International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 12, Issue 4-2025

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2025.12.04.001

Dehydrogenase activity in leaves

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Abstract

Plants are rich in dehydrogenases, which use different substrates for functioning as oxidoreductases. Succinate dehydrogenase, an enzyme bound to the inner mitochondrial membrane, is an important dehydrogenase with a dual role. The enzyme oxidizes succinate to fumarate in the citric acid cycle, and reduces ubiquinone during the electron transport chain. The present investigation aims at studying dehydrogenase activity in leaves and monitoring the progression of dehydrogenase activity using colorimetry. The monocot *(Tradescantia fluminensis)* and the dicot *(Portulaca umbraticola)* were selected as the experimental materials for a comparison of dehydrogenase activity. Reaction mixtures with and without succinic acid were maintained. Methylene blue, an artificial electron acceptor, was used as the dye to indicate the activity of dehydrogenases. The study showed that both the materials contained dehydrogenase as well as succinate dehydrogenase activity. However, *T. fluminensis* had higher dehydrogenase activity than *P. umbraticola.* Therefore, the progression of dehydrogenase activity was studied using leaves of *T. fluminensis* in the presence of succinic acid. The dehydrogenases were active, and one hour from incubation the dye reduction was nearly 90 %.

Keywords: Dehydrogenases, succinate dehydrogenase, methylene blue, colorimetry, *Portulaca umbraticola, Tradescantia fluminensis*

1. Introduction

Dehydrogenases are oxidoreductase enzymes. There are several dehydrogenases in the cell, such as glyceraldehyde-3-phosphate dehydrogenase involved in glycolysis and dark reaction of photosynthesis; pyruvate dehydrogenase that links citric acid cycle to glycolysis; isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase in citric acid cycle; NADH dehydrogenase complex in mitochondrial electron

transport chain; alcohol dehydrogenase and lactate dehydrogenase in fermentation; glutamate dehydrogenase in ammonium assimilation; and glucose-6-phosphate dehydrogenase in pentose phosphate pathway. Succinate dehydrogenase, a flavoprotein bound to the inner mitochondrial membrane of eukaryotes, is the only enzyme that participates in both the citric acid cycle and the electron transport chain. Succinate dehydrogenase oxidizes succinate to fumarate, thereby reducing FAD to $FADH_2$ in the citric acid cycle. The same enzyme referred to as Complex II in the mitochondrial electron transport chain also transfers electrons from succinate via $FADH_2$ through a group of three Fe-S proteins to the ubiquinone pool (Lehninger et al.,1993; Taiz and Zeiger, 1998).

Methylene blue has been used as the indicator dye and artificial electron acceptor to measure dehydrogenase activity. In the oxidized state the dye is blue and becomes decolorized on being reduced. The redox potentials of the oxidized and reduced forms of methylene blue are similar to that of the oxidized and reduced forms of ubiquinone; hence methylene blue competes with ubiquinone for getting reduced by succinate dehydrogenase (Plummer, 1978). The extent of reduction of methylene blue recorded using a colorimeter is a measure of the extent of dehydrogenase activity. Faster the reduction of methylene blue, lower will be the absorbance and more will be the dehydrogenase activity. When succinic acid, the substrate for succinate dehydrogenase, is provided in the reaction mixture containing the experimental material, the activity of succinate dehydrogenase will be promoted and the decolorization of methylene blue will be faster than in the reaction mixture without succinic acid.

Dehydrogenases are present in the different parts of a plant and during the various developmental stages of plant parts: for example, they are present in seeds, during seed germination, in leaves and in pollen grains (Moin et al., 2024; Subramanyan and Bahri, 2013). Succinate dehydrogenase is present in all cells respiring aerobically (Hederstedt and Rutberg, 1981). Leaves are sites of active metabolism. For a comparative analysis, leaves of a monocot and a dicot were selected. The experimental materials selected were easily available in summer in Delhi, and have value in traditional medicine. The fresh leaves of *Tradescantia fluminensis* Vell. (common name small-leaf spiderwort, family Commelinaceae) and *Portulaca umbraticola* Kunth (common name wingpod purslane, family Portulacaceae) were chosen for the study. The observations showed that the leaves of *T. fluminensis* had higher dehydrogenase activity than the leaves of *P. umbraticola.* Therefore, the progression of dehydrogenase activity was studied using the leaves of *T. fluminensis.*

2. Materials and Methods

2.1 Detection of dehydrogenase activity

Dehydrogenase activity was studied in fresh healthy leaves of *Portulaca umbraticola* and *Tradescantia fluminensis* collected from the college garden. The test tubes were set up as given in Table 1. The substrate succinic acid (0.05 M, 6 mL) was added in all the test tubes except the controls. Then 20 µL of 1 % methylene blue (aqueous) was added. The leaves were weighed (0.5 g), placed on a microslide and sliced with the help of a blade. The materials were placed in a Petri dish lined with moist filter paper till transfer to the reaction mixtures. For the boiled and cooled control, the weighed material was first sliced and then boiled in distilled water taken in a boiling tube, and then cooled by holding the tube under a running water tap; the water in the boiling tube was drained and the material was taken out with a spatula, blotted and used in the experiment. The plant materials were added last into the test tubes. Control test tubes with distilled water instead of the substrate succinic acid; and without the experimental material but with the substrate and methylene blue, or with distilled water and methylene blue were maintained for comparison. All the test tubes were incubated in the dark. One hour from incubation the first reaction mixture was shaken, and then carefully decanted into a clean and dry cuvette and the absorbance was recorded at 600 nm using a colorimeter.

Darkness has been reported to favour succinate dehydrogenase activity (Popov et al., 2010).The same procedure was followed for all the reaction mixtures. The colorimeter was set for zero absorbance at 600 nm using succinic acid for tubes 1, 2, 4, 5, and 8; and using distilled water for tubes 3, 6 and 7 (Table1). The experiment was repeated six times and the average absorbance values have been taken for interpreting the results.

2.2 Progression of dehydrogenase activity in *T. fluminensis*

The previous experiment showed that dehydrogenase activity was more in *T. fluminensis* than in *P. umbraticola.* Therefore, leaves of *T. fluminensis* were used to study the progression of dehydrogenase activity over a period of time (Table 2). The colorimeter was set for zero absorbance at 600 nm using succinic acid. The absorbance of the reaction mixtures was recorded at 15-minute time intervals up to 1 hour from incubation. The experiment was repeated four times and the average absorbance values and percent decrease in absorbance were calculated.

3. Results

3.1 Detection of dehydrogenase activity

Both the leaf materials showed dehydrogenase activity (Table 1, Figure 1). However, in the presence of succinic acid the dehydrogenase activity was more compared to the activity in the absence of succinic acid. This showed that the leaves of both the species contained good succinate dehydrogenase activity: in the presence of the substrate succinic acid in the reaction mixture the electron and hydrogen ion transfer from succinic acid to methylene blue is rapid and, therefore, the fading of the intensity of blueness of methylene blue. Both in the presence and absence of succinic acid, the dehydrogenase activity in *T. fluminensis* was more than that in *P. umbraticola* as observed by the corresponding lower absorbance values in *T. fluminensis.* In both species the boiled and cooled sample of leaf material showed higher absorbance compared to the absorbance when fresh leaf material was present. The controls with no plant material and with or without succinic acid showed nearly the same absorbance.

Table 1. Dehydrogenase activity in leaves of *P. umbraticola* and *T. fluminensis.*

S. No.	Plant	SA* (mL)	DW** (mL)	MB*** (µL)	Absorbance (600 nm)						
					Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Avg
1	*P. umbraticola*	6	_	20	0.06	0.09	0.14	0.17	0.08	0.15	0.115
2	*P. umbraticola* B&C	6	_	20	0.34	0.20	0.24	0.28	0.20	0.25	0.252
3	*P. umbraticola*	_	6	20	0.17	0.12	0.17	0.18	0.10	0.18	0.153
4	*T. fluminensis*	6	_	20	0.02	0.03	0.02	0.03	0.01	0.02	0.022
5	*T. fluminensis* B & C	6	_	20	0.12	0.13	0.10	0.11	0.12	0.10	0.113
6	*T. fluminensis*	_	6	20	0.09	0.08	0.09	0.08	0.07	0.08	0.082
Controls without the plant material											
7	_	_	6	20	0.20	0.29	0.20	0.21	0.21	0.20	0.218
8	_	6	_	20	0.23	0.19	0.19	0.22	0.19	0.19	0.202

SA*: Succinic Acid; DW**: Distilled Water; MB***: Methylene Blue; B & C: Boiled & Cooled.

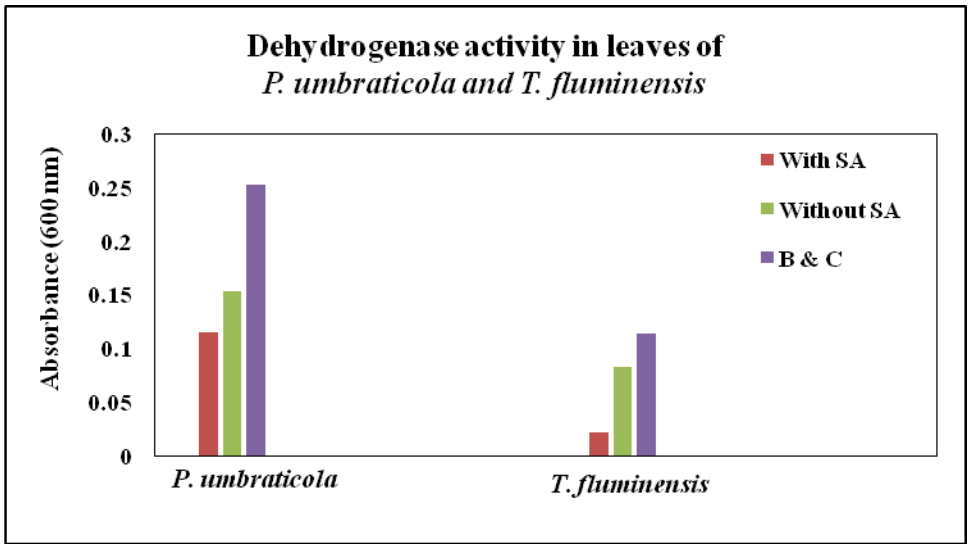

Figure 1. Dehydrogenase activity in leaves of *P. umbraticola and T. fluminensis.* The absorbance of the reaction mixtures with succinic acid, without succinic acid, and with boiled and cooled leaf material. SA: Succinic Acid; B & C: Boiled & Cooled.

3.2 Progression of dehydrogenase activity in *T. fluminensis*

The absorbance of the reaction mixture decreased rapidly and in one hour from incubation the decrease in absorbance was close to 90 % (Table 2, Figure 2). As the period of incubation increased, methylene blue in the reaction mixture gradually got reduced and there was a concomitant decrease in the absorbance (Table 2, Figure 2). Thirty and 60 minutes from incubation the decrease in absorbance was nearly 52 % and 88%, respectively (Table 2).

Table 2. Progression of dehydrogenase activity in *T.fluminensis* leaves.

Time (min)	SA* (mL)	MB** (µL)	Absorbance (600 nm)					
			Replicate No.				Average	% Decrease in absorbance
			1	2	3	4		
0	6	20	0.28	0.28	0.28	0.28	0.280	--
15	6	20	0.23	0.23	0.24	0.24	0.235	16.07
30	6	20	0.13	0.14	0.13	0.14	0.135	51.79
45	6	20	0.08	0.09	0.09	0.10	0.090	67.86
60	6	20	0.03	0.03	0.03	0.05	0.035	87.50

SA*: Succinic Acid; MB**: Methylene Blue

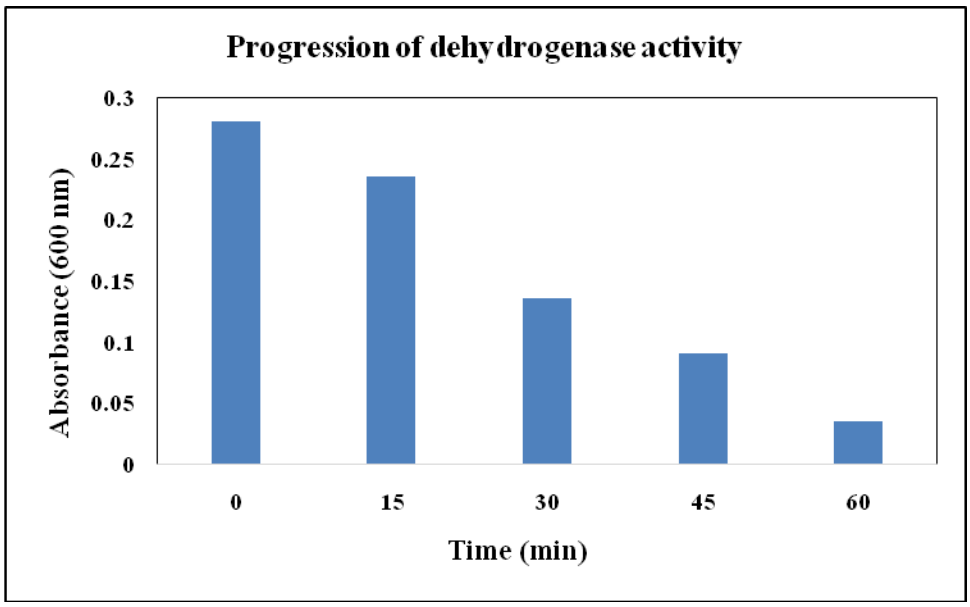

Figure 2. The absorbance of the reaction mixtureof *T. fluminensis* at 0, 15, 30, 45 and 60 minutes from incubation.

4.Discussion

4.1 Detection of dehydrogenase activity

When the leaves were boiled and used, the dye reduction could not take place because of the denaturation of dehydrogenases (Table 1, Figure 1). However, the control with boiled and cooled leaves of *T. fluminensis* showed less absorbance than that of *P. umbraticola.* It is quite likely that the low absorbance in *T. fluminensis* was because the hydrogen ions and electrons already present in the sliced leaf material were at concentrations sufficient enough to reduce some of the methylene blue causing a decrease in absorbance. Also, in case of *P. umbraticola,* which is low in dehydrogenase activity, slicing the leaf which is succulent causes turbidity and hence the increase in absorbance. Figueroa et al. (2001) showed that succinate dehydrogenase is coded by three nuclear genes in *Arabidopsis thaliana*; two of the genes code nearly identical proteins (SDH2-1 and SDH2-2) and were expressed in all tissues analysed. However, the highest expression of the two genes was detected in flowers and inflorescences. The third gene coding for SDH2-3 was 67% similar to SDH2-1 and SDH2-2, and was expressed at a low level (Figueroa et al., 2001). In another study the expression of succinate dehydrogenase in *A. thaliana* was studied using isolated mitochondria obtained from leaves exposed to varying illumination (Popov et al., 2010). The enzyme activity increased briefly when transferred to light from darkness; however, the activity then decreased to half of the original activity: and the red-far red photoreversibility of the effect was also observed. It was concluded that phytochrome A regulates mitochondrial respiration through its effect on SDH expression (Popov et al., 2010).

Plants contain 14 families of aldehyde dehydrogenases which indicates the functional versatility of the enzyme (Islam and Ghosh, 2022), and the enzyme glucose-6-phosphate dehydrogenase has various roles in plants, namely in seed germination, bud dormancy, nitrogen assimilation, and response to abiotic stresses (Jiang et al., 2022). At least six malate dehydrogenases have been localized to the cytosol, mitochondria, plastids, and peroxisomes of land plants. The enzyme has an important role in photosynthesis, photorespiration, lipid metabolism, and NH_4^+ metabolism (Baird et al., 2024). Malate can be shuttled through membranes

via translocators, and the interconversion of malate and oxaloacetate is carried out by malate dehydrogenases. Malate-oxaloacetate interconversions are important in photorespiration, fatty acid β-oxidation, inter-organelle signalling and in CO_2-concentrating mechanisms in photosynthetic eukaryotes (Dao et al., 2022). Recently it has been realized that during stress, chloroplast NADPH dehydrogenase complex is essential for plant growth and development, and according to Ma et al. (2021) a better understanding of the NADPH dehydrogenase during photosynthesis may help improve crop yield. It is interesting to observe that *P. umbraticola* shows less dehydrogenase activity than *T. fluminensis* although the former has been reported to be a C_4 plant based on Kranz anatomy, expression of the key C_4 enzyme NADP-malic enzyme, and leaf carbon isotope values (Voznesenskaya et al., 2010). Betaine aldehyde dehydrogenase is an important enzyme in the biosynthesis of glycine betaine, an osmo-protectant, and tolerance to a variety of abiotic stresses has been improved by introducing the gene coding for betaine aldehyde dehydrogenase into different plant species (Niazian et al., 2021). The chloroplast $NADP^+$-malate dehydrogenase in C_3 plants is a light-activated enzyme and catalyzes the export of malate from the chloroplast only when the NADPH to $NADP^+$ ratio is high, thereby balancing the NADPH to ATP ratio required for optimal carbon reduction in the presence of light (Scheibe, 1987).

4.1 Progression of dehydrogenase activity in *T. fluminensis*

As the period of incubation increased the methylene blue molecules were gradually reduced by dehydrogenases present in the sliced leaf material added to the reaction mixture (Table 2, Figure 2). The reduced methylene blue is colourless, and hence the fading of blue colour was observed with time. The oxidation of succinic acid present in the medium by succinate dehydrogenase provided an additional source of electrons and hydrogen ions enhancing the reduction of methylene blue.

5. Conclusion

Both *P. umbraticola* and *T. fluminensis* contain dehydrogenases. However, *T. fluminensis* showed higher dehydrogenase as well as succinate dehydrogenase activity than *P. umbraticlola.* One hour from incubation the reduction of methylene blue by *T. fluminensis* leaves was nearly 90 %.

6. Acknowledgments

We sincerely thank Professor Bijayalaxmi Nanda, Principal, Miranda House, University of Delhi, Delhi, and Professor Monika Tomar, Coordinator, D S Kothari Centre for Research and Innovation in Science Education, Miranda House, University of Delhi, Delhi, for organizing the Workshop Flavor of Research and providing us the facilities to conduct research during the summer vacations of 2023. The laboratory staff of the Department of Botany gave us full support and cooperation and we thank them.

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DOI:10.22192/ijarbs.2025.12.04.001

How to cite this article:
Janaki Subramanyan, Ilma Moin, Lenthoibi Thokchom, Muani Mizo and Prachi Patidar. (2025). Dehydrogenase activity in leaves. Int. J. Adv. Res. Biol. Sci. 12(4): 1-7.
DOI: http://dx.doi.org/10.22192/ijarbs.2025.12.04.001