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Comparative proteomic analysis of gamma-aminobutyric acid responses in hypoxia-treated and untreated melon roots

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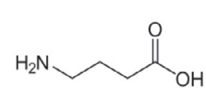
ABSTRACT

Hypoxia is one of the main environmental stresses that accounts for decreasing crop yield. To further investigate the mechanisms whereby exogenous GABA alleviates hypoxia injury to melon seedlings, a comparative proteomic analysis was performed using roots subjected to normal aeration and hypoxia conditions with or without GABA (5 mM). The results indicated that protein spots on gels after hypoxia and hypoxia + GABA treatment were significantly changed. Three "matched sets" were analyzed from four treatments, and 13 protein spots with large significant differences in expression were identified by MALDI-TOF/TOF mass spectrometry. Exogenous GABA treatment enhanced the expression of protein in cytosolic phosphoglycerate kinase 1, exaA2 gene product, dnaJ and myb-like DNA-binding domain-containing proteins, as well as elongation factor-1 alpha and hypothetical proteins in hypoxia-induced roots. However, the hypoxia + GABA treated roots had a significantly lower expression of proteins including malate dehydrogenase, nucleoside diphosphate kinase, disease resistance-like protein, disulfide isomerase, actin, ferrodoxin NADP oxidoreductase, glutathione transferase, netting associated peroxidase. This paper describes the effect of GABA on melon plants under hypoxia-induced stress using proteomics, and supports the alleviating function of GABA in melon plants grown under hypoxic conditions.

1. Introduction

Hypoxia, is a common environmental stress that occurs throughout plant lifespans and leads to decreased crop yields and economic loses due to flooding, excessive irrigation, poor soil aeration, wetlands or hydroponics (Bailey-Serres and Voesenek, 2008). However, hypoxia also occurs in ways related to season and weather, where it is usually periodic and of short duration. Short-term hypoxia can cause physiological changes in plants, such as inhibiting oxidative phosphorylation, switching to anaerobic respiration, reducing nucleoside triphosphates (NTPs), and increasing the ratio of NADH/NAD^+ (Morard et al., 2004). Furthermore, normal physiological metabolism in plants is perturbed due to reduction of energy and carbohydrates under hypoxia-induced stress (Li et al., 2012). In order to clarify how plants endure the harm of hypoxia stress, previous papers reported some tolerance mechanisms including physiological and biochemical changes in melon seedlings subjected to hypoxia (Song et al., 2012; Wang et al., 2014). Melon (*Cucumis melon* L.), an important horticultural crop worldwide, is susceptible to effects caused by water (Gao et al., 2011). However, melons require an appropriate amount of water for growth, and they are highly sensitive to oxygen deficiency. Consequently, hypoxia in the rhizosphere frequently causes large losses in yield and substantial economic loss (Biais et al., 2010).



γ-Aminobutyric acid (1), GABA

γ-Aminobutyric acid (GABA) (1) is a significant component in most prokaryotic and eukaryotic organisms as a four carbon non-protein amino acid (Guo et al., 2012). It plays a dual role in

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regulating C:N balance and nitrogen metabolism. It is also involved in response to many aspects of physiological metabolism, such as carbon flux in the tricarboxylic acid (TCA) cycle, antioxidant effects and regulation of signal transduction (Bouche and Fromm, 2004). Following exposure to various different stresses, plant tissues can rapidly accumulate high levels of GABA (1) because of the effect of glutamic acid (Glu) decarboxylation and polyamine degradation (Shelp et al., 2006). Research increasingly proves that GABA (1) accumulation in plants is associated with hypoxic stress resistance by regulating cytoplasmic pH, supporting nitrogen/carbon fluxes, antioxidant stress and maintaining the TCA cycle (Fait et al., 2008). Several papers have reported that GABA (1) application promotes plant growth, improves plant resistance, which is closely related to physiological metabolism regulation by increasing enzyme activities in nitrate uptake and transformation (Yokdang et al., 2011), enhancing alanine content against stress injuries (Rocha et al., 2010; Valderrama et al., 2006), and preventing production of reactive oxygen species (ROS) and programmed cell death (Reggiani and Bertani, 2003). Previously it was reported that exogenous GABA (1) could alleviate injury resulting from hypoxia-induced stress in melons by improving nitrate uptake and metabolism (Song et al., 2012), as well as by promoting polyamine synthesis (Wang et al., 2014). However, the precise molecular mechanisms of its involvement in plant responses to stress are largely unknown.

Since cellular proteins largely regulate and alter cell metabolism and structure, proteomics is a powerful tool to use in the elucidation of plant tolerance mechanisms to environmental stress (Li et al., 2012). Indeed, a considerable amount of knowledge about specific proteins in various biological process is available based on proteomics research (Finnie et al., 2002). However, only a limited number of published reports have studied melon proteomics. These studies include the analysis of phloem exudates (Gómez et al., 2005), different resources (Fei and Liu, 2011), melon responses to viral infection (Malter and Wolf, 2011), and drought stress (Yoshimura et al., 2008). The application of exogenous GABA (1) could alleviate hypoxia-induced damage, but there are no reports regarding the precise mechanism of how it regulates plant responses to hypoxia. To better understand how the melon proteome is affected by GABA (1) under hypoxia, this investigation studied differentially expressed proteins in melon roots subjected to either hypoxia or normal conditions with and without GABA (1). The identified GABA (1) and hypoxia-specific proteins would offer novel insights for further research at the molecular and biochemical levels.

2. Results and discussion

2.1. Effect of GABA (1) on growth of melon seedling treated by hypoxic stress

The root volume, leaf area, and fresh or dry mass of the root and shoot of 40 different seedlings were assessed on four days following different treatments (Table 1). The growth of roots and shoots was inhibited significantly by hypoxic stress. As a result, root volume, leaf area, fresh and dry mass of roots and shoots were all significantly lower than values of control samples. Exogenous GABA (1) application resulted in a noticeable improvement in seedling growth under hypoxic conditions and, as a result, the root volume, leaf area and root dry mass treated by hypoxia + GABA could reach the level of the untreated control, with growth parameters all significantly higher than the hypoxic stress condition. However, the growth of the seedlings did not exhibit any significant change with GABA (1) application under normoxic condition.

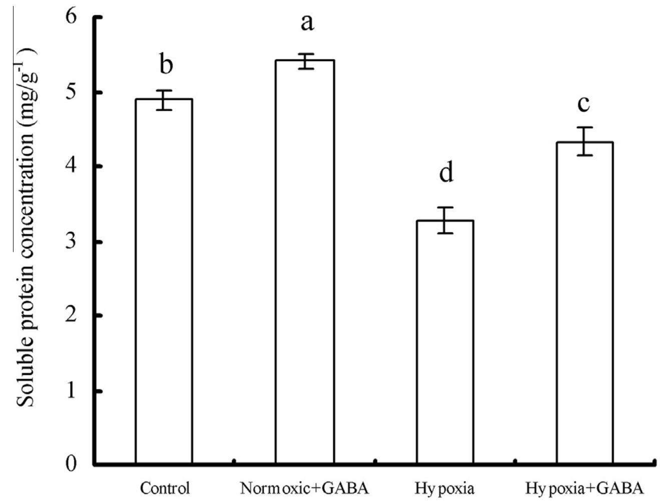

Fig. 1. Changes of protein contents in roots of melon seedlings, which were treated for 4 d under one of four conditions. (1) Control, normal aeration without GABA (1); (2) Normoxic + GABA, normal aeration with 5 mM GABA (1); (3) Hypoxia, lower oxygen concentration without GABA (1); (4) Hypoxia + GABA, lower oxygen concentration with 5 mM GABA (1). The dissolved oxygen concentration (DOC) was controlled using a dissolved oxygen analyzer, and the DOC values of normal aeration and low oxygen concentration were set as 8 ± 0.2 mg L^{-1} and 2 ± 0.2 mg L^{-1} respectively. The expressed data are the mean ± SE ($n = 4$) using four different seedlings.

2.2. Effect of GABA (1) on soluble protein concentration under hypoxia stress

Based on the protein concentration data and electrophoresis gel images, the soluble protein concentrations were determined to be significantly decreased in the roots of the hypoxia treatment group, but exogenous GABA (1) increased soluble protein concentrations under normoxic conditions and hypoxic stress (Figs. 1 and 2). Additionally, from regions 1 and 2 in Fig. 2, significantly more protein bands in the hypoxia and hypoxia + GABA-treated roots were observed than in the control roots. Furthermore, the highest number of protein bands and the highest protein concentrations were observed from about 38 KD to 55 KD in the hypoxia + GABA-treated roots. This result indicated that hypoxia and exogenous treatment with GABA (1) could induce changes in the type and concentration of protein.

Table 1
Change in growth of melon seedlings during either hypoxia or non-hypoxia with and without exogenous application of GABA (1) for 4 d.

Treatment	Root volume (mm^3)	Leaf area (cm^2)	Root fresh mass (g/plant)	Root dry mass (g/plant)	Shoot fresh mass (g/plant)	Shoot dry mass (g/plant)
Control	22.26 ± 1.90 a	58.83 ± 5.36 a	3.01 ± 0.16 a	0.16 ± 0.02 a	11.65 ± 0.79 a	0.81 ± 0.08 a
Normoxic + GABA (1)	23.25 ± 2.84 a	63.36 ± 3.65 a	3.06 ± 0.18 a	0.16 ± 0.01 a	12.47 ± 0.58 a	0.84 ± 0.06 a
Hypoxia	16.94 ± 1.28 b	46.88 ± 2.36 b	1.87 ± 0.13 c	0.10 ± 0.01 c	6.29 ± 0.47 c	0.42 ± 0.04 c
Hypoxia + GABA (1)	21.52 ± 1.81 a	55.94 ± 4.82 a	2.58 ± 0.15 b	0.15 ± 0.01 ab	8.96 ± 0.69 b	0.60 ± 0.04 b

Note: data are the mean ± SE of 40 independent samples. Different letters indicate significant differences between treatments ($P < 0.05$).

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