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Influence of Cultivar and Germination on Bioactive Amines in Soybeans (*Glycine max* L. Merril)

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The levels of amines in soybeans as affected by cultivar in two consecutive years and by germination were investigated. Spermidine, spermine, putrescine, agmatine, and cadaverine were detected, whereas tyramine, histamine, tryptamine, serotonine, and phenylethylamine were not. Spermidine was the predominant amine followed by spermine. High concentrations of these amines confirmed soybean as a rich source. Cadaverine was confirmed to be inherent to soybean. The percent contribution of spermidine and spermine to total levels was not affected by cultivar in either years. However, amine levels were affected by cultivars in different ways in the consecutive years. Cadaverine was affected more by the cultivar, whereas spermidine, spermine, and agmatine were affected by harvest year. During germination the levels of amines from soybean increased significantly, except for agmatine. Spermidine and spermine accumulated in the cotyledon, whereas cadaverine and putrescine accumulated in the radicle and hypocotyl.

KEYWORDS: Bioactive amines; polyamines; soybeans; germination; cultivars; spermidine

INTRODUCTION

It has long been known that certain amines fulfill a number of important metabolic and physiological functions in living organisms. They are formed during normal metabolic processes and are, therefore, present in foods. Bioactive amines are organic bases of low molecular weight. They can be classified on the basis of the number of amine groups, chemical structure, and biosynthesis or physiological functions. The latter is the most widely used. On the basis of this criterion, amines are classified as polyamines and biogenic amines. The polyamines spermine and spermidine play an important role in cell division, organogenesis, response to stress, and inhibition of lipid peroxidation. The biogenic amines histamine, tyramine, tryptamine, serotonine, putrescine, cadaverine, and phenylethylamine are neuroactive or vasoactive. Biogenic amines are mainly formed by decarboxylation of precursor amino acids. Polyamine synthesis is a more complex process, although the first few steps also include decarboxylation reactions in the formation of putrescine, which is an obligate intermediate (*1*−*3*).

Few studies investigating several amines simultaneously in fruits and vegetables have been performed. Early studies were undertaken to investigate specific amines in foods. Examples are studies by Smith (*4*) and by Wheaton and Stewart (*5*) on the levels of tryptamine and aromatic amines, respectively, in different plant species. Most of the recent studies, however, are focused on the polyamines (*6*, *7*). Most fruits and vegetables contain small amounts of polyamines, with spermidine as the predominant one (*6*). Some biogenic amines are of specific occurrence in the plant kingdom and, therefore, can be used as a tool for taxonomic studies. Other biogenic amines can be introduced during production and processing because of decarboxylase activity from microorganisms (*8*−*10*).

Knowledge of the levels of amines in food products is necessary. Polyamines are required in cellular metabolism and growth and, particularly, in rapidly growing tissues. Similarly, during periods of wound healing, regeneration, and compensatory growth, requirements are also high (*1*, *2*, *17*, *18*). Polyamine deprivation can be beneficial in reducing tumor growth (*2*, *16*). High levels of biogenic amines can cause distinct pharmacological and toxic effects, especially when detoxifying enzymes are impaired (*19*). However, information is still lacking on the levels of amines in foodstuffs and on the influence of many factors including plant species and variety, type of tissue, germination, conditions of growth, stage of development, degree of ripening, and processing and storage conditions.

Soybean is one of the most widely used ingredients in the food industry. It can be used naturally or as sprouts and fermented products. It is well-known that germination can

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improve the sensory acceptance and nutritional quality of seeds by increasing the contents and availability of essential nutrients, increasing digestibility, and decreasing the levels of antinutrients (*11*, *12*). However, there are few and contradictory studies on the influence of germination on the levels of bioactive amines in seeds. Reasons for the controversy could be associated with the moist environment prevalent during germination, which affects the moisture content of the seed and also favors proliferation of microorganisms with amino acid decarboxylases. Information is needed on the influence of cultivar and germination on the levels of bioactive amines in soybean.

The objective of this study was to investigate the influence of cultivar and germination on the profile and levels of bioactive amines in soybeans. The specific objective was to determine the profile and levels of amines in soybeans as affected by (i) different promising soybean cultivars from the state of Minas Gerais, Brazil, in two harvest years and (ii) germination.

MATERIALS AND METHODS

Samples. Soybeans, *Glycine max* L. Merril, grown in Triangulo Mineiro, the western part of the state of Minas Gerais, Brazil, and harvested in the years 2003 and 2004, were used in this study. Three different lots of seven cultivars were selected: (A) Vencedora (BRSMG-68), (B) CS-935142, (C) Conquista MG-BR-46, (D) CS-201, (E) Monarca, (F) Liderança BRSMG, and (G) Garantia (BRSMG). These cultivars were selected because they are popular and good-yielding cultivars grown by soybean producers.

Reagents. Bioactive amine standards were purchased from Sigma Chemical Co. (St. Louis, MO). They included spermine tetrahydrochloride, spermidine trihydrochloride, putrescine dihydrochloride, agmatine sulfate, cadaverine dihydrochloride, serotonine hydrochloride, histamine dihydrochloride, tyramine, tryptamine, and 2-phenylethylamine dihydrochloride.

All reagents were of analytical grade, except HPLC reagents that were of LC grade. Ultrapure water was obtained from a Milli-Q system (Millipore Corp., Milford, MA). The mobile phases were filtered in HAWP and HVWP membranes (47 mm diameter and 0.45 μm pore size, Millipore Corp.), used for aqueous and organic solvents.

Influence of Soybean Cultivars on Bioactive Amines Profile and Levels. Three lots of the seven cultivars of soybean listed above (A−G) from two consecutive years (2003 and 2004) were used in this experiment. The samples were ground in an electric grinder, sieved through a 12-mesh sieve, and analyzed for bioactive amines and moisture content.

Germination of Soybean. The soybean cultivar Monarca harvested in 2003 was selected for this experiment. The seeds were applied onto humidified layers of paper towel by means of a perforated plate containing 50 orifices. The paper towel was wrapped into a coil that was placed in a vertical position for germination. The germination chamber (type Magelido) was kept at 24.5 ± 0.5 °C and at 92 ± 2% relative humidity in the presence of light (*20*). Samples were collected at 24 h intervals for up to 4 days. Two experiments were conducted. In the first, the whole germinated soybean was ground and homogenized for analysis. In the second experiment, the germinated samples were, when possible, separated into cotyledon, hypocotyl, and radicle. The respective tissues were ground, homogenized, and analyzed for bioactive amines and moisture content.

Determination of Bioactive Amines. Amines were extracted from samples (1 g) with 7 mL of 50 g/L trichloroacetic acid (TCA). After agitation for 10 min in a vortex mixer, the slurry was centrifuged at 10000*g* at 4 °C, and the supernatant was collected. The solid residue was extracted twice with volumes of 7 and 6 mL of TCA. Supernatants were combined and filtered through a 0.45 μm pore size filter. The amines were separated by ion-pair reverse phase HPLC and quantified by fluorescence after postcolumn derivatization with *o*-phthalaldehyde as described by Cirilo et al. (*21*).

Liquid chromatography was performed with an LC-10AD system connected to a RF-551 spectrofluorometric detector at 340 and 445 nm of excitation and emission, respectively, and to a CBM-10AD controller (Shimadzu, Kyoto, Japan). A reversed-phase μBondapak C18 column, 300 × 3.9 mm i.d., 10 μm, was used with a μBondapak C18 Guard-pak insert (Waters, Milford, MA). The mobile phases were (A) a solution of 0.2 M sodium acetate and 10 mM 1-octanesulfonic acid sodium salt adjusted to pH 4.9 with acetic acid and (B) acetonitrile. The flow rate was set at 0.8 mL/min and the gradient was as follows: 13 min at 11% B, 19 min at 30%, 24 min at 11%, and 45 min at 11%. The postcolumn derivatization reagent was delivered at 0.4 mL/min. It consisted of 1.5 mL of Brij-35, 1.5 mL of mercaptoethanol, and 0.2 g of *o*-phthalaldehyde dissolved in a 500 mL solution of 25 g of boric acid and 22 g of KOH (pH adjusted to 10.5 with 30 g/L KOH). The column and postcolumn reaction apparatuses were kept at 23 ± 1 °C.

The identification of the amines was performed by comparison of the retention times of amines in the samples to those of standard solutions and also by addition of the suspected amine to the sample. Quantification was accomplished by direct interpolation in the standard curves ($R^2 \geq 0.9926$). The determination limits were 0.02 mg/100 g for spermidine, spermine, agmatine, putrescine, cadaverine, histamine, tyramine, and phenylethylamine and 0.04 mg/100 g for serotonine and tryptamine.

Determination of Moisture Content. The ground samples were analyzed for moisture content by drying to constant weight in a forced draft oven at 105 ± 2 °C (*22*). The moisture content was used to calculate and express bioactive amine levels on a dry weight basis.

Statistical Analysis. The results were submitted to analysis of variance, and the means were compared by the Duncan test at 5% of probability.

RESULTS AND DISCUSSION

Profile and Levels of Bioactive Amines in Different Soybean Cultivars in Two Consecutive Years. Overall, among the 10 amines investigated, only 5 were detected in the soybean cultivars analyzed: spermidine, spermine, agmatine, putrescine, and cadaverine. Four of these amines had been reported previously, and one (cadaverine) was confirmed for the first time. The presence of spermidine, spermine, agmatine, and putrescine in soybean was described in the literature (*2*, *6*, *15*, *23*, *24*). Okamoto et al. (*6*) detected a mixture of cadaverine and histamine in soybean. The method of analysis used by these investigators could not separate the two amines; therefore, they could not ascertain whether one or both of them were present.

The presence of spermidine and spermine in soybean was expected, as these polyamines are ubiquitous in the plant kingdom, together with their diamine precursor, putrescine. According to the literature (*8*, *16*, *25*, *26*), polyamines play a critical role in several processes, among them, root growth, somatic embryogenesis, control of intracellular pH, flower and fruit development, and response to abiotic stress, such as potassium deficiency, osmotic shock, drought, and pathogen infection. Furthermore, polyamines are associated with cell wall and membrane, preventing deterioration because of radical scavenging properties.

Agmatine is a product from the decarboxylation of arginine. It can be metabolized to form putrescine in a two-step conversion. Agmatine iminohydrolase catalyzes the formation of *N*-carbamoylputrescine, which is converted to putrescine by *N*-carbamoylputrescine amido hydrolase (*8*, *24*). The presence of agmatine in the samples analyzed suggests that the formation of polyamines in soybean can also be accomplished via arginine.

The presence of cadaverine was reported in soybean for the first time. Cadaverine is a diamine that can be formed by decarboxylation of lysine. It has restricted occurrence, being found mainly in species of the Leguminosae family (*6*, *8*, *9*, *23*). Its presence in soybean, which belongs to this botanic family, is in accordance with the literature.

Table 1. Levels of Bioactive Amines in Different Cultivars of Soybean from Minas Gerais, Brazil, from Two Consecutive Years (2003 and 2004)

	amine levels,[a] mg/100 g of dw (coefficient of variation, %)									
	SPD		SPM		PUT		AGM		CAD	
cultivar	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
A	12.98bc (12)	19.41 (62)	3.20c (7)	5.22 (60)	0.51 (19)	0.80b (47)	1.40 (15)	1.67b (81)	1.28ab (2)	1.64ab (72)
B	12.28bcy (38)	38.89x (42)	4.28bcy (35)	11.41x (37)	0.98 (44)	1.40a (35)	0.63y (8)	3.86ax (40)	0.74bc (8)	2.83a (40)
C	9.92cy (5)	18.64x (14)	2.78cy (9)	7.83x (12)	0.45 (8)	0.55b (11)	0.83y (5)	1.36bx (7)	1.35a (14)	2.13ab (8)
D	13.28bcy (21)	19.91x (17)	3.17cy (20)	9.09x (19)	0.57y (24)	1.68ax (2)	1.21x (18)	0.58by (26)	0.61cy (16)	1.02bcx (17)
E	11.09cy (8)	26.04x (2)	2.99cy (5)	7.66x (4)	0.37y (15)	1.48ax (12)	1.07y (17)	2.24bx (4)	1.09abcy (12)	2.22abx (7)
F	18.74a (22)	–	8.35a (18)	–	1.07 (16)	–	2.32 (62)	–	1.53a (24)	–
G	18.15ab (27)	25.84 (13)	5.20b (31)	7.11 (16)	0.97 (86)	1.36a (13)	1.03y (6)	2.08bx (18)	1.43a (47)	0.69c (5)
mean	13.77	24.79	4.28	8.05	0.70	1.21	1.21	1.96	1.14	1.75

[a] Mean values with different letters in the same column (a–c) or in the same line (x, y) for each amine are significantly different (Duncan, $p \leq 0.05$). dw, dry weight basis; –, sample was not available; SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; CAD, cadaverine.

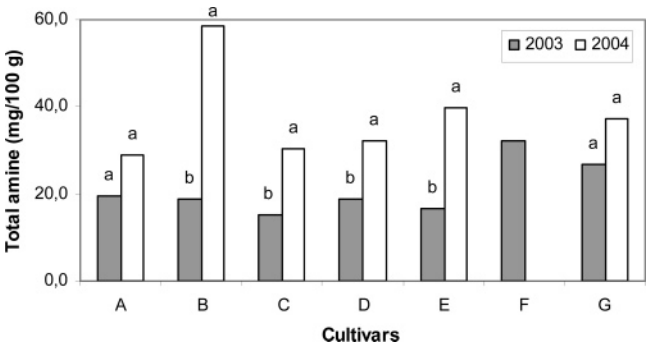

Figure 1. Total bioactive amine levels in different soybean cultivars harvested in 2003 and 2004. Mean values with different letters for the same cultivars are significantly different (Duncan, $p \leq 0.05$).

The total levels of amines in the different soybean cultivars harvested in 2003 and 2004 are indicated in **Figure 1**. Because the moisture content of the different cultivars varied significantly (from 7.50 to 8.51 g/100 g for 2003 and from 5.96 to 6.90 g/100 g for 2004), amine levels were expressed on a dry weight basis. Total amine levels in cultivars from 2003 varied from 15.33 to 26.78 mg/100 g with an average of 21.12 mg/100 g. There was significant difference among cultivars, with higher total levels in cultivars F and G and lower levels in cultivars C and E. However, even though total amines levels in cultivars from 2004 varied from 30.62 to 61.79 mg/100 g (average of 40.0 mg/100 g), no significant difference was observed in the levels among cultivars. When total amines of samples of the same cultivar but from different years were compared, samples from 2004 contained higher levels, except for cultivars A and G. Samples from cultivar F were not available in 2004.

The levels of the individual amines in the different cultivars are indicated in **Table 1**. There was significant difference in the levels of spermidine, spermine, and cadaverine among cultivars harvested in 2003. Significantly higher spermidine levels were observed for cultivars F and G; higher spermine levels were detected in cultivar F, and lower cadaverine levels were found in samples B, D, and E. There was no significant difference in the levels of putrescine and agmatine among the different cultivars.

Different from what was observed for 2003, there was no significant difference in spermidine and spermine levels for the different cultivars harvested in 2004. Significant difference among cultivars was observed for only putrescine, agmatine, and cadaverine. Cadaverine was the only amine affected by the cultivar in both years. However, the higher levels were not observed in the same cultivars in both years.

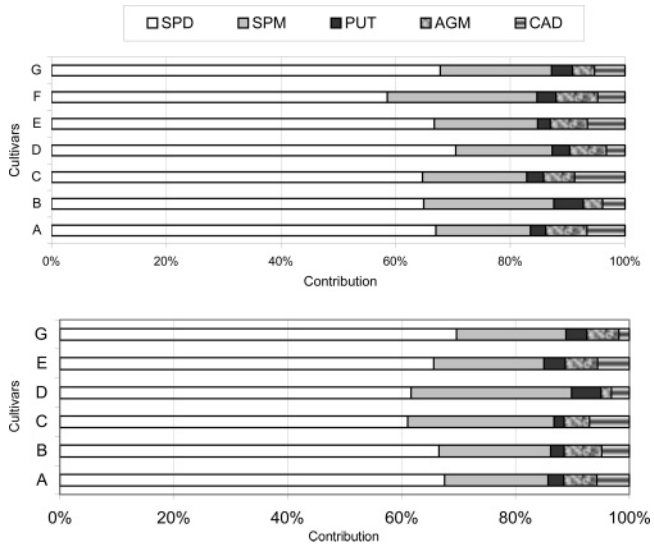

Figure 2. Contribution of individual amines to total levels in different soybean cultivars from Minas Gerais, Brazil, from 2003 and 2004. SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; CAD, cadaverine.

According to **Table 1**, spermidine, spermine, and agmatine were the amines most sensitive to the year of harvest, whereas putrescine and cadaverine were the most resistant. Cultivar A was the least sensitive, whereas cultivars D and E were the most sensitive to changes caused by the year of harvest.

The contributions of each amine to total levels in samples from 2003 and 2004 are indicated in **Figure 2**. Overall, in every cultivar of soybean analyzed, independent of the year of harvest, spermidine was the predominant amine, followed by spermine. A spermidine-to-spermine ratio of ~3.3 was observed for every cultivar analyzed. The mean contribution of the other amines to total levels indicated the predominance of agmatine and cadaverine, followed by putrescine. However, when the cultivars were considered individually, the contribution of the amines present in smaller amounts to total levels varied. Therefore, the contributions of the amines present in smaller amounts—agmatine, cadaverine, and putrescine—to total levels were affected by the cultivar and also by the year of harvest. These results suggest that there are other factors which can affect the profile and levels of amines besides the cultivar.

The higher contribution of spermidine over spermine to total levels in soybean was also observed by Bardócz et al. (*2*) and Okamoto et al. (*6*). The levels of spermidine in the different cultivars were similar to those reported in the literature, but the levels of spermine were higher and those of putrescine lower

(*2*, *6*, *23*). The higher spermidine levels observed in soybean compared to other vegetables corroborate findings that spermidine levels are high in seeds, which are responsible for the preservation of the species (*2*, *6*, *8*−*10*, *18*, *27*).

Even though it has been reported that the profile and levels of amines in plants can be affected by different cultivars (*8*, *21*, *26*, *28*), this study was the first to attempt to determine the influence of different cultivars on the profile and levels of amine in soybeans. However, because the results indicated that there can be other factors affecting amine levels, more controlled experiments should be undertaken to determine the role of cultivar on amine levels in soybean.

According to the literature, pre- and postharvest factors affect amine levels in vegetables. Various types of stress can affect amine levels, among them, water availability (*29*), mineral deficiency (*9*), acid-, herbicide-, and ozone-caused damages (*30*), osmotic shock, temperature or variation in altitude, and chilling injury (*31*, *32*). These reports have demonstrated that most of the stress conditions resulted in an increase in polyamine and putrescine levels. Ripening conditions have also been observed to affect polyamine (*25*, *33*) and biogenic amine levels (*34*). According to Moret et al. (*16*) and Simon-Sarkadi et al. (*35*), storage conditions can affect spermidine and putrescine levels in leaf vegetables.

The samples were obtained from the western area of the state of Minas Gerais, so it is unlikely that geographical conditions could have affected amine levels in the different cultivars. However, cultivation practices and postharvest factors could have varied. The results indicated significantly higher spermidine, spermine (polyamines), and putrescine levels in most of the cultivars in 2004. On the basis of the fact that the levels of these amines increase under stress conditions, soybeans harvested in 2004 could have been exposed to more stressful conditions than those harvested in 2003. Studies are necessary to investigate the influence of these parameters on bioactive amines in soybeans.

In conclusion, five bioactive amines were detected in soybeans—spermidine, spermine, putrescine, agmatine, and cadaverine. Spermidine was the predominant amine, followed by spermine, in every cultivar analyzed. High concentrations of polyamines were found in soybeans, confirming that this seed is a rich source of the polyamines. Cadaverine was observed to be inherent to soybeans, a specific characteristic of plants from the Leguminoseae family.

The predominance of spermidine over spermine was not affected by the cultivar or by the year of harvest. However, the contribution of agmatine, cadaverine, and putrescine to total amine levels varied significantly with the cultivar and year of harvest.

The levels of amines in soybean were affected by both cultivar and year of harvest. However, the influence of the cultivar and the year of harvest on the levels of bioactive amines in soybeans could not be established. Therefore, experiments under more controlled conditions should be performed to elucidate the role of cultivars and climate, as well as cultivation, harvest, and storage conditions on the levels of bioactive amines in soybeans.

Influence of Soybean Germination on Amine Levels. Pictures of the soybean during the early stages of germination are shown in **Figure 3**. In the first day (24 h), the seeds were turgid because of water absorption. However, no germination structures were seen. In the second day (48 h), it was possible to see a radicle coming out of the cotyledon. After the third day (72 h), the soybean seedling could be divided into radicle (or root, depending on the stage of development), hypocotyl, and cotyledon.

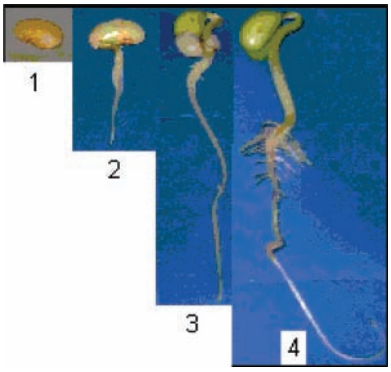

Figure 3. Photographs of soybean during 4 days of germination at 24.5 ± 0.5 °C.

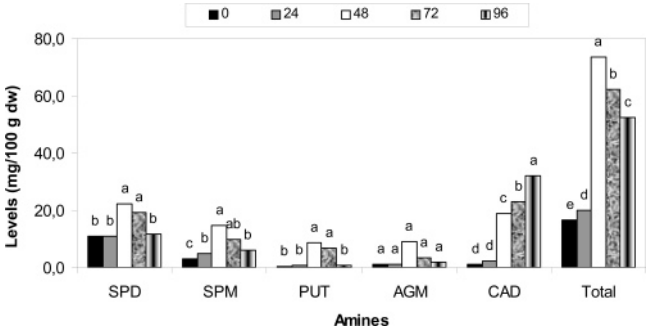

Figure 4. Levels of bioactive amines during soybean germination at 24.5 ± 0.5 °C for 96 h. dw, dry weight basis; SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; CAD, cadaverine. Mean values with different letters for each amine are significantly different (Duncan test, $p \leq 0.05$).

The moisture content of soybean increased significantly during germination. In the first day, the moisture had increased from 6.28 to 55.14 g/100 g, reaching 62.16 g/100 g in the second day and 68.84 g/100 g in the third day of germination. No significant difference was observed in the moisture content of the sprout between the third and fourth days. When the different structures are considered, the moisture content increased significantly in the radicle, reaching 91.78 g/100 g in the third day. No significant difference was observed in moisture content between the radicle and the hypocotyl in the third day of germination. The cotyledon contained similar moisture contents in the second and third days of germination (~65 g/100 g). Such a change in the moisture content during germination reinforces the need to express results on a dry weight basis when amine changes are investigated.

The soybean used in the germination studies contained five amines, with total levels of 16.61 mg/100 g. There was predominance of spermidine, followed by spermine, putrescine, agmatine, and cadaverine. As indicated in **Figure 4**, there was a significant change in amine levels during the early stages of germination. Total levels increased significantly in the first 48 h, reaching 73.68 mg/100 g, which is 6.6 times higher than the original levels. However, this level later decreased to 52.30 mg/100 g at 96 h. The same pattern of change was observed for spermidine, spermine, and putrescine; significantly higher levels were observed during 48−72 h of germination, decreasing afterward. Cadaverine levels increased significantly throughout the 96 h of germination. There was no significant change in the levels of agmatine during the germination of soybeans for 96 h.

Table 2. Levels of Bioactive Amines in Different Parts of Soybean during Germination at 24.5 ± 0.5 °C for 72 h

		mean amine levels,[a] mg/100 g of dw (coefficient of variation, %)					
day	part	SPD	SPM	PUT	AGM	CAD	total
1 (24 h)		11.09ab (8)	2.99ab (5)	0.37c (15)	1.07a (17)	1.09c (12)	16.61b (6)
2 (48 h)	cotyledon	13.55a (37)	3.84a (33)	0.35c (34)	1.39a (59)	0.43c (19)	19.6b (37)
	radicle	7.29bc (18)	0.89c (27)	2.15a (20)	0.75a (59)	99.7a (18)	111a (18)
3 (72 h)	cotyledon	9.82ab (11)	2.51b (14)	0.43c (2)	0.59a (20)	0.01d (17)	13.4b (10)
	hypocotyl	4.74c (31)	0.73c (38)	0.90bc (26)	0.50a (57)	26.6b (21)	33.4b (23)
	radicle	3.62c (21)	0.06c (17)	2.49a (17)	0.31a (79)	108a (15)	114a (15)

[a] Mean values with different letters in the same column are significantly different (Duncan, $p \leq 0.05$). dw, dry weight basis; SPD, spermidine; SPM, spermine; PUT, putrescine; AGM, agmatine; CAD, cadaverine.

The increase in total bioactive amines observed during germination was previously reported in different seeds. According to Simon-Sarkadi and Holzapfel (*12*) and Shalaby (*13*), amines are endogenously produced during the germination process. The significantly higher levels of spermidine, spermine, and putrescine observed in 48−72 h suggest that this is the period with the greatest cellular multiplication and growth. The concentrations of these amines are expected to increase in tissues with a high rate of development, as demonstrated in this experiment.

The presence of cadaverine in lentil, mung bean, and radish sprouts was reported by Simon-Sarkadi and Holzapfel (12). Shalaby (*13*) observed an increase in cadaverine levels during the germination of some leguminous seeds (bean, chick pea, and lupine), reaching a maximum value on the fifth day. Moret et al. (*16*) detected cadaverine and other biogenic amines in soybean sprouts. The presence of cadaverine in the sprouts could be associated with its role in the elongation of the root, for example, the increase in cell size (*8*). However, Simon-Sarkadi and Holzapfel (*12*) also observed a significant increase in Enterobacteriaceae and Pseudomonas spp. and reasoned that these microorganisms could also play a vital part in the metabolism of biogenic amines, especially putrescine and cadaverine. Germination occurs in a warm and moist environment, conducive to the rapid proliferation of microorganisms (*13*). Therefore, the increase in cadaverine levels could be explained by these two factors.

Analysis of the different structures of the seedlings indicated a heterogeneous distribution of amines during germination (**Table 2**). At 48 h of germination, two distinct structures were observed—cotyledon and radicle. There was a significant difference in the levels of most of the amines between the two structures, except for agmatine. The radicle contained significantly higher levels of putrescine and cadaverine, whereas the cotyledon contained higher levels of the polyamines spermidine and spermine.

At 72 h of germination, a third structure was observed—the hypocotyl. There was a significant difference in the levels of amines among the structures, except for agmatine. Significantly higher spermidine and spermine levels were detected in the cotyledon. The radicle contained the highest levels of putrescine and cadaverine followed by hypocotyl and cotyledon.

The accumulation of cadaverine in a specific part of the sprout weakens the hypothesis that it was produced by microbial contamination, which would have affected the whole seedling. Moreover, the significantly higher concentration of cadaverine in the radicle and in the hypocotyl supports the hypothesis of the importance of the cadaverine in cellular elongation (*36*).

In conclusion, during the first 96 h of germination, there was a significant change in bioactive amines in soybeans, reported on a dry weight basis. The levels of spermidine, spermine, and putrescine were significantly higher in 48 h of germination, whereas cadaverine levels increased significantly up to 96 h. The distribution of amines in soybean seedlings was heterogeneous. Significantly higher spermidine and spermine levels were present in the cotyledon, whereas putrescine and cadaverine accumulated in the radicle. Therefore, germination for 48 h can be used to increase polyamine levels in soybean. The interference from cadaverine can be eliminated by removing the radicle from the cotyledon. Germinated soybean cotyledon, which is richer in polyamine compared to soybean, could be used in food products or feeds in which high polyamine levels are desired.

LITERATURE CITED

(1) Glória, M. B. A. Amines. In *Handbook of Food Science*; Hui, H., Nollet, L. L., Eds.; Dekker: New York, 2005.

(2) Bardocz, S.; Grant, G.; Brown, D. S.; Ralph, A.; Pusztai, A. Polyamines in food—implications for growth and health. *J. Nutr. Biochem.* **1993**, *4*, 66−70.

(3) Maccarrone, M.; Baroni, A.; Finazzi-Agró, A. Natural polyamines inhibit soybean (*Glycine max*) lipoxygenase-1, but not lipoxygenase-2 lysozyme. *Arch. Biochem. Biophys.* **1998**, *1* (1), 35−40.

(4) Smith, T. A. Phenethylamine and related compounds in plants. *Phytochemistry* **1977**, *16*, 9−18.

(5) Wheaton, T. A.; Stewart, I. The distribution of tyramine, *N*-methyltyramine, hordenine octopamine and synephrine in higher plants. *Lloydia* **1970**, *33* (2), 244−254.

(6) Okamoto, A.; Sugi, E.; Koizumi, Y.; Yanagida, F.; Udaka, S. Polyamine content of ordinary foodstuffs and various fermented foods. *Biosci., Biotechnol., Biochem.* **1997**, *61* (9), 1582−1584.

(7) Torrigiani, P.; Bregoli, A. M.; Ziozi, V.; Scaramagli, S.; Ciriaci, T.; Rasori, A.; Biondi, S.; Costa, G. Pre-harvest polyamine and aminoethoxyvinyglycine (AVG) applications modulate fruit ripening in stark red gold nectarines (*Prunus persica* l. Batsch). *Postharvest Biol. Technol.* **2004**, *33*, 293−308.

(8) Flores, H. E.; Protacio, C. M.; Signs, M. W. Primary and secondary metabolism of polyamines in plants. *Phytochemistry* **1989**, *23*, 329−393.

(9) Smith, T. A. Polyamines. *Annu. Rev. Plant Physiol.* **1985**, *36*, 117−143.

(10) Starling, M. F. V. Perfil e teores de aminas biogênicas em hortaliças. M.Sc. Dissertation, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, 1998.

(11) Adjei-Twum, D. C. Studies on the germination, growth, and development of soybean (*Glycine max* Merr. L.) used as a vegetable. Ph.D. Thesis, University of Illinois, Urbana-Champaign, IL, 1976.

(12) Simon-Sarkadi, L.; Holzapfel, W. H. Biogenic amines and microbial quality of sprouts. *Z. Lebensm.-Unters. Forsch.* **1995**, *200*, 261−265.

(13) Shalaby, A. R. Changes in biogenic amines in mature and germinating legume seeds and their behavior during cooking. *Nahrung* **2000**, *44*, 23−27.

(14) Nout, M. J. R.; Ruikes, M. M. W.; Bouwmeester, H. M.; Beljaars, P. R. Effect of processing conditions on the formation of biogenic amines and ethyl carbamate in soybean tempe. *J. Food Saf.* **1993**, *13*, 293−303.
(15) Kim, J. H.; Kim, D. H.; Ahn, H. J.; Park, H. J.; Byun, M. W. Reduction of the biogenic amine contents in low salt-fermented soybean paste by gamma irradiation. *Food Control* **2004**, *16*, 1, 43−49.
(16) Moret, S.; Smela, S.; Populin, T.; Conte, L. S. A survey on free biogenic amine content of fresh and preserved vegetables. *Food Chem.* **2004**, *89* (3), 355−361.
(17) Buts, J. P.; Keyser, N. D.; Raedemaeker, L. D.; Collette, E.; Sokal, E. M. Polyamine profiles in human milk, infant artificial formulas and semi-elemental diets. *J. Pediatr. Gastroenterol. Nutr.* **1995**, *21* (1), 44−49.
(18) Kalac, P.; Krausová, P. A review of dietary polyamines: formation, implications for growth and health and occurrence in foods. *Food Chem.* **2005**, *90*, 219−230.
(19) Lima, A. S.; Glória, M. B. A. Aminas bioativas em alimentos. *Bol. SBCTA* **1999**, *33* (1), 70−79.
(20) Association of Official Seed Analysts. Rules for testing seeds. *Seedling Evaluation Handbook*; AOSA: Lincoln, NE, 1999.
(21) Cirilo, M. P. G.; Coelho, A. F. S.; Araújo, C. M.; Gonçalves, F. R. B.; Nogueira, F. D.; Glória, M. B. A. Profile and levels of bioactive amines in green and roasted coffee. *Food Chem.* **2003**, *82*, 397−402.
(22) Instituto Adolfo Lutz. *Normas Analíticas do Instituto Adolfo Lutz*, 3rd ed.; IAL: São Paulo, Brazil, 1985.
(23) Kalac, P.; Krizek, M.; Pelikánová, T.; Langová, M.; Veskrna, O. Contents of polyamines in selected foods. *Food Chem.* **2005**, *90*, 561−564.
(24) Matsuzaki, S.; Hamana, K.; Isobe, K. Occurrence of N6-methylagmatine in seeds of leguminous plants. *Phytochemistry* **1990**, *29* (4), 1313−1315.
(25) Gonzalez-Aguilar, G. A.; Zacarias, L.; Lafuente, M. T. Ripening affects high-temperature-induced polyamines and their changes during cold storage of hybrid fortune mandarins. *J. Agric. Food Chem.* **1998**, *46*, 3503−3508.
(26) Walters, D. R. Polyamines and plant disease. *Phytochemistry* **2003**, *64* (1), 97−107.
(27) Eliassen, K. A.; Reistad, R.; Risoen, U.; Ronning, H. F. Dietary polyamines. *Food Chem.* **2002**, *78*, 273−280.
(28) Glória, M. B. A.; Watson, B. T.; Simon-Sarkadi, L.; Daeschel, M. A. A survey of biogenic amines in Oregon Pinot Noir and Cabernet Sauvignon wines. *Am. J. Enol. Vitic.* **1998**, *49* (3), 279−282.
(29) Coelho, A. F. S.; Gomes, E. D.; Sousa, A. P.; Glória, M. B. A. Effect of irrigation level on yield and bioactive amine content of American lettuce. *J. Sci. Food Agric.* **2005**, *85*, in press.
(30) Conca, R.; Bruzzoniti, M. C.; Mentasti, E.; Sarzanini, C.; Hajos, P. Ion chromatographic separation of polyamines: putrescine, spermidine and spermine. *Anal. Chim. Acta* **2001**, *439*, 107−114.
(31) Serrano, M.; Martines-Romero, D.; Guillén, F.; Valero, D. Effects of exogenous putrescine on improving shelf life of four plum cultivars. *Postharvest Biol. Technol.* **2003**, *30*, 259−271.
(32) Valero, D.; Martínez-Romero, D.; Serrano, M.; Riquelme, F. Postharvest gibberellin and heat treatment effects on polyamines, abscisic acid and firmness in lemons. *J. Food Sci.* **1998**, *63* (4), 611−615.
(33) Rodrigues, S. D. C.; López, B.; Chaves, A. R. Changes in polyamines and ethylene during the development and ripening of eggplant fruits (*Solanum melongena*). *J. Agric. Food Chem.* **1999**, *47*, 1431−1434.
(34) Adão, R. C.; Glória, M. B. A. Bioactive amines and carbohydrates changes during ripening of banana cv 'Prata' *Musa acuminata* × *M. balbisiana*. *Food Chem.* **2004**, *90*, 705−711.
(35) Simon-Sarkadi, L.; Holzapfel, W. H.; Halász, A. Biogenic amine content and microbial contamination of leafy vegetable during storage at 5 °C. *J. Food Biochem.* **1994**, *17*, 407−418.
(36) Wuebker, E. F.; Mullen, R. E.; Koehler, K. Flooding and temperature effects on soybean germination. *Crop Sci.* **2001**, *41*, 1857−1861.

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