Research and Application of Lipoic Acid in Plants

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Abstract. Lipoic acid is a kind of small molecular compound with strong oxidizing properties. It has been widely used in medicine and has achieved good results since its discovery. However, it is less used in plants, and the biosynthetic pathway is not clear. The content in the plant is mainly measured by high-performance liquid chromatography (HPLC). At present, it is mainly used as an additive to the culture medium for plant tissue culture and Agrobacterium-mediated plant genetic transformation, in order to reduce the browning rate of explants, improve Agrobacterium-mediated genetic transformation efficiency.

1. Introduction
Lipoic acid (LA) is a small molecule of disulfide, which is the coenzyme of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase in mitochondria. It was first discovered in 1937 and isolated from the liver By Reed in 1951 [1]. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), have powerful antioxidant properties, which could remove free radicals and reactive oxygen species, weaken oxidative stress, chelate metal ions, promote endogenous antioxidants’ regeneration such as glutathione, coenzyme Q10 and its own [2-4]. Meanwhile, lipoic acid is the only known antioxidant, has both water-soluble and liposoluble properties. Contrast with water-soluble antioxidants VC (only act on the cytoplasm), liposoluble antioxidants VE (only act on the cell membrane), lipoic acid can function simultaneously in the cytoplasm and cell membrane, plays a double protection role [5]. Lipoic acid has been the focus of medical research because of its powerful multifunctional protection and is widely used in the treatment of diabetes, liver disease, heart disease, rheumatism, Alzheimer's disease, alcoholism and other related diseases, considered as the ‘universal antioxidant’ [6-8]. At present, lipoic acid is limited to the field of medical research, its universal antioxidant properties gets very good expression in animals, this artical for the review of research and application of lipoic acid in plants, to provide some reference for the promoting application of lipoic acid’s high antioxidant properties in plant genetic transformation.

2. Oxidative Metabolism of Lipoic Acid and Its Synthetic
In eukaryotes, lipoic acid is linked to a lysine residue of five different mitochondrial proteins by covalent bonds [9], catalyzes the oxidative decarboxylation of pyruvate into acetyl-CoA and α-ketoglutarate into succinyl-CoA, respectively. In the pyruvate decarboxylation reaction, the lipoic acid combines with the enzyme by linking to the lysine on the side chain of the transacetylase to form the lipoamyllysine. The plants also contain chlorophyll pyruvate dehydrogenase [10]. The final lipoamide
moiety is found as the H-protein, part of the glycine cleavage system, called glycine decarboxylase complex in plants, catalyzing the conversion of glycine into CO2, ammonium, and methylene tetrahydrofolate [11].

Usually, antioxidant substances possess antioxidant properties in their reduced form. LA is unique, because it retains protective functions in both its reduced and oxidised forms. Antioxidant activity is a relative concept, which depends on the kind of oxidative stress and oxidized substrate [12]. When we evaluate the antioxidant potential of a compound, criteria such as (a) specificity of free radical scavenging, (b) interaction with other antioxidants, (c) metal-chelating activity, (d) effects on gene expression, (e) bioavailability, (f) location (in aqueous or membrane domains, or both), and (g) ability to repair oxidative damage have to be taken into consideration. An antioxidant needs only to meet a few of the previous mentioned criteria to play an important role in the detoxification of oxidative stress. An ‘ideal’ antioxidant would fulfil all the above criteria. The LA/DHLA redox couple approaches the ideal. It can be considered the ‘universal antioxidant’ [13].

It has been proved that both plants and animals can synthesize lipoic acid from octanoic acid (an essential fatty acid) [14]. It is reported that mitochondria can synthesize fatty acids, which may be involved in the synthesis of lipoic acid [15]. But the complete biological pathway is still unknown.

3. Quantitative Determination of Lipoic Acid in Plants
Lipoic acid is found in asparagus, wheat and potatoes, but there is little data on its quantification in plants. In plants, lipoic acid is in the form of binding to the protein mostly, the content main determined by the lipoyllysine. One method of determining the content of lipoic acid is to convert the conjugate to acid, alkaline or enzymatic hydrolysis to release lipoic acid; the other is to establish an enzyme immunoassay that does not require the release of lipoic acid. But the enzyme immunoassay is not sensitive, it can’t distinguish the two forms of lipoic acid [16]. Hydrolysis of lipoyl-N-e-lysine amide bond can be determined by colorimetric method, but this method is not sensitive and specific, or microbial determination, but takes more time and effort [17-19]. Endogenous lipoic acid in isolated lipoate-containing proteins was measured in form of lipoyllysine by enzymatic hydrolysis, using HPLC equipped with an ultraviolet (UV) detector (330 nm). This method has the advantage of increasing the recovery of endogenous lipoic acid. But the UV detection system is not sensitive to the detection of small amounts of lipoamide at 330nm [20]. In addition, there are HPLC based on electrochemical detector colorimetric detection, almost 100% of the interaction with the analyte [21].

Compared with the animal containing only the pyruvate dehydrogenase complex in the mitochondria, the plant contains two pyruvate dehydrogenase complexes, which are present in the mitochondria and chloroplasts, and the two isomers have different immunological differences and molecular weights, Which is related to the number of lipoic acid domains [10]. The content of lipoyllysine in plant tissues is mainly determined by the content of mitochondria and chloroplasts [11]. Spinach is currently the highest, with about 3.15 mg per gram of dry weight, besides, cauliflower, tomato, pea and cabbage also contains large amounts of lipoyllysine. Grams of dry weight accounted for 0.4-1.0 mg [11].

4. Application of Lipoic Acid in Plant Tissue Culture and Genetic Transformation
Browning is a common phenomenon observed in plant tissue culture [22]. It is thought that browning is related to polyphenol oxidase (PPO) in tissues [23]. There are three explanations for enzymatic browning: the regional distribution hypothesis of phenol, phenolase, free radical damage hypothesis and protective enzyme system hypothesis. The three hypotheses mainly explain that, the polyphenols are distributed in the vacuoles of cells, the phenols are distributed in the cytoplasm, and this regional distribution separates the substrate from the phenolic membrane from the plasma membrane. When the explants in the mechanical damage or other adversity, the cell membrane structure has been destroyed, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were dysfunctional, and too much free radicals was accumulated. After the reaction of reactive oxygen balance system broken, the plant cells hurt [24].
Plant tissue culture technology is widely used in the genetic transformation of plants, and the commonly method for cultivating transgenic plants is Agrobacterium-mediated method. However, this method has a problem that the genetic transformation efficiency is low and the transgenic plant regeneration is difficult. The genetic transformation rate of dicotyledonous plants was below 5%, and monocotyledonous plants were about 1% [25]. Agrobacterium tumefaciens is a kind of plant pathogen, explant infection by pathogen will occur oxygen explosion reaction, producing a large number of reactive oxygen species, causing the phenomenon of the cells browning and necrosis, low rate of transformation of transgenic buds and transformation 'stubborn'[26,27].

In 2004, Dan et al. Showed that plant transformation media containing lipoic acid has been found to improve the efficiency of transformation or regeneration. [28]. In 2009, Dan et al. Used lipoic acid as an antioxidant in a variety of plants Agrobacterium tumefaciens-mediated tissue culture. In soybean and tomato, the efficiency of transformation was significantly increased from 0.6% to 3.7% and 29.8% to 87%, respectively, the conversion efficiency of wheat was increased from 2.8% to 5.7%. On cotton, the resistance rate of glyphosate increased from 41.4% to 61.2%. Lipoic acid successfully affecting the number of transgenic plants Three main problems: transformation of tissue browning, necrosis, tissue transformation regeneration difficulties, and shoot escape [26]. Recently, researchers have applied lipoic acid to Agrobacterium tumefaciens-mediated transformation of the low efficiency of Mexican lime, lipoic acid through the promotion of callus development and Agrobacterium tumefaciens-mediated epicotylation of stem segments, greatly improving the conversion efficiency of Mexican lime [29].

5. Summary and perspectives
Lipoic acid as an antioxidant is powerful, its application in the plant still has very large development space. It can solve the problems about explants, such as browning necrosis and the low transformation efficiency of Agrobacterium tumefaciens-mediated. We can explore its application in different plant tissue culture of the optimal concentration, It can also be applied to plant tissue culture production, as an additive in the plant medium, to reduce costs and increase production.

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