Role of Aminolevulinic Acid in Stress Adaptation and Crop Productivity

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A B S T R A C T

Aminolevulinic acid (ALA) is found as one of the potential plant growth regulator which is involved in the development of heme, chlorophyll, bilins, vitamin B and other specialized plant products. ALA has a significant role in stress adaptation in plants through regulating several key physiological processes associated with plant adaptations such as seed germination, reduced Na^+ uptake, altered light reactions, improved scavenging of reactive oxygen species, enhanced photosynthetic assimilation and maintenance of nutrients status. ALA also acts as a potential biodegradable herbicide and its herbicidal activity enhances accumulation of different chlorophyll intermediates, such as protochlorophyllide, protoporphyrin IX and Mg-protoporphyrin IX. Therefore, there are immense possibilities to increase crop productivity through application of ALA under different stress conditions.

Introduction

5-aminolevulinic acid (ALA) is a key precursor in the biosynthesis of all porphyrin compounds, including heme, phytochrome, chlorophyll and vitamin B12. Now, it is emerging as a new phytohormone which also serves as new plant growth regulating substance (PGR). In recent years, ALA has received wide attention due to its potential use as herbicide (Xu *et al.,* 2015), insecticide (Yang *et al.,* 2011), plant stimulator under different stress conditions (Kosar *et al.,* 2015), antimicrobial drug (Malik *et al.,* 1990) and photosensitizer for photodynamic therapy (Wachowska *et al.,* 2011). When used in low concentration (Sasikala and Ramana, 1995), it has been considered an effective mean of minimizing various abiotic stresses and their various adverse effects in a number of agricultural crops by regulating a variety of physiobiochemical processes such as photosynthesis, stimulating fixation of CO_2 in light, chlorophyll biosynthesis, nutrient uptake, antioxidant metabolism, and protein synthesis, which are directly or indirectly involved in the mechanism of stress tolerance. Thus, ALA could be used as a biofertilizer as it has a promotive effect on the growth and yield of several crops and vegetables under various environmental factors, salinity, drought, and extreme temperatures.

Biosynthesis of ALA

C_4 and C_5 pathway: the routes for ALA production

The formation of the key intermediate for ALA biosynthesis proceeds via two different pathways (Fig. 1) (Sasaki *et al.,* 2002). One is the Shemin pathway (C_4 pathway) that is present in mammals, birds, yeast, protozoa and the purple non-sulfur photosynthetic bacteria. Another pathway is the glutamate pathway (C_5 pathway) that exists in higher plants, algae, archaea and many bacteria, such as *Escherichia coli* and *Salmonella typhimurium* (Kang *et al.,* 2011b). A few microorganisms have both C_4 and C_5 pathways e.g., *Euglena gracilis* (Weinstein and Beale, 1985) and *Rubrivivax benzoatilyticus* PS-5 (Sattayasamitsathit and Prasertsan, 2013).

Microbial production of ALA

ALA was mainly synthesized through chemical methods (Sasaki *et al.,* 2002). However, chemical synthetic methods involve complicated processes and relatively give low yields with high costs. Therefore, microbial synthesis appears to be a good alternative in the view of promising applications of ALA with its numerous advantages. One such example includes the fact that ALA production can be naturally carried out through the biosynthetic pathway of organisms (especially microorganisms) in their cell factories which shortens the ALA production process (Liu *et al.,* 2014). The microbial synthesis of ALA in cells is tightly regulated by feedback inhibition at the level of ALA formation. In most microorganisms, synthesis of ALA for cell growth and metabolism requires limited carbon sources (Table 1). *R. sphaeroides* produced the highest ALA concentration (4.2 mM) in media by anaerobic-light cultivation under optimized conditions. However, this concentration is still low for economic consideration. The lengthy fermentation time and light requirement during culture growth also make *R. sphaeroides* undesirable for industrial production.

Applications

ALA is reported to have the ability to promote plant growth, productivity and enhance stress tolerance, such as chilling (Korkmaz *et al.,* 2010), drought (Liu *et al.,* 2013), and salinity (Youssef and Awad, 2008; Naeem *et al.,* 2012). The ALA also suppresses the respiration and the release of CO_2 under dark (Hotta *et al.,* 1997a, b). The appropriate applications of ALA showed 10-60% promotive effect over the control on radish, kidney beans, barley, watermelon, potatoes, garlic, rice and corn (Sasikala *et al.,* 1994). Therefore, 5-aminolevulinic acid (ALA), is known to be effective in counteracting the injurious effects of various abiotic stresses in plants.

Salt adaptation

Salinity is one of the major threats to agricultural crop production worldwide. Therefore, many approaches have been used to alleviate salinity stress of various crops; one of which is the use of 5- aminolevulinic acid plant growth regulators (PGRs). Research conducted on ALA has mainly focused on photosynthesis (Liu *et al.,* 2013), chlorophyll accumulation (Memon *et al.,* 2009), the antioxidant system (Sun *et al.,* 2009), and ion uptake (Naeem *et al.,* 2010). Although ALA has been reported to be capable of enhancing the salt tolerance of various plants, data on the molecular mechanism of ALA application are limited. Naeem *et al.,* (2010) observed that spraying ALA on oilseed rape improved leaf water relations under salinity stress. They found that ALA could regulate the transcription of aquaporins in cucumber leaves

and enhance the salt tolerance of the plants (Yan *et al.,* 2014). Therefore, analyzing the effects of ALA on the expression of aquaporins in tomato seedlings and to determine the interaction between aquaporin expression and water content of tomato leaves under different salinity conditions. Yang *et al.,* (2014) showed the exogenous application of ALA was effective for mitigating salinity damages in creeping bentgrass. The effects of ALA in creeping bentgrass were associated with the suppression of Na+ toxicity and enhanced water retention, photosynthetic activities, antioxidant metabolism, and the accumulation of organic acids, amino acids, and sugars involved in osmotic regulation and respiratory pathways, and protein metabolism, as well as stress protection from membrane damages. Zhao *et al.,* (2015) also studied that exogenous ALA application significantly improved net photosynthetic rate (*Pn*), total chlorophyll content, and plant biomass accumulation of tomato seedlings under salinity stress.

Mechanism involved salt stress

Salt stress is known to adversely affect endogenous levels of phytohormones that influence a variety of processes in plants such as reducing seed germination, seedling growth, ion uptake, stomata opening, and photosynthetic rate (Iqbal *et al.,* 2010). In Sorghum, Netondo *et al.,* (2004) observed that maximum quantum yield of photosystem II (Fv/Fm), photochemical quenching coefficient (qP) and electron transport rate (ETR) significantly decreased, but non-photochemical quenching (NPQ) increased substantially under saline conditions. Sensitivity to salt stress in cereals might, thus, be associated with both reduction in PSII photochemical efficiency and enhanced NPQ to dissipate excess energy. Consequently, saline soil has detrimental effects on plant growth and yield. Ashraf and Harris (2004)

reported reactive oxygen species (ROS) such as singlet oxygen ($1O_2$), superoxide anion (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), are produced in normal aerobic metabolism, but their levels are increased under stress. In plants, changes in antioxidative enzyme activities such as catalase (CAT), ascorbic peroxidase (APx), glutathione reductase (GR) and superoxide dismutase (SOD) involved in the detoxification of ROS, are often observed under salt stress.

ALA has been reported to increase ascorbate–glutathione cycle activity and to increase the level of antioxidant enzymes such as SOD, CAT, and APx (Wongkantrakorn *et al.,* 2009). It has been repeatedly demonstrated that the problems caused by salinity can be counteracted by use of ALA (Naeem *et al.,* 2011). There have been a number of demonstrations that ALA at low concentrations can promote plant growth (Zhen *et al.,* 2012; Ali *et al.,* 2013) and their exogenous application of ALA is very effective in minimizing the salt-induced adverse effects in various crops, e.g. spinach (*Spinaciaoleracea*) (Nishihara *et al.,* 2003), pakchoi (*Brassica campestris*) (Wang *et al.,* 2005), potato (*Solanum tuberosum*) (Zhang *et al.,* 2006), date palm (*Phoenix dactylifera*) (Youssef and Awad, 2008) and oilseed rape (*Brassica napus*) (Naeem *et al.,* 2011). Unfortunately, commercial ALA is too expensive for many common agricultural applications. The use of microorganisms that directly produce ALA may be a lower cost.

Cold adaptation

Low temperature may impose stress on plant growth in a two-fold manner: by the effects of low temperature alone, and by dehydration of the cells and tissues when cellular water freezes (Beck *et al.,* 2007). Low temperatures are deleterious to many crops of the tropics and subtropics which cannot acclimatize to

cold. This kind of damage on the plant has been termed 'chilling' (Sakai and Larcher, 1987). These effects include damaged membranes (Xing and Rajashekar, 2001), reduced cellular respiration (Lee *et al.,* 1997), increased abscisic acid levels (Nayyar *et al.,* 2005), cryoprotectants and increased reactive oxygen species (Lee *et al.,* 1997) which arises from imbalances of electron transport rate and the metabolic consumer activity of the reductive power (Beck *et al.,* 2007). Most temperate plants can cold-acclimate. They acquire tolerance to extracellular ice formation in their vegetative tissues, while many important crops, such as rice, maize, soybean and cotton are chilling sensitive and incapable of cold acclimation. Recently, it was found that ALA has a promotive effect on growth and yield of several field crops (Watanabe *et al.,* 2000). Zhang *et al.,* (2006) observed that ALA increased cold and salt tolerance in rice and potato. It has been reported that ALA biosynthesis in plants is inhibited by low and high temperatures (Hodgins and Oquist, 1989; Tewari and Tripathy, 1998). From previous studies, exogenous application of ALA could enhance the antioxidant level in spinach and pakchoi (Memon *et al.,* 2009) and enhance plant's tolerance to cold stress (Wang *et al.,* 2004).

ALA pre-treatment also increased reduced glutathione (GSH), ascorbic acid (AsA), total glutathione, and total ascorbate concentrations, and the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), whereas decreased the contents of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), and superoxide radical ($O_2 N_2$) release in germinating seeds under cold stress. The results indicate that ALA considered as an endogenous plant growth regulator could effectively protect *E. nutans* seeds from cold-induced oxidative damage during germination without any adverse effect. Manafi *et al.,* (2015) evaluated the effect of exogenous ALA enhancing cold stress tolerance of young soybean plants (*Glycine max*). Therefore, exogenous application of ALA is a novel strategy and has been considered as an effective means of minimizing the cold induced adverse effects.
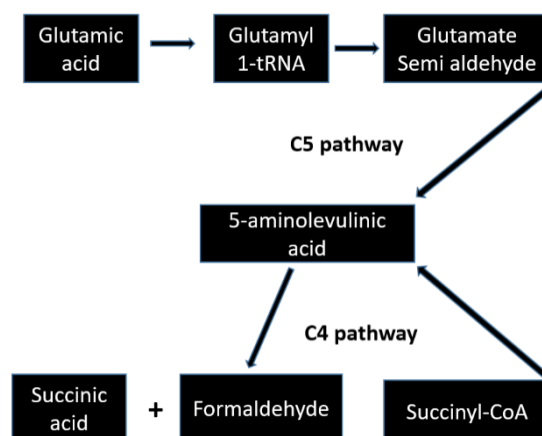

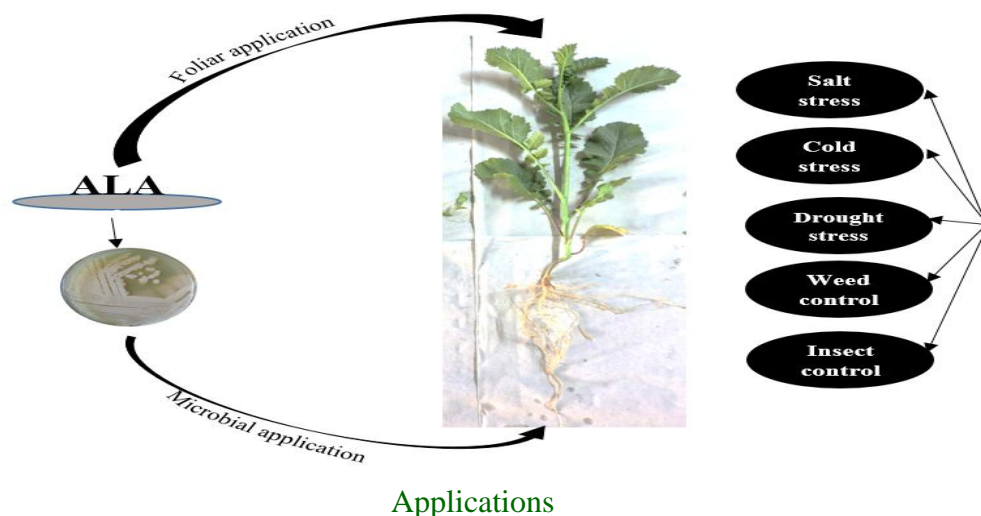**Fig.1** C_5 and C_4 pathways for ALA biosynthesis

Applications

Table.1 Production of δ-aminolevulinic acid by bacteria

Microorganisms	Carbon source	ALA (µM)	References
Rhodopseudomonas palustris	Glycine and succinate	2.61	Kantha *et al.,* 2015
Rhodovulum sp.	Glutamate, glycine	2.0	Noparatnaraporn *et al.,* 2000
Rubrivivax benzoatilyticus	Glycine, succinate and levulinic acid	190.74	Sattayasamitsathit and Prasertsan, 2013
Rhodopseudomonas sp.KL9	Malate, succinate and acetate	67.2	Hyun and Song, 2007
Rhodobacter capsulatus	Glutamate effluent	40	Chaikritsadakarn *et al.,* 2004
Rhodopseudomonas palustris KG31	Glutamate, acetate	0.18	Saikeur *et al.,* 2009
R. sphaeroides	Glucose, glycine, levulinic acid	20.0	Nishikawa *et al.,* 1999
R. sphaeroides CR720	Glucose, glycine	27.5	Kamiyama *et al.,* 2000

Drought adaptation

Water is the key requirement for plant growth and productivity. Drought stress results in the generation and accumulation of active oxygen species, peroxidation of membrane lipids and impairment of nitrogen metabolism, photosynthesis, growth, and development, causing a substantial decline in yield and quality (Li *et al.,* 2014). Li *et al.,* (2011) reported promoting effect of ALA in a cucumber variety (*Cucumis sativus* L. cv.

Yuexiu no. 3). Seeds were pretreated with 3 µM ALA for 2 days and then were exposed to drought stress induced by 10% polyethylene glycol (PEG) 6000. After 2 days of ALA pretreatment, no changes were observed in plant growth indexes including plant heights and the fresh and dry weights of shoots and roots and the second leaves. Drought stress increased the activities of superoxide dismutase (SOD, EC 1.15.1.1), guaiacol peroxidase (GPX, EC 1.11.1.7), glutathione peroxidase (GSH-Px, EC

1.11.1.9), ascorbate peroxidase (APX, EC 1.11.1.11), glutathione reductase (GR, EC 1.6.4.2), and monodehydroascorbatereductase (MDHAR, EC 1.6.5.4) in ALA-pretreated leaves. The results indicated ALA pretreatment protects cucumbers from drought stress.

Liu *et al.,* (2011) evaluated the effects of 5-aminolevulinic acid (ALA, 0, 0.1, 1 and 10 mg l^{-1}) on the growth of oilseed rape (*Brassica napus* L. cv. ZS758) seedlings under water-deficit stress induced by polyethylene glycol (PEG 6000, 0 and −0.3 MPa). Water-deficit stress imposed adverse effects on seedling growth by reducing shoot biomass, cotyledon water potential, chlorophyll content and non-enzymatic antioxidants (glutathione and ascorbic acid) levels.

Application of ALA at lower dosages (0.1 and 1 mg l^{-1}) improved shoot growth and chlorophyll contents and whereas moderately higher dosage of ALA (10 mg l^{-1}) hampered the growth. The study also indicated that 1 mg l^{-1} ALA improved chlorophyll content, but reduced malondialdehyde (MDA) content and ROS production significantly under water-deficit stress. Lower dosages of ALA (0.1 and 1 mg l^{-1}) also enhanced reduced/oxidized glutathione ratio (GSH/GSSG) and reduced/oxidized ascorbic acid (ASA/DHA) as compared to the seedlings under water-deficit stress. The antioxidant enzymes (ascorbate peroxidase, peroxidase, catalase, glutathionereductase and superoxide dismutase) enhanced their activities remarkably with 1 mg l^{-1} ALA treatment under water-deficit stress. These results indicated that 0.1–1 mg l^{-1} ALA could enhance the water-deficit stress tolerance of oilseed seedlings through improving the biomass accumulation, maintaining a relative high ratio of GSH/GSSG and ASA/DHA, enhancing the activities of the specific antioxidant enzymes and inducing the expression of the specific antioxidant enzyme genes.

Bioherbicide

ALA has received great attention as a biodegradable herbicide (Chon, 2003). However, herbicidal activity of ALA on several plants was affected by the application methods. At low concentration (0.01-10 mg/L), ALA showed growth promoting effects on yield of several crops (Hotta *et al.,* 1997b), whereas it suppressed plant growth at higher concentrations (>2 mM). Its application is harmless to crops, humans and animals (Edwards *et al.,* 1984; Beck *et al.,* 2007; Bhowmick and Girotti, 2010; Mikolajewska *et al.,* 2010; Kang *et al.,* 2012). Zhang *et al.,* (2006) reported that ALA at low concentrations of 0.3–3 mg/L promoted development and growth of potato microtubers *in vitro* and enhanced protective functions against oxidative stresses, but ALA at 30 mg/L and higher concentrations may induced oxidative damage.

ALA has photodynamic herbicide properties under appropriate treatment conditions. An immediate dark-incubation period after spraying ALA is an essential step to accumulate tetrapyrrole within the plants. ALA serves as a building block of tetrapyrrole accumulation, while a group of modulators, *O*-phenanthroline, ethyl nicotinate and 2, 2'-depyridyl (DP), were found to affect the pattern of tetrapyrrole accumulation and acted in concert with ALA. DP is a cheap chemical and it is selected to mix with ALA to enhance the accumulation of tetrapyrrole. During the daylight period, the excess tetrapyrrole produces active oxygen (singlet oxygen), which oxidizes the unsaturated fatty acid on the cell surface (lipoprotein component), thus setting in motion a greatly damaging free-radical chain reaction. The cell membranes become leaky

and this, in turn, resulted in a rapid and severe dehydration, bleaching and collapse of the leaf and/or hypocotyl tissue (Rebriz *et al.,* 1984). Within 24 h, the green plant tissue turns into a brownish desiccated mass of dead tissue (Rebriz *et al.,* 1990). On the other hand, treated plants kept for the same period of time in darkness were unaffected. The accumulation of tetrapyrrole in plant leaves causes very severe photodynamic damage and the leaves die within a few hours while the cotyledons, stem and growing point remain unaffected. Dicotyledonous weeds such as redroot pigweed, purslane and lambquarter are highly susceptible to the tetrapyrrole induced photodynamic damage. Monocots such as corn, wheat, oats and barley were not adversely affected by the spray (Rebriz *et al.,* 1984). The death of plants depended on the age of plant, ALA concentration, type of modulators, ratio of ALA and modulator, light intensity and varieties of plant treated (Kobayashi and Haque, 1971).

Perspective and Future aspects

Due to serious problems such as environmental pollution, energy crisis, global warming and an increasing demand of sustainable development, bio-based production of industrial chemicals, pharmaceuticals and biofuels, have been received much attention and intense research. In the past decades, many good characterized native strains for ALA production were screened and isolated. Further research at molecular levels may be necessary to identify molecular and metabolic pathways conferring ALA effects on stress mitigation/herbicidal effect in different plant species.

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