AMADH inhibitor optimization and its effects on GABA accumulation in soybean sprouts under NaCl–CaCl₂ treatment

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Abstract

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) was the suitable inhibitor for aminoaldehyde dehydrogenase (AMADH) compared with *N*-ethylmaleimide (NEE) and iodoacetamide (IAM). EDC exhibited the most obvious inhibition effect on AMADH activity, while its inhibition on glutamate decarboxylase (GAD) was insignificant. Compared with the control, AMADH activity reduced by 70.4% with 0.5 mM EDC, and γ -aminobutyric acid (GABA) content declined by 44.3% in soybean sprouts at 4 days of germination. AMADH activity reduced by 80.62, 67.61 and 72.02% in the 4-day sprouts with 1 mM EDC under NaCl, CaCl₂ and NaCl + CaCl₂ treatment, respectively, and GABA content decreased by 43.56, 38.84 and 35.53%, respectively. EDC is a proper inhibitor for AMADH and it could be used to quantify the contribution of polyamine degradation pathway on GABA formation. In soybean sprouts, the presence of CaCl₂ under NaCl stress decreased the contribution of polyamine degradation pathway on GABA accumulation.

Keywords GABA · AMADH · Germination · Soybean sprouts

Introduction

Soybean (*Glycine max* L.) contains an amount of protein, oil and other functional components including γ -aminobutyric acid (GABA) with the function of lowering blood pressure, regulating heart rate, etc. (Ma et al. 2015). In the dried soybean seed, GABA content is low (Abe and Takeya 2005), and cannot satisfy the daily demand. After germination, GABA is accumulated significantly in soybean sprouts (Xu and Hu 2014). Especially under hypoxia (Yang et al. 2016), NaCl (Yin et al. 2014b) and CaCl₂ (Wang et al. 2016) treatment, GABA accumulation in sprouts is activated. There

are two pathways for GABA synthesis including GABA shunt and polyamine degradation pathway in plant. Glutamate decarboxylase (GAD, EC 4.1.1.15) catalyzes the α -decarboxylation of glutamate (Glu) in an irreversible reaction to form GABA directly in GABA shunt. In polyamine degradation pathway, diamine or polyamines including putrescine (Put), spermidine (Spd) and spermine (Spm) are catalyzed by diamine oxidase (DAO, EC 1.4.3.6) or polyamine oxidases (PAO, EC 1.5.3.3) to form γ -aminobutyraldehyde. Then, γ -aminobutyraldehyde is catalyzed by aminoaldehyde dehydrogenase (EC 1.2.1.19, AMADH) to produce GABA (Shelp et al. 2012). Hence, in the polyamine degradation pathway, AMADH is the rate-limiting enzyme for GABA synthesis.

Previous researchers had quantified the contribution of the two pathways to GABA accumulation in germinated soybean (Guo et al. 2012) and fava bean (Yang et al. 2013). They used aminoguanidine (AG) as the inhibitor of DAO to calculate the contribution of the polyamine degradation pathway on GABA accumulation (Xing et al. 2007; Yang et al. 2013). Because DAO is not a specific enzyme for catalyzing polyamines, the inhibition of DAO activity is not appropriate to quantify the contribution of the polyamine degradation pathway on GABA synthesis in plant. Furthermore, DAO and PAO are formed prior to AMADH and they

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are unable to represent the ability of GABA production completely through the polyamine degradation pathway. Lately, a publication showed the results of using aminooxyacetate to inhibit GAD activity to quantify the contribution of GABA shunt on GABA accumulation in fava bean under salt stress (Yin et al. 2018). However, there is an issue of reliability for aminooxyacetate as an inhibitor of ethylene synthesis, as it can inhibit plant growth (Broun and Mayak 1981). Hence, there is no specific inhibitor for AMADH to investigate the contribution of polyamine degradation pathway on GABA synthesis in plant tissues at present.

Publications implied that *N*-ethylmaleimide (NEE) (Patel et al. 1980), iodoacetamide (IAM) (Matsuda and Suzuki 2004) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (Brauner et al. 2003) are the potential inhibitors of AMADH activity in vitro. This study attempted to screen the specific inhibitor from these three chemicals for AMADH via testing its activity, sprouts growth performance and malondialdehyde (MDA) content to quantify the contribution of polyamine degradation pathway on GABA accumulation in plant tissues more accurately. Thereafter, the effect of the inhibitor on GABA accumulation was assessed in soybean sprouts under NaCl, CaCl₂ and NaCl + CaCl₂ treatment.

Materials and reagents

Dry seeds of soybean (cv. Yunhe-NJ) were purchased from Jilin province of northeast China and stored at -20°C prior to use. GABA, NEE (E3876-5G), IAM (424331-5G), EDC (424331-5G), Put, Spd and Spm were purchased from Sigma (St. Louis, MA, USA). Acetone, benzoyl chloride, HClO₄ and phenol were purchased from China Pharmaceutical Group Shanghai Chemical Reagent Company (Shanghai, China). Other reagents were of analytical grade or otherwise specified.

Pretreatment of soybean seeds

Dry soybean seeds were sterilized with 1% (v/v) sodium hypochlorite for 10 min and washed and steeped with distilled water at 30°C for 6 h. The soaked seeds were then placed in a sprouting machine and germinated in a dark incubator at 30°C for 4 days with a culture solution containing different chemicals (0.5 mmol/L of NEE, IAM, and EDC).

Chemical treatments

During soybean germination, 0.5 mmol/L of NEE, IAM and EDC were applied to select the proper inhibitor for AMADH, and distilled water treatment was defined as the control. Different concentrations of EDC (0, 0.5, 1.0, 2.0,

3.0 and 4.0 mmol/L) were applied. Finally, the 1.0 mmol/L of EDC was used to quantify the polyamine degradation pathway on GABA accumulation in soybean sprouts under 60 mmol/L NaCl, 2 mmol/L CaCl₂ and 60 mmol/L NaCl + 2 mmol/L CaCl₂ treatment, respectively. The concentration of NaCl and CaCl₂ was optimized in advance. Soybean sprouts were treated with the solution for 4 days. The culture solution was replaced every 12 h.

Determination of sprout length, and fresh and dry weight

Sprout length was directly measured using a centimeter ruler and expressed as mean $\pm$ SD of at least 20 soybean sprouts. Fresh weight of sprout was determined directly using an electronic scale. For the determination of dry weight, sprout samples were harvested after treatment, then heated at 105°C for 15 min and kept at 55°C until constant weight. After cooling to room temperature, the dry weight was determined.

Determination of MDA content

The extent of lipid peroxidation in terms of MDA formation was measured following the reported method (Wael et al. 2015).

Determination of GABA content, glutamate and free polyamines content

GABA and glutamate were extracted and purified according to the method of Bai et al. (2009) and then analyzed by HPLC (Agilent1200 LC, Santa Clara, California, USA) with a ZORBAX Eclipse AAA reversed-phase column (3.5 μm), 4.6×150 mm id, at 425 nm by UV-vis diode-array absorbance detection (DAD).

Polyamines' content was determined according to Yang et al. (2015a).

GAD, DAO and AMADH activity assay

GAD activity was determined according to Bai et al. (2009). The GABA content in the reaction solution was analyzed by the standard method (Bai et al. 2009). One unit of GAD activity was defined as the release of 1 μmol of GABA produced from glutamate per 60 min at 40°C . The GAD activity of plant tissues is defined as units of 1 g fresh weight. DAO activity was determined according to the reference (2011). AMADH activity was determined according to the previous study (Yin et al. 2013).

Statistical analysis

Each value was expressed as mean $\pm$ SD of the three independent experiments. All data were analyzed by GraphPad 7.0 software, and significance was determined by two-tailed *T* test or one-way ANOVA. A probability level of $p < 0.05$ was considered as statistically significant.

Results

Effects of chemicals on the physiologic changes of soybean sprouts

After 4 days of germination, the growth of soybean sprouts under IAM treatment was completely inhibited, and sprout length was inhibited by 32.09% under EDC treatment compared with the control (Fig. 1a). The fresh weight of soybean did not change with germination under NEE and IAM treatment, while it increased significantly under EDC treatment (Fig. 1b). Compared with the control, the dry weight of sprouts increased under NEE treatment 2 days of germination, while it increased under IAM at 4 days of germination (Fig. 1c). MDA content increased significantly under IAM treatment, followed by NEE treatment (Fig. 1d). Therefore, it was suggested that the soybean cell

membrane was seriously damaged under IAM and NEE treatments.

Effect of chemicals on GABA content and AMADH activity of soybean sprouts

GABA content decreased significantly under treatment of inhibitors at 2 or 4 days of germination (Fig. 2a), compared with the control. It was reduced by 44.3, 11.0 and 54.8% under EDC, IAM and NEE treatments at 4 days of germination, respectively. AMADH activity decreased significantly after the treatment with three chemicals (EDC, NEE and IAM) (Fig. 2b), while there were no significant differences for the degree of inhibition among the treatments at 4 days of germination. The result demonstrated that the three inhibitors were efficient in restraining GABA content and AMADH activity in the germinated soybean. However, EDC was the appropriate inhibitor of AMADH in soybean, since it had the least effect on the growth of sprouts.

Effects of different concentrations of EDC on the physiology of soybean sprouts

EDC was selected for the subsequent trials based on the inhibition results. With the increase of EDC concentration, the

Fig. 1 Effects of chemicals on the physiology of soybean sprouts. The concentration of EDC, NEE and IAM was 0.5 mmol/L. The data were analyzed by one-way ANOVA. Different lowercase letters indicate the significant difference among different treatment days for each treatment. Asterisk indicates the significant difference between the chemical treatments and the control on the same germination day ($p < 0.05$)

Fig. 2 Effects of chemicals on GABA content (a) and AMADH activity (b) of soybean sprouts. The concentration of EDC, NEE and IAM was 0.5 mmol/L. The data were analyzed by one-way ANOVA. Different lowercase letters indicate the significant difference among different chemical treatments ($p < 0.05$). Seeds were germinated for 2 or 4 days

Fig. 3 Effects of different concentrations of EDC on the physiology of soybean sprouts. The data were analyzed by one-way ANOVA. Different lowercase letters indicate the significant difference among different concentrations on the same day ($p < 0.05$)

length of soybean sprouts decreased, and the sprout length of each treatment was significantly different from that of the control after 4 days of germination (Fig. 3a, b). The inhibition effect was mainly on the root. The fresh weight of soybean sprouts was not affected by 0.5 and 1.0 mmol/L of EDC, but it decreased significantly at a higher concentration. The dry weight had no obvious changes compared with the control (Fig. 3c, d).

Effects of different concentrations of EDC on GABA content and AMADH activity

GABA content decreased by 32.2% under 1 mmol/L EDC treatment compared with the control (Fig. 4a). With further increased concentration, GABA content increased slightly at 4 days of germination. At 2 days of germination, GABA reached the lowest level at 2 mmol/L EDC

Fig. 4 Effects of different concentrations of EDC on GABA content (a) and AMADH activity (b). Different lowercase letters indicate the significant difference among different concentrations on the same day ($p < 0.05$)

treatment and increased slightly at higher EDC concentration (4 mmol/L EDC). This might be related to the stress from the high concentration of EDC. With the increased concentration, AMADH activity decreased significantly and was maintained at a low level with no significance from 1.0 to 4.0 mmol/L (Fig. 4b). The AMADH activity inhibition rate was up to 87.9% under 1 mmol/L of EDC at 4 days of germination, and GABA content decreased by 34.1% under the same condition.

Glu and PAs content in germinating soybean under EDC treatment

There was no significant difference in Glu content in soybean sprouts under 1 mmol/L of EDC treatment after 4 days of germination compared with the control (Fig. 5a). Under EDC treatment, Put content increased by 69.7% compared with the control, while Spd and Spm content remained unchanged (Fig. 5b). This demonstrated that EDC blocked the Put degeneration pathway significantly, but had no significant impact on Glu content.

Effects of EDC on DAO and GAD activities of soybean sprouts

GAD activity was not affected by EDC treatment in 2-day and 4-day germinated soybean (Fig. 6a). DAO activity showed no difference after 2 days of germination, but decreased significantly in 4-day germinated soybean (Fig. 6b). The results indicated that at the early stage of germination, EDC did not inhibit polyamine degradation. However, after the inhibition of AMADH activity, polyamine metabolism was impacted.

Fig. 5 Effects of 1 mmol/L of EDC on Glu and PAs content of soybean sprouts. Soybean germinated for 4 days. The data were analyzed by two-tailed *T* test. Different lowercase letters in each index indicate the significant difference between EDC and the control ($p < 0.05$)

Fig. 6 Effects of 1 mmol/L of EDC on GAD (a) and DAO (b) activity of soybean sprouts. Soybean germinated for 4 days. The data were analyzed by two-tailed *T* test. Different lowercase letters indicate the significant difference between EDC and the control at the same germination time ($p < 0.05$)

Effects of EDC on GABA accumulation in soybean sprout under NaCl, CaCl₂ and NaCl + CaCl₂ treatment

Effects of EDC treatment on the content of GABA, Glu and polyamines

Compared with the control, GABA content in soybean sprouts increased by 26.2, 7.2 and 29.2% under NaCl, CaCl₂ and NaCl + CaCl₂ treatment, respectively. After the application of EDC, GABA content of these treatments declined, and it decreased by 43.56, 38.84 and 35.53%, respectively. The precursor of GABA, Glu content increased under EDC treatment (Fig. 7a). EDC addition based on NaCl, CaCl₂ and NaCl + CaCl₂ treatment

Fig. 7 Effects of EDC treatment on the content of GABA and Glu (a) and polyamines (b) under NaCl, CaCl₂ and NaCl + CaCl₂ treatments. Soybean germinated for 4 days. The data were analyzed by one-way ANOVA. Different lowercase letters indicate the significant difference among the treatments for each index ($p < 0.05$)

did not affect polyamines content except for that of Spd (Fig. 7b).

Effects of EDC on the activity of AMADH, GAD and DAO

Compared with the control, NaCl treatment increased the AMADH, GAD and DAO activity of soybean sprouts by 39.7, 28.4 and 21.2%, respectively. CaCl₂ treatment only increased the GAD activity. NaCl + CaCl₂ treatment increased AMADH, GAD and DAO activity by 39.0%, 36.7% and 19.6%, respectively. AMADH activity was significantly inhibited by EDC, but GAD and DAO activities were not affected. After adding EDC based on NaCl, CaCl₂ and NaCl + CaCl₂ treatment, AMADH activity was inhibited by 80.62, 67.61 and 72.02%, respectively (Fig. 8).

Fig. 8 Effects of EDC on AMADH, GAD and DAO activity under NaCl, CaCl₂ and NaCl+CaCl₂ treatments. Soybean germinated for 4 days. The data were analyzed by one-way ANOVA. Different lowercase letters indicate the significant difference among the treatments for each index ($p < 0.05$)

Discussion

This study screened the inhibitor of AMADH activity to supply an effective method for quantifying the contribution of the polyamine degradation pathway on GABA accumulation in soybean sprouts. Compared with the previous study using the inhibitor of DAO (2011, Yang et al. 2013), the inhibitor selected felicitously for AMADH in this study was more appropriate since AMADH converts γ -aminobutyraldehyde into GABA directly in the polyamine degradation pathway. The results showed that EDC inhibitory effect was the most effective among the three chemicals, and the optimum concentration of EDC was 1 mM for soybean sprouts (Figs. 2 and 4). Compared with the other two chemicals, EDC had effective inhibition on AMADH activity, while it had no significant inhibition effect on sprouts growth (Fig. 1). Meanwhile, EDC did not affect GAD activity at the designed concentration level (Fig. 6a). Hence, EDC was optimized as the appropriate inhibitor for AMADH in the polyamine degradation pathway in this study. After the inhibition of AMADH activity, polyamine degradation was impacted and resulted in the accumulation of Put (Fig. 5b). Interestingly, in the early stage of germination (2 days), EDC did not affect DAO activity, but at 4 days of germination, EDC decreased DAO activity as well. This might be due to the inhibition of polyamine degradation after the inhibition of AMADH activity. As a result, DAO upstream of AMADH in the polyamine degradation pathway and its activity were also reduced (Fig. 6a). In addition, after EDC treatment, GABA

content decreased but Put content increased, and it had no impact on Spd and Spm content (Fig. 5b). This indicated that EDC inhibited the degradation of Put and GABA synthesis from the polyamine degradation pathway (Yang et al. 2013).

Under NaCl treatment, GABA in soybean sprouts was accumulated significantly in this study (Fig. 7a), which was consistent with our former study (Yin et al. 2014c). Reports showed that Ca²⁺ could enhance GABA content under NaCl stress and alleviate the inhibition of NaCl effect on the growth of soybean sprouts (Yin et al. 2014a). After adding EDC based on NaCl, CaCl₂ and NaCl+CaCl₂ treatment, GABA content and AMADH activity decreased significantly (Figs. 7a and 8). However, the inhibition effect under NaCl, CaCl₂ and NaCl+CaCl₂ treatment was different. Interestingly, the application of CaCl₂ decreased the inhibition effect of EDC on GABA accumulation and AMADH activity, significantly. This might be relevant to the improvement of growth contributed by Ca²⁺ under NaCl stress (Yin et al. 2014a). At the same time, the results indicated that CaCl₂ treatment decreased the contribution rate of the polyamine degradation pathway on GABA accumulation in soybean sprouts under NaCl stress.

In legumes, L-arginine content is relatively high and can be converted into polyamines (Vuosku et al. 2006) and then oxidized to GABA (Alcazar et al. 2010). Hence, the polyamine degradation pathway is very important for GABA accumulation in legumes. By the inhibition of DAO activity, Xing et al. (2007) reported that the polyamine degradation pathway supplied 39% of GABA in soybean seedling root under NaCl stress. Under non-stress condition or hypoxia stress, the polyamine degradation pathway provided about 30% of GABA formation in the germinated fava bean (Yang et al. 2015b). However, aminoguanidine could not block the polyamine degradation pathway completely, although it is a specific inhibitor of DAO. The 5.0 mmol/L of AG inhibited 77.77% and 90.34% of DAO activity in cotyledon and embryo of germinating fava bean, respectively. Meanwhile, inhibition by 25.0% and 29.3% for GABA was observed accordingly (Yang et al. 2015b). Although AMADH activity was not inhibited completely as well in the present study, the GABA content decreased more compared with the reported results (Yang et al. 2013). Besides, researchers used aminooxyacetate to inhibit GAD activity to evaluate the contribution of GABA shunt on GABA accumulation in hypoxia–NaCl stressed fava bean sprouts (Yin et al. 2018). It was an acceptable method seemingly. However, aminooxyacetate is the inhibitor of ethylene synthesis in plants, and it significantly impacts the ethylene synthesis and the growth of the sprouts (Broun and Mayak 1981). Aminooxyacetate is also considered as a plant growth inhibitor (Gladon and Koranski 1986). Hence, it is inadvisable for investigating the contribution of GABA shunt on GABA accumulation in

plants. As described above, inhibiting AMADH activity is more appropriate than DAO activity with aminoguanidine, and GAD activity with aminoguanidine, to investigate the GABA metabolism in plants.

Conclusion

EDC was the optimum as the appropriate inhibitor of AMADH activity among the three chemicals to qualify the contribution of the polyamines degradation pathway on GABA accumulation in soybean sprout, since it did not affect the sprout growth and GABA shunt metabolism. EDC inhibited the degradation of Put, and GABA synthesized by polyamine degradation pathway was mainly from Put. Under NaCl, CaCl₂ and NaCl + CaCl₂ treatment for 4 days, the polyamine degradation pathway contributed at least 43.56, 38.84 and 35.53% to GABA formation, respectively. CaCl₂ treatment decreased the contribution of the polyamine degradation pathway on GABA accumulation in soybean sprouts under NaCl stress.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Abe T, Takeya K (2005) Difference in γ -aminobutyric acid content in vegetable soybean seeds. *Nippon Shokuhin Kogyo Gakkaishi* 52(11):545–549
- Alcazar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio AF (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta* 231(6):1237–1249
- Bai QY, Chai MQ, Gu ZX, Cao XH, Li Y, Liu KL (2009) Effects of components in culture medium on glutamate decarboxylase activity and gamma-aminobutyric acid accumulation in foxtail millet (*Setaria italica* L.) during germination. *Food Chem* 116(1):152–157
- Brauner F, Šebela M, Snégaroff J, Peč P, Meunier J-C (2003) Pea seedling aminoaldehyde dehydrogenase: primary structure and active site residues. *Plant Physiol Biochem* 41(1):1–10
- Broun R, Mayak S (1981) Aminoxyacetic acid as an inhibitor of ethylene synthesis and senescence in carnation flowers. *Sci Hort* 15(3):277–282
- Gladon RJ, Koranski DS (1986) Aminoxyacetic acid (AOA) as a plant growth inhibitor. Patent Number: 4608076, US
- Guo Y, Yang R, Chen H, Song Y, Gu Z (2012) Accumulation of γ -aminobutyric acid in germinated soybean (*Glycine max* L.) in relation to glutamate decarboxylase and diamine oxidase activity induced by additives under hypoxia. *E Food Res Technol* 234:679–687
- Ma P, Li T, Ji F, Wang H, Pang J (2015) Effect of GABA on blood pressure and blood dynamics of anesthetic rats. *Int J Clin Exp Med* 8(8):14296–14302
- Matsuda H, Suzuki Y (2004) γ -Guanidinobutyraldehyde dehydrogenase of *Vicia faba* leaves. *Plant Physiol* 76:654–657
- Patel RN, Hou CT, Derelanko P, Felix A (1980) Purification and properties of a heme-containing aldehyde dehydrogenase from *Methylosinus trichosporium*. *Arch Biochem Biophys* 203(2):654–662
- Shelp BJ, Bozzo GG, Trobacher CP, Zarei A, Deyman KL, Brikis CJ (2012) Hypothesis/review: contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. *Plant Sci* 193:130–135
- Vuosku J, Jokela A, Laara E, Saaskilahti M, Muilu R, Sutela S, Altabella T, Sarjala T, Haggman H (2006) Consistency of polyamine profiles and expression of arginine decarboxylase in mitosis during zygotic embryogenesis of Scots pine. *Plant Physiol* 142(3):1027–1038
- Wael MS, Mostafa MR, Taia EM, Saad MH, Magdi TA (2015) Allel-viation of cadmium toxicity in common bean (*Phaseolus vulgaris* L.) plants by the exogenous application of salicylic acid. *J Pomol Hort Sci* 90(1):83–91
- Wang X, Yang R, Jin X, Shen C, Zhou Y, Chen Z, Gu Z (2016) Effect of supplemental Ca²⁺ on yield and quality characteristics of soybean sprouts. *Sci Hort* 198:352–362
- Xing SG, Jun YB, Hau ZW, Liang LY (2007) Higher accumulation of gamma-aminobutyric acid induced by salt stress through stimulating the activity of diarnine oxidases in *Glycine max* (L.) Merr. roots. *Plant Physiol Biochem* 45(8):560–566
- Xu JG, Hu QP (2014) Changes in γ -aminobutyric acid content and related enzyme activities in Jindou 25 soybean (*Glycine max* L.) seeds during germination. *LWT Food Sci Technol* 55(1):341–346
- Yang R, Chen H, Gu Z (2011) Factors influencing diamine oxidase activity and γ -aminobutyric acid content of fava bean (*Vicia faba* L.) during germination. *J Agric Food Chem* 59(21):11616–11620
- Yang R, Guo Q, Gu Z (2013) GABA shunt and polyamine degradation pathway on γ -aminobutyric acid accumulation in germinating fava bean (*Vicia faba* L.) under hypoxia. *Food Chem* 136(1):152–159
- Yang R, Wang S, Yin Y, Gu Z (2015a) Hypoxia treatment on germinating faba bean (*Vicia faba* L.) seeds enhances GABA-related protection against salt stress. *Chil J Agr Res* 75(2):184–191
- Yang R, Yin Y, Gu Z (2015b) Polyamine degradation pathway regulating growth and GABA accumulation in germinating fava bean under hypoxia-NaCl stress. *J Agric Sci Technol* 17:311–320
- Yang R, Feng L, Wang S, Yu N, Gu Z (2016) Accumulation of γ -aminobutyric acid in soybean by hypoxia germination and freeze-thawing incubation. *J Sci Food Agric* 96(6):2090–2096
- Yin Y, Yang R, Wu J, Gu Z (2013) Partial purification, characterization and cDNA cloning of aminoaldehyde dehydrogenase in germinated soybean (*Glycine max* L.). *Eur Food Res Technol* 237:731–738
- Yin Y, Yang R, Gu Z (2014a) Calcium regulating growth and GABA metabolism pathways in germinating soybean (*Glycine max* L.) under NaCl stress. *Eur Food Res Technol* 239(1):149–156

- Yin Y, Yang R, Gu Z (2014b) Organ-specific proteomic analysis of NaCl-stressed germinating soybeans. *J Agr Food Chem* 62(29):7233–7244
- Yin Y, Yang R, Guo Q, Gu Z (2014c) NaCl stress and supplemental CaCl₂ regulating GABA metabolism pathways in germinating soybean. *Eur Food Res Technol* 238(5):781–788
- Yin Y, Cheng C, Fang W (2018) Effects of the inhibitor of glutamate decarboxylase on the development and GABA accumulation in germinating fava beans under hypoxia-NaCl stress. *RSC Adv* 8(36):20456–20461