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Research article

Polyamine biosynthesis and degradation are modulated by exogenous gamma-aminobutyric acid in root-zone hypoxia-stressed melon roots

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ABSTRACT

We detected physiological change and gene expression related to PA metabolism in melon roots under controlled and hypoxic conditions with or without 5 mM GABA. Roots with hypoxia treatment showed a significant increase in glutamate decarboxylase (GAD) activity and endogenous GABA concentration. Concurrently, PA biosynthesis and degradation accelerated with higher gene expression and enzymes activity. However, endogenous GABA concentrations showed a large and rapid increase in Hypoxia + GABA treated roots. This led to a marked increase in Glu concentration by feedback inhibition of GAD activity. Hypoxia + GABA treatment enhanced arginine (Arg), ornithine (Orn) and methionine (Met) levels, promoting enzyme gene expression levels and arginine decarboxylase (ADC), ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAMDC) activities in roots. Hypoxia + GABA treatment significantly increased concentrations of free putrescine (Put), spermidine (Spd) and spermine (Spm) from day two to eight, promoting the PA conversion to soluble conjugated and insoluble bound forms. However, PA degradation was significantly inhibited in hypoxia + GABA treated roots by significantly decreasing gene expression and activity of diamine oxidase (DAO) and polyamine oxidase (PAO). However, exogenous GABA showed a reduced effect in control compared with hypoxic conditions. Our data suggest that alleviating effect of exogenous GABA to hypoxia is closely associated with physiological regulation of PA metabolism. We propose a potential negative feedback mechanism of higher endogenous GABA levels from combined effects of hypoxia and exogenous GABA, which alleviate the hypoxia damage by accelerating PA biosynthesis and conversion as well as preventing PA degradation in melon plants.

1. Introduction

Melon (*Cucumis melon* L.) is an important horticultural fruit that is widely cultivated in China. According to the FAO, the cultivation area and production of melons in China in 2009 were approximately 590,000 ha and 14.62 million tons, accounting for 46 and 53% of worldwide values, respectively (http://www.fao.org). However, hypoxic stress in melons often causes enormous economic losses, because of this species' high sensitivity to oxygen deficiency (Biais et al., 2010).

Hypoxia, i.e., low oxygen concentration, is a common environmental challenge which occurs in plants throughout their lifespan, causing serious injury to terrestrial plants as a consequence of poor soil drainage, soil compaction, irrigation and certain conditions in natural wetlands or hydroponic cultures. Short-term hypoxia leads to the inhibition of oxidative phosphorylation and transfer to anaerobic respiration, resulting in a marked decrease in NTPs and a dramatic increase in the NADH/NAD^+ ratio (Bailey-Serres et al., 2012). More severe hypoxia can interfere with proteins, nucleic acids and the cytoskeleton, making hypoxia a potential growth inhibitor and limiting crop yield. Some plants have evolved defense mechanisms to cope with potential damage from hypoxia. Normally, a respiration shift from the oxidation of glucose to carbon

Abbreviations: ADC, Arginine decarboxylase; Arg, Arginine; DAO, diamine oxidase; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; Glu, glutamate; Met, Methionine; NTP, nucleotide triphosphates; ODC, Ornithine decarboxylase; Orn, Ornithine; PA, polyamine; PAO, polyamine oxidase; PBS, phosphate buffered solution; PLP, pyridoxal phosphate; Put, putrescine; SAMDC, S-adenosylmethionine decarboylase; Spd, spermidine; Spm, spermine.

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dioxide and respiration-driven ATP production to fermentative reduction of pyruvate to lactate plays an important role in the maintenance of energy and membrane lipid integrity (Rocha et al., 2010). Recent studies have demonstrated that the nitrogen metabolism involved in the defense response in higher plants to hypoxia or anoxia (Shi et al., 2008), resulting from high rates of amino acid interconversion, are independent of the N source, polyamines synthesis, or protein degradation and synthesis (Gao et al., 2011). How the nitrogen metabolism exerts a beneficial effect on the root hypoxia tolerance in hypoxia plants is not clearly understood.

γ-aminobutyric acid (GABA), a four-carbon non-protein amino acid, is rapidly produced in response to biotic and abiotic stresses (Fait et al., 2008). Two routes of synthesis of GABA have been described as being the decarboxylation of glutamate (Glu) from the catalytic action of glutamate decarboxylase (GAD) (EC 4.1.1.15) and the degradation of polyamine (PA) from the catalytic action of diamine oxidase (DAO) (EC 1.4.3.6) and polyamine oxidase (PAO) (EC 1.5.3.3) (Bhatnagar et al., 2001). Therefore, a close relationship exists between GABA and PA (Fig. 1). Recent investigation indicated that approximately 30% of GABA formed in germinating fava beans (*Vicia faba*, Fabaceae) under hypoxic conditions was supplied by the polyamine degradation pathway (Yang et al., 2013). Research suggests that GABA accumulation is closely related to defense against hypoxic stress. GABA synthesis from GAD can regulate the cytoplasmic pH by consuming protons and GABA production may act as a source of nitrogen and/or carbon for supporting plant growth under oxygen deficiency (Shelp et al., 2012a). Moreover, GABA metabolism (GABA shunt), has been associated with various physiological responses, including carbon fluxes in the TCA cycle, protection against oxidative stress and signaling transduction (Fait et al., 2008). Recently, research has shown that treatment with exogenous GABA improved plant growth, enhancing plant tolerance to stress by modulating the activities of enzymes involved in primary nitrogen metabolism and nitrate uptake (Barbosa et al., 2010), increasing the accumulation of alanine against stress damage (Miyashita and Good, 2008) and preventing the accumulation of reactive oxygen intermediates and cell death (Bouche et al., 2003). A closed relationship exists among GABA, Glu and PA. However, whether PA synthesis and degradation in plants are modulated by exogenous GABA under hypoxia stress is unclear.

Polyamines are ubiquitous low-molecular-weight aliphatic amines that are involved in regulating plant growth and development during abiotic stress including hypoxia (Gill and Tuteja, 2010). The most commonly found polyamines in higher plants, putrescine (Put), spermidine (Spd) and spermine (Spm) may be present in free, soluble conjugated and insoluble bound forms. Put can be synthesized directly by decarboxylation of either ornithine or arginine catalyzed by Ornithine decarboxylase (ODC) (EC 4.1.1.17) and Arginine decarboxylase (ADC) (EC 4.1.1.19) respectively. Spd and Spm are synthesized from Put by the successive addition of aminoprogyl groups from decarboxylated S-adenosylmethionine derived from SAM by the action of S-adenosylmethionine decarboylase (SAMDC) (EC 4.1.1.50) (Gardiner et al., 2010). Polyamine degradation is catalyzed by diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.3) (Fig. 1). Suggestions have been made that PA could alleviate hypoxia injury in roots and increase the root-zone hypoxia tolerance. The relationship between the ability to tolerate hypoxia and capacity to accumulate PA, including in PA synthase activity have been observed in seedlings of many plants (Hussain et al., 2011). The application of exogenous PA partially increased the survival of plants by inducing lactate fermentation, increasing H^+-ATPase activities of plasma membrane (Nada et al., 2004), decreasing the reactive oxygen species injury (Gao et al., 2011), stimulating the activity of NR and nitrate reduction to maintain the redox and energy status under hypoxia stress (Shi et al., 2008). The role for polyamines in protection against the stress-induced cellular damage has been demonstrated in transgenic plants that accumulate high levels of polyamines through the over-expression of key biosynthetic enzymes in the polyamine biosynthetic pathway (Alcázar et al., 2010).

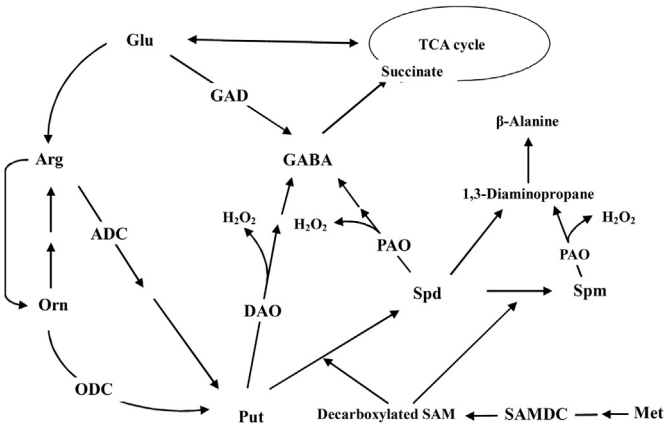

Fig. 1. The biosynthetic and degradation pathway for GABA and major PA (Put, Spm and Spd) in plants.

PA degradation is one of the GABA synthesis pathways, with a close relationship existing between endogenous GABA and PA in the plant cell. However, it is currently unknown what PA or related metabolites accumulate during exogenous GABA under hypoxia stress. We previously demonstrated that exogenous GABA could enhance nitrate uptake and transformation (Song et al., 2012) and increase the activity of antioxidant enzymes involved in reactive oxygen species metabolism (Luo et al., 2011). In our study, the effect of exogenous GABA treatment on PA metabolism has been examined for PA and metabolite concentrations, gene expression, PA biosynthesis and degradation activity in roots under aerated and hypoxic stress conditions with or without the exogenous GABA. The objective of this study was to elucidate the mechanisms of exogenously GABA on PA metabolism under hypoxia stress and to determine if a close relationship existed between exogenous GABA increasing the tolerance of melon seedlings and PA metabolism.

2. Materials and methods

2.1. Plant cultivation and experimental treatments

Melon (*Cucumis melo* L. var. *reticulatus* Naud) 'Xiyu No.1' seedlings, a sensitive variety (Gao et al., 2011), were cultivated in an environmentally controlled greenhouse (25–30 °C and 15–18 °C day/night, respectively) in quartz sand constantly supplied with liquid nutrient (1/2 Hoagland solution). When the melon seedlings were at the three-leaf stage, all uniform seedlings were divided into four groups and transplanted into plastic troughs containing 30 L of full-strength Hoagland solution (pH 6.5 ± 0.1, electrical conductivity 2.0–2.2 $mScm^{-1}$). The nutrient solution was aerated using an air pump at 20 min intervals to maintain the dissolved oxygen level at 8.0 ± 0.2 mg L^{-1}. We use a dissolved oxygen analyzer (Pisco DO500, Germany) to monitor the dissolved oxygen in the nutrient solution. The dissolved oxygen for the control was set at 8 ± 0.2 mg L^{-1}, while the dissolved oxygen for the hypoxia treatment was set at 2 ± 0.2 mg L^{-1}. The dissolved oxygen was automatically assessed and controlled by an air and N_2 pump every 5 min. The solution was maintained at 20–25 °C.