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RESEARCH ARTICLE

# THE DISTRIBUTION OF ACTIVITY OF L-GALACTOSE DEHYDROGENASE (L-GALDH) IN L-ASCORBATE PATHWAY IN VASCULAR AND NON-VASCULAR PLANTS

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**ABSTRACT**

L-galactose dehydrogenase (L-GalDH) in photosynthetic eukaryotes is acting as a key enzyme in the pathway of L-ascorbate biosynthesis. This enzyme is widely distributed in most lineages of photosynthetic eukaryotes. From this study, multiple sequence alignment inferred that there is an evolutionary relationship amongst the L-GalDH sequences in different species of photosynthetic eukaryotes because they have sequence similarities and identities to each other. Secondly, Phylogenetic tree showed that L-GalDH activity is widely evolved in a great variety of eudicots and monocots in comparison to eukaryotic algae. The tree revealed that gene duplication played a major role in evolution of multiple paralogous copies of this within a species called within-species in-paralogs and different species called between-species out-paralogs because the common ancestral gene of this enzyme in vascular and non-vascular plants is diverged from each other at the earliest stage of evolution. Moreover, speciation events can lead to an emergence of different L-GalDH proteins in different species known as orthologs. Thirdly, biochemical and enzymatic tests reveal the presence and absence of the activity of the L-GalDH enzyme in a green plant (Pisum sativum), Chlorophyte (Blindingia minima) and Rhodophyta (Porphyra dioica). The results showed that Blindingia minima has a L-GalDH function but it is very low but porphyra dioica lack it.



## INTRODUCTION

Ascorbic acid (AA), known as L-ascorbate and vitamin c, is a major active antioxidant component and also a key metabolite in photosynthetic eukaryotes (Puskas *et al.*, 1998 and Smirnoff *et al*., 2003). The content of AA is significantly different amongst the species of photosynthetic eukaryotes and even amongst the tissues of the same species. This can result in the reason why some fruits have an extremely high content (Puskas *et al*., 1998 and De Tullio, *et al*., 2007). Moreover, it is discovered in all sub-cellular compartments such as the Apoplast, Mitochondria, Peroxisomes, Cytosol, Nuclei, Vacuole and Chloroplast stroma.

Loewus and his colleagues demonstrated in 1956, the pathway of Mannose/L-galactose called as L-ascorbate pathway is the key source of AA formation in vascular and non-vascular plants, but alternative minor pathways are also present. In these plants, the biosynthetic pathway of L-ascorbate includes several biochemical reactions and is carried out by several key enzymes. One of these enzymes is L-galactose dehydrogenase abbreviated as L-GalDH. L-GalDH (EC 1.1.1.316) plays a key role in catalyzing a step in L-ascorbate rout in photosynthetic eukaryotes. It belongs to family of Aldo keto reductase and its value of pfam represents PF00248 (Schade, *et al*., 1990). The biological function of this enzyme is to catalyze a step in the biosynthetic pathway of ascorbate and is the only recognized activity of L-GalDH in photosynthetic eukaryotes (Foyer and Lelandais, 1996). The reaction of L-galactose + $NAD^+$ = L-galactono-1,4-lactone + NADH + $H^+$ in L-ascorbate pathway is conducted by this. This reaction has been found in Embryophyta, Chlorophytes, Haptophytes, Oomycetes, Alveolates. Research studies show that the L- GalDH enzyme in different species has similar function and specificity (Gatzek *et al*., 2002 and De Tullio, *et al*., 2013). It is hypothesized that this enzyme in different lineages of photosynthetic eukaryotes has an evolutionary relationship with each other.

To verify this hypothesis, I have used Muscle Alignment Tool to assess Multiple Sequence Alignments (MSA). In addition, phylogenetic analysis has been carried out to determine the direction of evolution and the evolutionary relationship between different L-GAlDH sequences in photosynthetic eukaryotes. Moreover, Biochemical test has been used to determine the

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presence or abscence of activity of the L-GAlDH in *Blidingia minima* (green algae) and *porphyra dioica* (red algae).

## MATERIALS AND METHODS

### *Plant and Seaweed materials*

*Pisum sativum* was grown on a compost, Scotts Levington F2 Seed & Modular. After that, in a growth room, these were protected at 22 Centigrade. For growing, about 20 hrs electronic light was provided and then we collected leaves for the experiment. However, seaweeds like *Blidingia minima and Porphyra* dioica were gathered from the beach of Exmouth town and then they were protected in a growth room at 22 Centigrade with 20 hrs of electronic light. The water of sea and the oxygen through an air pump were supplied.

### *Assemble and align protein databases*

In order to infer and estimate evolutionary relationships among L-GalDH sequences in different photosynthetic plants, phylogenetic tree will be constructed. First of all, Because the L-GalDH sequences of *Arabidopsis thaliana* were already available in the genomic sequences of National Center for Biotechnology Information (NCBI web site), These L-GalDH sequences were used as Blastp query sequences to search for the available database (original dataset). After that, the whole homologous sequences for this, which is available in the publicly original database, were downloaded.

## RESULT AND DISCUSSION

### *Multiple Sequence Alignment (MSA) for the phylogenetic analysis of L-GalDH*

The L-GalDH protein belongs to Aldo-Keto reductase family protein (pfam= PF00248). Biochemical function of this is oxidureductase activity. It consists of inter protein domain/s: Aldo-keto reductase and domain. Because the different L-GalDH proteins found in different green plants have similar sequences and the same function, the amino acid sequence of their domains, for example Aldo-keto reducatase, may undergo less rapid changes. Gene duplication and speciation events which are the events of molecular evolution have played an important role in the formation of similar domains in different species where they have the same biological function. MUSCLE alignment algorithm present in Molecular Evolutionary Genetics Analysis version 6 (MEGA6) was used to align and assess 93 homologous L-GalDH sequences. Because the amino acid sequences are too long, the most interesting part of multiple sequence alignment (MAS) was displayed (Figure-1). Here, the MSA shows that L-GalDH sequences in different photosynthetic eukaryotes have a very close similarity to each other; therefore, these sequences have an evolutionary relationship with each other. Furthermore, these sequences have identical positions at their respective positions. The conserved amino acids include Glutamine (Q), Proline (P), Leucine (L), Glycine (G), Glycine (G), Proline (P), Serine (S), Tyrosine (Y), Cysteine (C) and Tryptophan (W) (Figure-1). The cases of mutation such as point mutations (residue variations) can be detected as distinct traits in more than one alignment column. Moreover, deletion or insertion mutations can be detected as hyphens (gaps) in more than one alignment sequences. The similarities and identities of the L-GalDH sequences can suggest an evolutionary relationship. These can help to discover the common ancestor.

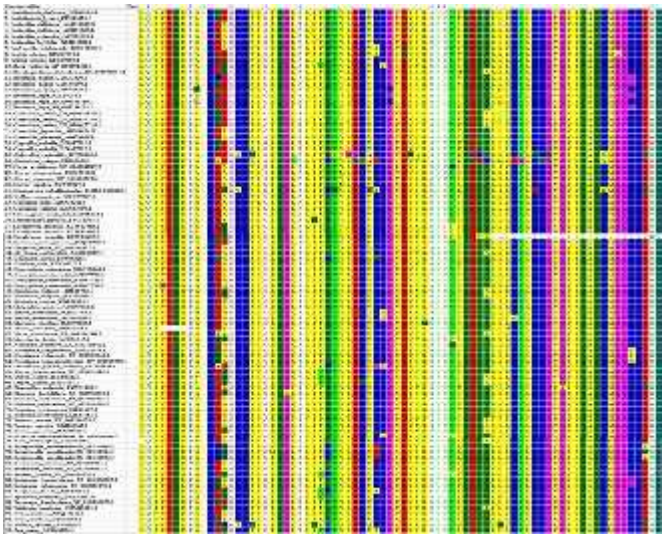

**Figure 1,** A part of multiple sequence alignements (MSAs) of L-GalDH was demonstrated as an instance. Representation of an example of MSAs of L-GalDH generated with MUSCLE algorithm. 93 homologous sequences from protein database are collected from (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using *Arabidopsis thaliana* L-GalDH as a query sequence. The colours correspond to amino acid conservations according to the presence of residual frequencies in each column and the features. Notice that more than one completely conserved amino acid such as Glutamine (Q), Proline (P), Leucine (L), Glycine (G), Glycine (G), Proline (P), Serine (S), Tyrosine (Y), Cysteine (C) and Tryptophan (W) signed with an asterisk at the top of the alignment.

### *Distribution and Phylogenetic analysis of L-GalDH enzymes within the lineages of photosynthetic eukaryotes*

Our dataset consisted of 93 homologous L-GalDH sequences in 69 species of classes and families of Photosynthetic eukaryotes (Table-1). Maximum likelihood (ML) phylogeny was reconstructed to show an evolutionary relationship between them. It can be seen that bootstrap value are shown on the branch lengths. The values at the branches represent bootstrap values used to estimate the reliability of phylogenetic tree. Bootstrap analyses were conducted on the basis of 1000 re-samplings of the sequence alignment. The phylogenetic tree result shows that the sequences in various species of the photosynthetic eukaryotes have an evolutionary relationship with each other because they have a close sequence similarity to each other (Figure-2). They are also descanted from a single common ancestor via events of gene evolution. Gene duplication and speciation which are two events of evolution play a key role in the evolution of the L-GalDH sequences, especially gene duplication. Gene duplication at the early stage of evolution could rapidly happen before the functional and structural divergences of common ancestor of the L-GalDH enzyme (Figure-2). The tree is diverged into two major groups (Group I and Group II). Within the Group I the majority of species belong to the group of vascular plants. Within the vascular plants, there are two major groups; Eudicots and Monocots. It appears, different L-GalD Hs within the various species of eudicot have a close evolutionary relationship with each other because they are evolved from a single common ancestor. They show the fewest amino acid variations; therefore, they are clustered together in several black subclades. Similarly, the members of monocot are grouped together in a big blue

**Table 1**, Photosynthetic eukaryotes having L-GalDH are listed. Class and family are shown. Thiers Gene names encoding L-GalDH.

| Species | Classes | Families | Genes names |
|---|---|---|---|
| | | | LOC104716785 |
| *Camelina sativa* | Eudicot | Brassicaceae | L-GalDH |
| *Arabidopsis thaliana* | Eudicot | Brassicaceae | L-GalDH |
| *Arabidopsis lyrata* | Eudicot | Brassicaceae | LOC106423369 |
| *Brassica napus* | Eudicot | Brassicaceae | LOC103862253 |
| *Brassica rapa* | Eudicot | Brassicaceae | CARUB_v10005247mg |
| *Capsella rubella* | Eudicot | Brassicaceae | EUTSA_v10025723mgL |
| *Eutrema salsugineum* | Eudicot | Brassicaceae | OC104807192 |
| *Tarenaya hassleriana* | Eudicot | Brassicaceae | AALP_AA7G204100 |
| *Arabis alpine* | Eudicot | Brassicaceae | L484_000890 |
| *Morus notabilis* | Eudicot | Moraceae | LOC102621043 |
| *Citrus sinensis* | Eudicot | Rutaceae | GDH |
| *Citrus unshiu* | Eudicot | Rutaceae | CICLE_v10028842mg |
| *Citrus clementina* | Eudicot | Rutaceae | GSCOC_T00014656001 |
| *Coffea canephora* | Eudicot | Rubiaceae | LOC101495571 |
| *Cicer arietinum* | Eudicot | Fabaceae | LOC103495364 |
| *Cucumis melo* | Eudicot | Cucurbitaceae | LOC101206435 |
| *Cucumis sativus* | Eudicot | Cucurbitaceae | GDH |
| *Spinacia oleracea* | Eudicot | Amaranthaceae | LOC104904801 |
| *Beta vulgaris* | Eudicot | Amaranthaceae | EU683447.1 |
| *Malpighia glabra* | Eudicot | Malpighiacea | F383_18998 |
| *Gossypium arboretum* | Eudicot | Malvaceae | LOC105763009 |
| *Gossypium raimondii* | Eudicot | Malvaceae | GDH |
| *Vitis vinifera* | Magnoliophyta | Vitaceae | LOC18442443 |
| *Amborella trichopoda* | Eudicot | Amborellaceae | GalDH |
| *Moringa oleifera* | Eudicot | Moringaceae | LOC104607573 |
| *Nelumbo nucifera* | Eudicot | Nelumbonaceae | LOC105635996 |
| *Jatropha curcas* | Eudicot | Euphorbiaceae | LOC102599558 |
| *Solanum tuberosum* | Eudicot | Solanaceae | LOC101254135 |
| *Solanum lycopersicum* | Eudicot | Solanaceae | LOC104103548 |
| *Nicotiana tomentosiformis* | Eudicot | Solanaceae | LOC104219974 |
| *Nicotiana sylvestris* | Eudicot | Solanaceae | L-Galdh-1 |
| *Nicotiana langsdorffii* | Eudicot | Solanaceae | LOC104453016 |
| *Eucalyptus grandis* | Eudicot | Myrtaceae | GDH |
| *Myrciaria dubia* | Eudicot | Myrtaceae | LOC105129751 |
| *Populus euphratica* | Eudicot | Myrtaceae | POPTR_0009s08490g |
| *Populus trichocarpa* | Eudicot | Myrtaceae | GDH |
| *Camellia japonica* | Eudicot | Theaceae | FJ772089 |
| *Actinidia eriantha* | Eudicot | Actinidiaceae | AY176585 |
| *Actinidia deliciosa* | Eudicot | Actinidiaceae | GDH |
| *Actinidia latifolia* | Eudicot | Actinidiaceae | GDH |
| *Camellia sinensis* | Eudicot | Theaceae | LOC105055920 |
| *Elaeis guineensis* | Eudicot | Arecaceae | LOC101299720 |
| *Fragaria vesca* | Eudicot | Rosaceae | LOC103334968 |
| *Prunus mume* | Eudicot | Rosaceae | GDH |
| *Prunus persica* | Eudicot | Rosaceae | LOC103436244 |
| *Malus domestica* | Eudicot | Rosaceae | LOC103938180 |
| *Pyrus x bretschneideri* | Eudicot | Rosaceae | KC782563 |
| *Rosa roxburghii* | Eudicot | Rosaceae | PHAVU_002G216600g |
| *Phaseolus vulgaris* | Eudicot | Papilionaceae | LOC100809400 |
| *Glycine max* | Eudicot | Fabaceae | glysoja_034550 |
| *Glycine soja* | Eudicot | Fabaceae | LOC105949697 |
| *Erythranthe guttata* | Eudicot | Phrymaceae | M569_09951 |
| *Genlisea aurea* | Eudicot | Papilionaceae | LOC105169328 |
| *Sesamum indicum* | Monocot | Papilionaceae | LOC103707620 |
| *Phoenix dactylifera* | Monocot | Arecaceae | LOC103979808 |
| *Musa acuminate* | Monocot | Musaceae | LOC101762145 |
| *Setaria italic* | Monocot | Poaceae | GalDH |
| *Hordeum vulgare* | Monocot | Poaceae | TRAES_3BF183000020C |
| *Triticum aestivum* | Monocot | Poaceae | D_c1 |
| *Oryza sativa* | Monocot | Poaceae | OsI_38334 Os12g0482700 |
| *Oryza brachyantha* | Monocot | Poaceae | LOC102719064 |
| *Brachypodium distachyon* | Monocot | Poaceae | unknown |
| *Oncidium hybrid* | Monocot | Poaceae | FJ237037 |
| *Zea mays* | Monocot | Poaceae | ZEAMMB73_081419 |
| *Sorghum bicolor* | Monocot | Poaceae | Sb08g014630 |
| *Selaginella moellendorffii* | Lycophyta | Selginellaceae | SELMODRAFT_124085 |
| *Coccomyxa subellipsoidea* | Chlorophyta | Chlorophyaceae | COCSUDRAFT_18370 |
| *Volvox carteri* | Chlorophyat | Chlorophyaceae | VOLCADRAFT_93987 |
| *Chlorella variabilis* | Chlorophyat | Chlorophyaceae | CHLNCDRAFT_25289 |
| *Chondrus crispus* | Rhodophyta | Rhodophyaceae | CHC_T00010069001 |

subclade because they have very close sequence similarities to each other. Interestingly, Eudicots and Monocots are combined together within a large clade because they show the fewest residual changes. It can be seen that *Amborella trichopoda* which belong to the class Amborella is clusterd with the eudicots because the L-GalDH sequence of this is more similar to eudicot members than to monocot members. Most importantly, the tree reveals that the biological function of this enzyme is occurred in lower plants such as Lycophyta (fern ally) and Chlorophyte (green algae). *Selaginella moellendorffii* which belongs to Lycophyta is more similar to monocots rather than either is to eudicots. The tree infers that L-GalDH sequences in the members of Lycophyta, Monocots and Eudicots show the fewest residual variations; so, they are combined with each other in the group I. However, group II small group only includes eukaryotic algae (seaweeds). It can be seen that they have an evolutionary relationship with each other because they are descended from a common ancestor (Figure-2). Within the eukaryotic algae there are two main class, Chlorphyta and Rhodophyta. The ML tree demonstrated that there is a historical relationship between enzymatic sequences in Eudicots, Monocots, Lycophytas and eukaryotic algae because they are derived from a common ancestor gene in a single species.

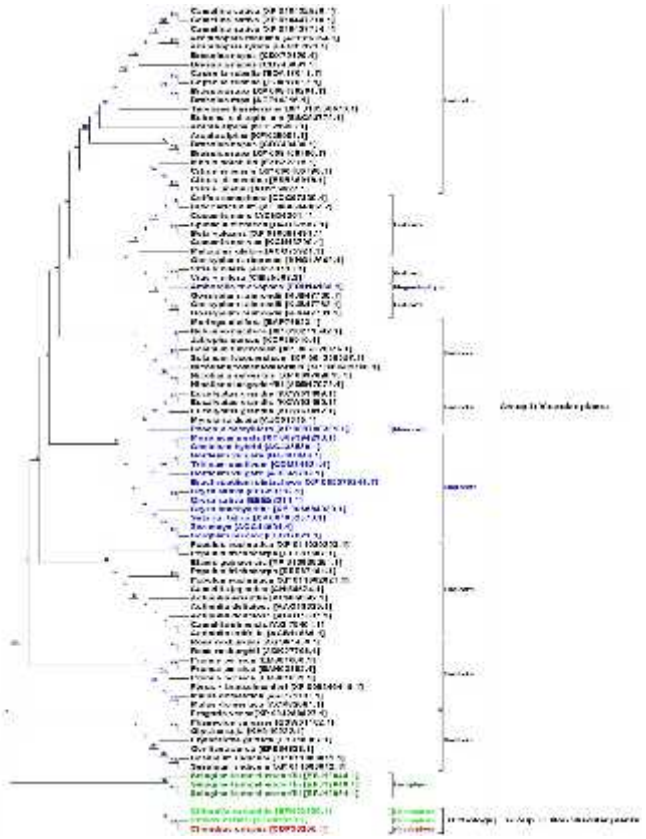

**Figure 2**, Phylogenetic tree of L-GalDH sequences. The tree was reconstructed with the maximum likelihood method, using the MEGA software, Version 6.06 (Tamura et al., 2013). The tree shows that L-GalDH function is evolved with each other and It is moredistributed in classes of eudicots and monocots (vascular plants) than eukaryotic algae (nonvascular plants). The numbers at the branches represent bootstrap values used to estimate the reliability of phylogenetic tree. Bootstrap analyses were conducted on the basis of 1000 re-samplings of the sequence alignment.

***How can L-GalDH evolve within the species of photosynthetic eukaryotes?***

It appears that there are two types of homology between different L-GalDH sequences which are descended from a single ancestral gene in one ancestral species. Maximum likelihood tree shows that Gene duplication and speciation events from this tree have played an important role in the production of multiple homologous L-GalDH proteins, especially gene duplication events (Figure-3). Bootstrap values are shown on the branch length. The gene duplication can lead to the formation of out-paralogs and In-paralogs. The out-paraologs are paralogs that were duplicated before the speciation events, but they are not important in the same species. These L-GalDH enzymes are descended in the common ancestor of the pair of species. These may have arranged in separate species through the loss of the reciprocal partner. This event can be mostly detected between subclades (Figure-3). However, the in-paralogs are paralogs that were duplicated after the speciation events (Dale *et al*., 2012). These L-GalDH enzymes in the same species may be evolved from a common ancestor via duplication events. This event can be detected in some species such as *Camelina sativa, Capsella rubella, Brassica rapa, Vitis vinifera , Gossypium raimondii Selaginella Moelendroffii etc.*

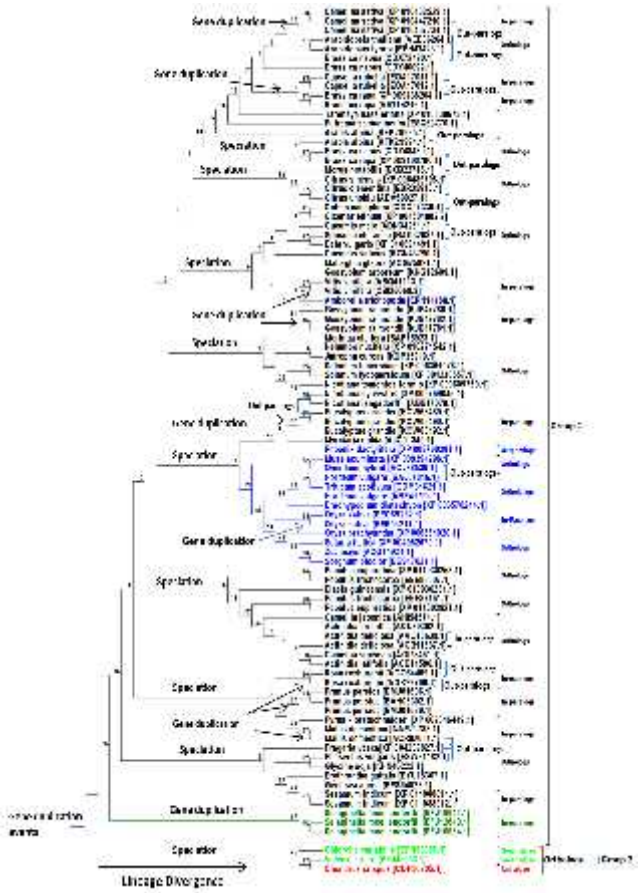


**Figure 3,** Maximum likelihood phylogeny reconstructed using an alignment of 93 L-GalDH sequences of photosynthetic eukaryotes. The result shows that evolution of L-GalDH within the species of photosynthetic eukaryotes occurred via Gene duplication events (lead to the formation of In-paralogs and out-paralogs) and speciation events (play a key role in production of orthologs). Bootstrap scores for all nodes are displayed.

However, within the group I and group II, there are a number of orthologs created by speciation events. The orthologs are different L-GalDH proteins in different species are descendent from a common ancestor gene where they may have the same function and specificity. This event can be seen in most subclades (Figure-3).

## MATERIALS AND METHODS

### *Samples collection*

Pea seedlings were bought from Edwin Tucker & Sons LTD and planted on a compost, Scotts Levington F2 Seed & Modular. After that they were kept at 20 centigrade in a growth chamber that provides 16 hours light periods for germinating and growing. The intermediate age leaves were tested for the knowing of L-GalDH activity. Moreover, Chlorophyta (*Blindingia minima*) and Rhodophyta (*Porphyra purpurea*) were brought from Exmouth beach and kept in a growth chamber at 20 degrees C with 16 hours of light period for growing. The artificial sea water from Reef Crystals was used and the oxygen was provided through an air pump.

### *Enzyme assay methods*

L-GalDH was extracted using procedure as elucidated by (Smirnoff *et al*., 1998). These enzymes were read by TECAN® Infinite M200 PRO.

### *Ascorbic acid (AsA) assay and its dilution*

0.088g of ascorbic acid AA powder was dissolved in 10 ml Milli-Q water to prepared AA solution. Following this, a diluted ascorbic acid (stock solution) was prepared from the AsA solution. The diluted AA was composed of 10µl of the AA solution and 990 µl of molecule water. 25 µl of this solution was added to each sample prior to the commencement of the process of reading the activity of L-GalDH enzymes.

### *Preparation of sample homogenate:*

Homogenate is a resultant fluid that is produced by using homogenizers (buffer solutions). In this experiment, plant leaves of each sample were homogenised through a specific yet standard procedure (Smirnoff *et al*., 1998). 0.500 mg of plant leaves was weighed and then grounded via mortar whilst homogenizers were used to break up the leaf cells. This releases the cytoplasm and the organelles from the large pieces of tissue debris. Thus, it is obvious that each enzyme needs a specific homogenizer to release the protein from the other parts of cells. Eventually, microtube volume 1.5 ml was used to collect the resultant fluid.

### *Centrifugation*

Centrifugation is useful method for removing insoluble debris. The preparation of the homogenate from each sample was subjected to a centrifuge machine. The homogenates were centrifuged at 4 degrees C with speed of 16100g for 10minutes. Then, supernatant fluid of each sample was accurately separated from the sediment layer.

### *Preparation of microplate reader*

Microplate reader (known as plate reader) is a necessary instrument for observing biological event of samples in absorbance reader. a certain volume of different buffer solution was added together with 5 to 10 µl of extracts that was added to a plate well to read the activity of the L-GalDH.

### *Absorbance reading for preparing samples*

TECAN® Infinite M200 PRO was used to measure the absorbance of the sample materials. It was used to read Corning 60 well UV microplate at 524nm, every 30 minutes for 10 hours . Furthermore, this type of a spectrophotometer is capable of calculating the absorbance sample that passed through the sample solution. It can also record then the value of radiations in an excel page. To confirm the presence of the activity of the enzymes, the standard curve was immediately designed to the numerical absorbance. It can be observed on the standard curve that the activity of the enzymes is gradually and substantially decreasing because the enzymes were influenced via the measuring of the absorbance with the spectrophotometer (Figure-5).

### *Determination of L-GalDH activity*

The activity of the L-GalDH can be determined by the boost in the absorption of the enzyme assay. The L-GalDH activity of *Pisum sativus* leaves, *Blidingia minima* and *Porphyra dioica* are demonstrated in the figure-6 . The control is revealed in blue which only water is added. The adding galactose, and fucose are shown in red and green, respectively. In *Pisum sativus* leaves after adding sugar, galactose shows a rise in absorbance while others, control, fucose and glucose, show no changes in absorbance (Figure-4.1). These characteristics are also seen in *Blidingia minima* in which only an absorbance of galactose is increased (Figure-4.2).

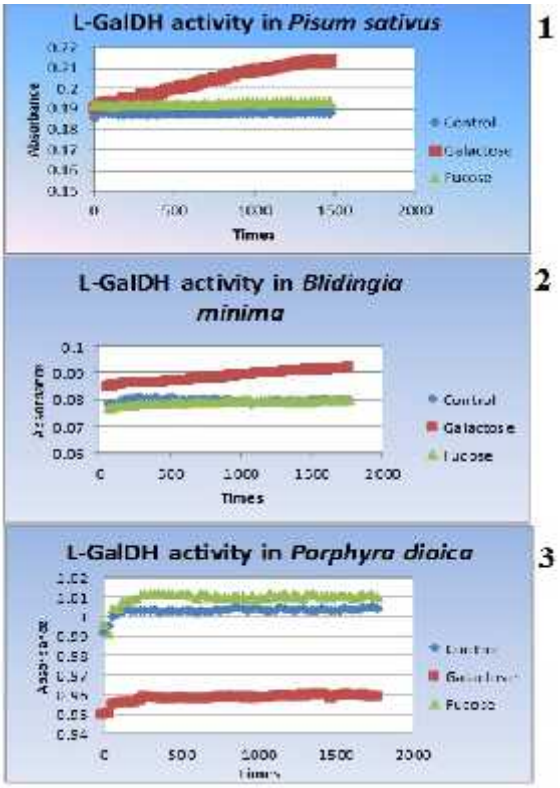


**Figure 4**, The graphs illustrate the activity of L-GalDH in 1) *Pisum sativus*, 2) *blidingia minima*, and 3) *Porphyra dioica* at the absorbance of 340nm.The pink colour shows the activity of the control which contain only water. The orang and red colours show the activity after adding galacatose and fucose, repectively

It appears that the L-GalDH activity is present in this but its concentration is significantly low. unlike *Blidingia minima*, both galactose and fucose in *Porphyra dioica* display no difference in absorbance, only constant straight lines parallel to control. This lack L-GalDH (Figure-4.3).

## DISCUSSION

In this research study multiple sequence alignement (MSA) used to indicate the similarity and identity of 93 L-GalDH sequences. The MSA shows that L-GalDH sequences within the species of Photosynthetic eukaryotes are structurally similar to each other. The L-GalDH enzyme appears to be unique in the photosynthetic eukaryotes (Smirnoff, 1996 and 2000). Furthermore, the MSA infers that these enzymes in their structures have a conserved domain known as Aldo-Keto reductase. Biological function of this domain is specific for L-galactose. It catalyses $NAD^+$-dependent oxidation of L-galactose at C1 to form L-galactono-1, 4-lactone (Gatzek *et al*., 2002). It is involved in the pathway of ascorbate biosynthesis.
Maximum likelihood (ML) phylogeny was reconstructed for 93 homologous L-GalDH sequences to determine the distribution of this enzyme within classes of photosynthetic eukaryotes. The tree result infers that L-GalDH activity is found in a great variety of species belonged to vascular plants; especially eudicots and monocots. Wheeler and his colleagues (1998) reported that L-GalDH enzyme in higher plants have the same function and similar properties. This enzyme has a high concentration in these plants, incomaprison with lower plants such as ferns, mosses and seaweeds. Furthermore, the activity of this enzyme is hardly found in lycophyta (ferns) and eukaryotic algae. Some species of seaweeds and ferns have the activity of L-GalDH but a very low concentration (Smirnoff, 20001 and 2003). He reported that L-GalDH activity observed in species of seaweeds (non-vascular plants) is similar to those which found in vascular plants. The minority of the species studied in this analysis belong to lycophyta and eukaryotic algae. lycophyta includes only *Selaginella moellendroffii* containing three in-paralogous L-GalDH. My result is significantly similar to his result.

Within the eudicots and monocots there, Howevere, are two types of homology. Most species contain one L-GalDH enzyme known as orthologs. The orthologs denote that different L-GalDHs in different species are evolved by speciation (Dale *et al*., 2012). Wheeler and his colleagues (1998) reported that the L-GalDH in *pisum* sp., *Arabidopsis* sp., *Orayza* sp, *Nicotiana* sp., *Solanum* sp., *Triticum* have a similar biological function and structure. However, some species contain more than one L-GalDH known as paralogs. However, gene duplication played a major role in evolution of multiple paralogous copies of this within a species called within-species in-paralogs and different species called between-species out-paralogs(Dale *et al*., 2012). In a research study, more than one L-GalDH was observed in *Brassica* sp. and they might have similar functions (Gatzek *et al*., 2002). According to another research conducted by Mieda and his colleagues (2004), The L-GalDH enzymes observed in *Camelina sativa* have the highest sequence similarity to each other. This result is very similar to my result because they are clustered together in a clade.

Regarding the biochemical tests, the result showed that a low level of L-GalDH activity is present in *Blidingia minima*, in comparison to *Pisum sativum*. However, the low L-GalDH activity could be resulted from low L-GalDH concentration, meaning the *Porphyra* dioica lacked L-GalDH activity. In *Porphyra*, both galactose and fucose show no difference in absorbance, only constant straight lines parallel to control.

## CONCLUSION

L-GalDH activity considerably pervades the compartments of cell of photosynthetic eukaryotes. A large number of this enzyme is structurally and functionally analysed and their sequence data are available in NCBI's datasets. Maximum likelihood (ML) phylogenies are reconstructed to determine the historical and evolutionary relationships between homologous L-GalDH sequences. They infer that the events of gene duplication and speciation could lead to the distribution of this among the lineages of eukaryotic photosynthetic organisms; eudicots, monocots, lycophyta, chlorophyta and rhodophyta. The evolutionary tree reconstructed with maximum-likelihood method shows that gene duplication played a major role in evolution of multiple paralogous copies of this within a species called within-species in-paralogs and different species called between-species out-paralogs. Moreover, speciation events can contribute to the production of orthologs. However, the presence and absence of activity of L-GalDH are biochemically experimented in *Pisum sativa*, *Blidingia minima* and *Porphyra dioica*. The activity was found to be present in *Blidingia minima* and *Porphyra dioica* but they are very small levels were observed compared to *pisum sativa*. It is expected that they may be functionally and structurally similar with other homologous L-GalDH enzymes. Finally, after a closer examination of the comparative protein trees with species trees, it can be suggested with a considerable degree of certainty that paralogs and orthologs of each enzyme can provide useful information in phylogenetic analysis and taxonomic classification of molecules and photosynthetic species.

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