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Factors Influencing Diamine Oxidase Activity and γ-Aminobutyric Acid Content of Fava Bean (*Vicia faba* L.) during Germination

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ABSTRACT: Factors (germination time, spectra, temperature, pH, and chemical inhibitors) influencing diamine oxidase (DAO, EC 1.4.3.6) activity and γ-aminobutyric acid (GABA) content of fava bean (*Vicia faba* L.) during germination were investigated in this study. DAO activity significantly increased in germinating seeds but varied with different organs. The enzyme activity was higher in shoot than that in cotyledon, hypocotyl, and radicle. When seeds were germinated in the dark, DAO activity was 2.35-, 2.00-, 2.36-, 4.40-, and 1.67-fold of that under white, red, blue, green, and yellow spectra, respectively. The optimum germination temperature and pH value for increasing DAO activity were 30 °C and 3.0, respectively. The DAO activity was inhibited significantly by aminoguanidine and sodium ethylenediamine tetracetate, while it was activated by $CuCl_2$ and $CaCl_2$. Germinating at an appropriate temperature and pH, 30% of GABA formation was supplied by DAO. Calcium was related to the regulation of DAO activity and GABA accumulation.

KEYWORDS: Fava bean, diamine oxidase, γ-aminobutyric acid, seed germination

INTRODUCTION

Diamine oxidase (DAO, EC 1.4.3.6) is ubiquitous in microorganisms, plants, and mammals.[1,2] In legumes and animals, there is copper-containing amine oxidase with 2 mol of Cu^{2+} per mol enzyme.[3,4] In monocotyledon, however, flavin adenine dinucleotide (FAD) serves as a cofactor with 4 mol of FAD to 1 mol of enzyme.[5] During seed germination, the DAO activity increases, and the etiolated seedlings possess significantly higher enzyme activity than those growing under the light,[3] and the enzyme activity increased sharply in the embryo during the time period of germination.[6]

Fava bean (*Vicia faba* L.) is one of the common legumes, which is rich in dietary proteins, carbohydrates, B group vitamins, and minerals. However, its nutritional value is limited by the presence of antinutritional components such as vicine,[7] convicine, tannins,[7,8] phytic acid,[9] trypsin inhibitors,[10] etc. The presence of nutrients and antinutrients in cereals and legumes can be modified by germination. After germination, moreover, some new functional components like γ-aminobutyric acid (GABA) are generated in fava bean,[11] with an improved utilization of minerals simultaneously.[12]

Putrescine (Put) is converted by DAO into GABA via a Δ^1-pyrroline intermediate formation.[13] Reports have shown that polyamine and GABA contents in the plants increase to adapt adverse environmental conditions under a stress (water, hypoxia, heat, or drought stress).[14,15] However, previous studies of GABA accumulation in plants were mainly focused on GABA shunt[3,6] and polyamine oxidation to a lesser extent.[16]

Except for being an inhibitory neurotransmitter in the brain and spinal cord of mammals,[17] GABA also has other important physiological functions, such as regulation of blood pressure and heart rate and alleviation of pain and anxiety.[18] DAO is an essential enzyme for GABA formation. The objectives of this study are to investigate the key factors and explore their biochemical effects on DAO activity and GABA content in germinating fava beans, which can contribute useful information and new knowledge to science and technology of agri-food and biochemistry sectors and benefit functional food researches and production practices in the future.

MATERIALS AND METHODS

Materials and Reagents. Dried fava beans (Qi Bean 2), harvested in 2010, were supplied by Jiangsu Academy of Agricultural Sciences (Nanjing, China) and stored at −20 °C before the experiments. Horseradish peroxidase, Put, aminoguanidine (AG), 4-aminoantipyrine and *N*,*N*-dimethylaniline were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents were analytical grade.

Seed Germination. Fava bean seeds were sterilized using 10% (v:v) of sodium hypochlorite for 30 min, then washed, and steeped in distilled water at 30 ± 1 °C for 8 h. Before germination, soaked seeds were put in a culturing pallet (25 cm in length × 20 cm in width × 5 cm in height) with sterilized quartz sand (2 cm in thick) and then covered by fresh-keeping films with eyelets. A culture solution or distilled water was given every 8 h for seed germination. After germination, seeds were collected and washed with distilled water to measure DAO activity as well as protein and GABA contents.

Enzyme Extraction. Seed tissues (2.0 g) were ground with mortar and pestle at 4 °C in 6.0 mL of 70 mmol/L sodium phosphate buffer (pH 6.5) containing 10% glycerol (v/v). Homogenates were centrifuged at 6000*g* for 20 min at 4 °C (GL-20G-II, Shanghai Anting Scientific Instrument Factory, China). The supernatants were stored at −20 °C for further analyses.

Assay on DAO Activity. The DAO activity was determined according to Xing et al.[16] Reaction solutions (2.9 mL) contained 2.0 mL of 70 mmol/L sodium phosphate buffer (pH 6.5), 0.5 mL of

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Table 1. Procedure of GABA Gradient Elution in This Study

elution time (min)	mobile phases A (%)	mobile phases B (%)
0	70	30
10	70	30
15	60	40
22	60	40
30	30	70
35	70	30
40	70	30

crude enzyme extracts, 0.1 mL of horseradish peroxidase (250 U/mL), and 0.2 mL of 4-aminoantipyrine/*N*,*N*-dimethylaniline. The reaction was initiated by adding 0.1 mL of 50 mmol/L Put. The absorbance at 555 nm was read on UV-2802 UV−visible spectrophotometer (Unico, United States). A 0.01 value of the changes per minute in absorbance at 555 nm was regarded as one activity unit (U) of the enzyme. The DAO activity was expressed as "U per milligram protein".

GABA Measurement. GABA was extracted and purified according to Bai et al.[19] Then, the samples were dissolved with 2 mL of 1 mol/L $NaHCO_3$ (pH 9.0) and centrifuged at 6000*g* for 10 min. GABA was determined by HPLC (Agilent 1200, United States) with a ZORBAX Eclipse AAA reversed-phase column (3.5 μm), 4.6 mm × 150 mm i.d. as described by Syu et al.[20] The amino acid solution (1 mL, pH 9.0) was mixed with 1 mL of dabsyl chloride (2 mg/mL, in acetone) and reacted at 67 °C for 10 min. Then, the reaction was stopped by putting the tubes into an ice bath and was detected at 425 nm using UV−vis diode-array absorbance detection (G 1314B, Agilent). Mobile phases A and B were 0.045 mol/L CH_3COONa (pH 4.0) and acetonitrile, respectively. The flow rate was 1 mL/min. The procedure of gradient elution is shown in Table 1. The allowed time of GABA separation was within 30 min at 30 °C.

Determination of Protein. The protein content was measured from the supernatant (enzyme extraction) using a protein-dye reagent (Coomassie blue G-250) with bovine serum albumin as a standard according to Li et al.[21]

Effect of Spectra on DAO Activity of Germinating Fava Bean. After pretreatment as described in the section of "Seed Germination", the culturing pallets were placed into illumination boxes with white, red, blue, green, and yellow spectra, respectively. The seeds were subsequently collected and washed with distilled water to measure DAO activity and protein content.

Determination of Germination Temperature. Seeds were germinated over a range of 20−40 °C for 5 days according to the method described earlier. Distilled water (pH 6.8) was given every 8 h for seed germination. Thereafter, the DAO activity and GABA content were determined as described earlier. The terminal activity was expressed as U per milligram protein.

Assay on pH. The seeds were germinated over the pH range of 3.0−8.0 (citric acid buffer) for 5 days referring to the method described earlier. Buffer solution was given per 8 h to fulfill the sufficient needs for the seeds germination. After germination, the DAO activity and GABA content were measured. The terminal activity was expressed as U per milligram protein.

Assay on Chemical Inhibitors. The effect of chemicals on enzyme activity was determined by premixing the tested chemicals to the culture solution. After germination, the residual activity of DAO and GABA content were determined. Aminoguanidine: AG (2.5, 5.0, 7.5, 10.0, and 12.5 mmol/L), $CuCl_2$ (80, 160, 240, 320, and 400 μmol/L), $CaCl_2$ (0.05, 0.1, 0.5, 1.0, and 2.0 mmol/L), and sodium ethylenediamine tetracetate: EDTA-Na_2 (3.0, 6.0, 9.0, 12.0, and 15.0 mmol/L) were used for assay. Controls were prepared by germinating the seeds in the distilled water (pH 6.8). The remaining enzyme activity and GABA content were measured using the method described earlier.

Statistical Analysis. Three replicates for the experiment and measurements. Average values and standard deviations were computed in accordance with experimental data. Statistical analyses were performed by Fisher's *F* test at a significant level of $p < 0.05$.

RESULTS AND DISCUSSION

Effect of Germination Time on DAO Activity. The DAO activity of fava beans increased significantly ($p < 0.05$) during germination (Table 2), and the enzyme activity was about 123-fold of that in nongerminated seeds. During germination, however, the change in DAO activity of the cotyledon was insignificant ($p > 0.05$). It was not detectable in either shoot or radicle during the first 2 days of germination. In hypocotyl, the enzyme activity was also not detectable in the first 3 days of germination but increased significantly with germination time ($p < 0.05$). After germination, the enzyme activity in shoot was 390.86-, 7.51-, and 5.89-fold of that in cotyledon, hypocotyl, and radicle, respectively. The enzyme activity in shoot was significantly higher than that in other organs.

Effect of Spectra on DAO Activity. Germinating in the dark was helpful for enhancing DAO activity of the whole seeds (Table 3). However, a totally opposite result was obtained under green light. Red light decreased DAO activity in shoot. Green light restricted DAO activity in cotyledon, hypocotyl, and radicle. Yellow light increased the enzyme activity in hypocotyl and radicle.

Commonly, light is essential for plant carrying out photosynthesis. When plants grow under a stress condition, the polyamine level increased and induced the elevation of DAO activity.[16] During the germination of mung bean and lentil seeds, the DAO activity increased, and the etiolated seedlings showed a significantly higher enzyme activity than those grew under the light conditions.[3,22,23] The present result suggests that dark environment is a type of stress for fava bean germination. It also showed that green light significantly restricted DAO activity in germinating fava bean seeds, with a decrease in germination speed and radicle elongation, simultaneously. The result may be caused by the reflection of green light by the green seed casing of seeds (Qi Bean 2). On the contrary, during Agave seedlings development, green filter decreased the increase of weight.[24] These facts revealed that the effects of green light on DAO activity of germinating seeds varied with different species.

Effects of Temperature on DAO Activity and GABA Content. The present results showed that the DAO activity increased with temperature from 20 to 30 °C, then decreased with that from 30 to 40 °C (Figure 1). The optimum temperature for fava bean germination was 30 °C, with a DAO activity of 7.46 U/mg protein. The GABA content showed a similar trend, indicating the importance of DAO activity for GABA formation. In this study, it was observed that the optimum germination temperature for increasing DAO activity of fava bean was 30 °C. A paper reported that DAO activity of chickpea seedlings was higher when it germinated at 25 °C, and the enzyme activity in the cotyledons and the embryonic axis was sharply inhibited when germinated at higher temperatures (30−35 °C).[25] It suggests that the DAO in different plants has different optimum germination temperatures. The reason may be related to the size of the seeds that are germinated. The effect of germination temperature on GABA content of the same cultivar has been reported by Li

Table 2. Effect of Germination Time on DAO Activity (U/mg Protein) in Fava Bean Organs[a]

time (days)	seed	cotyledon	shoot	hypocotyl	radicle
0	0.12 ± 0.03 g	0.12 ± 0.05 c	NA	NA	NA
1	0.25 ± 0.04 f	0.29 ± 0.04 ab	NA	NA	NA
2	0.26 ± 0.04 f	0.26 ± 0.04 ab	NA	NA	NA
3	0.57 ± 0.06 e	0.33 ± 0.12 a	4.13 ± 0.08 e	NA	1.92 ± 0.15 d
4	1.99 ± 0.07 d	0.30 ± 0.07 ab	18.38 ± 0.36 d	0.53 ± 0.13 d	1.93 ± 0.46 d
5	6.36 ± 0.24 c	0.36 ± 0.08 a	52.93 ± 2.83 c	1.12 ± 0.19 c	5.33 ± 0.65 c
6	13.56 ± 0.12 b	0.20 ± 0.02 bc	65.87 ± 3.19 b	5.43 ± 0.78 b	8.79 ± 0.72 b
7	14.76 ± 0.31 a	0.21 ± 0.09 bc	82.08 ± 2.52 a	10.93 ± 0.50 a	13.93 ± 0.75 a

[a] Results are means of three replicates ± their SDs. NA means no activity of DAO. Mean values in the same column followed by different letters are significantly different at $p < 0.05$.

Table 3. Effect of Spectra on DAO Activity [U/(mg Protein)] in Germinating Fava Bean Organs[a]

spectrum	seed	cotyledon	shoot	hypocotyl	radicle
dark	6.11 ± 0.14 a	0.36 ± 0.08 a	52.94 ± 2.83 b	1.12 ± 0.19 d	5.33 ± 0.65 c
white	2.60 ± 0.26 d	0.13 ± 0.03 c	48.33 ± 4.37 b	1.56 ± 0.26 c	7.31 ± 0.48 a
red	3.05 ± 0.23 c	0.14 ± 0.03 c	25.90 ± 1.00 d	2.96 ± 0.27 b	4.70 ± 0.28 c
blue	2.59 ± 0.34 d	0.21 ± 0.03 b	51.63 ± 2.70 b	2.24 ± 0.25 b	6.76 ± 0.37 ab
green	1.39 ± 0.14 e	0.00 ± 0.00 e	30.95 ± 1.05 c	0.00 ± 0.00 e	2.40 ± 0.16 d
yellow	3.65 ± 0.68 b	0.04 ± 0.00 d	62.90 ± 0.23 a	8.37 ± 0.27 a	6.91 ± 0.28 a

[a] Seeds were germinated for 5 days at 200 lux except those in dark. Data are means of three replicates ± their SDs. Mean values in the same row followed by different letters are significantly different at $p < 0.05$.

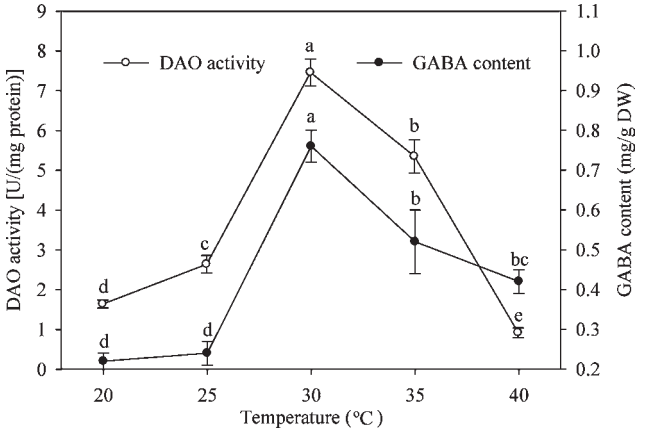

Figure 1. Effects of temperature on DAO activity and GABA content in germinating fava beans. Values are the means of triplicate analyses. Error bars show the standard deviation. Those with different lowercase letters in the same curve are significantly different at $p < 0.05$.

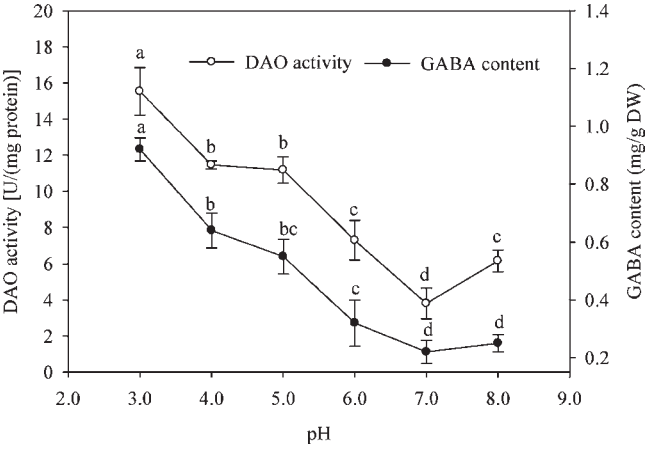

Figure 2. Effects of pH on DAO activity and GABA content of germinating fava beans. Values are the means of triplicate analyses. Error bars show the standard deviation. Those with different lowercase letters in the same curve are significantly different at $p < 0.05$.

et al.,[11] which showed that GABA content was 2.41 mg/g DW when germinating at 33.6 °C, 3.17-fold of the present result (0.76 mg/g DW). Differently, the previous study was carried out under hypoxia conditions. Indicating hypoxia treatment increased the GABA level for more than three times at least in fava bean.

Effects of pH on DAO Activity and GABA Content. The present experiments showed that DAO activity significantly decreased with the increase of pH from 3.0 to 7.0, but it increased when the pH continuously rose from 7.0 to 8.0 (Figure 2). Hence, the DAO activity was lowest at pH 7.0. The GABA content also showed the same trend. A previous study reported that GABA content in fava bean was significantly accumulated when germinating under hypoxia treatment.[11] In fact, a higher or lower pH is also an environmental stress for seeds germination.

Effects of Chemical Inhibitors on DAO Activity and GABA Content. The effects of AG, $CuCl_2$, $CaCl_2$, and EDTA-Na_2 on DAO activity and GABA content of germinating fava bean are shown in Figure 3A–D. AG inhibited DAO activity significantly ($p < 0.05$), and the inhibition was most significant when the concentration was greater than 7.5 mmol/L, and the inhibition remained a steady level. The maximum inhibitory rate was 89.79%. The GABA content showed a similar trend, but its maximum in-

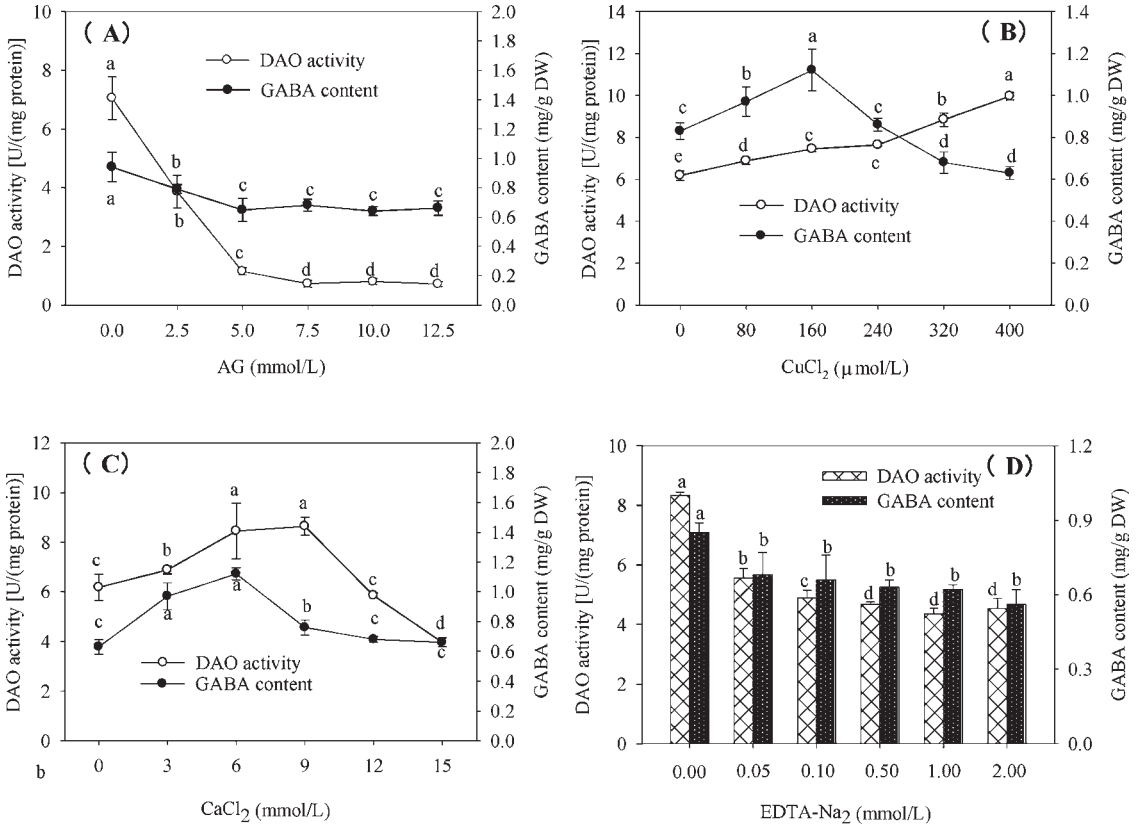

Figure 3. Effects of AG (A), $CuCl_2$ (B), $CaCl_2$ (C), and EDTA-Na_2 (D) on DAO activity and GABA content of germinating fava beans. Values are the means of triplicate analyses. Error bars show the standard deviation. Those with different lowercase letters in the same curve are significantly different at $p < 0.05$.

hibitory rate was 31.2% only (Figure 3A). $CuCl_2$ increased the DAO activity significantly ($p < 0.05$) with the increase of concentration. The GABA content increased significantly at 0−160 μmol/L $CuCl_2$ but decreased with a further addition of $CuCl_2$ (Figure 3B). Figure 3C illustrates the effect of $CaCl_2$ on DAO activity and GABA content of germinated seeds. The enzyme activity was stronger at 6−9 mmol/L $CaCl_2$ than at either a higher or a lower concentration. The GABA content showed a similar trend but presented a maximum content at 6.0 mmol/L of $CaCl_2$. An addition of EDTA-Na_2 inhibited DAO activity significantly ($p < 0.05$). The enzyme activity was inhibited by 33.33, 41.37, 44.00, 47.72, and 45.56% at 0.05, 0.10, 0.50, 1.00, and 2.00 mmol/L of EDTA-Na_2, respectively (Figure 3D). The GABA content significantly decreased when EDTA-Na_2 was added, but no significance ($p > 0.05$) was observed between 0.05 and 2.00 mmol/L in this experiment. A very low level of EDTA-Na_2 ($p < 0.05$ mmol/L) was capable of inhibiting DAO activity (Figure 3 D). Hence, GABA formation was sensitive to EDTA-Na_2.

It has been noted that AG is a specific inhibitor of DAO, and it can inhibit 99% of DAO activity of soybean seedlings[26] by in vitro determination. In this study, AG was also a strong inhibitor for DAO activity of germinating fava beans. It inhibited 31.2% of GABA formation, indicating that GABA produced from polyamine oxidation could account for at least 30% of the GABA, as GABA produced from DAO activity could not be inhibited completely by the addition of AG. The remains (70% of GABA) were supplied by the GABA shunt. Cu^{2+} is necessary for the formation of DAO in leguminous plant.[3,4] The addition of $CuCl_2$ provided germinating seeds with Cu^{2+} to synthesize DAO during germination. Therefore, the addition of $CuCl_2$ significantly increased DAO activity in germinating fava beans. However, the GABA content was decreased by a high concentration of $CuCl_2$. As known to all, copper is a heavy metal, which can result in the deactivation of glutamate decarboxylase (GAD, EC 4.1.1.15).[27] The latter is a key enzyme for converting glutamate to GABA in the GABA shunt, whereas the GABA shunt contributes to more than 2/3 of GABA formation as shown in this study (Figure 3A). Hence, the GABA content decreased significantly with an increase of $CuCl_2$ concentration (Figure 3B). As a second messenger, Ca^{2+} is important for the metabolic regulation in plant organs. It participates in the signal transmission and regulates enzyme activity. Research has reported that there are some Ca^{2+} binding sites (inside or outside the enzyme) in *Escherichia coli* copper-containing amine oxidase, and it is very important for DAO activity.[28] Therefore, the GABA content was also affected by an addition of $CaCl_2$ (Figure 3C). EDTA-Na_2 is a Ca^{2+} selective chelator. It decreased DAO activity significantly ($p < 0.05$) in this study (Figure 3D). Previous research reported that the inhibition provided by EGTA was almost reversed by exogenous $CaCl_2$.[25] An addition of 3−9 mmol/L $CaCl_2$ stimulated the enzyme activity (Figure 3C), indicating that there are calcium binding sites in DAO of germinating fava bean.

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REFERENCES

(1) Cooper, R.; Knowles, P.; Brown, D.; McGuirl, M.; Dooley, D. Evidence for copper and 3,4,6-trihydroxyphenylalanine quinone cofactors in an amine oxidase from the gram-negative bacterium *Escherichia coli* K-12. *Biochem. J.* **1992**, *288*, 337–340.

(2) Suetsugu, T. Enzymic properties of diamine oxidase from hog kidney. *Jpn. J. Pharmacol.* **1974**, *24* (1), 141–149.

(3) Choudhary, A.; Singh, R. P. Distribution of Cu^{2+}-diamine oxidase during ontogeny of seedlings of *Vigna radiata* cultivars. *Biol. Plant. (Prague)* **1997**, *40* (3), 449–452.

(4) Zhang, Q.; Mashima, Y.; Noda, S.; Imamura, Y.; Kudoh, J.; Shimizu, N.; Nishiyama, T.; Umeda, S.; Oguchi, Y.; Tanaka, Y.; Iwata, T. Characterization of AOC2 gene encoding a copper-binding amine oxidase expressed specifically in retina. *Gene* **2003**, *318*, 45–53.

(5) Chaudhuri, M. M.; Ghosh, B. Purification and characterization of diamine oxidase from rice embryos. *Phytochemistry* **1984**, *23* (2), 241–243.

(6) Scoccianti, V.; Torrigiani, P.; Bagni, N. Distribution of diamine oxidase activity and polyamine pattern in bean and soybean seedlings at different stages of germination. *Physiol. Plant* **1990**, *80* (4), 515–519.

(7) Vilariño, M.; Métayer, J. P.; Crépon, K.; Duc, G. Effects of varying vicine, convicine and tannin contents of faba bean seeds (*Vicia faba* L.) on nutritional values for broiler chicken. *Anim. Feed Sci. Technol.* **2009**, *150* (1−2), 114–121.

(8) Goyoaga, C.; Burbano, C.; Cuadrado, C.; Varela, A.; Guillamón, E.; Pedrosa, M. M.; Muzquiz, M. Content and distribution of vicine, convicine and l-DOPA during germination and seedling growth of two *Vicia faba* L. varieties. *Eur. Food Res. Technol.* **2008**, *227* (5), 1537–1542.

(9) Oomah, B. D.; Luc, G.; Leprelle, C.; Drover, J. C. G.; Harrison, J. E.; Olson, M. Phenolics, phytic acid, and phytase in Canadian-grown low-tannin faba bean (*Vicia faba* L.) genotypes. *J. Agric. Food. Chem.* **2011**, *59* (8), 3763–3771.

(10) Guillamon, E.; Pedrosa, M. M.; Burbano, C.; Cuadrado, C.; Sanchez, M. D. C.; Muzquiz, M. The trypsin inhibitors present in seed of different grain legume species and cultivar. *Food Chem.* **2008**, *107* (1), 68–74.

(11) Li, Y.; Bai, Q.; Jin, X.; Wen, H.; Gu, Z. Effects of cultivar and culture conditions on γ-aminobutyric acid accumulation in germinated fava beans (*Vicia faba* L.). *J. Sci. Food Agric.* **2010**, *90* (1), 52–57.

(12) Luo, Y.; Gu, Z.; Han, Y.; Chen, Z. The impact of processing on phytic acid, in vitro soluble iron and Phy/Fe molar ratio of faba bean (*Vicia faba* L.). *J. Sci. Food Agric.* **2009**, *89* (5), 861–866.

(13) Bhatnagar, P.; Glasheen, B. M.; Bains, S. K.; Long, S. L.; Minocha, R.; Walter, C.; Minocha, S. C. Transgenic manipulation of the metabolism of polyamines in poplar cells. *Plant Physiol.* **2001**, *125* (4), 2139.

(14) Bouchereau, A.; Aziz, A.; Larher, F.; Martin-Tanguy, J. Polyamines and environmental challenges: recent development. *Plant Sci.* **1999**, *140* (2), 103–125.

(15) Fait, A.; Fromm, H.; Walter, D.; Galili, G.; Fernie, A. R. Highway or byway: The metabolic role of the GABA shunt in plants. *Trends Plant Sci.* **2008**, *13* (1), 14–19.

(16) Xing, S. G.; Jun, Y. B.; Hau, Z. W.; Liang, L. Y. Higher accumulation of [gamma]-aminobutyric acid induced by salt stress through stimulating the activity of diamine oxidases in *Glycine max* (L.) Merr. roots. *Plant Physiol. Biochem.* **2007**, *45* (8), 560–566.

(17) Manyam, B. V.; Katz, L.; Hare, T. A.; Kaniefski, K.; Tremblay, R. D. Isoniazid-induced elevation of CSF GABA levels and effects on chorea in Huntington's disease. *Ann. Neurol.* **1981**, *10* (1), 35–37.

(18) Mody, I.; De Koninck, Y.; Otis, T.; Soltesz, I. Bridging the cleft at GABA synapses in the brain. *Trends Neurosci.* **1994**, *17* (12), 517–525.

(19) Bai, Q. Y.; Chai, M. Q.; Gu, Z. X.; Cao, X. H.; Li, Y.; Liu, K. L. 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.* **2009**, *116* (1), 152–157.

(20) Syu, K. Y.; Lin, C. L.; Huang, H. C.; Lin, J. K. Determination of theanine, GABA, and other amino acids in green, oolong, black, and pu-erh teas with dabsylation and high-performance liquid chromatography. *J. Agric. Food Chem.* **2008**, *56* (17), 7637–7643.

(21) Li, H. S.; Sun, Q.; Zhao, S. J.; Zhang, W. H. *The Experimental Principle of Plant Physiology and Biochemistry*, 1th ed.; Chinese Higher Education Press: Biejing, 2002.

(22) Choudhary, A.; Singh, R. P. Cadmium-induced changes in diamine oxidase activity and polyamine levels in *Vigna radiata* Wilczek seedlings. *J. Plant Physiol.* **2000**, *156* (5−6), 704–710.

(23) Maccarrone, M.; Rossi, A.; Avigliano, L.; Finazzi Agro, A. Activity and expression of diamine oxidase in lentil seedling under different growth conditions. *Plant Sci.* **1991**, *79* (1), 51–55.

(24) Maiti, R.; Wesche-Ebeling, P.; Sanchez-Arreola, E.; Ramirez-Bravo, O. Effect of different lights on germination and seedling growth of Agave species. *Crop Res.-Hisar* **2005**, *30* (1), 90–95.

(25) Matilla, A. J.; Garcia, S.; Bueno, M. Diamine oxidase activity during the germinative and post-germinative growth of the embryonic axis in chickpea seeds. *Biol. Plant.* **2002**, *45* (4), 551–556.

(26) Suzuki, Y. Some properties of the amine oxidase of soybean seedlings. *Plant Cell Physiol.* **1973**, *14* (2), 413–414.

(27) Bai, Q.; Zeng, B.; Gu, Z. Study on enzymatic properties of glutamate decarboxylase from germinated *Setaria italica* (L.) Beauv. *J. Anhui Agric. Sci.* **2011**, *39* (3), 1268–1270.

(28) Smith, M. A.; Pirrat, P.; Pearson, A. R.; Kurtis, C. R. P.; Trinh, C. H.; Gaule, T. G.; Knowles, P. F.; Phillips, S. E. V.; McPherson, M. J. Exploring the roles of the metal ions in *Escherichia coli* copper amine oxidase. *Biochemistry* **2010**, *49* (6), 1268–1280.