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Research Article

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Dehydrogenase activity in germinating barley grains and green gram seeds

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Abstract

Dehydrogenases are common enzymes which use different substrates for carrying out the oxidoreductase reaction. Succinate dehydrogenase is a dehydrogenase found in all aerobic organisms. The enzyme has two functions: the first is to oxidize succinate to fumarate in the tricarboxylic acid (TCA) cycle and the second function is to reduce ubiquinone during the electron transport chain. In the present study dehydrogenase activity was measured using colorimetry during germination in a monocot and a dicot, namely in grains of barley (*Hordeum vulgare*) and seeds of green gram (*Vigna radiata*) three and five days from sowing. The artificial electron acceptor methylene blue was used as the indicator of dehydrogenase activity. Both *H. vulgare* and *V. radiata* contain dehydrogenases and in presence of the substrate succinic acid the dehydrogenase activity increased. As germination progressed the dehydrogenase activity increased: 5-day-old seedlings of both taxa studied showed more total as well as succinate dehydrogenase activity than the respective 3-day-old seedlings. In 3-day-old seedlings, both total dehydrogenase and succinate dehydrogenase activity in *H. vulgare* was more than that in *V. radiata*. However, in 5-day-old seedlings although the total dehydrogenase activity in *H. vulgare* was more than the activity in *V. radiata*, the succinate dehydrogenase activity was more in *V. radiata* than the activity in *H. vulgare*. The study shows that dehydrogenases in general and succinate dehydrogenase in particular have an important role during seed germination.

Keywords: Dehydrogenases, succinate dehydrogenase, seed germination, methylene blue, colorimetry, *Hordeum vulgare, Vigna radiata*

1. Introduction

Dehydrogenases are oxidoreductases present in the different parts of plants (Moin et al., 2024; Subramanyan and Bahri, 2013; Subramanyan et al., 2025). There are as many as 14 families of aldehyde dehydrogenases in plants. The large number is because of genome duplication and expansion, and shows the versatile nature of the enzyme (Islam and Ghosh, 2022). Malate dehydrogenases, which catalyse the interconversion of oxaloacetate and malate, are involved in the TCA cycle, photosynthesis, photorespiration, lipid metabolism and ammonium metabolism; all major groups of land plants contain at least six malate dehydrogenases present in the cytosol, mitochondria, plastids and peroxisomes (Baird et al., 2024). Succinate dehydrogenase is present in all aerobes (Hederstedt and Rutberg, 1981). The enzyme is a flavoprotein having a dual role: participating in the TCA cycle as well as the electron transport chain. During the TCA cycle succinate is oxidized to fumarate and FAD is reduced to $FADH_2$. In the electron transport chain succinate dehydrogenase, also referred to as Complex II, 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).

In the present investigation methylene blue has been used as the artificial electron acceptor and indicator dye to determine dehydrogenase activity. Methylene blue is blue in the oxidized state and turns colourless on being reduced. Both methylene blue and ubiquinone compete with each other for getting reduced by succinate dehydrogenase because the redox potentials of the two compounds are similar in their oxidized and reduced states (Plummer, 1978). The fading of methylene blue in the reaction mixtures and hence less absorbance as recorded by a colorimeter is a measure of the extent of dye reduction as a consequence of dehydrogenase activity. Addition of succinic acid, the substrate for succinate dehydrogenase, to the reaction mixtures with the experimental materials will promote succinate dehydrogenase activity and the decolorization of methylene blue will be faster as compared to the reaction mixtures without succinic acid.

Seed germination is crucial to achieve optimal crop productivity, both for agronomic and economic reasons (Farooq et al., 2021). Seeds require water, oxygen and a suitable temperature for germination. Once radicle emergence occurs, the stored food reserves start getting mobilized by the hydrolytic enzymes such as amylase in cereals and pulses (Subramanyan et al., 2023). The respiration is anaerobic when the seed is resting and during the initial stages of germination (Oaikhena et al., 2013).The imbibition of water by seeds causes resumption of metabolic activity: initially the already existing enzymes such as the TCA cycle enzymes which are sufficient are utilized. The pre-existing mitochondria which are few and poorly differentiated may undergo repair and become fully functional, and biogenesis of new cell organelles including mitochondria occurs (Bewley et al., 2000). In the present study *Hordeum vulgare* L., a major cereal crop of the temperate regions; and *Vigna radiata* (L.) R. Wilczek, an important pulse crop in Asia, were selected as the monocot and dicot, respectively, for comparing dehydrogenase activity in 3- and 5-day-old seedlings.

2. Materials and Methods

A comparative study on dehydrogenase activity during germination was carried out using 3-day-old and 5-day-old germinated seedlings of *Hordeum vulgare* L. (common name barley, family Poaceae, a monocot) and *Vigna radiata* (L.) R. Wilczek (common name green gram or mung bean, family Fabaceae, a dicot).

2.1 Raising the seedlings

H. vulgare grains and *V. radiata* seeds, around 50 g each, were taken in separate beakers, rinsed thoroughly with tap water, and then soaked in tap water for 3 hours. A muslin cloth was taken and folded in the form of a triangle, the soaked barley grains were rinsed and then placed on the cloth.

The cloth was knotted and made into a loose pouch and then tied to a tap. Water was allowed to trickle very slowly through the pouch. In this manner the soaked grains remained wet and received sufficient water for germination. Dry

sand was taken in a tray and the soaked seeds of green gram were spread uniformly on the sand. Sand was spread on the seeds as a thin layer. Then tap water was gently sprinkled on the sand and the tray was placed under diffused light near a window. Every day water was sprinkled over the sand in order to keep the germinating seeds moist. The *H. vulgare* and *V. radiata* seedlings were harvested 3 and 5 days from sowing for the experiments.

2.2 Detection of dehydrogenase activity

The test tubes were readied according to Table 1. Succinic acid (0.05 M, 6 mL) was used as the substrate for succinate dehydrogenase. Then methylene blue (1 % aqueous, 20 µL) was added with the help of a micropipette. The seedling axis was used as the experimental material. The axis included the fibrous roots, mesocotyl, coleoptile and leaf in *H. vulgare*; and the primary root, plumule and leaves in *V. radiata*. The remnant of the grain and the cotyledons were not taken in *H. vulgare* and *V. radiata*, respectively. The weighed experimental material (0.5 g) was sliced using a blade. Controls with boiled and cooled experimental material, without the substrate succinic acid and without an experimental material were maintained. The plant material was added last into the test tubes, and the test tubes were incubated for one hour in darkness. It has been reported that darkness favours succinate dehydrogenase activity (Popov et al., 2010). When one hour had lapsed, the first reaction mixture was taken out and shaken, and then gently decanted into a clean and dry cuvette and the absorbance was recorded at 600 nm using a colorimeter. Similarly, the absorbance of all the reaction mixtures were recorded one hour from incubation. For setting the colorimeter for zero absorbance at 600 nm, succinic acid was used as the blank for tubes 1, 2, 4, 5, and 8, and distilled water was used as the blank for tubes 3, 6 and 7 (Table 1). All experiments were conducted thrice and the average absorbance values were used to discuss the results.

3. Results

3.1 Seedling morphology

The grains of *H. vulgare* and seeds of *V. radiata* showed good germination. In 3-day-old *H. vulgare* seedlings the fibrous roots and coleoptile had emerged; and in some seedlings the primary leaf had emerged by rupturing the coleoptile. In 5-day-old seedlings the coleoptiles had elongated further and the emerging leaf was partially visible in many seedlings (Figure 1 A, B). *V. radiata* seeds showed epigeal germination; in 3-day-old seedlings the lateral roots had begun to initiate in the radicle, and in most seedlings the first pair of leaves had emerged although the plumular hook had not opened in all seedlings. Five days from sowing most seedlings showed fully expanded leaves and the cotyledons had shrivelled (Figure 1 C, D).

3.2 Comparison of dehydrogenase activity

Both 3- and 5-day-old seedlings of *H. vulgare* and *V. radiata* contain dehydrogenases and the enzyme activity is more in presence of the substrate succinic acid as compared to the enzyme activity in the reaction mixture without succinic acid (Table 1). The absorbance values of the reaction mixtures with boiled and cooled sample of plant materials were similar to or more than the control absorbance values where no plant material was taken. Five-day old seedlings of *H. vulgare* and *V. radiata* showed more total as well as succinate dehydrogenase activity than the respective 3-day old seedlings. The total dehydrogenase activity as well as succinate dehydrogenase activity in 3-day-old seedlings was more in *H. vulgare* than that in *V. radiata.* Contrastingly, 5-day-old seedlings of *V. radiata* showed more succinate dehydrogenase activity than the activity in *H. vulgare.* However, the total dehydrogenase activity in 5-day-old seedlings of *H. vulgare* was more than the activity in *V. radiata.* The controls without the plant material, and in the presence or absence of succinic acid in the reaction mixture showed nearly the same absorbance (Table 1).

Figure 1. Representative 3- and 5-day-old seedlings of *H. vulgare* (A, B) and *V. radiata* (C, D).

Table 1. Dehydrogenase activity in the axes of 3- and 5-day-old seedlings *H. vulgare* and *V. radiata*.

S. No.	Plant*	SA (mL)	DW (mL)	Absorbance (600 nm)								Increase in enzyme activity (%)
				3-day-old seedlings				5-day-old seedlings				
				Rep 1	Rep 2	Rep 3	Avg	Rep 1	Rep 2	Rep 3	Avg	
1.	*H. vulgare*	6	_	0.15	0.09	0.13	0.123	0.14	0.08	0.14	0.120	2.44
2	*H. vulgare* (B & C)	6	_	0.35	0.17	0.25	0.257	0.24	0.15	0.23	0.207	--
3	*H. vulgare*	_	6	0.16	0.14	0.17	0.157	0.13	0.12	0.12	0.123	21.66
4.	*V. radiata*	6	_	0.22	0.08	0.17	0.157	0.11	0.07	0.09	0.090	42.68
5.	*V. radiata* (B & C)	6	_	0.37	0.17	0.24	0.260	0.31	0.23	0.29	0.277	--
6.	*V. radiata*	_	6	0.25	0.15	0.19	0.197	0.16	0.12	0.14	0.140	28.93
Controls without the plant material												
7.	_	_	6	0.20	0.29	0.20	0.230	0.21	0.21	0.20	0.207	--
8.	_	6	_	0.23	0.19	0.19	0.203	0.22	0.19	0.19	0.200	--

*The seedling axis was used. Each reaction mixture contained 20 µL methylene blue.
SA: Succinic Acid; DW: Distilled Water; B & C: Boiled and Cooled plant material.

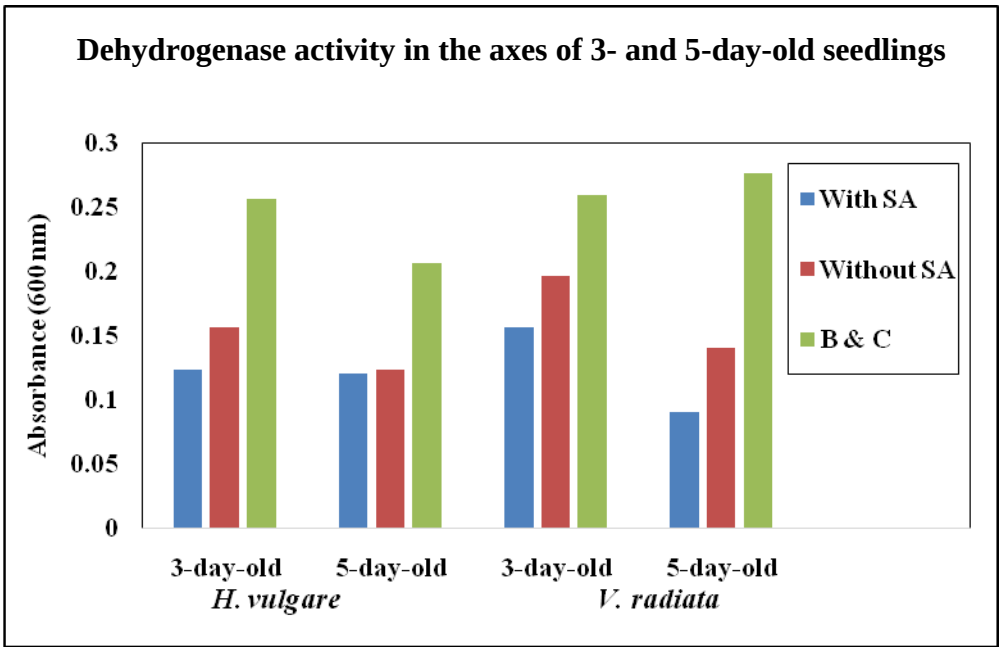

Figure 2. Dehydrogenase activity in the axes of 3- and 5-day-old seedlings of *H. vulgare* and *V. radiata*. SA: Succinic Acid; B & C: Boiled and Cooled plant material.

4. Discussion

4.1 Seedling morphology

Both the soaked *H. vulgare* grains and *V. radiata* seeds received continued hydration which allowed germination and further growth. The seedlings grew using the stored food reserves, namely the abundant starch (Subramanyan et al., 2023). Additionally, storage proteins also supported growth; *V. radiata* was far richer in proteins than *H. vulgare*. The available light allowed the greening of the emerging leaves in both taxa and the cotyledons in *V. radiata*.

4.2 Dehydrogenase activity

The presence of the substrate succinic acid specifically enhances succinate dehydrogenase activity (Table 1, Figure 2). More the enzyme activity more is the methylene blue reduction and the consequent decrease in absorbance. Maximum absorbance and, therefore, least reduction of methylene blue was observed in reaction mixtures with boiled and cooled sliced seedling axes. When the fresh sliced axes were used, intermediate dye reduction was observed in the absence of succinic acid, and maximum dye reduction when succinic acid was added to the reaction mixture. The reason for increased absorbance in three of the reaction mixtures with boiled and cooled plant material was the turbidity caused because of slicing the axes prior to boiling. Boiling the material denatures dehydrogenases and prevents methylene blue reduction.

Total dehydrogenases increase by nearly 22 % and 29 % in *H. vulgare* and *V. radiata* 5-day-old seedlings, respectively, when compared to 3-day-old seedlings (Table 1). However, when succinic acid was present in the reaction mixture the increase in enzyme activity was only 2.44 % in *H. vulgare* in 5-day-old seedlings when compared to 3-day-old seedlings. Contrastingly, *V. radiata* showed nearly 43 % increase in enzyme activity in 5-day-old seedlings compared to 3-day-old seedlings. This can be explained on the basis of the fact that *V. radiata* seedlings had put forth the first pair of leaves three days from sowing. In 5-day-old seedlings of *V. radiata* the leaves were fully expanded. On the other hand, in *H. vulgare* the primary leaf had emerged a little by rupturing the coleoptile only is some 3-day-old seedlings. In 5-day-old seedlings although the coleoptiles had elongated significantly the primary leaf had either not emerged yet or had emerged only a little beyond the coleoptile tip. The high total and succinate dehydrogenase activity in 5-day-old

V. radiata seedlings compared to 3-day-old seedlings can be explained because of the seedlings becoming photosynthetic owing to the presence of leaves. The leaves will also contain numerous mitochondria and thereby contribute to the increase in succinate dehydrogenase activity.

Our study clearly showed that the dehydrogenases including succinate dehydrogenase play an important role during seed germination, and later on as the seedlings establish themselves and growth progresses. In cowpea *(Vigna unguiculata)* seeds, an initial burst of dehydrogenase activity which had an important role in the breakdown of stored food during the anaerobic phase of seed germination has been reported; the pattern of enzyme activity observed was lactate dehydrogenase > alcohol dehydrogenase > succinate dehydrogenase in a 60>31>9 percent ratio, respectively (Oaikhena et al., 2013). In a study on *Arabidopsis thaliana* using isolated mitochondria from leaves exposed to varying illumination showed that succinate dehydrogenase activity initially increased briefly when transferred to light from darkness, followed by a decrease in the enzyme activity to half of the original activity (Popov et al., 2010). Also, red-far red photoreversibility of the effect was observed; and it was concluded that succinate dehydrogenase expression and, therefore, mitochondrial respiration, is regulated by phytochrome A (Popov et al., 2010).

A proteome analysis of mitochondria in *Arabidopsis* seeds within the first 0–24 h of germination showed that glutamate dehydrogenase 1 or 3, glyceraldehyde-3-phosphate dehydrogenase, and succinate-semialdehyde dehydrogenase were upregulated, whereas the flavoprotein subunit of succinate dehydrogenase remained at a constant level (Farooq et al., 2021). In *Arabidopsis* and many other crops, four plastidal and two cytosolic isoforms of glucose-6-phosphate dehydrogenases (G6PDH) have been reported. G6PDHs oxidize glucose-6-phosphate to 6-phosphogluconolactone, and regulate seed germination. Reactive oxygen species (ROS) are required for breaking seed dormancy as well as preventing excessive damage to the radicle. It has been proposed that cytosolic G6PDH controls seed germination by modulating ROS homeostasis and hormonal signalling (Jiang et al., 2022). ROS are primarily produced in the mitochondria, the peroxisome, and by the NADPH oxidases of the plasma membrane (Farooq et al., 2021).

Conclusion

Three- and 5-day-old seedlings of *H. vulgare* and *V. radiata* contain dehydrogenases including succinate dehydrogenase; and 5-day old seedlings of both taxa showed more enzyme activity than the respective 3-day old seedlings. When 3-day-old seedlings were compared, the total dehydrogenase as well as succinate dehydrogenase activity in *H. vulgare* was more than in *V. radiata.* But in 5-day-old seedlings the succinate dehydrogenase activity was more in *V. radiata* than the activity in *H. vulgare.* Dehydrogenases play a pivotal role in seed germination and seedling establishment which are pre-requisites for good crop productivity of cultivated plants.

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