Scientia Horticulturae 240 (2018) 249–257

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

β-Aminobutyric acid treatment confers decay tolerance in strawberry fruit by warranting sufficient cellular energy providing

Abbasali Jannatizadeh[a], Morteza Soleimani Aghdam[a,*], Boukaga Farmani[b], Filippo Maggi[c], Mohammad Reza Morshedloo[d]

[a] Department of Horticultural Science, Imam Khomeini International University, Qazvin, Iran
[b] Department of Food Science and Technology, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Tabriz, Iran
[c] School of Pharmacy, University of Camerino, Camerino, Italy
[d] Department of Horticultural Sciences, University of Maragheh, Maragheh, Iran

ARTICLE INFO

Keywords:
Strawberry fruit
β-Aminobutyric acid
ROS scavenging enzymes activity
cellular energy providing
fungal decay
phenylalanine ammonia lyase

ABSTRACT

In this experiment, the mechanisms employed by β-aminobutyric acid (BABA) treatment to confer postharvest decay tolerance in strawberry fruit stored at 4 °C for 12 days were explored. Notably, BABA treatment at 25 mM conferred remarkably decay tolerance in strawberry fruit which was accompanied by higher membrane integrity representing by lower malondialdehyde (MDA) accumulation. Strawberry fruit treated with BABA exhibited remarkably higher cellular energy providing arising from higher H^+-ATPase, Ca^{2+}-ATPase, cytochrome c oxidase (CCO), and succinate dehydrogenase (SDH) enzymes activity. Additionally, strawberry fruit treated with BABA exhibited remarkably higher superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzymes activity resulting in lower H_2O_2 accumulation. Also, higher phenylalanine ammonia lyase (PAL) enzyme activity may be accountable for higher phenols and anthocyanins accumulation in strawberry fruit treated with BABA leading to superior DPPH scavenging capacity. Finally, strawberry fruit treated with BABA exhibited remarkably lower membrane degrading enzymes phospholipase D (PLD) and lipoxygenase (LOX) activity. According to our results, postharvest 25 mM BABA applying may be considered as a favourable strategy not only for conferring decay tolerance of strawberry fruit by warranting sufficient cellular energy providing, triggering H_2O_2 scavenging enzymes activity, enhancing phenols and anthocyanins accumulation and hampering membrane degrading enzymes activity which not only are vital for preserving membrane integrity, but also are crucial for keeping nutritional quality of strawberry fruit during postharvest cold storage.

1. Introduction

During postharvest life of fruits and vegetables, fungal decay development is accompanying by membrane integrity losing representing by higher electrolyte leakage and malondialdehyde (MDA) accumulation arising from insufficient cellular energy providing, overactivity of membrane degrading phospholipase D (PLD) and lipoxygenase (LOX) enzymes and ineffective reactive oxygen species (ROS) scavenging system activity leading to higher H_2O_2 and O_2^- accumulation (Lin et al., 2017a; Sun et al., 2018; Zhang et al., 2017a,b; Zhang et al., 2018). Conferring postharvest decay tolerance in litchi and longan fruit treated with exogenous ATP (Yi et al., 2008, 2010; Lin et al., 2017a; Zhang et al., 2017a), loquat fruit treated with methyl jasmonate (MeJA; Cao et al., 2014a), pear fruit treated with acibenzolar-S-methyl (ASM; Ge et al., 2017) and strawberry fruit treated with melatonin (Aghdam and Fard, 2017) has been ascribed to warranting sufficient cellular energy providing by higher H^+-ATPase, Ca^{2+}-ATPase, cytochrome c oxidase (CCO) and succinate dehydrogenase (SDH) enzymes activity and upregulating GABA shunt pathway activity. Warranting sufficient cellular energy providing plays crucial role in triggering ROS scavenging system activity which diminishes O_2- and H_2O_2 accumulation, hampering membrane degrading enzymes PLD and LOX activity, and promoting shikimate and phenylpropanoid pathways activity resulting in higher phenols accumulation, all are crucial for maintaining membrane integrity representing by lower electrolyte leakage and MDA accumulation (Aghdam et al., 2018). Warranting sufficient cellular energy providing by employing postharvest strategies is a promising approach to confer postharvest decay tolerance accompanying by preserving sensory and nutritional quality of fruits and vegetables (Aghdam and Fard, 2017; Cao et al., 2014a; Chen et al., 2014; Ge et al.,

* Corresponding author.
E-mail addresses: jannatizadeh@ut.ac.ir (A. Jannatizadeh), aghdamm@ut.ac.ir (M.S. Aghdam), bfarmani@tabrizu.ac.ir (B. Farmani), filippo.maggi@unicam.it (F. Maggi), morshedloo@ut.ac.ir (M.R. Morshedloo).

https://doi.org/10.1016/j.scienta.2018.06.048
Received 4 May 2018; Received in revised form 6 June 2018; Accepted 13 June 2018

2017; Yi et al., 2010; Yi et al., 2008; Aghdam et al., 2018).

β-Aminobutyric acid (BABA) is a four-carbon non-proteinogenic amino acid acting as a safe priming molecule able to confer postharvest fungal decay tolerance in fruits and vegetables (Yan et al., 2015). Recently, Wang et al. (2016) pointed out that the BABA treatment at 10 mM conferred tolerance to postharvest gray mold rot caused by *Botrytis cinerea* in strawberry fruit. BABA treatment employed higher chitinase, β-1,3-glucanase and PAL enzymes activity along with higher *FaPR1*, *FaChi3*, *Faβglu*, and *FaPAL* genes expression. Also, BABA treatment at 10 mM displayed direct antifungal activity through spore's membrane disintegration and leakage of soluble proteins and carbohydrates (Wang et al., 2016).

The aim of this work was to explore the mechanisms employed by BABA treatment to confer postharvest decay tolerance in strawberry fruit stored at 4 °C for 12 days by assessing cellular energy providing enzymes activity, ROS scavenging enzymes activity, membrane degrading enzymes activity along with PAL enzyme activity responsible for phenols and anthocyanins accumulation.

2. Materials and methods

2.1. Fruit treatment with BABA

Strawberry fruit (*Fragaria* × *anannasa* cv. Selva), at commercial ripeness (> 75% of the surface showing red color), were harvested from a commercial production greenhouse in Qazvin (Iran) and transported to the postharvest laboratory at Imam Khomeini International University. Fruit were selected for size and uniformity of color and fruit with injuries, disease or infections were discharged. For postharvest treatment, 1080 fruit were selected and divided into 6 lots of 180 fruit (60 fruit examined in triplicate for each lot) which were soaked in a BABA solution at 0 (control), 1, 5, 10, 25 and 50 mM at 20 °C for 5 min and then air-dried at room temperature for 2 h. Sixty fruit for each replicate were put in plastic jars and stored at 4 ± 0.5 °C with 90–95% RH for 12 days. Fruit were taken out of storage after 4, 8 and 12 days and cellular energy providing enzymes activity, membrane degrading enzymes PLD and LOX activity, antioxidant enzymes activity and PAL enzyme activity responsible for phenols and anthocyanins accumulation and their contribution to conferring fruit decay tolerance was evaluated during a shelf life of 2 days at 20 ± 0.5 °C.

2.2. Fruit decay evaluation

Every 4 days of storage at 4 ± 0.5 °C followed by 2 days of shelf life at 20 ± 0.5 °C, the percentages of decayed strawberry fruit were recorded. Strawberry fruit showing surface mycelia development were considered decayed (Romanazzi et al., 2002).

2.3. Fruit malondialdehyde (MDA) assay

MDA content was measured by thiobarbituric acid (TBA) method according to Hodges et al. (1999). Fruit tissue (2 g) was homogenized with 25 mL of 5% (w/v) trichloroacetic acid (TCA). After centrifugation for 10 min at 10,000 ×g, TBA reactivity was assayed by adding 2.5 mL of 0.5% TBA in 15% TCA to 1.5 mL of supernatant. The reaction solution was held for 30 min in boiling water, then cooled quickly and finally centrifuged at 12,000 ×g for 10 min to clarify the solution. Absorbance was measured at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm, calculated with an extinction coefficient of 1.55 nmol $L^{-1}m^{-1}$. MDA content was expressed as nmol g^{-1} fresh weight (FW).

2.4. Fruit energy metabolism enzymes assay

Fruit mitochondria were extracted according to Zhou et al., (2014). Fruit tissue (10 g) was ground with 50 mL of 50 mM Tris–HCl buffer (pH 7.5), containing 0.25 M sucrose, 0.3 M mannitol, 1 mM EDTA and 0.5 g L^{-1} PVP at 4 °C. The extracts were then homogenized and centrifuged at 4000 ×g for 10 min at 4 °C. The supernatants were pooled and centrifuged at 20,000 ×g for 20 min at 4 °C. After washing twice with washing buffer (10 mM Tris–HCl buffer, containing 0.25 M sucrose, 0.3 M mannitol and 1 mM EDTA), the final precipitate was dissolved with washing buffer for gaining mitochondria.

H^+-ATPase and Ca^{2+}-ATPase enzymes activity were assayed by measuring the inorganic phosphorus liberated after catalytic hydrolysis of ATP to ADP. For H^+-ATPase activity, 3 mL of reaction mixture containing 0.3 mL of mitochondria, 30 mM Tris–HCl buffer (pH 8.0), 3 mM magnesium sulfate, 0.1 mM sodium orthovanadate, 50 mM sodium nitrate, and 0.1 mM ammonium molybdate. For Ca^{2+}-ATPase activity, 3 mL of reaction mixture containing 0.3 mL of mitochondria, 30 mM Tris–HCl buffer (pH 8.0), 3 mM magnesium sulfate, 0.1 mM sodium orthovanadate, 50 mM sodium nitrate, 3 mM calcium nitrate and 0.1 mM ammonium molybdate. The reaction was initiated by the addition of 100 µL of 30 mM ATP-Tris (pH 8.0) and stopped with 30 mM TCA after 20 min of incubation at 37 °C. One unit of H^+-ATPase and Ca^{2+}-ATPase enzymes activity were expressed as the release of 1 mmol of phosphorus per second (Jin et al., 2013). CCO activity was assayed according to Jin et al. (2013). The reaction was carried out at 37 °C for 3 min in a reaction mixture containing 0.2 mL of mitochondria, 0.2 mL of 0.3 mM cytochrome *c* solution, and 20 mM dimethyl phenylenediamine. One unit of CCO activity was defined as an increase of 0.01 in absorbance per second. SDH activity was measured according to Acevedo et al. (2013). The reaction was carried out at 30 °C for 5 min in a reaction mixture containing 0.3 mL of mitochondria, 3 mL of 0.2 mM potassium phosphate buffer (pH 7.4), 1 mL of 0.2 mM sodium succinate, 0.1 mL of 1 mM di(p-chlorophenyl) methyl carbinol, and 0.1 mL of 10 mM methyl sulfanyl phenazine. One unit of SDH activity was defined as an increase of 0.01 in absorbance per second. Protein content was determined according to Bradford (1976), using BSA as a standard. H^+-ATPase, Ca^{2+}-ATPase, CCO and SDH enzymes activity was expressed as U mg^{-1} protein.

2.5. Fruit energy status

ATP, ADP, and AMP contents were assayed according to Yi et al. (2008). Fruit tissue (2 g) was homogenized with 6 ml of 0.6 M perchloric acid. After centrifugation at 16,000 ×g for 15 min at 4 °C, the supernatant (3 ml) was quickly neutralized to pH 6.5–6.8 using 1 M KOH, diluted to 4 ml and passed through a 0.45 µm filter. ATP, ADP, and AMP were analyzed by HPLC with a 4.6 mm × 250 mm C18 column and a UV detector at 254 nm. Mobile phase A consisted of 0.06 M K_2HPO_4 and 0.04 M KH_2PO_4 dissolved in deionized water and adjusted to pH 7.0 with 0.1 M KOH. Mobile phase B was acetonitrile. ATP, ADP, and AMP contents were expressed as µg g^{-1} FW. Adenylate energy charge (EC) was calculated by [ATP + 1/2 ADP] / [ATP + ADP + AMP] (Yi et al., 2008).

2.6. Fruit membrane degrading PLD and LOX enzymes activity

PLD enzyme activity was assayed by choline Reinecke salt precipitation method described by Liu et al. (2011). Fruit tissue (1 g) was homogenized in 10 mL of 50 mM Tris–HCl (pH 8.0) containing 10 mM KCl, 200 mM sucrose, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 2% PVP (w/v). After centrifuging at 10,000 ×g for 30 min at 4 °C, the supernatant was used for PLD activity assay. By using 1,3-phosphatidylcholine, the substrate emulsion was prepared. The reaction mixture contained 1 mL of supernatant and 3 mL of 0.4 mg/mL substrate. The control contained 1 mL of 0.1 M sodium acetate buffer and 3 mL of 0.4 mg/mL substrate. The enzymatic reaction was performed for 1 h at 28 °C. One unit of PLD activity was defined as the enzyme that caused 0.1 of the absorbance change per h. The method of Lin et al. (2016) was employed for assessing the LOX enzyme activity.