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Effect of supplemental Ca^{2+} on yield and quality characteristics of soybean sprouts

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ABSTRACT

Effects of supplemental Ca^{2+} on growth and selected qualities of soybean sprout were investigated. Ca^{2+}-treated sprouts had 40 ~ 47% higher length and 31 ~ 39% higher yield than water-treated ones. Increment of endogenous indoleacetic acid and gibberellin in Ca^{2+}-treated soybean sprouts possibly contributed to the improved growth. Metabolism of selected anti-nutritional factor and bioactive substances in soybean sprouts was strengthened by Ca^{2+}. Phytic acid content of Ca^{2+}-treated soybean sprouts was 33 ~ 49% lower than that of the control. Supplemental Ca^{2+} increased content of gamma-aminobutyric acid and isoflavone by improving activity of diamine oxidase and isoflavone synthetase, respectively. Soybean sprouts produced by soaking Ca^{2+} and spraying Ca^{2+} contained more ascorbic acid and phenolics and hence exhibited enhanced antioxidant capacity compared to the control. Besides, no adverse change on protein content and amino acid composition was observed in Ca^{2+}-treated sprouts. These findings indicate that supplemental Ca^{2+} can increase soybean sprout yield and improve its nutrition qualities.

1. Introduction

Soybean sprout as a kind of year-round vegetable is popular in Asia. In recent years, the consumption of sprouts has been growing, because they are often perceived as a part of a healthy diet. First of all, soybean is an important source of protein and unsaturated fatty acids in Chinese diet. In addition, soybean sprouts are rich in health-promoting phytochemicals, such as isoflavone and gamma-aminobutyric acid (GABA), compared with their mature counterparts (Phommalth et al., 2008). Like other vegetables, the antioxidant compound (ascorbic acid, phenolics, etc.) content of soybean sprout is also high, which in turn can provide protection against oxidative damage during germination (Silva et al., 2013). Apart from beneficial substances, soybean is also an important source of antinutritional factors, such as phytic acid and trypsin inhibitor. Phytic acid is an active chelator of divalent metal ions in plant seeds, majority of which can be decreased by long- term germination (Kumar et al., 2010). In China, Zn, Fe, and Ca bioavailability of most soy products is inhibited by residual phytic acid (Ma et al., 2007), while little attention is focused on that of soybean sprouts. In addition, phytic acid can form complexes with proteins at low and high pH values. These complexes can alter protein structure, decrease protein solubility and inhibit protease activity, and hence result into the decrement of protein digestibility (Kumar et al., 2010).

Mature soybean seed contains a number of enzymes which are synthesized during its development (referred to as pre-formed enzymes). They are important for fundamental biology for ensuring the early progression of germination (Bau et al., 1997). The pre-formed enzymes will mobilize polymerized forms, such as concentrated starch and protein, into monomers which can be readily used by human body. This process improves the nutritional value of soybean significantly. Ca^{2+} is a central regulator of plant growth and development (Hepler, 2005). It has been reported that Ca^{2+} involved in the activation of some enzymes. A well-known example was that Ca^{2+} greatly facilitated the appearance of gibberellin-induced α-amylase in the medium, 20 mM of which stimulated an 18-fold increase compared to the water control (Jones, 1973). Besides, a Ca^{2+}-activated alkaline phytase was found in pollen of *Lilium longiflorum* (Scott and Loewus, 1986). The Ca^{2+}-stripped alkaline phytase of *Bacillus* sp. MD2 exhibited less than 2% of the original activity, but the activity was fully restored when the enzyme was re- charged with 1 or 5 mM Ca^{2+} (Tran et al., 2011). In contrast, some studies reported the decreased intestinal phytase activity in animals and microorganisms at high Ca^{2+} levels (Selle et al., 2009). To the most of our knowledge, apart from α- amylase,

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little information is available on the effect of Ca^{2+} on hydrolytic enzymes, such as protease and phytase, which are involved in catabolism during soybean germination. Soybean sprout is packed with key nutrients like GABA, isoflavone, and so on, which are synthesized in a large amount during germination, but less work has been done on the effect of Ca^{2+} on their synthesis.

Plant hormones have pivotal roles in plant growth, development, and response to biotic and abiotic stresses, each of which has characteristic biological effects (Miransari and Smith, 2014). In past researches, Ca^{2+} was found to inhibit cytokinin stimulation of synthesis of anthocyanin (Elliott, 1977) and betacyanin (Elliott, 1979). However, another study identified a Ca^{2+}/kinetin/ethylene connection and reported that Ca^{2+} stimulated kinetin metabolism and caused a striking increase of ethylene in mung bean hypocotyl segments (Lau and Yang, 1975). During germination, the accumulated indoleacetic acid (IAA) in seed cotyledon was the major source of IAA for seedling, transportation of which was reduced by low extracellular Ca^{2+} (Dela Fuente and Leopold, 1973). An supplemental Ca^{2+} research on tomato pedicel explants further confirmed that supplemental Ca^{2+} played a positive role in regulating enzyme activity and agronomic traits by mediating hormone balance (Xu et al., 2009). Thus, it is hypothesized that regulation of enzyme activity and growth by Ca^{2+} appears to need involvement of hormones.

At present, supplemental Ca^{2+} has been supplied on fruits and vegetables to keep them fresh. Balanced and timely application of calcium sources for fruit and vegetable crops during growing seasons and at postharvest stages, will improve the shelf life and nutritional quality of horticultural products (Aghdam et al., 2012). Regardless of places and seasons, soybean sprouts can easily be grown in a short period. Unlike other vegetables, the effects of Ca^{2+} on soybean sprout, such as yield and nutritional quality, have received little attention. Soybean has a high amount of calcium content, but most of which is unavailable for being chelated by phytic acid. In this study, supplemental Ca^{2+} was added during production of soybean sprouts, the effects of Ca on yield and nutrition characteristics were evaluated. In addition to Ca, the other factor, variety, was also been taken into account, soybean varieties cultivated in different regions were chosen as experimental materials.

2. Materials and methods

2.1. Materials and reagents

Two soybean genotypes, namely tianjiao and nannong (cultivated in Heilongjiang and Liaoning provinces of China, respectively, and harvested in October 2014) were purchased from Nanjing Agricultural Food Co. (Jiangsu, China) and stored at 4 °C until used. Pepsin, pancreatin, lipase and standard substance (gibberellin, IAA, zeatin, sodium phytate, GABA and isoflavones) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA). Acetonitrile, phosphoric acid, methanol, and formic acid were of high performance liquid chromatography (HPLC) grade. All other chemicals used were of analytical reagent grade. $CaCl_2$ (Calcium chloride) is naturally occurring, edible, and inexpensive, which has been approved by the China Food and Drug Administration for use in soy products. Hence, $CaCl_2$ was chose as the source of supplemental Ca^{2+} in this study.

2.2. Germination of seeds

Seeds were sterilized using 1% of sodium hypochlorite for 15 min, washed, and steeped in deionized water/$CaCl_2$ solution at a ratio 1:5 (w/v) at 30 °C for 12 h. The above soaked seeds were germinated at 30 °C for 96 h in a semi-automatic germination machine (Zhejiang Beixin Technology, Yongkang, China). They were automatically sprayed with deionized water/$CaCl_2$ solution for 2 min per h. The design of experiment was summarized in Table 1.

When germination ended, soybean sprouts were carefully collected and rinsed with distilled water. The fresh ones were used to determine length, diameter, yield, enzyme activities, antioxidant capacity, ascorbic acid, total phenolics, and hormones. The freeze dried ones were ground into 60-mesh flour, using a handheld laboratory flourmill (IKA, Staufen, Germany), and then used to determine content of phytic acid, inorganic P, protein, amino acid, GABA, isoflavone, and minerals.

2.3. Measurement of growth and quality parameters

2.3.1. Measurement of length, thickness, and yield

Length and thickness were measured using a ruler and micrometer caliper, respectively. Thirty sprouts were set as a sampling group for each measurement. Yield was expressed as the sprout weight germinated from 100 g of dry soybean seeds.

2.3.2. Determination of endogenous hormone content

The content of gibberellin, IAA, zeatin, and brassinosteroid in whole soybean sprout was assayed using the enzyme linked immunosorbent assay (ELISA) kits (Nanjing Senbeijia Biochemistry Biotech Inc., Jiangsu, China).

2.3.3. Phytase, protease, glutamate decarboxylase (GAD), diamine oxidase (DAO), isoflavone synthetase and antioxidant capacity determinations

Phytase activity was measured as described by Chiera et al. (2004). Soybean sprouts were frozen in liquid nitrogen and ground with a mortar and pestle. Total protein was extracted using a sodium acetate buffer (pH 4.5). The extraction solution was cleared by centrifugation and supernatant was collected. Total soluble protein content was measured according to Yang et al. (2013). Phytase assays were performed by incubating the protein extracts with sodium phytate, pH 5.5 at 55 °C for 15 min. After stopping phytase activity, the samples were analyzed for phosphorus (P) by measuring absorbance at 405 nm. Phosphorus concentrations were calculated from a standard curve determined using known concentrations of potassium phosphate. Activity (1 U) was defined as the release of 1 mol of P from sodium phytate per minute and expressed as U mg^{-1} protein.

Protease activity was assayed according to the method described by Li et al. (2010). Protease activity was routinely measured as the increase of absorbance at 275 nm in terminated enzyme reaction solution and calibrated against the absorbance of tyrosine at 275 nm. One unit of protease activity was defined as increase of one absorbance unit under the assay conditions.

GAD activity was determined according to Bai et al. (2009). Soybean sprout (≈1.00 g) was ground with 8 mL of potassium phosphate buffer on an ice bath. Then, the homogenate was centrifuged and the supernatant was crude GAD. The reaction mixture included crude enzyme liquid and substrate. The reaction solution was incubated at 40 °C for 2 h and then terminated at 90 °C for 5 min. GABA content of the centrifugal suspension was determined by the GABA determination method, as mentioned below. One unit of enzyme activity was defined as the release of 1 μmol of GABA produced per hour at 40 °C.

DAO activity was determined according to Yang et al. (2011). Germinated soybean (≈1.00 g) was ground with 8 mL of potassium phosphate buffer (pH 6.5). The centrifugal supernatant fluid was crude DAO. The reaction mixtures, including 2 mL of potassium phosphate buffer, 0.1 mL of peroxides solution, and 0.5 mL of enzyme extract, were incubated at 30 °C for 5 min. Then, 0.1 mL of putrescence solution was added to start enzymatic reaction.