunwala College, Ghatkopar, Mumbai 400086, M.S., India

(Received 12 June, 2023; Accepted 10 August, 2023)

ABSTRACT

Several studies have reported the presence of higher levels of enzymes in plants subjected to drought and salinity stress compared to plants grown in control environmental conditions. The enzyme levels are often measured to understand the mechanisms that plants use to cope with these stressors and adapt to changing environmental conditions. Oxalate oxidase enzyme (OXO, EC 1.2.3.4.) is a glycoprotein that catalyses the oxidation of oxalate to carbon dioxide and hydrogen peroxide (Whittaker and Whittaker, 2002). Oxalate oxidase was purified to homogeneity from *Portulacaria afra* leaf samples subjected to abiotic stress by sequential ammonium sulphate precipitation and Sephadex C-50 gel filtration chromatography. The enzyme was purified with a recovery of 53.88% in Control while 76.53% in long-term drought and 78.66 % in long term salinity stress plants. The higher enzyme activity levels in stressed plants indicate that the plant is actively responding to the abiotic stress. Understanding the mechanisms that plants use to cope with stress can help in developing strategies to improve crop productivity under adverse environmental conditions.

Key words: *Alarm photosynthesis, Enzyme Dialysis, Oxalate oxidase, Portulacaria afra, Sephadex Ion Exchange Chromatography.*

Introduction

Alarm photosynthesis is a term used to describe a unique process of photosynthesis in plants that occurs when the plant is under stress. Over time, plants evolved a variety of chemical and structural defences to deter herbivores from feeding on them. Oxalates are organic acids that can accumulate in various plant tissues, shown to play a role in plant defence against herbivores and pathogens (War *et al*., 2012). The oxalates can interfere with herbivore digestion and also contribute to the formation of calcium oxalate crystals, which can physically damage herbivore mouthparts or deter feeding. However, high levels of oxalates can also be toxic to the plant itself, especially under stress conditions.

This is where oxalate oxidase enzyme comes into play. Oxalate oxidase (OXO) is an enzyme found in various organisms, including plants, that catalyses the breakdown of oxalate into carbon dioxide and hydrogen peroxide (Sugiura *et al.,* 1979; Pietta *et al.,* 1982). Later research also showed that OXO is involved in various physiological processes, including photosynthesis (Franceschi and Nakata, 2005). The goal of the current study is to determine whether plants under drought and salinity stress have higher levels of the enzyme oxalate oxidase.

Material and Methodology

Cultivating *Portulacaria afra* (L.) Jacq.

Saplings of *Portulacaria afra* were imported from a

([1]Research Scholar, [2]Associate Professor)

nearby nursery and were allowed to grow naturally with temperatures ranging from 30-35 °C during the day and 20-25 °C at night, 65% relative humidity, 14h/16h of light, and 10h/8h of

darkness, respectively. During 120 days, the seedlings were allowed to acclimatise and grow, they were watered every third day. Clones were made from the horizontal branches For two months, the clones kept growing vegetatively. Drought and salt treatments were initiated at the end of two months. (Javkar and Avhad, 2023).

Establishing the stress conditions

Several groups of plants, including Control, Short Term Drought (STD), Long Term Drought (LTD), Short Term Salinity Stress (STS), and Long-Term Salinity Stress (LTS), were created. Five plants apiece were assigned to each group. Long- term drought plants were irrigated after every 15 days, while short-term drought plants received one watering at the end of every week. The prolonged stress experiment was carried out to look for any changes over a longer drought (Bastide B. *et al.,* 1993). Checking the threshold salt content was the first step in the salt treatment. Several salinity levels, including 100 mM, 300 mM, and 500 mM NaCl, were administered to plants to cause both short-term and long-term salt stress. For both short-term and long-term, this treatment was given for a total of 30 days and 60 days, respectively. Prior to each rewatering, the leaves were picked (Javkar and Avhad, 2023).

Crude Enzyme extraction

Portulacaria afra leaves were weighed and washed under running water, patted on blotting paper and air dried until the surface was complete moisture free. Solubilization of membrane-bound enzyme was done According to Lathika *et al.*, 1995. Plant sample of 100 g was crushed in a pre-chilled mortar and pestle in 100 ml of chilled lysis buffer containing Triton X- 100 and 0.01 M Tris HCl (pH 7.4). 1 mM PVPP was added to the supernatant right away in order to eliminate the action of polyphenol and other biomolecules. It was homogenized in a homogenizer and the further cell lysis was done in a sonicator. The mixture was filtered through a muslin cloth and then centrifuged for 30 mins at 15,000 x g at 4 °C. The supernatant was heated at 80 degree C for 5 mins and then centrifuged at 1000 x g for 15 mins at 4 °C, the resulting supernatant collected served as the source of crude enzyme. Its enzyme activity and specific activity were calculated. Proteins content was studied by Lowry's method, 1951.

Enzyme Purification by Dialysis Method

The Crude enzyme extract was further subjected to purification by enzyme dialysis method.

Step I Precipitation of ammonium sulphate (0-35%)

Solid ammonium sulphate was added to solubilized enzyme to achieve 0–35 percent saturation. The solution was mixed and centrifuged for 20 minutes at 10,000g. The pellet was removed from the supernatant and dissolved in 15 ml of 0.02 M PO4 buffer (pH 7.0) before being dialyzed against the same buffer (pH 7.0, 0.01 M) at 4 °C for 24 hours with constant stirring through a dialysis tube (UK Laemmli,1970). Sample E1 was created from the resulting solution.

Step II Precipitation of ammonium sulphate (35-80%)

The supernatant was then utilised to precipitate the solubilized protein in its entirety. To achieve a final

Fig. 1. *Portulacaria afra* experimental setup (Javkar and Avhad, 2023).

saturation of 35–80%, ammonium sulphate was added to the supernatant. The supernatant was discarded, and the pellet was dissolved in the same buffer following dialysis, yielding Sample E2.

Step III DEAE Sephadex ion exchange chromatography

Isolated dialyzed enzymes were placed onto a 1.5 x 100 cm, CM Sephadex C- 50 (weak cation exchange) column, which had already been pre-equilibrated with 0.05 M PO4 buffer pH 7.0. The column was eluted at a rate of 0.5 ml/min in the same buffer (Anjum *et al*., 2014). Protein and oxalate oxidase activities were measured in the fraction. The bound proteins were eluted using a linear NaCl (0-0.9 mM) gradient in 0.05 M PO4 buffer pH 7.0, and 3 ml were collected at a flow rate of 0.5 ml/min and assayed for protein and oxalate oxidase activity yet again. The specific activity of said active fractions were determined after they were pooled.

Table 1. Oxalate oxidase Enzyme Assay

	Blank (ml)	Reaction Mixture (ml)	Standard (ml)
MBTH	2.70	2.70	2.70
EDTA	0.10	0.10	0.10
D.W	0.10	0.10	0.10
Oxalic acid	0.02	0.02	0.02
Peroxidase	0.01	0.01	0.01
Oxalate oxidase	——	0.02	0.02

Oxalate Oxidase Enzyme Assay

Reaction mixture of 2.95 ml was prepared by adding 0.10 ml, 0.01ml and finally 0.02 ml Crude Oxalate oxidase (as std from Barley) which was replaced with

D.W for blank and *P. afra* enzyme extract for sample. Time course measurement at 600 nm for 5 min was performed for the above reaction mixture. Unit of enzyme One unit of enzyme is defined as the amount of enzyme required to generate 1 nmol H2O2/min under standard assay conditions. (Anjum *et al.*, 2014)

Results and Discussion

Extracts from pure leaf samples were tested for the presence of the enzyme oxalate oxidase. The results of this study lend support to the theory that plants that can tolerate stress exhibit an increase in oxalate oxidase as a defence mechanism, protecting themselves against herbivores by accumulating oxalates. Yet, an overabundance of oxalates can be deadly to plants, therefore they produce the oxalate oxidase enzyme to break down calcium oxalate crystals. It also supports the idea that stressed plants exhibit

Table 2. Oxalate oxidase Enzyme Assay in *Portulacaria afra* subjected to abiotic stress.

		Protein (mg)	Enzyme Activity (units)	Specific activity (Units/mg)	Fold Purification	Percentage Enzyme Yield%
Control	Crude	52.5351	1.837	0.035	2.304	53.88
	Purified	12.2869	0.990	0.081		
Short term drought	Crude	60.7335	2.451	0.040	3.795	71.78
	Purified	11.4887	1.759	0.153		
Long term drought	Crude	66.6936	2.907	0.044	4.600	76.53
	Purified	11.0949	2.225	0.201		
Short term Salinity 100 mm	Crude	53.6138	2.116	0.039	2.373	52.16
	Purified	11.7853	1.104	0.094		
Short term Salinity 300 mm	Crude	54.5307	2.389	0.044	4.186	66.45
	Purified	8.6570	1.588	0.183		
Short termSalinity 500 mm	Crude	61.5696	3.335	0.054	5.201	66.79
	Purified	7.9072	2.228	0.282		
Long term Salinity 100 mm	Crude	43.8242	2.145	0.049	3.061	51.99
	Purified	7.4434	1.115	0.150		
Long term Salinity 300 mm	Crude	36.2190	2.529	0.070	3.618	75.97
	Purified	7.6052	1.921	0.253		
Long term Salinity 500 mm	Crude	41.4509	3.487	0.084	3.766	78.66
	Purified	8.6570	2.743	0.317		

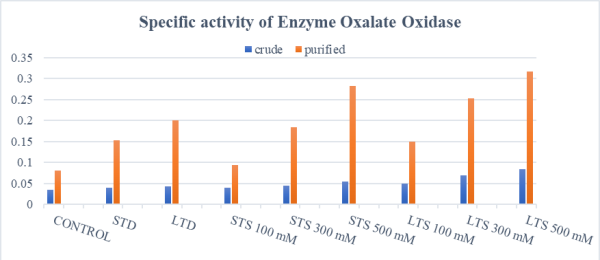

Fig. 2. Graphical representation of Specific activity of enzyme Oxalate oxidase

more pronounced calcium oxalate crystal disintegration, which calls for the Oxalate Oxidase enzyme to function in order to make up for the reduced CO2 fixation caused by stomatal closure.

Conclusion

Enzyme Oxalate Oxidase exhibits specific activity of 0.081 U/mg under control conditions, but increases to 0.153 U/mg and 0.201 U/mg under short and long- term drought conditions, respectively. Moreover, 71.78% and 76.53% of the enzyme yield are shown. By using enzyme dialysis to concentrate the enzyme extracts, the specific activity of the purified extracts was somewhat increased in comparison to the crude extracts. Similar to this, plant samples under short-term and long-term 500 mM salinity stress showed enzyme activity up to almost 0.282 U/mg and 0.317 U/mg. In comparison to the crude extracts, the enzyme was ultimately purified 3.766 times with a recovery rate of 78.66%.

Acknowledgments

The authors are grateful to The Principal of the institution, Biotechnology Research Lab, all colleagues for their valuable guidance and support.

Conflicts of interest

The authors declare that they have no potential conflicts of interest.

References

Anjum, S., Sundaram, S. and Kumar Rai, G. 2014. Banana peel oxalate oxidase- detection, purification, characterization and physiological role. *J Pharm Pharm Sci*. 7: 332-339.

Bastide, B., Sipes, D., Hann, J. and Ting, I.P. 1993. Effect of severe water stress on aspects of crassulacean acid metabolism in Xerosicyos. *Plant Physiology.* 103(4): 1089-1096.

Franceschi, V.R. and Nakata, P.A. 2005. Calcium oxalate in plants: formation and function. *Annu. Rev. Plant Biol*. 56: 41-71.

Javkar, R. and Avhad, A. 2023. Effect of drought and salinity stress on Calcium oxalate crystals of *Portulacaria afra.* (L.) Jacq. *Journal of Stress Physiology & Biochemistry*. 19(1):127-140.

Retrieved from: http://jspb.ru/index.htm

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 227(5259): 680-685.

Lathika, K.M., Sharma, S., Inamdar, K.V. and Raghavan, K.G. 1995. Oxalate depletion from leafy vegetables using alginate entrapped banana oxalate oxidase. *Biotechnology Letters*. 17: 407-410.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin reagent. *J Biol Chem*. 193: 275.

Pietta, P.G., Calatroni, A., Agnellini, D. and Pace, M. 1982. Improved purification protocol for oxalate oxidase from barley roots. *Preparative Biochemistry*. 12(4): 341-353.

Sugiura, M., Yamamura, H., Hirano, K., Sasaki, M., Morikawa, M. and Tsuboi, M. 1979. Purification and properties of oxalate oxidase from barley seedlings. *Chemical and Pharmaceutical Bulletin*. 27(9): 2003-2007.

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S. and Sharma, H.C. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling & Behavior*. 7(10): 1306-1320.

Whittaker, M.M. and Whittaker, J.W. 2002. Characterization of recombinant barley oxalate oxidase expressed by Pichia pastoris. *Journal of Biological Inorganic Chemistry*. 7(1-2): 136-145.