

Soybean Sprouts: A Review of Nutrient Composition, Health Benefits and Genetic Variation

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ABSTRACT Soybean [*Glycine max* (L.) Merr.] sprouts are highly digestible and a year around vegetable suitable for human consumption. Sprouting process causes a number of biochemical changes inside the seed, resulting in the accumulation of various primary and secondary metabolites. Due to such changes, sprouts contain high levels of health-promoting phytochemicals as compared to other vegetables. Sprouts are an excellent source of protein, amino acids, and vitamins, which provide numerous health benefits. Due to such advantages, soybean sprouts have been preferred as a part of daily diets in Korea and quality soybean sprouts are in high demand in the edible food market. To produce high quality soybean sprouts, several factors, including the choice of the variety, health benefits from sprout phytonutrients and inherent genetic variation for the sprout-related traits of the variety need to be considered. In this review, we have summarized literature on soybean sprout components, the health benefits, changes in nutritional factors during the sprouting process and the genetic variation among the cultivars developed for sprout usage. We have also reviewed procedures and factors like seed characteristics, temperature, chemical applications that influence the sprouting process. The information collectively presented here will be useful for understanding the progress of soybean cultivars developed for soybean sprout development and use.

Keywords Soybean, Sprout

INTRODUCTION

Sprouting, the germination stage of a seed, is a growing market for use of soybeans as an edible and nutritious vegetable. Crops like soybean [*Glycine max* (L.) Merr.], mungbean (*Vigna radiata*), alfalfa (*Medicago sativa*) are highly suitable for sprout production and human consumption (Silva *et al.* 2013). Sprouts can be prepared within 5-7 days from initial germination and can be available as a vegetable throughout the year (Silva *et al.* 2013). During the initial stages of germination, several biochemical changes occur inside the seed. For instance, macro-molecules such as proteins, polysaccharides and fats may break down into oligopeptides and free amino

acids, monosaccharides and oligosaccharides, and fatty acids, respectively (Bau *et al.* 1997). This process initiates the accumulation of several primary metabolites and increases the overall content of available nutrients, thereby improving the nutritive value of the sprouts.

Sprouts have several health benefits: 1) the sprouting process reduces the content of several unfavorable and anti-nutritional components like trypsin inhibitor and phytic acid related to problems in digestion (Doblado *et al.* 2007), 2) sprouts contain an increased amount of some of the bioactive compounds which have a role in protecting cells against damage caused by free radicals (Hochstein and Atallah 1988), 3) sprouting produces enzymes that aid in digestion, and 4) sprouting frees amino acids, fatty acids,

Received August 19, 2016; Revised October 30, 2016; Accepted October 30, 2016; Published November 30, 2016

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vitamins and minerals that otherwise would not be available for human nutrition.

Soybean sprouts (*Kongnamul* in Korea) have been preferred as a part of the daily diet in Korea, Japan and China over the years. The exact origin of *kongnamul* is unknown, but it is assumed that it has been eaten since the period of Three Kingdoms of Korea (B.C. 1-935) or the early Goryeo era (918-1392). Records of *kongnamul* can be found in the documents from the Goryeo era, *Hyangyak Gugeupbang* where cultivation of sprouts are mentioned. Taejo, the first King of Goryeo, and his soldiers were saved from starvation by growing bean sprouts in nearby streams. In the Joseon era (1392-1897) the document *Sallim gyeongje*, briefly refer to sprout cooking methods and in another Joseon era document *Seonghosaseol* it is stated that the poor used *kongnamul* to make *juk*. *Kongnamul* is again mentioned in *Cheongjangwanjeonseo* as the main food consumed during famines (<https://en.wikipedia.org/wiki/Kongnamul>).

There is increased demand for soybean varieties that efficiently produce high quality sprouts. Cataloguing information of the nutrient composition and health benefits of sprouts and genetic variation for sprout-related traits in soybean cultivars developed for sprout purposes would be highly useful for breeders and farmers. In this review, we conducted a comprehensive assessment of changes in nutritional factors during the sprouting process and their benefits for human health, factors affecting the sprouting process and the available genetic variation among the cultivars developed for soybean sprout production.

SOYBEAN AND SOYBEAN SPROUTS

Soybeans are an important part of the typical Korean diet due to the protein, oil and other components (Liu 1999). Soybean seed contains an average of 40% protein and 20% oil on a dry weight basis. The remaining 40% of the seed is comprised of carbohydrates, vitamins, phytochemicals, minerals and other minor elements. Different recipes like soymilk, soy sprouts, vegetable soybean and soy paste, served with rice are preferred among many oriental people. Among them, sprouts have been a common vegetable for centuries, particularly in areas where seasonal vegetables

were not available during the winter season (Shi *et al.* 2010; Yang *et al.* 2015). Soybean sprouts have been used in different recipes like soy sauce, mixing with rice, in soup and as a fresh salad. This diversity of consumption of soybean sprouts has increased demand of soybean varieties that produce high quality sprouts. Thick hypocotyls, high germination percentage, rapid water absorption, small seed size, and high yield are important factors in soybean sprout production (Kim *et al.* 1994; Park *et al.* 1994). Soybean sprouts have high nutritional value and are relatively easy to produce, making them a desirable vegetable (Lee *et al.* 2007a).

HEALTH BENEFITS AND NUTRITIONAL COMPOSITION OF THE SOYBEAN SPROUTS

Soybean sprouts contain nutritional components including isoflavones (Phommalth *et al.* 2008), riboflavin and niacin (Kyllen and McCready 1975) along with crude protein, amino acids and lipids (Mostafa and Rahma 1987). They also contain high amounts of macro- and micro-elements such as sodium, zinc, copper, potassium, iron, phosphorus, magnesium and manganese. These constituents are distributed in the hypocotyls and cotyledons in varied amounts depending on the soybean variety (Plaza *et al.* 2003; Youn *et al.* 2011). Consumption of soybean sprouts provide numerous health benefits like reducing the risk of cardiovascular diseases and cancer, due to the presence of several health-promoting phytochemicals with high antioxidant properties (Prakash *et al.* 2007). In addition, anti-nutritional factors like hemagglutinin, trypsin inhibitors, and lipoxygenase decrease during sprouting (Shi *et al.* 2010). Most of these compounds benefit human health and are discussed in details below.

Amino acid and protein content

Amino acids and proteins are the building blocks of life. Every cell in humans uses amino acids to synthesize proteins, which are essential to perform different functions like transport and storage of nutrients and development of cell structure, etc. (Zaman *et al.* 2010). Crude protein levels

of up to 46% have been reported in soybean sprouts (Bau *et al.* 1997), but levels vary during sprouting. Lee and Chung (1982) reported an increase in the crude protein content, whereas Choi *et al.* (2000b) reported a decrease in the crude protein levels after 5 days of sprouting.

Soybean sprouts contain high amounts of essential and non-essential amino acids (Oh *et al.* 2004; Suh *et al.* 2004; Oh *et al.* 2005; Park *et al.* 2005; Yun *et al.* 2005; Oh *et al.* 2006a; Oh *et al.* 2006b; Oh *et al.* 2007a; Oh *et al.* 2007b; Oh *et al.* 2007c; Oh *et al.* 2008; Phommalth *et al.* 2008; Cho *et al.* 2009; Oh *et al.* 2009a; Oh *et al.* 2009b; Kim *et al.* 2013) (Table 1). The concentration of the total amino acids varies across different soybean genotypes (Table 1) and during the days of sprouting (Song *et al.* 2000). For instance, sprouts from the variety Bosug contained higher amounts of amino acids than other cultivars. Another cultivar, Pungsannamulkong (Pungsannamul) (Suh *et al.* 1996) was found to contain 348 mg amino acids per gram of sprout (Oh *et al.* 2005). The cultivars Dagi and Sunam contained relatively lesser amounts of amino acids (Table 1). Free amino acid content was also found to vary during the sprouting process. Generally, most of the free amino acids increased during sprouting (Yang 1981). Soybean seeds collected from Japan, United States and China were

reported to have 437.2 mg, 452.2 mg and 367.2 mg/100 g of free amino acids and increased up to 12,768.8 mg, 10,845.9 mg, and 11,931 mg/100 g dry weight, respectively during the sprouting process (Mizuno and Yamada 2006). Particularly, the asparagine increased dramatically (Byun *et al.* 1977; Lee and Hwang 1996). Asparagine contents were 25% of dry weight after 15 days sprouting (Byun *et al.* 1977), and it increased from 42.9 mg/100 g in the seed to 7,423.3 mg/100 g in the sprout. The content of asparagine and aspartic acid varied among the different soybean varieties and showed positive correlation with sprouting days (Lee and Hwang 1996). Soybean sprout soup is one of best hangover foods in Korea because of its asparagine content, which is known to have detoxifying effects on acetaldehyde, a highly toxic metabolite produced in alcohol metabolism in humans (Lee and Hwang 1996). Significant increases have been observed in the concentrations of glutamate, histidine, alanine, proline, lysine, valine and isoleucine during sprouting (Friedman and Brandon 2001; Villaluenga *et al.* 2006). These reports show substantial variation for the amino acid content and composition, especially for the essential amino acids.

Table 1. Amino acid and isoflavone content in soybean sprouts in 17 soybean cultivars.

Cultivar	Amino acid (mg/g)		Isoflavone content ($\mu\text{g/g}$)				Reference
	Total	Essential	Daidzein	Glycitein	Genestein	Total	
Aga3	-	-	-	-	-	10,788	Phommalth <i>et al.</i> 2008
Anpyeong	44.5	-	-	-	-	2,679	Yun <i>et al.</i> 2005
Bosug	396	174	1,422	382	2,087	3,891	Oh <i>et al.</i> 2005
Dagi	42.8	-	1,073	774	1,722	3,569	Oh <i>et al.</i> 2004
Galchae	-	-	460	970	670	2,120	Oh <i>et al.</i> 2009a
Hoseo	133	56	1,160	-	1,240	2,600	Oh <i>et al.</i> 2008
Jangki	238	83	2,092	733	2,412	5,237	Oh <i>et al.</i> 2006a
Jonam	82	14	1,135	-	1,832	3,574	Oh <i>et al.</i> 2007a
Pungwon	169	69	2,587	2,959	-	5,935	Oh <i>et al.</i> 2007c
Sohwang	-	-	816	1,099	1,126	3,041	Cho <i>et al.</i> 2009
Sojin	46.6	-	-	-	-	3,765	Park <i>et al.</i> 2005
Sokang	295	127	1,612	777	2,129	4,518	Oh <i>et al.</i> 2006b
Sunam	43.5	-	391	93	1,160	1,644	Suh <i>et al.</i> 2004
Tawonkong	-	21	-	-	-	713	Kim <i>et al.</i> 2013
Wonheug	-	19	-	-	-	1,309	Kim <i>et al.</i> 2013
Wonhwang	174	63	2,052	764	2,554	5,370	Oh <i>et al.</i> 2007b
Wonkwang	104	46	1,493	-	1,685	3,481	Oh <i>et al.</i> 2009b

Oil and fatty acid content

Soybean oil is one of the most preferred vegetable oils used for food and other applications. Oil content ranges from 8.3% to 27.9%, with an average of 18.1% on a 13% moisture basis in soybean seed (Liu 1999; Wilson 2004). Soybean oil is a fat, and a soluble vitamin transporter throughout the body and helps adjust body temperature (Karasulu *et al.* 2011). Oil content decreased (Kim 1981) from 15% to 10% during sprouting (Shi *et al.* 2010). The quality of soybean oil is primarily associated with its fatty acid composition. Slight changes in fatty acid levels of sprouts have been reported. Mizuno and Yamada (2006) observed slight increases in palmitic acid, stearic acid and oleic acid contents but decreases in linoleic acid (LA) and α -linolenic acid (ALA) contents during sprouting. Lee *et al.* (2002) reported increase in the LA content, but a decrease in the ALA content in 5-day-old sprouts. Contrary to this, the ALA content increased (Dhakal *et al.* 2009) or did not change during sprouting (Dhakal *et al.* 2014). Similar results were found in sprouts of the soybean variety, Calland after 5 days of sprouting (Mostafa and Rahma 1987). Therefore, fatty acid composition of soybean sprouts depends not only on the cultivar, but also on other factors like the days of sprouting (Dhakal *et al.* 2009) and growth environment (Yang *et al.* 1982).

Isoflavone content

Isoflavones are a class of phytoestrogens, the plant-derived compounds that possess a wide range of biological activities (Shi *et al.* 2010). Although many plants are reported to contain isoflavones, four plants namely soybean, chickpea, onion and apple have been reported as the major sources of isoflavones (Dixon and Sumner 2003). Consumption of isoflavones provide various health benefits such as decreasing risks of cardiovascular disease, menopausal symptoms, protection from cancers, and bone resorption (Messina 2000; Allred *et al.* 2004; Prakash *et al.* 2007). Due to these advantages, selection for high isoflavones content in soybean seeds has been a goal of soybean breeders in recent years.

Total isoflavone content in soybean seed range from 0.05% to 0.5% of the dry weight (Lee *et al.* 2004). Several

studies have been conducted to reveal the type and quantities of isoflavones in soybean seed and sprouts (Messina *et al.* 1994; Anderson and Ganer 1997; Kim *et al.* 2003; Kim *et al.* 2006). Generally, the isoflavone content in soybean sprouts is higher than in the seed (Kim *et al.* 2003; Kim *et al.* 2004; Kim *et al.* 2006). Distribution of isoflavone content varies in roots, cotyledons and hypocotyls of soybean plants (Kim *et al.* 2003; Kim *et al.* 2006). High quantities of isoflavones have been observed in roots and hypocotyls compared to the other sprout components (Lee *et al.* 2007c). Large phenotypic variation for isoflavone content has been observed in soybean sprout genotypes (Table 1). For instance, 7-day-old sprouts produced from the cultivar Aga3 contained isoflavone concentration of 10,788 $\mu\text{g/g}$, compared to 3,556 $\mu\text{g/g}$ contained in the sprouts from Pungsannamul, the most favored sprout cultivar in Korea (Phommalth *et al.* 2008). The cultivars, Pungwon (Oh *et al.* 2007c) and Wonhwang (Oh *et al.* 2007b) had isoflavone contents of 5,935 $\mu\text{g/g}$ and 5,370 $\mu\text{g/g}$, respectively. Similarly, the cultivar Tawonkong (Kim *et al.* 1996) was reported to have low sprout isoflavone content (Kim *et al.* 2013). Isoflavone content in soybean seed and sprouts not only depends on the soybean cultivar, but it is also affected by the year of seed production, temperature, light and soybean field conditions (Hoeck *et al.* 2000; Xu *et al.* 2003; Seguin *et al.* 2004; Zhu *et al.* 2005). For instance, light caused an increase in the isoflavone content during sprouting (Chi *et al.* 2005).

Vitamin content

Vitamins are essential organic compounds having a high impact on human health. They are essential for plant and animal metabolism due to their role as enzymatic cofactors (Asensi-Fabado and Munné-Bosch 2010). Vitamin deficiencies may cause disorders which can be severe and even lethal in some cases. An adequate supply of vitamins can thus prevent such diseases and disorders (Kraemer *et al.* 2008). Since humans cannot synthesize vitamins, they must be acquired from their diets (Miret and Munné-Bosch 2014).

Soybean seeds are known to contain vitamin A, B1, E, and C (Collin and Sanders 1976). Many experiments have revealed that sprouting significantly improves content of

these vitamins (Plaza *et al.* 2003; Youn *et al.* 2011). For instance, Collins and Sanders (1976) reported that vitamin B1 content in soybean sprouts was 2-times higher than in the raw seeds. Also, vitamin C content increased 4 to 20 times during 4-5 days of germination (Plaza *et al.* 2003) (Table 2). Higher levels of vitamin C were observed in hypocotyls than in the cotyledons (Youn *et al.* 2011). Normally, the soybean seeds contains an average of 2 mg/100 g of vitamin C, but increased up to 11 mg/100 g after 5 days of sprouting (Liu 1999). Similarly, soybean seeds contained about 0.12 mg β -carotene/100 g, whereas 5-day-old sprouts contained about 0.2 mg/100 g (Liu 1999). Lee *et al.* (2013) reported an increase in the levels of the vitamins, lutein and β -carotene in whole sprouts of soybean varieties Pungsannamul and Bosug. They observed about a 20, 24 fold increase in the lutein levels and an 8, 17 fold increase in the β -carotene level, in Pungsannamul and Bosug, respectively. The average β -carotene content in soybean seed was 6.6 μ g/g whereas, in the 5-day-old sprouts, it was 33.3 μ g/g (Kang *et al.* 2012).

Saponin content

Saponins (glycosides) are the secondary plant metabolites, found in several plants and plant-derived foods

(Price *et al.* 1986). They provide several health benefits such as decreasing blood cholesterol levels (Lee *et al.* 2005), lowering blood glucose (Tanaka *et al.* 2006) and protecting against kidney diseases (Philbrick *et al.* 2003). Saponin content in soybean seed constitute about 0.5% of total dry weight (Fenwick and Oakenfull 1983) and range from 0.6% to 6.5% of dry weight (Tsukamoto *et al.* 1995). Oh *et al.* (2003) reported that crude saponin content in the cultivar Eunhakong (Shin *et al.* 1988) increased from 4.59 mg/g in the seeds to 5.33 mg/g in 6-day-old sprouts. Saponin content was highest in hypocotyls as compared to cotyledons and roots (Shimoyamada *et al.* 1990; Shimoyamada and Okubo 1991; Oh *et al.* 2003). Increases in levels of different kinds of saponins, like saponin group B (Oh *et al.* 2003; Jang and Han 2016), total soyasapogenol and soyasapogenol B (Kang *et al.* 2010) have been observed. Similarly, a decrease in the content of soyasapogenol A among 79 Korean cultivars was also observed (Kang *et al.* 2010). Such alterations in the saponin content seems to be highly influenced by the growing environments and may be determined by several factors like the type of cultivar, seed size, year of production, location grown and maturity (Rupasinghe *et al.* 2003). For instance, Yoshiki *et al.* (1998) reported increases in the saponin levels when the sprouting process was carried out in lighted

Table 2. Differences in the vitamin and mineral content in soybean seed and sprouts (Plaza *et al.* 2003).

Component		Dry seed	Sprouts ²⁾
Vitamins	Vitamin A (RE per 100 g d.w) ^{y)}	18.79	34.02
	Vitamin E (α -tocopherol μ g/g d.w)	0.89	5.91
	Vitamin B1 (thiamine μ g/g d.w)	0.47	2.01
	Vitamin B2 (riboflavin μ g/g d.w)	1.29	14.8
	Vitamin B6 (pyridoxal μ g/g d.w)	7.79	19.08
	Vitamin C (ascorbic acid μ g/g d.w)	99.51	316.44
	Minerals	Zn (μ g/g d.w)	23.93
Ca (μ g/g d.w)		810	2,770
Na (μ g/g d.w)		2,610	8,600
Mn (μ g/g d.w)		6.72	19.61
K (μ g/g d.w)		2,070	10,170
Cu (μ g/g d.w)		11.43	19.61
Mg (μ g/g d.w)		1,330	1510
Fe (μ g/g d.w)		48.87	35.29

²⁾Sprouts cultured for four days.

^{y)}Vitamin A content is calculated as retinol equivalents (RE= μ g β -carotene/6) per 100 g on dry weight (d.w) basis of seeds and sprouts.

conditions.

Sugar content

Soybean seed contains approximately 33% carbohydrates, up to 16.6% of which are soluble sugars (Hymowitz and Collins 1974). The soluble sugar fraction is mainly comprised sucrose, raffinose and stachyose (Yazdi-Samadi *et al.* 1977; Eldridge *et al.* 1979), which range from 41.3% to 67.5%, 5.2% to 15.8% and 12.1% to 35.2%, respectively (Yazdi-Samadi *et al.* 1977). Seed also contain glucose and fructose, whose concentration is <1% (Hymowitz and Collins 1974). Glucose, fructose and sucrose are considered desirable sugars due to the sweet taste and ease of digestion, but stachyose and raffinose are undesirable sugars, indigestible and cause flatulence and diarrhea (Wang *et al.* 2014). The sugar content in soybean seed is not stable across environments and wide variation has been observed among genotypes (Hartwig *et al.* 1997). The sugar content in soybean seed decreased during the sprouting process. Shi *et al.* (2010) found that sugar content in soybean seed was 19.9% but decreased to 14% after 7 days of sprouting. Similarly, Silva *et al.* (1990) reported more than 90% decreases in the stachyose and raffinose content in Brazilian soybean cultivar after four days of germination.

Mineral content

Soybean sprouts contain different minerals like Zn, Na, Fe, Ca, and others which are important for human nutrition. In sprouts, these minerals are largely distributed between hypocotyls and cotyledons in variable amounts (Youn *et al.* 2011). Differences in the soybean mineral concentrations between dry seed and sprouts are shown in Table 2. Contents of Zn, Ca, Na, Mn, K, and Cu increased significantly whereas Fe decreased during sprouting. Fe content in raw seed was 48.87 ($\mu\text{g/g}$ dry weight) whereas in 4-day-old sprouts, it was 35.29 ($\mu\text{g/g}$ dry weight) (Plaza *et al.* 2003). However, since the presence of minerals in soybean sprouts depend on soybean cultivar and sprouting conditions, slight variations may be observed (Bau *et al.* 1997).

FACTORS IMPORTANT FOR THE YIELD AND QUALITY OF THE SOYBEAN SPROUTS

Since the sprouting procedure is not unique and different approaches are used as a matter of convenience, numerous factors are known to affect the sprouting process. Generally, soybean sprouts are prepared as follows: well cleaned seeds are soaked in water to incubate for several hours at room temperature, and then placed in an appropriate vessel having small holes at the bottom for draining water. The vessels are kept in a dark place and watered several times a day. After 4-5 days, seedlings are washed thoroughly and de-hulled (Liu 1999; Lee *et al.* 2007a). During the sprouting process, many factors that affect growth and quality of the seedlings must be understood to produce sprouts with high quality and high yield. Some of the important factors are discussed below.

Seed size and quality

Seed size is one of the factors considered essential while choosing a soybean cultivar for sprout production. Small to medium-sized seeds are routinely preferred for sprouting because they usually have better and uniform germination. In addition, small seeded soybeans are known to produce sprouts with good taste and high yield (Liu 1999; Kim *et al.* 2000; Kim *et al.* 2004; Lee *et al.* 2007a). Soybeans having seed weight < 120 mg/seed are usually preferred for sprout production (Kwon *et al.* 1972). Soybeans having small seed size are known to have better water absorption, longer hypocotyls lengths, and a high germination percentage (Kim *et al.* 1994; Park *et al.* 1994). In addition to seed-size, other factors like seed coat color, purity and removal of broken/damaged or diseased seeds also affect sprout quality and length.

Light

Light can have negative effects on soybean sprout quality during germination. Light affects root elongation and initiates photosynthesis, turning the cotyledons a green color. Both, long roots and green cotyledons, are undesirable for soybean sprouts (Liu 1999; Shi *et al.* 2010). Generally, sprouts having shorter roots, white hypocotyls

of about 8-12 cm length and bright yellow cotyledons are greatly preferred by consumers and have high market value (Park *et al.* 1995; Lee *et al.* 2007a). Thus, performing the complete sprouting process in darkness, is crucial for minimizing undesirable sprouts.

Temperature and humidity

Air, water temperature and humidity have a profound effect on soybean germination, sprout quality and yield. Low temperatures cause negative effects on time of initial germination until sprout harvest, hypocotyl length, thickness and sprout quality (Tajiri 1980). A water temperature of more than 20°C is generally preferred during seed imbibition (Tajiri 1980; Lee *et al.* 2007a). Air temperature within the range of 20°C to 23°C during the incubation period (sprouting time) is recommended for good quality sprout production. Sprinkling sprouts with water after initiating germination is essential in reducing the temperature, removing the organic matter and providing oxygen inside sprout culture equipment. Therefore, water should be applied several times during sprouting (Park and Kim 1998). In addition, when the temperature of the water used for sprinkling is higher than 21°C, it may have some positive effects on hypocotyl length and diameter (Beeskow 1944). Koo *et al.* (2015) observed sprout whole lengths and hypocotyl lengths that were twice as long and higher in yield cultured at 25°C, as compared to those cultured at 20°C. The hypocotyl thickness was similar in the sprouts cultured at both the temperatures. Relative humidity of 80% is generally recommended to have an environment conducive to low seedling disease and uniform germination (Lee *et al.* 2007a).

Hypocotyls in soybean sprouts

The hypocotyl is a main part of sprouts along with cotyledons. The desirable hypocotyl length generally is in the range of 8-12 cm and hypocotyl thickness in the range of 2.0-2.2 mm at sprout harvest 5 days of sprouting, (Lee *et al.* 2007a). Many of soybean varieties developed for sprout purposes, have been reported to exhibit high phenotypic variation for the sprout characteristics like hypocotyl length and thickness. Characteristics of such cultivars developed for sprout usage since the year 2000 are listed in

the Table 3 (Baek 2001; Shin *et al.* 2002; Shin *et al.* 2003; Oh *et al.* 2004; Suh *et al.* 2004; Kim *et al.* 2005; Oh *et al.* 2005; Park *et al.* 2005; Yun *et al.* 2005; Oh *et al.* 2006a; Oh *et al.* 2006b; Oh *et al.* 2007a; Oh *et al.* 2007b; Oh *et al.* 2007c; Oh *et al.* 2008; Cho *et al.* 2009; Lee *et al.* 2009; Oh *et al.* 2009a; Oh *et al.* 2009b; Kim *et al.* 2013; Kim *et al.* 2014). Most of these varieties show hypocotyl lengths >8.8 cm and thickness >1.8 cm.

Sprout harvesting time

Harvesting time of the sprouts may vary depending on the factors such as germination rate and water temperature during imbibition and incubation. Also, seeds from old lots tend to show poor germination and poor growth, and may take more time to reach desired hypocotyl lengths. However, under most conditions, sprouts can be ready for harvest after 5-7 days after the start of germination. Delaying harvest may have negative impacts on sprout quality, often due to the undesired growth of lateral roots and leaves (Liu 1999; Silva *et al.* 2013).

Chemical treatment effects on soybean sprouts

Soybean sprouts may have a number of problems such as poor growth, low quality, excessive lateral root growth, and seedling rot, negatively affecting total yield and market value of sprouts. To control these factors, chemical compounds like hormones were tested for their effect on sprout quality and yield. Lee and Chung (1982) observed that soybean sprouts, treated with growth regulators like indole-3-acetic acid (IAA) and benzyladenopurine (BA) had relatively shorter roots than the controls, but fresh weight and diameter of the sprouts increased. In addition, substantial increases in crude protein, vitamin C and fat content of the sprouts were observed. Kang *et al.* (2004) reported increases in the hypocotyl to root length ratio, when soybean sprouts were treated with different BA concentrations. Inhibition of lateral roots in soybean sprouts treated with BA were observed by Kang *et al.* (1989). These results showed that treating soybean sprouts with growth regulators, IAA and BA produced sprouts with short root length, a desirable character favored in sprout production. Significant increases in useful sprout components like yield, weight, hypocotyl thickness, vitamin C

Table 3. Seed and sprout characteristics of 22 soybean varieties released from 2000 to 2010 used in sprout production.

Cultivar	Pedigree (maternal/paternal)	Year released	Soybean seed ²⁾				Soybean sprout ³⁾						Reference
			SC	HSW (g)	Yield (t/ha)	Lodging	HL (cm)	HD (mm)	Rate of rot (%)	RPG (%)	HSR (%)	SY (%)	
Saeyeolkong	Bukwangkong/Namhaekong	2000	Y	12.9	2.37	3.5	9.7	1.90	-	-	-	620	Baek <i>et al.</i> 2001
Sorogkong	Pureunkong/Namhaekong	2001	G	11.9	2.57	-	8.1	2.50	-	-	-	629	Shin <i>et al.</i> 2002
Anpyeong	Eunhakong/SS88034	2002	Y	11.6	2.53	Slight	-	-	-	-	-	638	Yun <i>et al.</i> 2005
Dagi	Namhaekong/D70-6545	2002	Y	11.7	2.63	2	8.9	2.37	0.01	-	-	621	Oh <i>et al.</i> 2004
Sunam	Kosuzu/Bukwangkong	2002	Y	9.3	2.22	3	8.8	2.22	0.02	1.4	-	658	Suh <i>et al.</i> 2004
Dachaekong	Hannamkong/Eunhakong	2002	Y	8.6	2.26	1	10.3	2.33	-	-	-	744	Shim <i>et al.</i> 2003
Bosug	Namhaekong/Camp	2003	Y	8.6	2.62	2	11.1	2.23	0.05	3.0	0	678	Oh <i>et al.</i> 2005
Sojin	Danyeopkong/SI93001	2003	Y	9.7	2.54	Slight	-	-	-	-	-	661	Park <i>et al.</i> 2005
Nogchae	Pureunkong/Milyang 44	2004	G	9.2	2.80	1	12.7	2.11	0.1	4.8	0	686	Kim <i>et al.</i> 2005
Sokang	Namhaekong/Camp	2004	Y	9.5	2.44	2	10.4	2.31	0.1	2.5	0	669	Oh <i>et al.</i> 2006a
Jangki	Eunhakong/MS91088	2005	Y	11.9	2.83	1	11.1	2.40	0.1	2.1	0	656	Oh <i>et al.</i> 2006b
Wonhwang	Camp/Myeongjunamulkong	2005	Y	10.0	2.80	2	10.3	2.20	0.1	2.4	0	668	Oh <i>et al.</i> 2007b
Jonam	Eunhakong/Jeonju-11	2006	Y	9.6	2.57	1	13.9	2.10	0	0.7	0	720	Oh <i>et al.</i> 2007a
Pungwon	SI93001/Suwon 164	2006	Y	10.9	3.04	1	11.4	2.20	0.2	0.6	0	704	Oh <i>et al.</i> 2007c
Hoseo	Camp/Nattosan	2007	Y	7.4	2.51	1	10	1.80	0.1	0.9	0	689	Oh <i>et al.</i> 2008
Wonkwang	Danyeopkong/MS91001	2007	Y	10.9	3.05	1	10.1	2.00	0.2	2.5	0	655	Oh <i>et al.</i> 2009b
Galchae	Ys1287/Jinju1	2008	Br	8.4	2.51	3	12.4	1.80	0	1.8	0	755	Oh <i>et al.</i> 2009a
Sohwang	Pungsannamul/Nattosan	2008	Y	8.5	2.69	2	10.1	1.90	0.2	1.5	0	713	Cho <i>et al.</i> 2009
Singang	Sowon/L29	2008	Y	11.0	2.78	2	8.8	2.00	-	-	-	635	Lee <i>et al.</i> 2009
Wonheug	Tawonkong/Jinju1	2009	B	8.8	2.34	3	11.4	1.90	-	3.6	0.1	717	Kim <i>et al.</i> 2013
Joyang 1	Pungsannamul/Suwon 187	2010	Y	11.6	3.03	1.7	11.2	2.20	1.6	0	0.2	547	Kim <i>et al.</i> 2014
Pungsannamul ³⁾	Bansakong/KLS87092	1996	Y	11.3	2.93	2.1	11.8	2.20	1.1	-	3.7	494	Kim <i>et al.</i> 2014

²⁾SC and HSW stand for seed coat color (B: black, Br: Brown, G: green, Y: yellow), and hundred seed weight, respectively. Lodging: 1-tolerance, 3-intermediate, 5-susceptible.

³⁾HL, HD, RPG, HSR, and SY stand for hypocotyl length, hypocotyl diameter, rate of poor germination, hard seed rate and sprout yield, respectively.

⁴⁾Pungsannamul is the most favored sprout cultivar in Korea and used as a check by the breeders for developing the new cultivars for sprout purpose.

and isoflavone contents were observed, when 6-day-old soybean sprouts were treated with carbonated water (pH 4.5) (Hwang 2012). Similar results were obtained when soybean sprouts were treated with chitosan, a linear polysaccharide and a natural antimicrobial compound (Lee *et al.* 1999b), and phosphate buffer (Choi *et al.* 2000a). Utilization of natural chemicals that act as plant growth regulators can help to enhance the efficiency of the soybean sprout production.

BREEDING SOYBEAN VARIETIES FOR SPROUT USAGE

Soybean is called the king among legume crops in terms of importance, agronomic traits, chemical composition, seed color, seed size and other characters (Liu 1999). Korea has a long history of inclusion of soybean sprouts in their food culture (<https://en.wikipedia.org/wiki/Kongnamul>). Before the start of modern breeding, several local varieties with small seed size and yellow colored seed coats were utilized for sprout production (Kwon *et al.* 1981; Lee *et al.* 1992; Jeong *et al.* 2007).

Breeding for sprout soybeans, breeders used several criteria such as, having a 100-seed weight of around 10 g, seed with good germination (more than 90% germination after harvesting) and seed vigor, bright hypocotyl color, yellow cotyledons, increased hypocotyl length, and sprout yield (sprout yield % = weight of sprout × 100 / initial seed weight). In 1970, the two cultivars, Danyeobkong (Essex) and Hill were introduced from the US to Korea for cultivation of soybean sprouts (Lee *et al.* 2015). The variety Eunhakong (Shin *et al.* 1988), first bred in Korea for soybean sprouts was released in 1986. Before the 1990s, only three varieties, Pangsakong (Hong *et al.* 1985), Eunhakong (Shin *et al.* 1988) and Namhaekong (Shin *et al.* 1989) were bred for sprout purposes (Lee *et al.* 2015). During the late 1990s soybean breeders expanded their breeding efforts to improve soybeans for sprout usage because of increased consumption and demand for sprouts as a vegetable. After the 1990s, about 41 varieties were developed for sprout production in Korea. Out of the 41 varieties, 16 varieties had small seeds and with 100-seed

weight ranging from 7.4-9.7 g. The other 25 varieties had small to medium sized seeds and their 100-seed weight ranged from 10.0-20.1 g. Selection of small seeded soybean for sprout production is highly essential (Kim *et al.* 2000). Other traits considered important in sprout breeding programs have been high nutritive value and resistance to lodging, shattering and diseases. Most commercial soybean varieties have yellow colored seed coats, which is highly preferred for sprout production and consumption. Some of the varieties with excellent traits for soybean sprout usage recently developed in Korea are given in Table 3. Eighteen the 21 varieties listed in the Table 3 are yellow seeded. Further, 11 out of 21 varieties had < 10 g of 100-seed weight and 10 out of 21 varieties had < 13 g per 100-seed weight. These data show a preference by breeders for traits like small seed, and yellow seed coats while developing cultivars for sprout usage. Varieties like Nogchae (Kim *et al.* 2005), which is short in height with fewer branches, are preferred for dense field cultivation. Many of these varieties have resistance to some bacterial and viral diseases. Nineteen out of 21 soybean varieties are resistant to the soybean mosaic virus. The varieties Joyang1 (Kim *et al.* 2014) and Wonheug (Kim *et al.* 2013), in addition to soybean mosaic virus also show resistance to the bacterial pustule disease.

GENETIC VARIATION IN THE SOYBEAN GERMPLASM

Wild (*Glycine soja*) and cultivated (*Glycine max*) soybeans exhibits great genetic diversity and show high phenotypic variation for several traits (Lee *et al.* 2007b). Korean soybean germplasm, especially the landraces show relatively high genetic variation (Cho *et al.* 2008). This diverse genetic resource may have beneficial alleles, which can be transferred to elite soybean cultivars to develop varieties for sprout production. Soybean seeds have been categorized on the basis of different characters like adaptation, variation in seed coat color, hilum color, leaf shape, flower color, maturity, and seed composition (Hong *et al.* 1988; Song *et al.* 1991). Cho *et al.* (2008) examined 2,765 Korean soybean landraces and categorized them in

different groups based on usage, maturity group, and adaptation. Of these, a set of 80 accessions were evaluated for genetic variation for agronomic and sprout traits (Kwon *et al.* 1981; Lee *et al.* 1992; Jeong *et al.* 2007). Kwon *et al.* (1981) evaluated 164 native sprout soybean lines from the Korean germplasm bank with small seed size (<15 g/100-seeds) and they showed genetic variation for hypocotyl length, germination percentage and sprout yield. Jeong *et al.* (2007) evaluated 783 soybean lines, which also included landraces distributed by the National Agrobiodiversity Center of Korea (<http://genebank.rda.go.kr/>), for sprout characteristics and reported substantial variation for sprout yield (range, 483%-550%; mean, 516%), whole sprout length (range, 15.3-16.6 cm; mean, 15.92 cm), root length (range, 5.4-6.4 cm; mean, 5.9 cm) and hypocotyl length (range, 9.6-10.5 cm; mean, 10.08 cm). They also observed that the range of 100-seed weight was 8.1-15.1 g with an average 11.6 g, and the distribution of seed coat color was 28% yellow, 11% green, 19% black, and 38% for mixed. Based on these characteristics, they selected 18 superior lines as breeding material for the development of high-quality soybean varieties for sprouts.

Phenotypic evaluation and selection of the sprout related traits, particularly at the germination stage may not be easy, as these are highly affected by the other factors mentioned above (Kim *et al.* 2000). Sprout related traits are quantitative in nature (Kim *et al.* 2000) and DNA-based markers or quantitative trait loci (QTL) controlling these traits can be identified and utilized in marker assisted selection for improvement of varieties for sprout usage. Although several QTL mapping studies for yield and other agronomical important traits have been reported in soybean, there are a few studies of mapping QTLs for sprout-related traits. Lee *et al.* (1999a) detected several restriction fragment length polymorphism markers associated with the sprout-related traits such as hypocotyl length and sprout yield in an F₂ derived population derived from a cross of Pureunkong (Pureun)×Jinpumkong 2 (Jinpum 2). In another study, Lee *et al.* (2001) assessed variation for the sprout-related traits in 100 F₂ lines from the cross of Pureun×Jinpum 2 and identified 10 markers associated with sprout yield, 7 markers linked to QTLs for

seed weight and 5 markers linked to QTLs for hypocotyl length. Apart from these reports, no considerable efforts have been made to identify QTLs controlling sprout-related traits. Little mapping information is available for sprout traits, QTL information for related traits like seed size, seed weight and seed coat color (www.soybase.org), would be useful to identify closely linked markers applicable in marker-assisted selection for superior sprout soybean cultivars.

CONCLUSION AND FUTURE PROSPECTS

Soybean sprouts are an important traditional vegetable food in Korea. Soybean sprouts have high nutritional value and are easy to produce. Optimum consumption of sprouts can provide the recommended dietary allowance of protein, vitamins, amino acids and isoflavones. Due to these benefits, there is steady demand for soybean sprouts in the market. Thus, it is essential to improve soybean cultivars that can produce high quality sprouts. The information presented in this review about the nutrients, health benefits and genetic variation for sprout characteristics will be helpful for breeders and farmers, especially in variety selection and improvement. Since soybeans with small seed are highly preferred in sprouting, the molecular markers/QTLs closely linked to the seed size can be utilized in the selection process. At present, favorable traits like long hypocotyls with short roots and high soybean sprout yield are being produced through different chemical applications in most small companies. Hence, to reduce chemical use, studies revealing the mode of gene action for hypocotyl elongation, hypocotyl thickness, and delayed lateral root formation need to be determined. Evaluation of soybean germplasm for the target traits and identification of QTLs/genes controlling sprout-related traits could help in the genetic improvement of varieties for sprout purposes. In addition, utilization of wild relatives, with favorable alleles for these traits would allow for inter-specific crosses between cultivated and wild soybeans to identify and explore novel alleles useful in improving soybeans for sprout production.

ACKNOWLEDGEMENTS

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development” (Project No. PJ01118303), Rural Development Administration, Republic of Korea.

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