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Biochemical, Pharmaceutical and Biotechnological Perspectives of the Ascorbate Synthesis Gene L-Galactono-1,4-Lactone Dehydrogenase

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ABSTRACT: Ascorbic acid is an antioxidant that is also considered an effective dietary supplement that our body can't synthesize; hence, our body's need for Vitamin C is fulfilled through fruits and vegetables, but the amount of vitamin C in them varies. In past decades, extensive information has been gathered about the genes involved in regulating ascorbate biosynthesis. Plethora of studies has unveiled that Smirnoff-Wheeler pathway plays an extensive role in ascorbic acid synthesis. The major challenge among the plant tissues remained the same that is the diversity and variability of L-ascorbic acid among different plant tissues. A diet deficient in vitamin C results in the inception of various diseases, which may show disastrous consequences. However, many studies have shown its implications in preventing multiple diseases, for instance, stroke, heart diseases, cancer, and various other neurodegenerative diseases. The most vital enzyme, L-Galactono-1,4-Lactone Dehydrogenase performs an essential function in the last step attributed to the ascorbic acid pathwayis discussed in this review. GLDH is a miniature form of complex I and is also known as complex I*. Many advancements have been made to regulate ascorbic acid production by overexpressing this enzyme. This review also focuses on cloning strategies and biological functions, structure, and active site of the respective enzyme.Other than this, cloning strategies and biological application have also been discussed.

Keywords: Ascorbic acid, Antioxidant, Photoprotection, Reactive oxidant species.

INTRODUCTION

Vitamin C,a familiar name as ascorbic acid, isessentialfor the health of a being because of its antioxidant property and solubility in water (Bendich *et al.,* 1986; Franke *et al.,* 2004). In many ways, it is used as an indicator for the quality check of nutrients at the time of processing food and its storage. Retention of ascorbic acid within foods indicates good retention of other nutrients as well (Uddin *et al*., 2002). For humans, it is considered to be an effective dietary supplement as their body can't synthesize it because of the mutation in L-gulono-1,4-lactone oxidase, the capacity to synthesize (Tareen *et al.,* 2015; Kaur *et al.,* 2021). Ascorbic acid is lost in numerous animals encompassing primates (Drouin *et al*., 2011). Vegetables like broccoli, peppers, cauliflowers and fruits like cherries, grapes, lemon, gooseberries, orange, strawberries, watermelon and papaya are common source of ascorbic acid for human consumption (Dumbrava *et al.,* 2016; Zhong *et al*., 2016; Najwa and Azrina, 2017). However, it is found in many fruits and vegetables, but the amount varies from plant to plant. No hypotheses have arisen regarding the loss as mentioned above. Even though humans are not able to synthesize vitamin C, the symptoms which are related to the deficiency of vitamin C are very much severe such as bleeding in gums, due to ruptured blood vessels, discolouration of the skin, weak joints, and all of these were observed and described by Vasco do Gama in early 1497 during his trip Africa to India.

Further, in 1747, James Lind illustrated that citrus fruit consumption prevents the symptoms that appeared due to scurvy. Due to all these factors, vitamin C was known by the name "Antiscorbutic factor". In 1928 vitamin C was ultimately isolated by Dr Szent Gyorgyi, though there was much speculation regarding its function. In the year 1932, crystallization of naturally isolated physiologically functional compounds identified antiscorbutic factor as vitamin C(Svirbely& Gyorgyi 1932; Tillmans & Hirsch 1932; Waugh & King 1932). They perform a crucial function in numerous livingmechanisms occurring in plants encompassing cell division and defence

mechanisms.(Smirnoff, 2011; Nehzo *et al*., 2015; Panahi and Dehdivan, 2017).

Ascorbate performs a crucial part in animals, like the formation of collagen and carnitine, which is considered an essential component in scar tissue, skin, ligament, tendon, and blood vessels (Levine 1986; Levine *et al.* 1995; Sies and Stahl 1995). Also,ascorbic acid plays a key role during wound healing and the maintenance and repair of bones, teeth, and cartilage. It also plays a role in shooting up the absorption of non-heme iron from the food, which is plant-based; it also manages cell division and cell (Landi *et al.,* 2015; Rodriguez-Ruiz *et al.,* 2017; Broad *et al*., 2019). Introduction growth as well as it is also involved in signal transduction(Pignocchi and Foyer 2003, Smirnoff and Wheeler 2000; Kerk and Feldman 1995; Noctor *et al.* 2000). The process of vitamin c formation can be used to determine its level within a plant. Reportedly there exists 4 Vit C synthesizing pathways- L-gulose pathway(Wolucka and Van Montagu, 2003), the galacturonate pathway (Agius *et al*., 2003), the myoinositol pathway(Lorence *et al*., 2004; Ishikawa *et al*., 2018), and the L-galactose pathway (Wheeler *et al*., 1998). An antioxidant ascorbic acid can interact directly with hydrogen peroxide, superoxide radicals, singlet oxygens, and hydroxyl radicals(Conklin, 2001, Halliwell *et al*., 2015, Smirnoff *et al.,* 2000; Conklin *et al*., 2004). The current review focusses on comprehending the current knowledge of the Ascorbic acid, different enzymes involved in it synthesis. Moreover the cloning strategy used for biosynthesis of ascorbic acid and their biotechnological application has also been discussed

BIOSYNTHESIS OF ASCORBIC ACID

In the human diet, vitamin C is vital, and mostly the foods which are of plant origin carry vitamin C in high amounts. Hence the nutritive value of plants can be increased by increasing the vitamin C content, and this has received significant consideration in recent years(Chen *et al.,* 2003; Hancock & Viola 2005; Naqvi *et al*., 2009) as it plays an essential function in plants, for example, it functions as a cofactor for numerous enzymes, also works as redox buffer(Pignocchi Foyer, 2003) along with its unique property of being an antioxidant(Smirnoff & Wheeler, 2000). It also performs its part in cell division, growth and development, and signal transduction(Pignocchi Foyer, 2003; Noctor *et al*., 2000; Kerk& Feldman, 1995).

Biosynthesis- Using the help of intermediates D-glucose-1-Phosphate, UDP-glucose, UDP-D-glucuronic acid, UDP-D-glucuronic acid-1-Phosphate, and D-glucuronic acid D-glucuronic acid is formed from D-glucose in mammals. D-Glucuronic acid is further changed to L-gulonic acid with the assistance of glucuronate reductase, Further converting to gulono-1,4-lactone with the help of aldono-lactonase(Burns, 1967). Gulono-1,4-lactone generated L-ascorbic acid with the use of gulono-1,4-lactone oxidase. In plants, the synthesis of ascorbic aciddramatically varies from the one occurring in animals. L-galactose forms L-ascorbic acid, too, via the Smirnoff-Wheeler pathway(Wheeler *et al.,* 1998). Mannose-1- phosphate forms L-galactose by converting guanosine diphosphate(GDP)-mannose into GDP-L-galactose with GDP-mannose-3,5-epimerase(Wolucka et al., 2001),which converts to L-galactose. L-galactose dehydrogenase which is a NAD-dependent compound further oxidizing L-galactose to L-galactono-1,4-lactone, which functions as ascorbic acid's straight away precursor further gets converted to L-ascorbic acid with the help of an enzyme Lgalactono-1,4-lactone dehydrogenase that lies in the within the membrane of mitochondria and that too on its outer side (Siendones *et al.,* 1999; Bartoli *et al*., 2000; Aboobucker *et al*., 2017).

All the steps that occur in the initial pace of the biosynthesis occur in cytosol; cytochrome c present in mitochondria is responsible for L-galactono-1,4-lactone oxidation, suggesting that the biosynthetic pathway also interacts with the energy metabolism as well as cellular redox state. In the biosynthetic process occurring in mammals, D-Glucuronic acid acts as an intermediate, which can be synthesized by inositol oxygenase in plants (Fig. 1).

KEY ENZYME (L-GALACTONO-1,4-LACTONE DEHYDROGENASE) AND ITS FUNCTION

In the photosynthetic eukaryotes for the formation of Ascorbic acid, the main enzymes that have a crucial part in the final steps of the pathway, i.e. ascorbate pathway, are L-Galactono-1,4-Lactone Dehydrogenase. Ascorbic acid is an antioxidant that additionally works as a cofactor (Mapson *et al.,* 1958). This ascorbic acid produced by nearly all the green plants is known to be formed in mainly every compartment of the cell, i.e. cytosol, chloroplast, apoplast, vacuoles, peroxisomes, mitochondria but the level of ascorbic acid varies among species as well as from compartment to compartment (Smienoff *et al.,* 2001). Subsequently, it paves the way for most fruits to have ascorbic acid (Miller *et al*., 2003). Many steps take part in Vit C synthesis,but the final stage where ascorbic acid is produced is by L-Galactono-1,4-lactone oxidation. L-Galactono-1,4-Lactone Dehydrogenase is the key enzyme that catalyzes this step. Also, a protein family favoured by Flavin Adenine Dinucleotide (FAD) linked enzyme of VAO, i.e. Vanily-1-Alcohol Oxidase utilizes type-c cytochrome as an electron acceptor. These enzymes have a crucial part in ascorbic acid synthesis, and also this enzyme, i.e. GLDH, is associated with respiratory complex I in mitochondria (Miller *et al.,* 2003). This enzyme activity is usually seen in flowering plants; still, some non-flowering plants also show its action, but the ascorbic acid content is shallow as the enzyme level is low. As this enzyme is

familiar in non-flowering plants, the main pathway of ascorbic acid formation is the oxidation of mannose/L galactose pathway (Shigeoka *et al.,* 1979; Helsper *et al.,* 1982; Grun *et al.,* 1984). However, L-Galactono-1,4-Lactone oxidation to Vit C in the laststage is observed in all the photosynthetic organisms via L-Galactono-1,4-Lactone Dehydrogenase enzyme; it is substrate-specific,i.e. specific for L-galactose (Leferink *et al.,* 2008; Majed 2016).

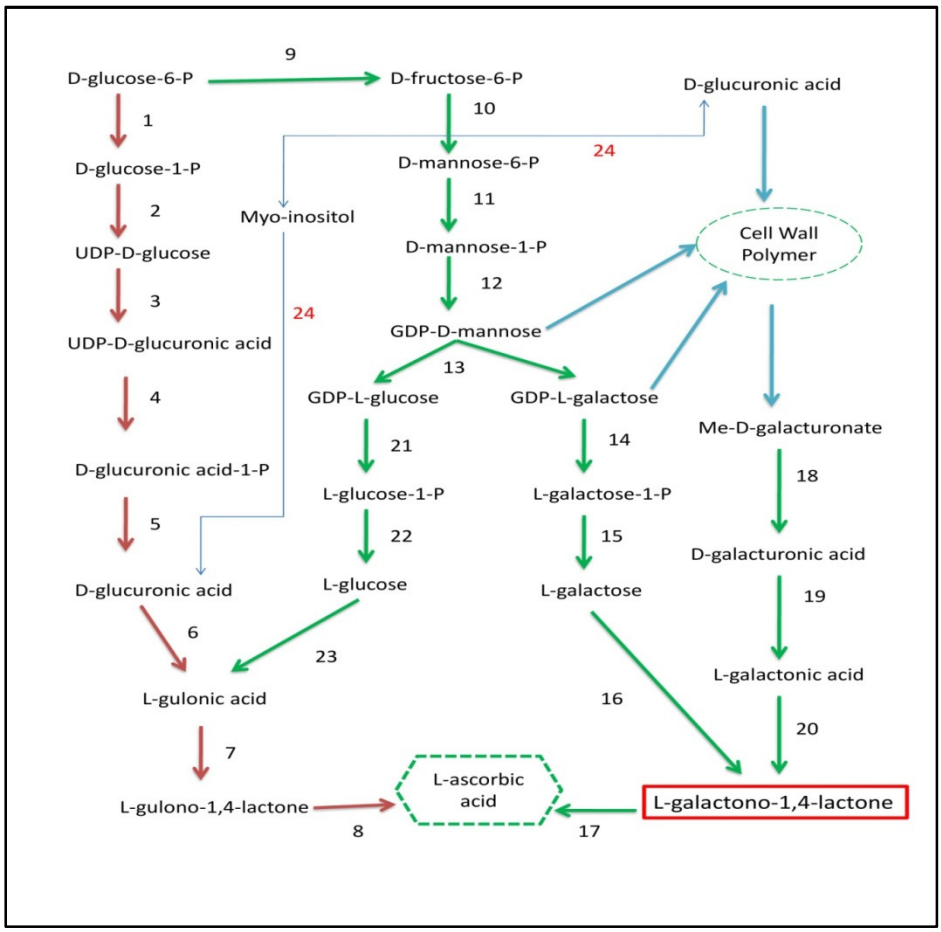

Fig. 1. Biosynthetic pathway for Ascorbic Acid. Reactions 1 to 8 show biosynthetic pathways in animals, whereas 9 to 24 show pathways in plants. Enzymes involved in the plant pathway include (9. glucose-6-phosphate isomerase, 10. mannose- 6-phosphate isomerase, 11. Phosphomannomutase, 12. GDP-mannose pyrophosphorylase (mannose-1-phosphate guanylyltransferase), 13 GDP-mannose-30,50-epimerase, 14. Phosphodiesterase, 15. sugar phosphatase, 16. L-galactose dehydrogenase, 17. L-galactono- 1,4-lactone dehydrogenase, 18. Methylesterase, 19. D-galacturonate reductase, 20. aldonolactonase, 21. Phosphodiesterase, 22. sugar phosphatase, 23. L-gulose dehydrogenase, 24. myo-Inositol oxygenase

Several plant species characterization plus purification of Galactono lactone dehydrogenase has been conducted (Mapson *et al.*, 1958; Oba *et al.,* 1995; Ostergaard *et al.,* 1997). The molecular mass of the primary Galactono lactone dehydrogenase translation product is 68 kDa, but after further processing, the molecular weight turns to 56-58 kDa. This mature protein is formed by removing the peptides from the N-terminal of at least 100 amino acids. This processoccurs when the dehydrogenase enzyme is transported to mitochondria (Ostergaard *et al.*, 1997; Leferink *et al.,* 2008). Galactono lactone dehydrogenase requires a noncovalently linked cofactor, i.e. FAD. Due to the absence of membrane-spanning regions, this mature protein, i.e. 56-58kDa galactono lactone dehydrogenase is attached to the mitochondrial membrane peripherally.

A. Structure and active site

Being present within the inner mitochondrial membrane GLDH is accountable for the finalstage of ascorbate synthesis, where ascorbic acid is generated by L-galactono-1,4-lactone oxidation in association with cytochrome c reduction (Bartoli *et al.,* 2000; Heazlewood *et al.,* 2003). Being avital component for ascorbate synthesisit is also essential for the proper arrangement of the respiratory complex I in plants andappropriate mitochondrial functioning (Alhagdow *et al.,* 2007; Pineau *et al.*, 2008). As part of VAO i.e. vanillyl-alcohol oxidase flavoprotein family, Gldh and

other related aldonolactone oxidoreductases thattake part in the synthesis of ascorbate share a topology of having two folded domains with the active site at their interface. However, the N-terminal is a conserved domain,plus it is a FAD-binding domain. In contrast, on the other hand, the substrate specificity is determined by the C terminal cap domain (Fraaije *et al.,* 1998; Leferink *et al*., 2008).

Galactono lactone dehydrogenase shows 25% sequence identity with alditol oxidase and 20% sequence recognition with cholesterol oxidase, respectively(Forneris *et al.,* 2008; Coulombe *et al*., 2001). These all belonging to VAO-family have a 3-dimensional structure with a Glu-Arg pair in the C terminal cap domain. As this domain is substrate-specific in AldO i.e. alditol oxidase, Glu320 shows hydrogen bonding towards polyol substrate (Forneris *et al*., 2008). In the case of CO, i.e., cholesterol oxidase loss of activity was observed due towithdrawal of the positive charge of Arg477 (Piubelli *et al*., 2008). This Glu-Arg pair in cholesterol oxidase works as a wicket and functions to manage the way into oxygen. Its location is at the bottom of the tunnel that scampers from the exterior to the active site (Coulombe *et al.,* 2001; Piubelli *et al*., 2008).

Therefore, the biochemical analysis of variants of both Arg388 and Glu386 disclosed their importance for stimulation of reaction. Withdrawal of Arg388 +ve charge resulted in inactivation of Galactono lactone dehydrogenase hence making it difficult to stabilize the opposite negative charge at the active site. There is a formation of GLDH variant predilect to L-gulono-1,4-lactone when Glu386 is mutated to Asp. (Fig. 1) (Leferink *et al*., 2009).

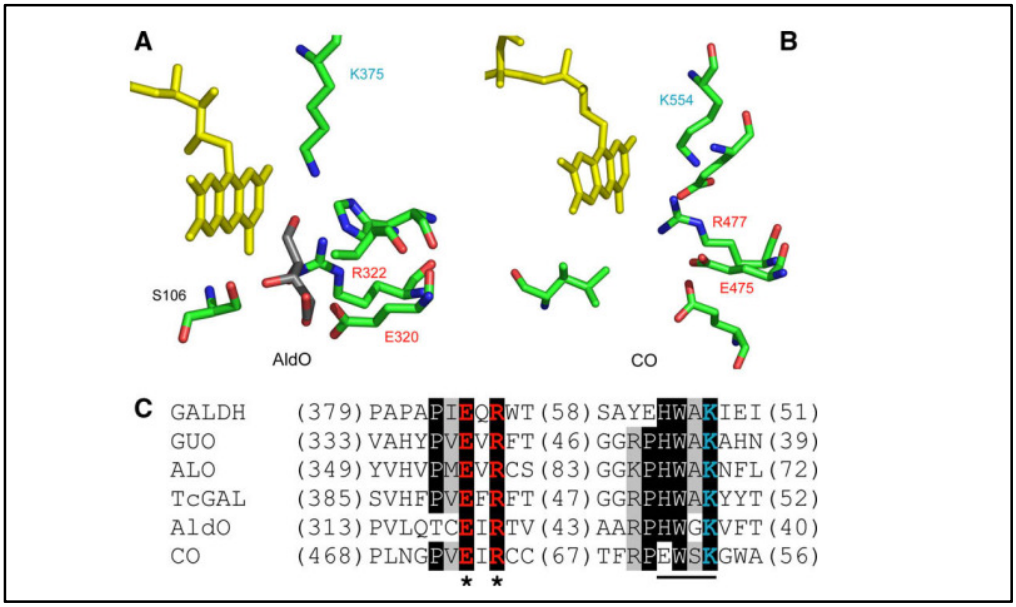

Fig. 2. The presumed active site residue of Galactono lactone dehydrogenase with other related members of the VAO family (a).Crystal structure of AldO active site bound to xylitol (pdb, 2VFS), (b). Crystal structure of CO active site (pdb, 1119), (c). One shaded in black represents the identical residue, the one in grey represents similar residue, and the one in red and marked asterisks indicatesa conserved Arg –Glu pair. These all represent the Clustal W multiple sequence alignment of the active site part of numerous aldonolactone oxidoreductases with related members of VAO-family. The no of residues in gaps and at terminals are noted in parentheses.

CLONING STRATEGY

The gene "L-galactono-1,4-lactone dehydrogenase" was cloned using Agrobacterium-mediated transformation technique for overexpression of this enzyme from Rosa roxburghii in tobacco plant to increase the accumulation of ascorbate ".

Transformation vectors were constructed using pBI121 and pCAMBIA1301. From the pBI121, i.e. the binary vector utilizing the help of restriction enzyme SacI/EcoRI and HindIII/BamHI respectively NOS terminator region as well as CaMV 35S promoter region were removed or excised further were added into the pCAMBIA1301 that to in its multiple cloning site. Therefore the cDNA encoding the respective enzyme galactono lactone dehydrogenase was isolated from the fruit Rosa roxburghii and then amplified using RT-PCR (An *et al.* 2007). This isolated cDNA was further added into the place named Kpn1 between promoter CaMV 35s and terminator NOS which too inthe sense orientation (Fig. 3).

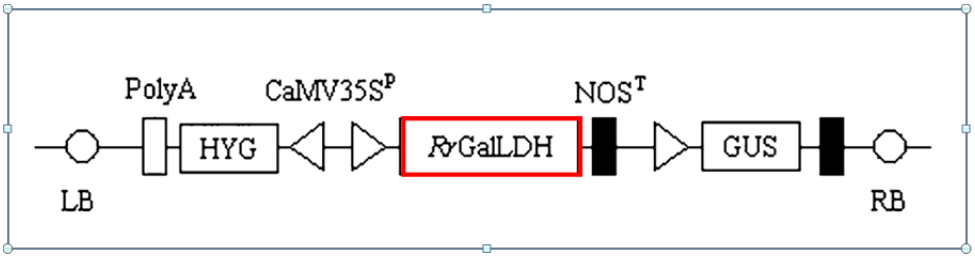

Fig. 3. Isolated cDNA coding for enzyme galactono lactone dehydrogenase was introduced in the site named Kpn1 between promoter CaMV 35s and terminator NOS in the sense orientation. This fig shows the red highlighted location of RrGalDH.

In the MS medium, a disc of a leaf was selected. This MS medium consisted of 6-benzyl amino purine (6-BA), a-naphthalene acetic acid (NAA), hygromycin and cefotaxime (sodium salt) for adventitious bud regeneration. Shoots were regenerated from the explants that were inoculated with Agrobacterium, which were further inoculated on MS medium containing Indole-3-butyric acid that too for rooting, under required photosynthetic photon flux and density under the day-night cycle necessary.

This vector named pCAMBIA-RrGalLDH was obtained as a result, which was further inserted into the strain LBA4404 of Agrobacterium tumefaciens and transformed further into tobacco. The gene expression was confirmed and quantified using PCR and Southern blotting using promoter-specific primer, i.e.3' forward primer and 5'reverse primer specific to RrGaldh. Other parameters for amplification include 94^{o}C for 5 min, further followed by a total of 35 cycles in thermocycler that underwent to 94^{o}C for about 30 seconds, 57^{o} C for about 40 seconds, 72^{o} C for about 40 seconds and at last the time of extension for about 10 mins at 72^{o}C. Separation of the PCR product was done on 1% agarose gel. For southern blotting, Probes were labelled with digoxigenin, i.e. DIG labelling system that too on the report of the manufacturer directions on the Dig detection kit.

Two lines, one untransformed, i.e. WT plant, and the other positive transgenic line, were used to extract RNA with the help of the CTAB-LiCl method (Chang *et al.*, 1993). Further, with the help of RT-PCR cDNA was synthesized using the extracted RNA; this whole process was carried out using the service of RNA PCR Kit Ver.2.1 using a forward primer specific to RrGalDH and reverse primer specific to 18S rRNA. The forward primer is 5'-CAAATTTCTGCCCTATCAAC -3', and 5'-CAAAATCCAACTACGAGCTT-3' is the Reverse primer. For amplification using PCR thermocycler, parameters taken under consideration were 94^{o}C for 5 min, further followed by a total of 35 cycles that underwent to 94^{o}C for about 30 seconds, 57^{o} C for about 40 seconds, 72^{o} C for about 40 seconds, and at last the time of extension for about 10 mins at 72^{o}C. Separation of the PCR result was done on 1% agarose gel.

FUNCTION AND PURPOSE OF VITAMIN C

A. Photoprotection

Being an antioxidant, vitamin C plays a very vitalpart in photoprotection, i.e. sound growth of plants even under high light conditions. As ascorbic acid also works in the xanthophylls cycle, it functions as a cofactor. This cycle involves the production of zeaxanthin from violaxanthin through the thylakoid membrane (Eskling *et al.*, 1997). The process in photosystem II is presumed to beengaged in quenching excess light energy non photochemically (Demmig-Adams & Adams 1990). Earlier it was known that under in vitro conditions, ascorbic acid is required because it performsa crucialpart in the conversion of violaxanthin to zeaxanthin, as it acts as a cofactor for the violaxanthin de-epoxidase that is responsible for this conversion(Hager 1969).*In vitro* study of ascorbic acid was further supported by data collected from an in-vivo study. Therefore, a mutant of Arabidopsis with deficiency of ascorbic acid showed a decreased level of enzyme de-epoxidase, leading to lower non-photochemical quenching.

B. Vitamin C: An Antioxidant

Ascorbic acid provides protection against oxidative stress due to its antioxidant property. Oxidative stress is caused due to numerous plant factors as well as due to some (ROS) reactive oxidant species, including superoxides, hydroxyl radicals, and hydrogen peroxide. Due to the photoreduction of oxygen and photosynthesis taking place by singlet oxygen formation, the generation of ROS occurs under normal conditions. Such conditions occur when extreme temperature, drought, and nutrient deficiency are combined with high light. UV-B, phytotoxic metals (like Zn, Cd, Cu), redox-active weedkillers (like paraquat) and air adulterants(likesulphur dioxide, ozone) are some factors of the plant environment that lead to a condition of increased ROS. The oxidative burst that occurs at the time of pathogen infection (Lamb & Dixon 1997) leads to the emergence of ROS, further inducinga hypersensitive response (Chen *et al.*, 1993; Levine *et al.* 1994). With multiple mechanisms, a network of antioxidants interconnected with each other control the level of ROS. Vit Cis capable enough of removingvarious types of ROS, such as superoxide,

singlet oxygen, and hydroxyl radicals (Padh 1990). Alpha-tocopherol, a membrane-bound antioxidant, is also maintained in its reduced state by the ascorbic acid (Packer *et al.,* 1979; Liebler *et al.,* 1986) by the activity of ascorbic acid peroxidase hydrogen peroxide is eliminated indirectly (Foyer & Halliwell 1976; Asada & Takahashi 1987; Asada 1992).

Due to increased inefficient regeneration plus oxidation, an increased amount of oxidized ascorbic acid pool is generated. There is the formation of monodehydroascorbate (MDA) due tothe loss of an electron from Ascorbic acid. The MDA, until it is re reduced and disproportionated to ascorbic acid and DHA, i.e. dehydroascorbate, remains unstable. By reductase activity, ascorbic acid can be formed by the MDA (monodehydroascorbate) and DHA (dehydroascorbate) reduction (Foyer & Halliwell 1976; Jablonski & Anderson 1981; Hossain & Asada 1984). On exposure to ROS like cyanide, drought, senescence, high light, and redox-active herbicide paraquat, there occurs an increase in MDA radicals in various plant species (Heber *et al.*, 1996).

C. Ascorbate Peroxidase

The leading consumer of ascorbic acid is ascorbate peroxidase. Devaluation (reduction) of hydrogen peroxide to water forms MDA, i.e. monodehydroascorbate, which plays a crucial function in removing hydrogen peroxide. Isoenzymes of ascorbate peroxidase are present in cytosol, mitochondria, peroxisome and chloroplast (Mittler & Zilinskas 1991; Asada 1992; Jimenez *et al.* 1997). The steady-state amount of ascorbate peroxidase increased as an increase in ROS. In Arabidopsis, exposure to high light leads to photooxidative stress that, in turn, leads to the elevation of two cytosolic ascorbate peroxide mRNA (Karpinski *et al.* 1997). Ascorbate peroxidase activity increases during deficiency of copper and salt, whereas mRNA level stays constant or declines (Savouré *et al.,* 1999).

In tobacco infected with TMV, i.e. tobacco mosaic viruses, there appeared post-transcriptional control of ascorbate peroxide during the process of programmed cell death (Mittler *et al.*, 1998). The decline in the quantity of ascorbate peroxidase was noticedat the moment when a corresponding cytosolic ascorbate peroxidase encoded by transcript accumulated ata high level. After attaching ascorbate peroxidase mRNA with the ribosome, there occurs a reduction in the expression of ascorbate peroxidase, salicylic acid, and jasmonate, their importance in this regulation (Mittler *et al.*, 1998).

D. Synthesising Protein Rich in Hydroxyproline

The presence of hydroxyproline rich glycoproteins (HRPGs) in plants are the cell wall key components and are taken understudies extensively. These glycoproteins are thought to be produced in response (simultaneously with prolyl hydroxylation reaction) to wounds, ethylene, and pathogens, hence displaying a considerable part in supporting the structural integrity of cell walls (Sommer *et al.*, 1997).

Aprofound change in the architecture of the cellular zone of roots was caused due to peptidyl-prolyl hydroxylases (De Tullio *et al.*, 1999). Increased demand for ascorbic acid is seen when there is the trigger of HRGP synthesis. There is seen dramatic MDA reductase mRNA accumulation caused due to wounds in different tissues of tomatoes (Grantz et al., 1995). This accumulation leads to the maintenance of ascorbic acid levels,which is essential for systematic hydroxylation. As a result of wounding, ROS are formed and being an antioxidant ascorbic acid demand also increases; all these factors regulate reductase activity (Grantz et al., 1995). Shreds of evidence show the undeviatingparticipation of ascorbic acid in triggering HRGP (Conklin 2001).

E. Cell Division

Pieces of evidence suggest that in plants, ascorbic acid participates in the process of mitosis. In general, the quantity of ascorbate in the meristematic tissue is lofty. In contrast, the amount seems to be lower in the zones with very little operating cell division, for example,the quiescent centre in the roots of maize (Smirnoff 1996). In the cells that are non-dividing, ascorbate oxidase mRNA and activity are high, but these both lower down on transition of the cell to active cell division (Kerk& Feldman 1995; Kato & Esaka 1999). Furthermore, it was observed that cells competent to cell division showed an instant increase in the cell division, i.e. from G1phase to S phase on any treatment with ascorbic acid exogenously(Citterio *et al.*, 1994). It shows the participation of Vit Cin cell division in plants, yet there is still an absence of complete mastery of the procedure involved in this process. There is seen the advancement of ascorbic acid's role in cell division(Smirnoff 1996). In the case of the pea plant, it was observed that ascorbate played a crucialpart in the progression of cell division ina competent cell but didn't show any induction of cell division in non-competent cells(Citterio et al., 1994). In the protoplast of the tobacco plants, there is seen inhibition of cell division because of prolyl hydroxylation inhibition (Cooper *et al*., 1994). In addition to this, it even causes the onion root's cellsto metaphase (De Tullio *et al.* 1999). However, in the development of the cell cycle, the involvement of ascorbic acid appears to be unlikely, but it is studied to be a vitalfactor in the division of cells(Cocklin 2001).

BIOLOGICAL FUNCTIONS OF ENZYME L-GALACTONO-1,4-LACTONE DEHYDROGENASE (GLDH)

Functioning as a vital assembly factor attributedto mitochondrial complex I (e.g. Arabidopsis)- Being an essential part of the final pace of the Vit C biosynthesis path, it also plays a significant role in mitochondria. Many findings have shown its essential role in

association with complex I of mitochondria, stating the importance of the firmness of complex I. Moreover, the activity of GLDH has also been seen within the smaller complexes of protein that too within the mitochondrial membrane. (Bartoli *et al.*, 2000; Millar *et al.,* 2003). GALD is known to be both an integral and peripheral protein (Siendones *et al.,* 1999; Bartoli *et al.,* 2000). As the function of GLDH is linked to the transferring of an electron to cytochrome c, the association of ascorbic acid synthesis is connected to the respiratory chain in mitochondria. Many reports suggest the linkage of complex I, i.e. (ubiquinone oxidoreductase: NADH) to GLDH.

Rotenone is the one that inhibits the activity of GLDH when complex I is engaged. Hence rotenone is known to be its inhibitor (Millar *et al.,* 2003). According to a study conducted, analysis of multiprotein complexes of the mitochondria using blue-native-PAGE resulted that GLDH being a miniature form of complex I and can be called by the name complex I* (Heazlewood *et al.,* 2003). According to the lately conducted experiment GLDH in-gel activity staining was performed, and the activity of GLDH was observed in 3 different complexes of the membrane, which includes complex I* also (Schertl *et al.,* 2012). Hence the study concluded that GLDH plays a crucial part in complex I accumulation, as GLDH mutant lacked complex I.

Complex I, the part of the electron transfer chain, plays a role in NADH oxidation, further leading to the shifting of electrons to ubiquinone, concurrently pumping protons through the inner membrane of mitochondria. This, in turn, leads to the birth of a gradient of proton that is further used to produce ATP by ATP synthase[79,80]. This complex has about 40 subunits. Therefore, the largest complex in the electron transport chain. It has two arms with a different function;the transfer of electrons occurs by the matrix arm, whereas the proton translocation takes place by the membrane arm[81]. This complex I has a size of 100kDa in plants comprising 44 subunits. During blue native gels screening, there has been observed 850kDa complex known to be a smaller type of complex I and is known as complex I**(Meyer *et al.* 2011; Heazlewood et *al.,* 2003; Schertl *et al.*, 2012; Pineau *et al.,* 2008) In accordance to study complex I* works the same as complex I, i.e. it can also oxidize NADH. But according to the genetic screening, it was observed that this complex I* was inactive in vivo and active in vitro. This complex I* is the same overall as complex I, but there is one subunit extra, i.e. GLDH. According to a study, co-localization of complex I with GLDH were observed, its primary role is yet to be confirmed, but it's known to function as an assembly factor of complex I (Schimmeyer *et al.*, 2016).

Gene of GLDH corresponds with an enhanced concentration of ascorbic acid plus reducing browning of leaves (e.g.*Lactus Sativa*)- As ascorbic acid is known for its property, i.e. natural antioxidant, in addition to this it is also browning of leaves after being treated or after cutting. According to a study conducted, overexpression of GLDH enzyme leads to elevated accretion of ascorbic acid and reduces the process of browning. A study concluded that about 19 fold overexpressed GLDH had more than 30 per cent ascorbic acid concentration compared to wild-type plants. Hence ascorbic acid biosynthesis manipulation is one of the most vital approaches to getting a proper insight into the research field, which can be applicative or essential research field (Landi *et al.,* 2015).

Transgenic plants (GLDH over-expressed) showed changed plant growth (e.g. rice plant)- A study was conducted demonstrating the effect of overexpressed GLDH and suppressed GLDH. For about a month in the greenhouse, both the plants were grown, and the plants with suppressed GLDH showed a reduced growth rate. It showed a reduction in the height of the plant to 56.9, length of roots to 41.6, the weight of leaf 16.5, and weight of roots 18.2% to that of the wild type. In some plants, no change in growth rate was observed. Nevertheless, the ascorbic acid level was increased to about 20 to 40% compared to wild-type plants. As a result, it remains concluded that the plants with suppressed GLDH showed depleted growth of the plant as well as seed set, but the plants with over-expressed GLDH showed a similar phenotype, but in comparison to wild type, they showed an increase in the level of seed set (Liu *et al.*, 2011).

Overexpressed galactono lactone dehydrogenase leads to increased ascorbic acid accumulation plus tolerance to abiotic stress (e.g. tobacco plant)- A study was conducted where about four transgenic lines were taken with control, i.e. the wild type. This study showed that transgenic lines 3 and 4 revealed an increased ascorbic acid content in the leaves compared towild type and that too by 2.1 folds higher. Hence, it was also observed that the GLDH gene in tobacco increased its activity 2 to 3 folds; in addition, about 30% increased content of ascorbic acid was seen. Several such studies were conducted, and as a result, every study stated that over-expression of GLDH in plants could be an efficient way to increase the production of ascorbic acid.

Further checking for tolerance to abiotic stress, studies were conducted, where culturing of transgenic plants with wild type plants was done in a medium having a high amount of NaCl for creating salt stress. Observation showed no apparent morphological effect or damage to the plant, but the shoot length and tested lines' biomass were affected. The transgenic plants showed higher weight in comparison to the wild type. As a result of the studies conducted, it was declared that over-expression of this gene enables tolerance to salt stress through elevated ascorbic acid content. Hence, this can be vital in generating salt-tolerant crops (Liu *et al.*, 2013).

Over-expression leads to increased ascorbic acid content, phenolics and increased antioxidant activity

(e.g. lettuce). Studies were conducted that showed a rise in the ascorbic acid amount on over-expression of GLDH gene. As a result of over-expression, the transgenic plants manifested an increase in Vit C amount 2.5 to 3.2 folds higher in comparison to wild type. Even the phenolic level was remarkably elevated in transgenic plants comparedto wild type. It was about 1.8 folds higher. The total antioxidant activity observed was about 1.5 folds higher than wild type (Guo *et al.*, 2013).

PHARMACEUTICAL APPLICATIONS

Vit-C topical articulation - L-ascorbic acid among numerous active forms of vitamin C L-ascorbic acid, among multiple active forms of Vitamin C seems to be highly functional (biologically), plus it is a well-studied example. The stratum corneum, i.e. the epidermis's outermost layer, is hydrophobic, making this unstable hydrophilic ascorbic acid molecule challenging to penetrate the skin. Charge on ascorbic acid is also a limiting factor to its penetration into the skin. Hence there is an effective method by lowering the pH of ascorbic acid to 3.5, making it stable and ameliorating its permeability. This transformation from an uncharged molecule to a charged molecule considerably assists in penetrating the skin. There are certain esterified forms of Vit C, such as MAP (magnesium ascorbyl phosphate) and ascorbyl-6-palmitate; both are lipophilic and they are highly stable at neutral pH. One such example of the products with ascorbic acid within is L'Oreal, New York. The by-product of filaggrin in the skin is transuranic acid. The photons of the solar radiation, mainly UV, act as a chromophore, further resulting in dioxygen formation. This all, in turn, initiates a cascade of processes forming ROS, i.e. reactive oxygen species or what one can call free radicals. Being unstable and very dangerous, they can lead to nucleic acid destruction and the cell wall.

Free radicals produced through UV initiate a cascade of signal transduction, causing decreased expression of nuclear factor-B and overexpression of a protein, i.e. (AP-1) activation protein-1 &atomic factor-B, inclusively leading to an overexpression of metalloproteinases, i.e. MMPs. Solar elastosis, coarse texture, photo pigmentation, deep wrinkles and telangiectasias are caused as a result. All these free radicals produced by getting exposed to UV get blocked partially by sunscreens. It has appeared AP-1 activation can be hampered by Vit C. As a result, MMP production is diminished; along with it, damage to collage is also reduced (Al *et al.*, 2017).

CONCLUSION AND FUTURE SCOPE

The review gives a brief about the enzyme L-Galactono-1,4-Lactone Dehydrogenase. GLDH is present in the inner mitochondrial membrane responsible for the final step of ascorbate biosynthesis, where it is produced through L-galactono-1,4-lactone oxidation in association with cytochrome c reduction. This paper also gives an overview of the active sites of this enzyme. Many steps associate in the formation of Vit C, but the final pace where ascorbic acid is produced is by L-Galactono-1,4-lactone's oxidation. L-Galactono-1,4-Lactone Dehydrogenase is the key enzyme that catalyzes this step. Also, a protein family favoured by Flavin Adenine Dinucleotide (FAD), a linked enzyme of VAO i.e. Vanily-1-Alcohol Oxidase, utilizes type-c cytochrome as an acceptor of an electron. These enzymes have a hand in the ascorbic acid synthesis, and also, this enzyme, i.e. GLDH is connected to respiratory complex I in mitochondria. The report also mentions the cloning strategies to increase the accumulation of ascorbate, which adds to resistance to oxidative stress.

Ascorbate's significance in various crucial and critical cellular processes is being investigated; the chemical is intriguing for new research. From cell division and growth to offering plant tolerance to environmental stress, ascorbate is engaged in various cellular functions. Current, Vit C research is mainly focused on increasing ascorbate accumulation or overexpression in ascorbate plants to meet the need for vitamin c content. Advanced genomic technologies will significantly impact the research of ascorbate regulation, assembly, and recycling in the future. The application of genetic engineering will enable us to combine two or more biosynthetic gene in expression model organism to regulate the biosynthesis of ascorbic acid and further exploration might be done to understand the effect of different metabolic products in synthesis process. Moreover, the impact of genes clone could also be studied in different model organism and their organs.

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