 doi:10.20944/preprints202210.0283.v1

# Exogenous γ-Aminobutyric acid (GABA) treatment suppresses ethylene biosynthesis in hardy kiwifruit (*Actinidia aruguta*) during postharvest storage

**Haiqiang Dong[1,3], Qingqing Lan[1], Yuwen Zhou[1], Yufei Zhou[1], Zeyang Bao[1], Qingde Wen[1], Chongjian Xiang[1], Caixia Zheng[1,2*], Xu Li[1*]**

[1]College of Biological and Environmental Sciences, Zhejiang Wanli University, Ningbo 315100, China

[2]College of Agriculture and Bioengineering, Heze University, Heze 274000, China

[3]Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University, Wuhan 430070, China

*Corresponding authors: ybulixu@163.com; lixu@zwu.edu.cn

**Abstract**: γ-Aminobutyric acid (GABA) plays an important role in plant development and postharvest properties of fruits. However, studies on regulation of kiwifruit ripening by GABA are scarce. Here, we evaluted physicochemical characters and the expression profile of genes related to ripening in hardy kiwifruit treated with exogenous GABA compared to control during postharvest storage period. The results showed that (1) exogenous GABA treatment not only improved the fruit quality but also inhibited respiration rate and ethylene production, as well as reduced the enzyme activities of ACC oxidase (ACO) and ACC synthase (ACS), via down-regulating the expression of *AaACO1* and *AaACO3*, *AaACS1* and *AaACS2* during kiwifruit storage period; (2) on the one hand, overexpression of *AaGAD1* or *AaGAD4* gene which is involved in GABA biosynthesis inhibited ethylene production by reducing the enzyme activities of ACO and ACS, whereas silencing of *AaGAD1* or *AaGAD4* gene led to the reverse effect. On the other hand, overexpression of *AaGAD1* or *AaGAD4* decreased the expression levels of *AaACO1* and *AaACO3*, *AaACS1* and *AaACS2*, whereas silencing of *AaGAD1* or *AaGAD4* gene increased the expression levels of these four genes. Above all, our findings demonstrate that exogenous GABA treatment could improve the storage quality and extend the shelf life of kiwifruit, Additionally, the content of GABA was participated in the regulation of ethylene biosynthesis at the

 doi:10.20944/preprints202210.0283.v1

molecular level.

**Keywords：** GABA; hardy kiwifruit; respiration rate; ethylene; storage

## 1. Introduction

Hardy kiwifruit (*Actinidia arguta*) belongs to the family Actinidiaceae and is also called kiwiberry or mini kiwi. It is gaining growing popularity as a domesticated fruit species with increasing commercial production worldwide, and is widely distributed in China, Italy, New Zealand and Greece [1-4]. It has a unique appealing flavor and smooth hairless fruit surface, and unique characteristics make it to be popular with the public [5]. More importantly, it is also a berry fruit with high nutritional values, containing a lot of vitamin C, lutein, phenolics and some minerals, especially P, Ca, Fe and Zn [6-8].

Although hardy kiwifruit is well-known and deep enjoyed by consumers owing to it has smooth, edible skins, it still faces tough challenges in storage conditions as the same as else kiwifruit species. As fruits would be too soft to package and ship when ripened, they are usually picked when physiologically mature and firm [9]. However, the fatal industrial problem of hardy kiwifruit is the rapid softening of fruit after harvest, which seriously restricts large-scale industrial production [1]. ‘Hayward’ can be stored under refrigeration (0°C, 90-95% relative humidity) for 4-6 months with good quality at the end of storage period [10-11], whereas the shelf life of hardy kiwifruit is only 1-2 months in this manner [9]. Consequently, hardy kiwifruit tends to be in short supply on the market. Thus, it is of great significance to search for effective ways to extend the storage freshness period of hardy kiwifruit.

Kiwifruit is typical climacteric fruit, and produces autocatalytic ethylene during ripening [12]. A few days later when kiwifruit are harvested, there is a quick increase in its respiration rate, which exhibits the ripening process; meanwhile, the release of ethylene induces rapid flesh softening and deterioration at a faster rate, and narrows the shelf life of kiwifruit [13]. For decades, researchers have taken many measures to extend storage life of kiwifruit by suppressing ethylene production and respiration rate. Previous study showed that exogenous 1-MCP that is a cyclic olefin which inhibits the action of ethylene suppressed ethylene, delayed softening and decreased soluble solid content (SSC) in ‘Hayward’ kiwifruit [14]. Similarly, Lim et al. [15]

 doi:10.20944/preprints202210.0283.v1

reported that exogenous 1-MCP treatments retarded the ripening process, inhibited the action of ethylene, decreased respiration rate, and extended storage life of hardy kiwifruit. Melatonin treatment also retarded the fruit softening by inhibiting respiration rate and ethylene release in kiwifruit during the later storage. In addition, edible coatings could retard ethylene release and inhibit respiration rate for preventing discoloration and softening [16].

GABA is mainly metabolized via a short pathway called GABA shunt because it bypasses two steps of tricarboxylic acid (TCA) cycle [17]. It is firstly biosynthesized through the consumption of proton and glutamate, which is irreversibly catalyzed by glutamate decarboxylase (GAD) [18]. GABA is one of the main signaling molecules, which influences diverse aspects of both preharvest and postharvest physiology of many horticultural products [19]. It plays crucial role in plant growth and development, such as cells osmoregulation, cell nitrogenous providing, and free radical scavenging especially in biotic and abiotic stress condition [19-20]. A few reports have stated that exogenous GABA treatment could improve storage quality and extend the shelf life of several kinds of fruits [21-24]. However, little information is available about the effect of exogenous GABA treatment as a factor that affects storage life and quality in kiwifruit.

In this study, we are trying to utilize exogenous GABA to extend storage life of kiwifruit by inhibiting respiration rate and ethylene release. We found via multiple tests that the application of exogenous GABA treatment is a safe approach to prolong storage life due to suppression of respiration rate and ethylene release, as well as enhancing nutritional values of postharvest kiwifruit fruit, which could be applied for commercial purposes.

## 2. Materials and Methods

### *2.1. Plant Materials and Treatments*

Fruits of hardy kiwifruit (*Actinidia aruguta* ‘Longcheng No.2’) were harvested from an orchard in Dandong, Liaoning, China, at commercial maturity (~80% mature, according to the growers’ recommendations). A total of 180 fruits were selected for the treatment experiment. The fruits of uniform size, smooth surface, and free of disease and physical damage were criteria in treatment experiment. One group (90

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 October 2022 doi:10.20944/preprints202210.0283.v1

fruits) was immersed in 0, 5, 10, 20 mM GABA (Solarbio Life Sciences, Beijing, China) for 15 min, and the other group (90 fruits) was immersed in distilled water for 15 min as control. Storage in an incubator at 20 ± 0.1°C for 20 days at 85-90% relative humidity. Samples were taken at 0, 5, 10, 15 and 20 days after storage and three biological replicates were evaluated at each time point. Fruit hardness, SSC, titratable acid (TA), respiration rate, and ethylene release were determined at each sampling time. Samples for gene expression analysis, ACO, ACS enzyme activity, and ascorbic acid (VC) content were stored in the ultralow temperature of -80°C for subsequent unified determination.

*2.2. Determination of SSC, and TA*

SSC was determined with a digital refractometer (Atago, Japan). For TA assay, 10 mL of kiwifruit flesh juice was titrated with 0.1 N NaOH to pH 8.0 and the result was expressed as percentage of tartaric acid [24].

*2.3. Measurement of VC and Firmness*

The kiwifruit samples (5 g) were dissolved in 10 mL acetonitrile containing 0.01 mL $L^{-1}$ $KH_2PO_4$ (5:95, pH 2.7) buffer, homogenized with this buffer up to a volume of 25 mL and centrifuged for 10 min at 12,000 × g under refrigerator temperature (4°C). The upper phase was diluted 1:5 with extraction buffer and passed through a 0.22-μm filter for water solvents (Michigan, USA). Liquid chromatography (LC) analysis was done by injection of filtered samples (10 μl) to a WondaSil C18 column (4.6 × 250 mm i.d., 5 μm). The LC system was equipped with a LC-15C series pumping system (Shimadzu) with an auto-sampler (SIL-10AF) and a SPD-15C series UV double wavelength detector (Shimadzu). Ultrapure acetonitrile (5%) and 0.01 mol $L^{-1}$ potassium dihydrogen phosphate (95%, pH 2.7) with a flow rate of 0.8 mL/min were used as solvents (mobile phase). VC was detected at 243 nm [25].

Whole fruit firmness (flesh rupture force) was determined with an Instron Universal Testing Machine (Model 4442, Canton, MA). Each fruit was placed on a stationary steel plate. On removal of the skin, two spots located on opposite sides of the fruit were punctured to a depth of 10 mm. Puncture tests involved the use of a 7.9 mm probe on a drill base with a crosshead setting of 50 mm $min^{-1}$. Ten individual fruits (two measurements per fruit; $n$ = 20) per treatment and date were evaluated

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 October 2022 doi:10.20944/preprints202210.0283.v1

[14].

*2.4. Measurement of Ethylene Production and Respiration Rate*

Ethylene production and respiration rate were measured according to the method of Han et al. [26] with some modifications. Ten fruits were enclosed in a 3.6-L airtight vessel for 1 h at 25 °C, and a 1-mL gas sample was collected with a syringe. Then, the gas sample was injected into a GC-14A flame ionization detector gas chromatograph (Shimadzu, Kyoto, Japan) to determine ethylene concentration. The ethylene production rate was expressed as μL $kg^{-1}$ $h^{-1}$. The respiration rate was measured using a $CO_2$ infrared gas analyzer (TEL7001; GE Telaire) and expressed as mL of $CO_2$ $kg^{-1}$ $h^{-1}$. Three biological replicates were used for each sample.

*2.5. qRT-PCR RNA Isolation and Transcript Analyses*

Total RNA was extracted using the modified CTAB method [12]. First-strand cDNA was synthesized using a Quantitect reverse transcription kit (Qiagen). Quantitative reverse transcriptase PCR (qRT-PCR) to determine expression level was performed using a LightCycler 480 (Roche), and the $2^{-\Delta\Delta CT}$ method for relative quantification [27]. The primers used for qRT-PCR are shown in Supplementary Table S1. The expression was normalized by internal control *Actin* (Genbank no. EF063572) [28].

*2.6. Extraction and Determination of GABA*

GABA was extracted as previously reported with some modifications [29]. Frozen flesh (500 mg) was ground in 1.0 mL of 20 mM HCl. The mixture was ultrasonically extracted at 80 W for 15 min and then centrifuged at 13000 × g for 10 min. The supernatant was passed through a 0.2-μm syringe filter into an Eppendorf tube. The detection was performed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) according to the method of Zhao et al. [30]. The MS/MS detection was carried out using an API 5500 Q-TRAP tandem MS instrument (AB SCIEX, Framingham, MA, U.S.A.). The results obtained are represented as mg/g FW.

*2.7. Phylogenetic Analysis*

Phylogenetic analysis was conducted using the MEGA 7.0 software. The tree was compiled with the Neighbor-Joining method. Statistical reliability of the internal branches was assessed using 1000 bootstrap replicates. Values at the nodes indicated

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 October 2022 doi:10.20944/preprints202210.0283.v1

the bootstrap percentages [31].

*2.8. Transient Overexpression and Silencing of AaGAD1 and AaGAD4 in Kiwifruit*

The full-length cDNA of *AaGAD1* and *AaGAD4* was cloned into the pRI101-AN by digestion with *Kpn1* and *BamHI*. The used primers are listed in Table S1. The 200-300 bp sequences selected of the full-length cDNA of *AaGAD1* and *AaGAD4* was cloned into the pTRV1+pTRV2 by digestion with *EcoR1* and *BamHI*. The used primers are listed in Table S1. The overexpression vectors *35S:AaGAD1* and *35S:AaGAD4*, as well as the silencing vectors *pTRV1+pTRV2-AaGAD1* and *pTRV1+pTRV2-AaGAD4* were transferred into *Agrobacterium tumefaciens* GV3101. Two empty vectors (pRI101 and pTRV1+pTRV2) were used as overexpression and silencing control, respectively. Fruit samples that were of similar size and free from physical injuries at 7 days before harvest were used for a transient expression assay, according to a previous study [32]. Each treatment contained 3 biological replicates of approximately 20 fruits. Then, the overexpression and silencing fruits were sampled for subsequent expression analysis and a series of physicochemical indexes measurement.

*2.9. ACS and ACO Activities*

The methods for ACS and ACO measurement were modified from those of Fan et al. [33] and Barry et al. [34], which were described in previous report [35]. Approximately 3.5 g and 2 g fruit flesh were used for ACS and ACO measurements, respectively. ACS and ACO activities were analyzed by measuring ethylene converted from the substrates. Ethylene measurement was carried out using a gas chromatograph, model SP 6800 (Lunan Chemical Engineering Instrument, Shandong, China), according to the above-mentioned description. All of the measurements were performed with 3 biological replicates.

*2.10. Statistical Analysis*

A completely randomized design was used in the experiments. Statistical analyses were performed using SAS (version 8.2, SAS Institute, Cary, NC, USA). The qRT-PCR results were analyzed using the $2^{-\Delta\Delta CT}$ method. Figures were prepared using OriginPro 9.0 (Microcal Software Inc., Northampton, MA, USA) and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Differences between means

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 October 2022 doi:10.20944/preprints202210.0283.v1

were evaluated with Student's t-test. ANOVA with Duncan's multiple range test was used to determine significant differences among multiple data sets.

## 3. Results

### *3.1. Effects of GABA Treatment on Fruit Quality during the Storage Period*

Changes in VC and firmness of GABA-treated kiwifruits and untreated control during storage are shown in Figure 1C-D. The changes in VC increased initially and then decreased during the storage period, and the 10 mM or 20 mM GABA treatment significantly maintained higher VC accumulation compared with the control. As shown in Figure 1D, the firmness decreased substantially in both GABA-treated and untreated fruits during the storage period. However, significantly higher firmness was observed in GABA-treated fruits and the inhibition of firmness decreased particularly at the 10 mM concentration of exogenous GABA.

Changes in SSC and TA of GABA-treated kiwifruits and untreated control during the storage period are shown in Figure 1A-B. The SSC increased constantly whereasTA decreased gradually until the end of the storage period in both treatment group and control group. As expected, 10 mM GABA treatment maintained higher content levels of SSC during the storage period. Taken together, these results showed that the 10 mM GABA treatment is beneficial for fruit quality during the storage period.

### *3.2. Effects of GABA Treatment on Fruit Ripening during the Storage Period*

To elucidate the role of GABA in fruit ripening, we first measured the endogenous GABA content of kiwifruit during development, The data showed that endogenous GABA content gradually decreased over the course of development (Figure S1A). Fruit samples were harvested every 20 d from 40 to 120 d after full bloom (DAFB), where 120 DAFB is the day of commercial harvest. We identified four homologous sequences, namely *AaGAD1*, *AaGAD2/5*, *AaGAD3*, and *AaGAD4*. To assess the extent of similarity of the four putative AaGAD amino acid sequences with those of *Arabidopsis thaliana*, we constructed a phylogenetic tree of amino acid sequences of the GAD family using MEGA 7.0. The results showed that four AaGAD amino acid sequences can be categorized into two groups. In the first group, AaGAD1, AaGAD3 and AaGAD4 were similar to AtGAD1; in the second group, AaGAD2/5 were similar

 doi:10.20944/preprints202210.0283.v1

to AtGAD2 and AtGAD5 (Figure S1B). In addition, four *AaGAD* genes (the rate-limiting enzyme for biosynthesis of GABA in kiwifruit) expression levels were consistent with the dynamic change of endogenous GABA content during fruit development, gradually decreased (Figure S1C). The relative expression levels of *AaGAD1* and *AaGAD4* were significantly higher than those of *AaGAD2/5* and *AaGAD3* during fruit development, so we speculated that either *AaGAD1* or *AaGAD4* gene is a key enzyme for GABA biosynthesis in kiwifruit. The decline of endogenous GABA during fruit development indicated that GABA might be a suppressor of fruit ripening.

To test this hypothesis, exogenous GABA treatment was performed for investigating the ethylene production and respiration rate that sign the most important indicator of climacteric fruit ripening during the storage period. Based on the above results, 10 mM GABA was used for further study. The results revealed that the ethylene production exhibited typical patterns of climacteric fruit where the peaks of ethylene production occurred at 15 days of storage in both control and GABA-treated fruits (Figure 2A). GABA treatments significantly inhibited ethylene production compared with untreated control fruits during the storage period (Figure 2A). Similarly, the GABA treatment led to a significantly lower respiration rate, especially during the climacteric peaks in parallel with the effects on ethylene production (Figure 2B). We also measured the activities of ACO and ACS that are important material basis for ethylene synthesis, and GABA treatments significantly inhibited the enzyme activity of ACO and ACS compared with untreated control fruits during the storage period (Figure 2C-D). Taken together, these results showed that exogenous GABA treatment inhibited ethylene release, thereby affecting fruit ripening.

*3.3. Ethylene Production was Inhibited by the Biosynthesis of Endogenous GABA that was Catalyzed via AaGAD1 or AaGAD4*

As *AaGAD1* or *AaGAD4* gene is likely key enzyme for GABA biosynthesis in kiwifruit (Figure S1C), we cloned the two genes and constructed expression vectors including the overexpression and gene silencing. The *AaGAD1* or *AaGAD4* expression was higher in overexpression fruits and lower in gene silencing fruits than untreated control by transient transformation (Figure 3A-B). Similarly, endogenous

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 October 2022 doi:10.20944/preprints202210.0283.v1

GABA was increased in *35S:AaGAD1* or *35S:AaGAD4* whereas decreased in *pTRV2-AaGAD1* or *pTRV2: AaGAD4* compared to the control (Figure 3C-D). In contrast, the ethylene production decreased in *35S: AaGAD1* or *35S: AaGAD4* and increased in *pTRV2-AaGAD1* or *pTRV2: AaGAD4* compared to the control (Figure 3E-F).

We also measured the activities of ACO and ACS that are important material basis for ethylene biosynthesis. Contary to the endogenous GABA, the activities of ACO and ACS were decreased in *35S: AaGAD1* or *35S: AaGAD4* and increased in *pTRV2-AaGAD1* or *pTRV2: AaGAD4* compared to the control (Figure 3G-J). In conclusion, these results indicated that the *AaGAD1* or *AaGAD4* is the key gene for endogenous GABA biosynthesis which is a suppressor of ethylene biosynthesis.

*3.4. Effects of Exogenous GABA Treatment on the Expression Patterns of Genes Related to Ethylene Biosynthesis*

To elucidate the internal relationship between exogenous GABA and ethylene biosynthesis during fruit ripening, we measured the expression of *AaCO1* and *AaACO3, AaACS1* and *AaACS2* [36-37]. As anticipated, the transcript levels of *AaACO1* and *AaACO3, AaACS1* and *AaACS2* were suppressed by GABA treatment during storage (Figure 4A-D). In contrast, the expression of *AaACO1*, *AaACO3* and *AaACS1*, *AaACS2* were decreased in *35S: AaGAD1* or *35S: AaGAD4* and increased in *pTRV2-AaGAD1* or *pTRV2: AaGAD4* compared to the control (Figure 4E-L). These results suggested that exogenous GABA participated in the inhibition of ethylene biosynthesis, thus maintaining the fruit quality during storage.

**4. Discussion and Conclusion**

GABA plays a crucial role in plant growth and development, especially in response to a series of abiotic and biotic stresses, including pathogen infection, hypoxia, drought, wound, cold and heat stress [20]. Recent studies have also confirmed that the GABA pathway plays an important role in citrate metabolism and the application of exogenous GABA contributes to an increase in the accumulation of citrate content and improves storage quality [22-23].

In this study, we found that exogenous GABA treatment was benefit to improve storage quality of fruit in hardly kiwifruit during postharvest storage. Although

 doi:10.20944/preprints202210.0283.v1

Changes in TA among 0 mM, 5 mM, 10 mM and 20 mM concentrations of GABA were not significant compared with the control during postharvest storage, we found that exogenous GABA treatment of 10 mM significantly increased content level of SSC, maintained VC accumulation, and decreased the decline of firmness compared with the control during postharvest storage (Figure 1). Therefore, GABA treatment of 10 mM significantly improved storage quality of fruit in hardy kiwifruit during postharvest storage. Except the reports on citrus, Shang et al. also found that exogenous GABA treatment could enhance storage quality of peach fruit via accumulating proline [21-23]. In addition, postharvest application of GABA also maintained internal quality of 'Sahebi' grape by increasing soluble sugars and organic acids [24]. Our results are consistent with previous findings in citrus, peach and grape, thereby exogenous GABA treatment could improve the storage quality and extend the shelf life of kiwifruit by increasing content level of SSC, maintaining VC accumulation, and decreasing the decline of firmness.

In general, GABA is an important metabolic material in TCAcycle, and mainly metabolized via a short pathway called GABA shunt [17]. Except a major influence on TCA cycle, our study revealed that exogenous GABA treatment exerted an important effect on the respiratory rate (Figure 2B). Previous study found that the content of GABA was generally higher in high-acid fruits than in low-acid fruits, while the respiration rate was significantly lower in high-acid fruits than in low-acid fruits during postharvest storage in citrus [23]. Similarly, another study indirectly showed that exogenous GABA treatment led to decline of the respiration rate, which resulted in reduction of the consumption of citrate and important amino acids in citrus [22-23]. Han et al. found that exogenous GABA treatment led to a significantly lower respiration rate compared with untreated control in apples [38]. Thereby, we generally concluded that the content of GABA was negatively correlated with the respiration rate.

In our study, exogenous GABA treatment not only inhibited the respiration rate but also significantly reduced the production of ethylene (Figure 2A), as well as the activities of ACO and ACS were also obviously lower in exogenous GABA treatment than untreated control (Figure 2C-D). *AaGAD1* or *AaGAD4* gene is likely key enzyme

 doi:10.20944/preprints202210.0283.v1

for GABA biosynthesis in kiwifruit, as mentioned above. On the one hand, overexpression of *AaGAD1* or *AaGAD4* gene inhibited ethylene production (Figure 3E-F) by reducing the activities of ACO and ACS (Figure 3G-J), whereas silencing of *AaGAD1* or *AaGAD4* gene led to the reverse effect (Figure 3 E-J). on the other hand, overexpression of *AaGAD1* or *AaGAD4* decreased the expression levels of *AaACO1* and *AaACO3*, *AaACS1* and *AaCS2* (Figure 4E-L), whereas silencing of *AaGAD1* or *AaGAD4* gene increased the expression levels of these four genes (Figure 4E-L). The above results suggested that in our study GABA participated in the regulation of ethylene biosynthesis and signal transduction at the molecular level. Although the potential relationship between GABA and ethylene synthetic pathways in fruit has not been directly described yet, it is not perfectly absent in plants. In fact, Han et al. also found that exogenous GABA treatment obviously inhibited the production of ethylene, and decreased the expression levels of *MdACS*, *MdACO* and *MdERF* that were key genes for ethylene biosynthesis and signal transduction compared with the control in apples during storage [38]. However, GABA enhanced ethylene production by inducing increases in ACS mRNA accumulation, ACC levels, ACO mRNA levels, and in vitro ACO activity in the cotyledons and leaves of sunflower [39]. Similarly, in poplar woody plant, exogenous GABA induced the crucial genes related to ethylene production and abscisic acid (ABA) biosynthesis in response to salt stress, suggesting that exogenous GABA promoted ethylene production and ABA biosynthesis [40]. Thereby, we concluded that interactions between GABA and ethylene biosynthesis may differ among plant species, and certainly confirmed that GABA participated in the regulation of ethylene biosynthesis and signal transduction at the molecular level.

In summary, our study demonstrated the following: (1) exogenous GABA treatment could improve the storage quality and extend the shelf life of kiwifruit; (2) the content of GABA was negatively correlated with the respiration rate; and (3) GABA participated in the regulation of ethylene biosynthesis and signal transduction at the molecular level. Therefore, the use of exogenous GABA treatment is a safe approach to prolong storage life and enhance nutritional values of postharvest kiwifruit fruit, which could be applied for commercial purposes.

**Declaration of Competing Interests**

The authors state that they have no conflicts of interest or personal ties that could have affected the research reported herein.

## Acknowledgments

This work was supported by the China Postdoctoral Science Foundation (Grant 2022M713004), the National Natural Science Foundation of China (Grant 32202443), Zhejiang Provincial Top Discipline of Biological Engineering (Level A) (Grant ZS2021003 and KF2022008) and Ningbo Public Welfare Fund Project (Grant 20211JCGY020055).

## References

1. Latocha, P.; Jankowski, P.; Radzanowska, J. Genotypic difference in postharvest characteristics of hardy kiwifruit (Actinidia arguta and its hybrids), as a new commercial crop Part I. Sensory profiling and physicochemical differences. *Food Res Int*. **2011**, *44*, 1936-1945.
2. Latocha, P.; Krupa, T.; Jankowski, P.; Radzanowska, J. Changes in postharvest physicochemical and sensory characteristics of hardy kiwifruit (*Actinidia arguta* and its hybrid) after cold storage under normal versus controlled atmosphere. *Postharvest Biol Tec*. **2014**, *88*, 21-33.
3. Wang, Y.H.; Xu, F.X.; Feng, X.Q.; MacArthur, R.L. Modulation of *Actinidia arguta* fruit ripening by three ethylene biosynthesis inhibitors. *Food Chem*. **2015**, *173*, 405-413.
4. Garcia, C.V.; Stevenson, R.J.; Atkinson, R.G.; Winz, R.A.; Quek, S.Y. Changes in the bound aroma profiles of 'Hayward' and 'Hort16A' kiwifruit (*Actinidia* spp.) during ripening and GC-olfactometry analysis. *Food Chem*. **2013**, *137*, 45-54.
5. Zhu, R.G.; Zhang, X.Y.; Wang, Y.; Zhang, L.J.; Zhao, J.; Chen, G.; Fan, J.G.; Jia, Y.F.; Yan, F.W.; Ning, C. Characterization of polysaccharide fractions from fruit of *Actinidia arguta* and assessment of their antioxidant and antiglycated activities. *Carbohyd Polym*. **2019**, *210*, 73-84.
6. Nishiyama, I.; Yamashita, Y.; Yamanaka, M.; Shimohashi, A.; Fukuda, T.; Oota, T. Varietal difference in vitamin C content in the fruit of kiwifruit and other Actinidia species. *J. Agric. Food. Chem*. **2004**, *52*, 5472-5475.
7. Fisk, C.L.; McDaniel, M.R.; Strik, B.C.; Zhao, Y. Physicochemical, sensory, and nutritive qualities of hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') as affected by harvest maturity

 doi:10.20944/preprints202210.0283.v1

and storage. *J. Food. Sci*. **2006**, *71*, 204-210.

8. Song, M.T.; Xu, H.R.; Xin, G.; Liu, C.J.; Sun, X.R.; Zhi, Y.H.; Li, B.; Shen, Y.X. Comprehensive evaluation of *Actinidia arguta* fruit based on the nutrition and taste: 67 germplasm native to Northeast China. *Food Sci Hum Well*. **2022**, *11*, 393-404.
9. Strik, B.; Hummer, K. 'Ananasnaya' hardy kiwifruit. *J. Am. Pom. Soc*. **2006**, *60*, 106–112.
10. McDonald, B. Precooling, storage, and transport of kiwifruit. In: Warrington, I.J., Weston, G.C. (Eds.), Kiwifruit: Science and Management. *Ray Richards Publisher, Auckland, NZ* **1990**, pp, 429-459.
11. Cheah, L.H.; Irving, D.E. Kiwifruit. In: Mitra, S.K. (Ed.), Postharvest Physiology and Storage of Tropical and Subtropical Fruits. *CAB International, New York* **1997**, pp, 209-227.
12. Cheng, J.; Zheng, A.; Li, H.; Huan, C.; Jiang, T.; Shen, S.; Zheng, X. Effects of melatonin treatment on ethanol fermenation and ERF expression in kiwifruit cv. Bruno during postharvest. *Sci Hortic*. **2022**, *293*,110696.
13. Sharma, R.R.; Pal, R.K.; Rana, V. Effect of heat shrinkable films on storability of kiwifruits under ambient conditions. *Indian J. Hortic*. **2012**, *69*, 404-408.
14. Boquete, E.J.; Trinchero, G.D.; Fraschina, A.A.; Vilella, F.; Sozzi, G.O. Ripening of 'Hayward' kiwifruit treated with 1-methylcyclopropene after cold storage. *Postharvest Biol Tec*. **2004**, *32*, 57-65.
15. Lim, S.; Han, S.H.; Kim, J.; Lee, H.J.; Lee, J.G.; Lee, E.J. Inhibition of hardy kiwifruit (*Actinidia aruguta*) ripening by 1-methylcyclopropene during cold storage and anticancer properties of the fruit extract. *Food Chem*. **2016**, *190*, 150-157.
16. Baldwin, E.A.; Nisperos-Carriedo, M.O.; Baker, R.A. Use of edible coatings to preserve quality of light (and slightly) processed products. *Crit Rev. Food. Sci*. **1995**, *35*, 509-524.
17. Bouché, N.; Fromm, H. GABA in plants: Just a metabolite? *Trends Plant Sci*. **2004**, *9*, 110-115.
18. Bown, A.W.; Shelp, B.J. The Metabolism and Functions of γ--aminobutyric Acid. *Plant Physiol*. **1997**, *115*, 1-5.
19. Aghdam, M.S.; Razavi, F.; Karamneghad, F. Maintaining the postharvest nutritional quality of peach fruits by γ-Aminobutyric acid. *Iran J. Plant. Physiol*. **2016**, *5*, 1457- 1463.
20. Shelp, B.J.; Bown, A.W.; Zarei, A. 4-Aminobutyrate (GABA): a metabolite and signal with practical significance. *Botany* **2017**, *95*, 1015-1032.

21. Shang, H.; Cao, S.; Yang, Z.; Cai, Y.; Zheng, Y. Effect of exogenous γ-Aminobutyric acid treatment on proline accumulation and chilling injury in peach fruit after long-term cold storage. *J. Agric. Food. Chem*. **2011**, *59*, 1264-1268.
22. Sheng, L.; Shen, D.; Luo, Y.; Sun, X.; Wang, J.; Luo, T.; Zeng, Y.; Xu, J.; Deng, X.; Cheng, Y. Exogenous gamma-aminobutyric acid treatment affects citrate and amino acid accumulation to improve fruit quality and storage performance of postharvest citrus fruit. *Food Chem*. **2017**, *216*, 138-145.
23. Sheng, L.; Shen, D.; Yang, W.; Zhang, M.; Zeng, Y.; Xu, J.; Deng, X.; Cheng, Y. GABA pathway rate-limit citrate degradation in postharvest citrus fruit evidence from HB pumelo (*Citrus grandis*) x Fairchild (*Citrus reticulata*) hybrid population. *J. Agric. Food. Chem*. **2017**, *65*, 1669-1676.
24. Asgarian, Z.S.; Karimi, R.; Ghabooli, M.; Maleki, M. Biochemical changes and quality characterization of cold-stored 'Sahebi' grape in response to postharvest application of GABA. *Food Chem*. **2022**, *373*, 131401.
25. Zheng, Y.J.; Duan, Y.T.; Zhang, Y.F.; Pan, Q.H.; Li, J.M.; Huang, W.D. Determination of organic acids in red wine and must on only one RP-LC-column directly after sample dilution and filtration. *Chromatographia* **2009**, *69*, 1391-1395.
26. Han, Y.; Zhu, Q.; Zhang, Z.; Meng, K.; Hou, Y.; Ban, Q.; Suo, J.; Rao, J. Analysis of xyloglucan endotransglycosylase/hydrolase (*XTH*) genes and diverse roles of isoenzymes during persimmon fruit development and postharvest softening. *PLoS One* **2015**, *10*, e0123668.
27. Bezrutczyk, M.; Hartwig, T.; Horshman, M.; Char, S.N.; Yang, J.; Yang, B.; Frommer, W.B.; Sosso, D. Impaired phloem loading in *zmsweet13a,b,c* sucrose transporter triple knock-out mutants in *Zea mays*. *New Phytol*. **2018**, *218*, 594-603.
28. Wang, W.; Moss, S.; Zeng, L.; Espley, R.V.; Wang, T.; Wang, K.; Fu, B.; Schwinn, K.E.; Allan, A.C.; Yin, X. 2022. The red-flesh of kiwifruit is differentially controlled by specific activation-repression systems. *New Phytol*. **2022**, *235*, 630-645.
29. Zhang, Y.; Li, P.; Cheng, L. Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp' apple flesh. *Food Chemistry*. **2010**, 123, 1013-1018.
30. Zhao, Y.; Tan, D.X.; Lei, Q.; Chen, H.; Wang, L.; Li, Q.T.; Gao, Y.N.; Kong, J. Melatonin

and its potential biological functions in the fruits of sweet cherry. *J. Pineal. Res*. **2013**, *55*, 79-88.

31. Gan, Z.; Yuan, X.; Shan, N.; Wan, C.; Chen, C.; Zhu, L.; Xu, Y.; Kai, W.; Zhai, X.; Chen, J. AcERF1B and AcERF073 positively regulate indole-3-acetic acid degradation by activating *AcGH3.1* transcription during postharvest Kiwifruit ripening. *J. Agr. Food. Chem*. **2021**, *69*, 13859-13870.
32. Gan, Z.; Fei, L.; Shan, N.; Fu, Y.; Chen, J. Identification and expression analysis of *Gretchen Hagen 3 (GH3)* in kiwifruit (*Actinidia chinensis*) during postharvest process. *Plants* **2019**, *8*, 473.
33. Fan, X.T.; Mattheis, J.P.; Fellman, J.K. A role for jasmonates in climacteric fruit ripening. *Planta* **1998**, *204*, 444-449.
34. Barry, C.S.; Blume, B.; Bouzayen, M.; Cooper, W.; Hamilton, A.J. Grierson, D. Differential expression ofthe 1-aminocyclopropane-1-carboxylate oxidase gene family of tomato. *Plant J*. **1996**, *9*, 525-535.
35. Zhang, Y.; Chen, K.S.; Zhang, S.L.; Ferguson, I. The role of salicylic acid in postharvest ripening of kiwifruit. *Postharvest Biol Tec*. **2003**, *28*, 67-74.
36. Wu, Y.; Liu, X.; Fu, B.; Zhang, Q.; Tong, Y.; Wang, J.; Wang, W.; Grierson, D.; Yin, X. Methyl jasmonate enhances ethylene synthesis in kiwifruit by inducing *NAC* genes that activate *ACS1*. *J. Agric. Food Chem*. **2020**, *68*, 3267-3276.
37. Gan, Z.; Yuan, X.; Shan, N.; Wan, C.; Chen, C.; Xu, Y.; Xu, Q.; Chen, J. AcWRKY40 mediates ethylene biosynthesis during postharvest ripening in kiwifruit. *Plant Sci*. **2021**, *309*, 110948.
38. Han, S.; Nan, Y.; Qu, W.; He, Y.; Ban, Q.; Lv, Y.; Rao, J. Exogenous γ-Aminobutyric acid treatment that contributes to regulation of malate metabolism and ethylene synthesis in apple fruit during storage. *J. Agric. Food. Chem*. **2018**, *66*, 13473-13482.
39. Kathiresan, A.; Tung, P.; Chinnappa, C.C.; Reid, D. M. γ-Aminobutyric acid stimulates ethylene biosynthesis in sunfIower. *Plant Physiol*. **1997**, *115*, 129-135.
40. Ji, J.; Yue, J.; Xie, T.; Chen, W.; Du, C.; Chang, E.; Chen, L.; Jiang, Z.; Shi, S. Roles of γ-aminobutyric acid on salinity-responsive genes at transcriptomic level in poplar involving in abscisic acid and ethylene-signalling pathways. *Planta **2018***, *248*, 675-690.

## Figure Captions

 doi:10.20944/preprints202210.0283.v1

**Figure 1.** Quality changes of hardy kiwifruits treated with different GABA concentrations. Changes in soluble solid content (A), titratable acidity (B), ascorbic acid content (C), and firmness of the control and GABA-treated kiwifruits (at 0, 5.0, 10.0, 15.0 and 20.0 mM) during storage at room temperature. The value measured before treatment was used as the initial value for all treatments at 0 time. Data are the mean ± standard error from three biological replicate assays. Different letters show significant differences ($p < 0.05$) for each sampling date among treatments.

**Figure 2.** Effects of different concentrations of GABA treatments on physicochemical characters in kiwifruit. Ethylene production (A), respiration rate (B), ACC oxidase activity (C), and ACC synthase activity (D) of the control and GABA-treated kiwifruits (at 0, 5.0, 10.0, 15.0 and 20.0 mM) during storage at room temperature. Data are means of three biological replicates ± SE. The different lowercase letters indicate significant differences ($p < 0.05$). Asterisks indicate a significant difference between the treatment and control, according to Duncan's multiple range tests (*$P < 0.05$, **$P < 0.01$).

**Figure 3.** Transient overexpression of *AaGAD1* and *AaGAD4* in kiwifruit. Changes in the transcripts of *AaGAD1* (A) or *AaGAD4* (B) were determined by qRT-PCR in the *AaGAD1* overexpression kiwifruits and silencing kiwifruits or the *AaGAD4* overexpression kiwifruits and silencing kiwifruits. GABA content (C and D), Ethylene production (E and F), ACC oxidase activity (G and H) and ACC synthase activity (I and J) in the *AaGAD1* overexpression kiwifruits or silencing kiwifruits and the *AaGAD4* overexpression kiwifruits or silencing kiwifruits. Data are means of three biological replicates ± SE. Asterisks indicate a significant difference between the treatment and control, according to Duncan's multiple range tests (*$P < 0.05$, **$P < 0.01$).

**Figure 4.** The relative expression levels of genes related to ethylene synthesis in the control and GABA-treated kiwifruits at days after full bloom, and transient overexpression of *AaGAD1* and *AaGAD4* in kiwifruit. Changes in the transcripts of *AaACS1* (A), *AaACS2* (B), *AaACO1* (C) and *AaACO3* (D) were determined by qRT-PCR in the control and GABA-treated kiwifruits (at 10.0 mM) during storage at room temperature. Relative expression levels of *AaACS1* (E), *AaACS2* (F), *AaACO1* (G), and *AaACO3* (H) in the *AaGAD1* overexpression kiwifruits or silencing kiwifruits. Relative expression levels of *AaACS1* (I), *AaACS2* (J), *AaACO1* (K), and *AaACO3* (L) in the *AaGAD4* overexpression kiwifruits or silencing kiwifruits. Data are means of three biological replicates ± SE. Asterisks indicate a significant difference between the treatment and control,

 doi:10.20944/preprints202210.0283.v1

according to Duncan's multiple range tests (*$P < 0.05$, **$P < 0.01$).