Soybean Peptide: Novel Plant Growth Promoting Peptide from Soybean

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1. Introduction

Soybean is one of the most important agricultural products and its global production was more than 200 million tons per year in 2005 (Table 1) (Ministry of Agriculture, Forestry and Fisheries (Japan), 2007; Uchida, 2007). Soybean is used mainly as a vegetable oil (31.6 million tons a year in 2005) and the production ratio is the highest (30%) among vegetable oils.

Soybean waste, which remains after extraction of vegetable oil, contains about 50% proteins, which consist of a well-balanced mix of amino acids. Therefore, soybean waste is a valuable biomass for animal feedstuffs. Soybean is used directly as food in Japan and several Asian countries but soybean proteins are used less widely elsewhere in the world.

Agricultural products	Production (Million tons)
Corn	710
Wheat	624
Rice	401
Soybean	214
Barley	153

Table 1. Amount of main agricultural production in 2005. (Ministry of Agriculture, Forestry and Fisheries (Japan), 2007)

Recently, investigations into utilization of proteins from soybean waste have been carried out for the development of high quality foods. Protein fractions, such as soy protein isolates (SPI) and whey protein are industrially produced, and these fractions are used as additives for the improvement of food nutrition (Malhotra & Coupland, 2004). Several soybean proteins have been purified and utilized as medicines for hypotension, rheumatism, and cholesterol control. Peptide inhibiting angiotensin I converting enzyme has also been developed by a protease treatment (Farzamirad & Aluko, 2008; Yonekura & Tanaka, 2003; Yonekura et al., 2004).

On the other hand, soybean waste was the most utilized N source for organic fertilizers prior to the 1940s (Okuda, 1961). Recently, utilization of organic fertilizers for the production of organic agricultural products is rising because healthcare and environmental concerns are increasing. Studies into utilization of soybean waste as a fertilizer and as

bioactive materials have been attempted (Kubo et al., 1997; Matsumiya et al., 2007; Shinano et al., 1991; Yamazaki & Roppongi, 1998).

This chapter describes utilization of soybean meal and the development of a bioactive peptide for plant growth. Moreover, mechanisms of novel bioactive peptides for root hair promotion are described in this chapter.

2. Isolation of soybean meal degrading bacteria and analysis of effectiveness of the degraded products as fertilizers

Because of increasing environmental concerns, the excessive utilization of chemical fertilizers has recently received increased attention. Therefore, the development of new fertilizers using natural materials, such as amino acids and natural nitrogen compounds, has become the focus of much research (Acea et al., 1988; Klopper et al. 1989; O'Sullivan et al., 1991).

Soybean meal, which is produced in large amounts as biomass, is rich in nitrogen compounds and has been utilized as fertilizer. Degradation and mineralization of soybean meal in the soil environment requires several reactions: proteins → peptides & amino acids → ammonia → nitrite → nitrate (Smith et al., 1977). The proteins → peptides & amino acids reaction is important for nitrogen mineralization in the soil environment (Kamimura & Hayano, 2000; Watanabe & Hayano, 1995). However, the degradation of soybean meal in the soil is too slow for mineralization.

Isolation of *Bacillus circulans* HA12, which degrades soybean meal efficiently and rapidly, is described here (Hasegawa et al., 2002; Kubo et al., 1994), and the plant growth promoting effects of the degraded soybean meal products (DSP) are also described in this section.

2.1 Isolation of soybean meal degrading bacteria

Soybean meal degrading bacteria were isolated using a 1% (w/v) soybean meal medium (Kubo et al., 1994), and about 50,000 bacteria were isolated. Protease production of all isolates were tested by LC agar medium (Matsumiya et al., 2004), resulting in 21 strains being isolated. Each isolate was further sub-cultured in soybean meal medium at 50°C for 48 h. As a result, the protease-producing bacterium HA12 was isolated. The procedure for the screening is shown in Fig. 1.

Strain HA12 was characterized and identified based on Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974). Because the strain was strictly aerobic, gram positive, catalase producing, and endospore forming, the strain belongs to genus *Bacillus*. The maximum temperature for the growth of strain HA12 was 55°C. The characteristics of the strain are listed in Table 2. Strain HA12 is identified as *B. circulans* and designated as *B. circulans* HA12.

2.2 Analysis of soybean meal degradation by *B. circulans* HA12

B. circulans HA12 formed a clear halo on LC agar medium and the strain degraded soybean meal efficiently. The protease activity was 550 U/ml after 16 h of cultivation (Kubo et al., 1997). The protease(s) from *B. circulans* HA12 was secreted into the medium and the optimum temperature of the protease was about 70°C.

Strain HA12 consumed dissolved soybean proteins for primary metabolism during the first stage. During the next stage, soybean meal was degraded and protein accumulated gradually in the medium. Subsequently, proteins were further digested to smaller molecules, including peptides and amino acids. The maximum concentrations of peptides

produced by degradation of soybean meal with *B. circulans* HA12 for 48 h were 6.5 mg/ml (1% w/v soybean meal medium) and 30 mg/ml (10% w/v soybean meal medium). The amino acid composition of DSP is shown in Table 3.
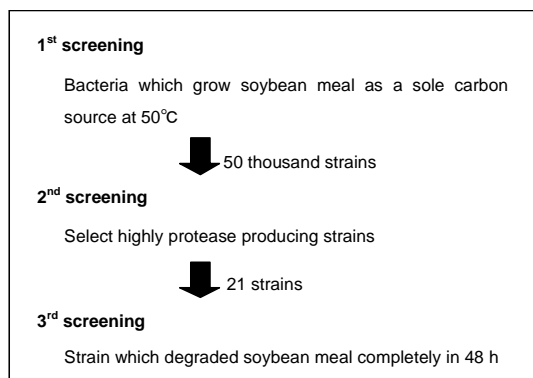

Fig. 1. Screening procedure for soybean waste-degrading bacteria.

Property	Growth	Property	Growth
Cell morphology	Rod	Growth in urease	+
Gram staining	+	Growth in NaCl 0%	+
Spore formation	+	3%	+
Motility	-	7%	-
Growth at: 25°C	+	Hemolysis	-
37°C	+	Decarboxylation from ornithine	-
Strict aerobic reaction	+	Decarboxylation from lysine	-
Oxidase reaction	+	Decarboxylation from arginine	+
Catalase reaction	+	Gas from glucose	+
Nitrate reduction	-	Gas from mannitol	+
H_2S production	-	Gas from lactose	+
Indole production	+	Gas from sucrose	+
Methyl red reaction	+	Gas from maltose	+

Table 2. Properties of strain HA12.

Amino acid	Mol. (%)	Amino acid	Mol. (%)	Amino acid	Mol (%)
Ala	5.84	His	1.37	Pro	6.74
Arg	3.35	Ile	4.75	Ser	4.27
Asp	13.33	Leu	6.29	Thr	3.32
Cys	0.00	Lys	7.66	Trp	0.00
Glu	18.40	Met	1.86	Tyr	4.64
Gly	6.61	Phe	6.25	Val	5.31

Table 3. Amino acid composition of degraded soybean meal products.

2.3 Effect of degraded soybean meal products on plant growth

Because DSP includes small molecules such as peptides, the plant growth promoting effects of DSP were investigated. The fresh weight of *Brassica rapa* was increased by 25% through addition of DSP (12 mg-peptides/kg-soil) (Table 4 & Fig. 2). The growth of *Solanum tuberosum* L., *Solanum lycopersicum*, and *Brassica juncea* were also promoted by addition of DSP. Moreover, DSP produced thicker roots than a chemical fertilizer (Fig. 3).

The total nitrogen, total phosphate (TP), and total potassium (TK) in DSP were 0.70, 0.11, and 0.28%, respectively. These TP and TK contents are not enough to act as a fertilizer in DSP, and moreover, DSP did not contain nitrate. Therefore, the plant growth promotion of DSP appears to be caused by bioactive peptides.

	Fresh weight (g)	Relative yield (%)
Water	44.2 ± 5.2	100
Chemical fertilizer	53.3 ± 6.6	121
DSP	55.1 ± 6.9	125

Table 4. Effect of degraded soybean meal products on growth of *Brassica rapa.*
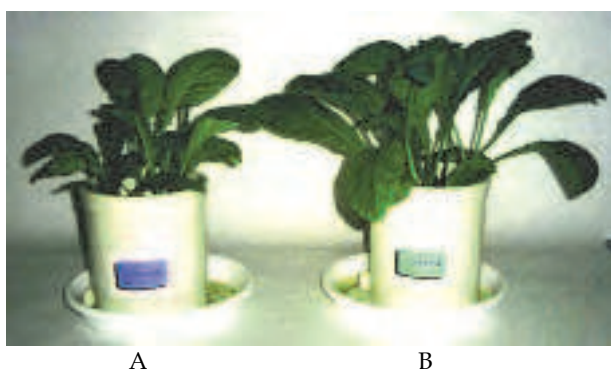

Fig. 2. Plant growth-promoting effect of degraded soybean meal products (DSP). A: *Brassica rapa* grown with chemical fertilizer, B: *B. rapa* grown with DSP.
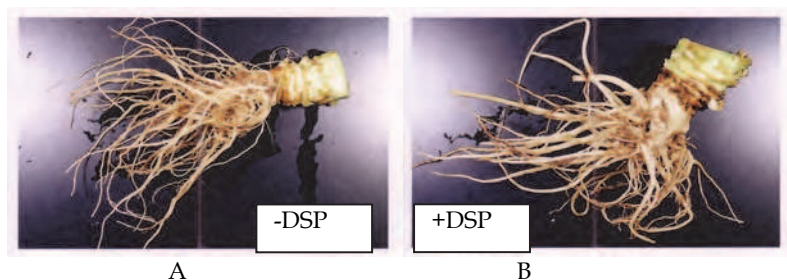

Fig. 3. Effect of degraded soybean meal products (DSP) on the root system of *Brassica juncea*. A: root of *B. juncea* grown in soil without DSP, B: *B. juncea* grown in soil with DSP.

2.4 Effect of DSP on root hair promotion

Recently, several bioactive peptides from plants have also been found to have phytohormone-like activities (Ito et al., 2006; Kondo et al., 2006; Matsubayashi and Sakagami, 1996; Matsubayashi et al., 1999; McGurl et al., 1992; Pearce et al., 1991; Schopfer et al., 1999; Suzuki et al., 1999). Phytosulfokine, systemin, SCR/SP11, and CLE are endogenous peptides produced in a variety of plants. The respective bioactivities of these peptides cause cell differentiation, protease inhibitor induction, cell division, and the pollen self-incompatibility response.

In order to analyze the plant growth promoting effect, the effect of DSP on root of *B. rapa* was analyzed. The number of root hairs was increased and elongated when DSP (30 µg/ml) was added (Fig. 4). DSP also promoted the root hair formation of *B. oleracea* L., *Lactuca sativa*, *Trifolium incarnatum* L., and *Gypsophila elegans*.

Root hair is an important plant organ for the absorption and transport of nutrients (Gilroy & Jones, 2000; Lauter et al., 1996). The enhancement of plant growth by DSP is caused by the increase of root hair numbers and length. Root hair promotion is observed with even 0.3 µg/ml of DSP, and the root hair promoting activity increases with DSP concentration.

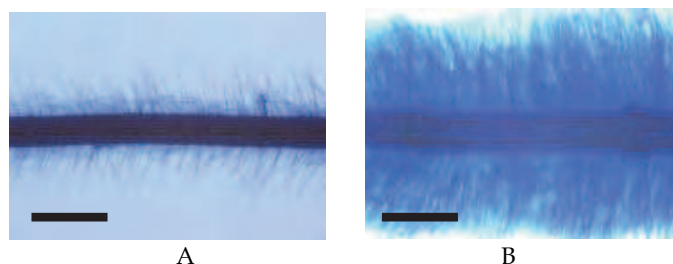A   B

Fig. 4. Root hair promoting effect of degraded soybean meal products (DSP). A: root of *Brassica rapa* grown in plant growth medium (Matsumiya et al., 2007), B: root of *B. rapa* grown with DSP in plant growth medium. Bar denotes 1 mm.

2.5 Comparison between bioactive effects of DSP and phytohormones

Ethylene, which is a phytohormone, also promotes root hair numbers and length. The bioactive effects of DSP and ethylene were compared (Fig. 5). The root hair promotion by DSP was similar to ethylene, but spiraling of the main root was observed in the case of ethylene.

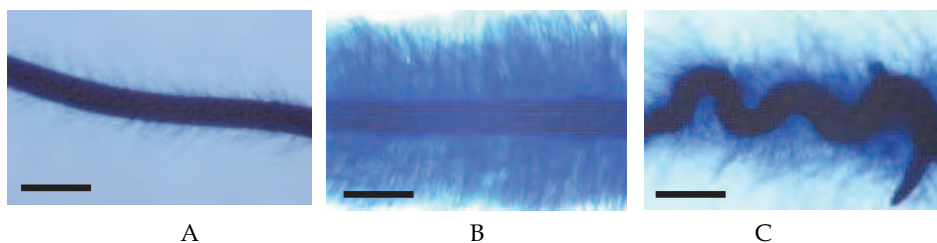A   B   C

Fig. 5. Effects for main root and root hairs of degraded soybean meal products (DSP) and ethylene against *Brassica rapa*. A: plant growth medium, B: plant growth medium + DSP, C: plant growth medium + ethylene. The bar shows 1 mm.

On the other hand, adventitious root formation by DSP and ethylene were analyzed (Fig. 6). Obvious adventitious root formation was observed in the case of DSP addition. DSP and ethylene showed different effects on main root and adventitious root formation, suggesting that DSP did not induce ethylene for root hair promotion.
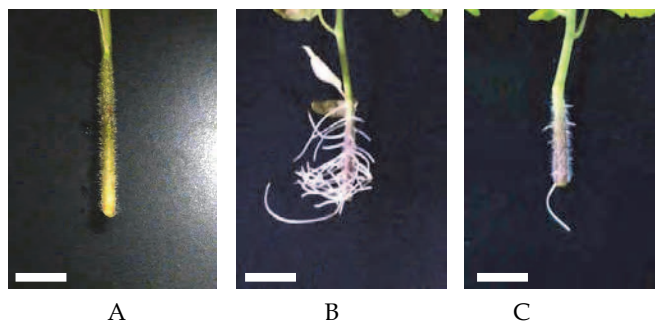

Fig. 6. Adventitious root formation with degraded soybean meal products (DSP) or phytohormones in *Lycopersicon esculentum.* The adventitious root formation assays were carried out using shoots of *L. esculentum* soaked in DSP and ethylene for 1 week at 25°C. A: water, B: water + DSP, C: water + ethylene. The bar denotes 2 cm.

	Root hair	Adventitious root	Shoot growth	Epinasty	Diapause	Callus induction	Leaf enlargement
Ethylene	+	+	-	+	-	-	-
Auxin	-	+	-	-	-	+	-
Cytokinine	-	-	-	-	-	-	+
Gibberelin	-	-	+	-	-	-	-
Abscisic acid	-	-	-	-	+	-	-
Brassinosteroid	-	-	+	-	-	-	-
Jasmoic acid	-	-	-	-	-	-	-
DSP	+	++	-	-	-	-	-

Table 5. Bioactive effects of degraded soybean meal products or phytohormones on plants.

The bioactive effects of DSP and phytohormones on plants were analyzed (Table 5) (Gaither, 1975; Masucci & Schiefelbein, 1994; Pitts et al., 1998; Tanimoto et al., 1995;). The effects of DSP on plants did not agree with those of phytohormones, and therefore DSP has different mechanisms of action on plant growth.

3. Exogenous bioactive peptides in DSP and the structural determination of the root hair promoting peptide

Systemin (McGurl et al., 1992; Pearce et al., 1991), phytosulfokine (Matsubayashi and Sakagami, 1996; Matsubayashi et al., 1999), SCR/SP11 (Schopfer et al., 1999; Suzuki et al., 1999), and CLV3 (Ito et al., 2006; Kondo et al., 2006) have been identified as endogenous peptide signals, which act as phytohormones. On the other hand, 2,3-butandiol, which is produced by several *Bacillus* strains, is known as an exogenous signal for plants (Ryu et al.,

2003). DSP seems to comprise an exogenous peptide signal and shows bioactivity for root hair promotion similar to that of phytohormones. In this section, the effect of DSP on roots and the structure of the root hair promoting peptide in DSP are described.

3.1 Effect of DSP on roots

To analyze the mechanism by which root hair numbers and length are increased by DSP, the number of trichoblasts (hair cells) and atrichoblasts (hairless cells) were counted. The trichoblast number in the presence of DSP (30 μg/ml) increased about 4.4 times over that without DSP, and the atrichoblast number also increased in response to DSP treatment by about 1.9 times (Table 6). The effect of DSP on the root hair seems to be similar to that of ethylene (phytohormone) (Dolan, 1996; Masucci, & Schiefelbein, 1994; Tanimoto et al., 1995;).

Ethylene led to an increase in root hair numbers by converting atrichoblasts to trichoblasts, while the localization pattern of the trichoblasts and atrichoblasts was not altered by addition of DSP (Fig. 7). DSP did not affect the balance of the endogenous phytohormones. DSP contains exogenous peptide signal(s) for root hair promotion and causes root hair promotion through a different mechanism than that of ethylene.

Treatment	Trichoblast number (cells/mm^2) (%)	Atrichoblast number (cells/mm^2) (%)	Length of root hair (mm) (%)	Thickness of root hair (μm) (%)	Surface area of root hair (mm^2/mm^2) (%)
- DSP	51.5 (100)	66.7 (100)	0.34 (100)	9.39 (100)	0.26 (100)
+ DSP	224.2 (435)	124.2 (186)	0.99 (290)	12.4 (132)	4.32 (1,660)

Table 6. Effects of degraded soybean meal products on root hair size and density in *Brassica rapa*.
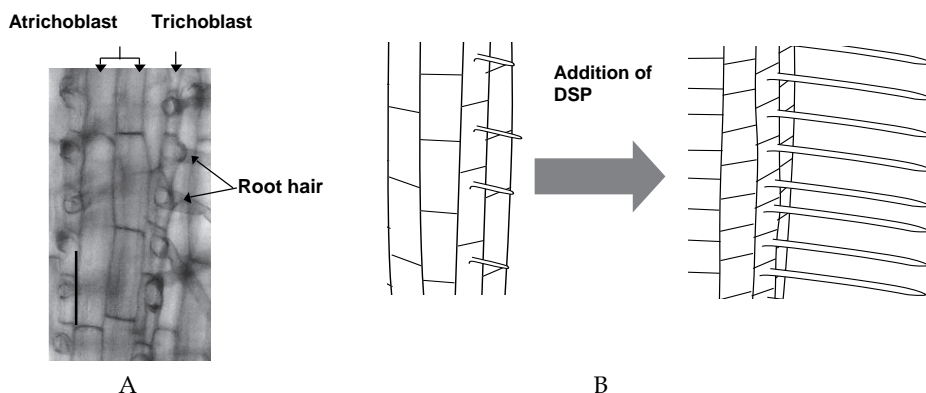

Fig. 7. Microscopic examination of root hairs grown in the presence of 30 μg/ml of degraded soybean meal products (DSP) (A) and a schematic model of the effect of DSP on trichoblast and atrichoblast (B).

3.2 Structure of exogenous peptide signal from DSP: analysis of protease from *B. circulans* HA12 for production of root hair promoting peptide(s)

The protease from *B. circulans* HA12 was purified and characterized. The N-terminal amino acid sequence (20 amino acids) of the protease produced by *B. circulans* HA12 was identical to subtilisin Carlsberg, derived from *B. licheniformis* (Jacobs et al., 1985; Jacobs, 1995). The molecular weight of the protease was about 30 kDa. The protease was inhibited by phenylmethylsulfonyl fluoride and its optimum pH was around 10. The protease from *B. circulans* HA12 was a subtilisin-like alkaline protease.

Fig. 8. Root hair promoting activity of various peptides.

Soybean meal was degraded by several proteases (pronase E, thermolysin, pepsin, trypsin, and subtilisin) and the root hair promoting activities of degraded products were analyzed. DSP by pronase E and thermolysin did not possess root hair promoting activity. Treatment by pepsin, trypsin, and subtilisin each showed root hair promoting activities, but these were lower than that of DSP (Fig. 8). The specific peptide(s) is produced by the degradation of soybean protein with an alkaline protease from *B. circulans* HA12.

3.3 Structure of exogenous peptide signal from DSP: identification of the root hair promoting peptide

Soybean contains various kinds of proteins, such as 7S globulin, 11S globulin, lectin and trypsin inhibitor (Brooks & Morr, 1985; Hamblin & Kent, 1973; Iibuchi & Imahori, 1978a; Iibuchi & Imahori, 1978b). The proteins were separated and purified by several steps, shown in Fig. 9. The separated soybean proteins were degraded by the alkaline protease from *B. circulans* HA12 and the root hair promoting activity of the degraded products from each fraction was analyzed (Table 7). Degraded products of Kunitz trypsin inhibitor (KTI), purified from whey protein, showed high root hair promoting activity, thus KTI was the origin protein for the root hair promoting peptide (Rackis et al., 1962).

The root hair promoting peptide from degraded products of KTI was purified by several chromatographic steps. The molecular mass was analyzed by matrix-assisted laser
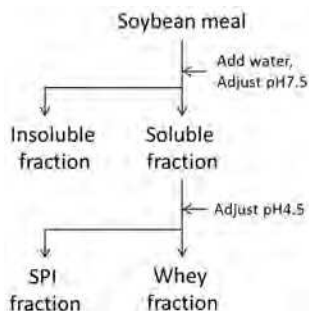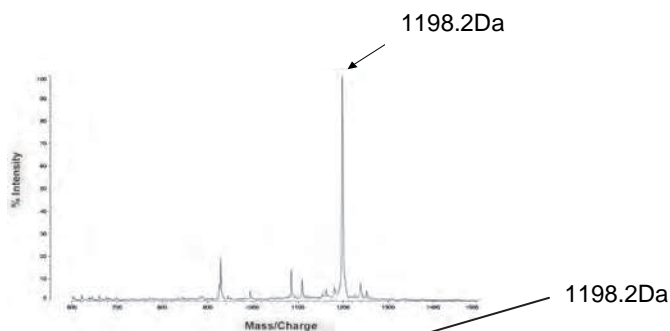
Fig. 9. Purification procedure of soybean meal proteins.

Treatment (peptides concentration)	Root hair promoting activity (%)
Without addition of peptides and amino acids	100.0
DSP (30 μg/ml)	331.1
Degraded soluble protein fraction (30 μg/ml)	337.1
Whey protein fraction (30 μg/ml)	335.5
KTI (10 μg/ml)	340.0

Table 7. Root hair promoting activity of degraded products from each protein fraction.

A

1198.2Da

1198.2Da

B

^{1}DFVLDNEGNPLENGGTYYILSDITAF**GGIRAAPTGNER**CP^{40}
^{41}LTVVQSRNELDKGIGTIISPSYRIRFIAEGHPLSLKFDSF^{80}
^{81}AVIMLCVGIPTEWSVVEDLPEGPAVKIGENKDAMDGWFRL^{120}
^{121}ERVSDDEFNNYKLVFCPQQREDDKCGDIGISIDDDGHTRR^{160}
^{161}LVVSKNKPLVVQFQKLDKESL^{181}

Fig. 10. Matrix-assisted laser desorption/ionization time–of-flight mass spectrometry spectra of root hair promoting peptide (A) and amino acid sequence of Kunitz trypsin inhibitor (B). The peptide sequence that is identical to 1198.2 Da is underlined.

desorption/ionization time–of-flight mass spectrometry (MALDI-TOF MS). The molecular weight of the bioactive peptide was 1,198.2 Da (Fig. 10A), and the molecular weight of the amino acid sequences in KTI was searched. Positions 27 - 38 in KTI (Gly-Gly-Ile-Arg-Ala-Ala-Pro-Thr-Gly-Asn-Glu-Arg) were identical to the molecular weight, and the peptide was thus designated root hair promoting peptide (RHPP) (Fig. 10B). RHPP was chemically synthesized and also shown to have root hair promoting activity (Fig. 11).
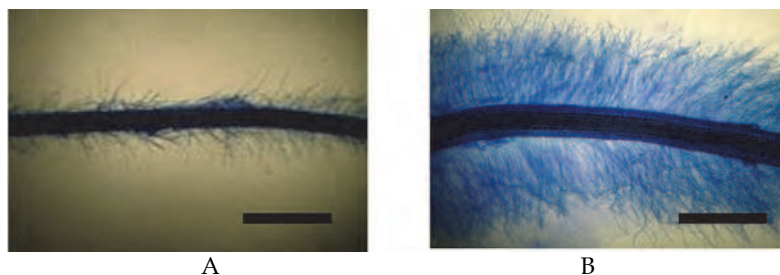
Fig. 11. Root hair promotion of chemically synthesized root hair promoting peptide (RHPP).
A: plant growth medium, B: plant growth medium + chemically synthesized RHPP.

3.4 Comparison of RHPP and other endogenous peptide signals

RHPP consists of 12 amino acids and is rich in Ala, Arg, and Gly residues. The amino acid sequences of the endogenous peptide signals are shown in Table 8, and the length of each ranges from 5 to 96. The amino acid sequences of exogenous peptide, RHPP, and endogenous peptides seem to have no relationship within their structure. The mechanisms of RHPP bioactivity (root hair promotion and adventitious root formation) seem to be different from those of endogenous peptide signals.

RHPP contains four residues of α-helix breaking amino acids and the root hair promoting activity was retained after heat treatment (121°C, 15 min). Thus, secondary and tertiary structures of the peptide are not required for root hair promoting activity. On the other hand, the root hair promoting activity of RHPP decreased when one residue of the C terminus was deleted, indicating that the 12 residues of RHPP might be the minimum unit for expressing root hair promoting activity.

Peptide	Role	Amino acid sequence	Reference
CLV3	Proliferation of cells in the apical meristem	MDSKSFVLLLLLFCFLFLHDASDLTQ AHAHVQGLSNRKMMMMKMESEW VGANGEAEKAKTKGLGLHEELRTVP SGPDPLHHHVNPPRQPRNNFQLP	(Kondo, et al. 2006)
Phytosulfokine	Stimulate the proliferation of plant cells	Y(SO$_3$H)IY(SO$_3$H)TQ	(Matsubayashi et al., 1999)
SCR/SP11	Self incompatibility	NLMKRCTRGFRKLGKCTTLEEEKCK TLYPRGQCTCSDSKMNTHSCDCKSC	(Suzuki et al., 1999)
Systemin	Activates the synthesis of proteinase inhibitors	AVQSKPPSKRDPPKMQTD	(McGurl, et al., 1992)
RHPP	Root hair promotion Adventitious root formation	GGIRAAPTGNER	(Matsumiya et al., 2007)

Table 8. Characteristics of peptide signals for plants.

4. Analysis of peptide uptake in DSP and accumulation of RHPP in plant roots

Inorganic nitrogen is one of the most important elements for plant growth. Plants usually absorb and utilize ammonia and nitrate as inorganic nitrogen for biosynthesis of proteins and nucleic acids. Lately, direct utilization of organic nitrogen, such as amino acids, peptides, and proteins, for plant growth has been found (Chapin et al., 1993; Kielland et al., 2006; Paungfoo-Lonhienne et al., 2008.). Growth of a rice plant in the presence of Gln was faster than that with nitrate.

On the other hand, L-methionine is known as a precursor of phytohormone. L-Met is absorbed into plant cells from root and stoma and converted to ethylene. Moreover, bioactivity of D-Met on roots has been also found (Hasegawa et al. 2002). DSP contains various kinds of peptides, and these seem to be utilized as nitrogen sources and/or bioactive compound(s). This section describes uptake of peptides in DSP and the accumulation of RHPP in plant roots.

4.1 Uptake of peptides in DSP by *B. rapa*

The peptide uptake was analyzed using *B. rapa* in the presence of DSP solution (Fig. 12). A decrease in peptide concentration was observed. *B. rapa* absorbed about 45% of the initial water volume, and the peptide concentration decreased by about 75%, indicating that the plant seemed to positively absorb peptides in DSP.
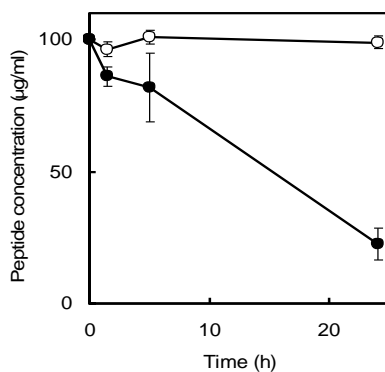

Fig. 12. Time course of peptides uptake from DSP solution by the root system of *B. rapa*. ○: without plant (control), ●: the root system of *B. rapa* was soaked in 100 μg/ml DSP solution.

Uptake of each peptide in DSP was analyzed by reversed phase HPLC (Fig. 13). All peaks decreased in intensity, suggesting that the plant absorbed many types of peptides (average uptake of peptides was 16.6%). Three specific peaks were markedly absorbed in the plant (peaks a, b, and c decreased by 54.5, 30.9, and 33.2%, respectively). Uptake of peptides by the plants seems to be influenced by the peptide lengths and amino acid sequences.

4.2 Accumulation of fluorescence labeled RHPP in roots

Carboxyfluorescein (FAM) labeled RHPP (FAM-RHPP) was synthesized for analysis of accumulation of RHPP. FAM-RHPP has root hair promoting activity at the same level as RHPP, so FAM-RHPP was used for further RHPP accumulation experiments.

Accumulation of the peptide was analyzed using a confocal laser scanning microscope. Fluorescence was observed over the whole epidermal cell (trichoblast and atrichoblast) after soaking of the root system in a FAM-RHPP solution for 24 hour (Fig. 14). The peptide was accumulated in both trichoblasts and atrichoblasts, and subsequently, FAM-RHPP seemed to increase trichoblast and atrichoblast numbers.
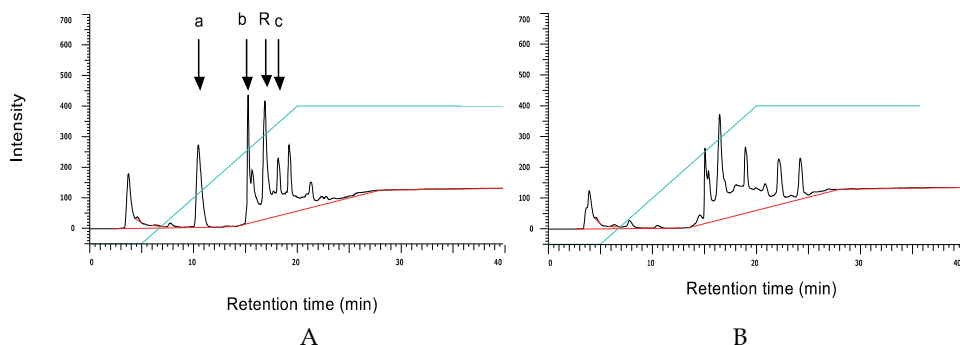

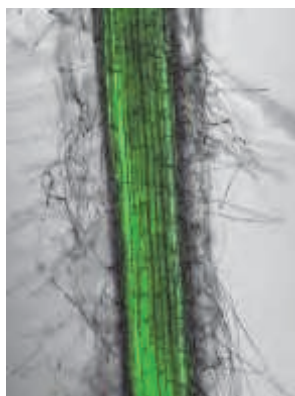Fig. 13. Peptide uptake from degraded soybean meal products solution by the root system of *Brassica rapa*.

Fig. 14. Analysis of carboxyfluorescein-RHPP uptake in the root system of *Brassica rapa* by confocal laser scanning microscope.

4.3 Hypothetical root hair promotion by RHPP

Many kinds of peptides were generated by the degradation of soybean meal by the alkaline protease from *B. circulans* HA12. Several peptides in DSP are specifically absorbed into plants from the root system. RHPP seems to be absorbed into the root of *B. rapa* and accumulated in trichoblasts and atrichoblasts. RHPP in the cytoplasm of the root may affect the expression of specific gene(s) for the promotion of root hair numbers and root hair length. The surface area of the root system is increased by RHPP and consequently plant growth is stimulated by enhancement of nutrient uptake from the root system.
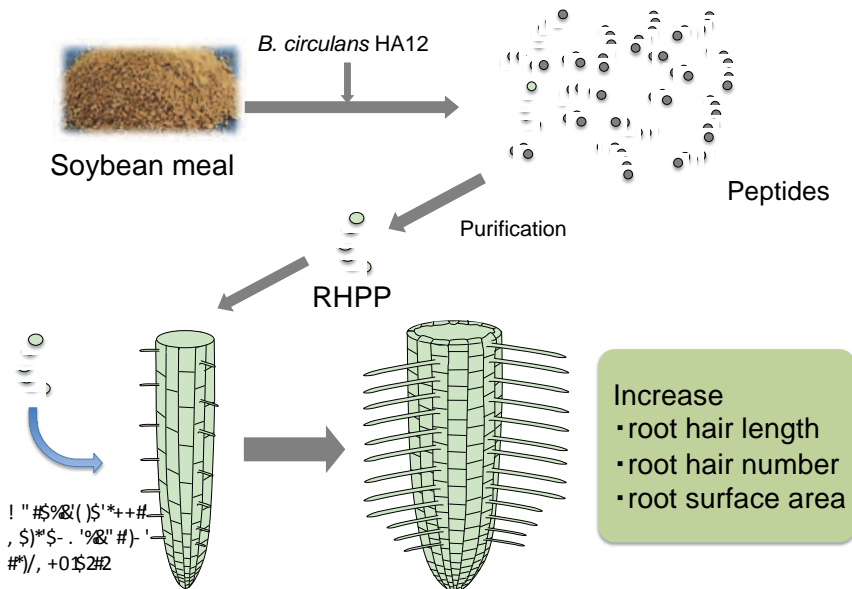
Fig. 15. Schematic model of internalization of the root hair promoting peptide.

5. Conclusion

This chapter describes utilization of soybean meal and the development of bioactive peptides for plant growth using soybean meal and the alkaline protease.

Section 1

A soybean meal degrading bacterium was isolated, identified, and designated as *B. circulans* HA12. The strain produced an alkaline protease. Soybean meal was degraded with *B. circulans* HA12, and DSP promoted various kinds of plant growth at low concentration. DSP increased root hair numbers for *B. rapa* and adventitious root was also formed from the stem of *L. esculentum* soaked in DSP solution. The bioactivity of DSP differs from that of phytohormones.

Section 2

DSP increased the number of epidermal cells without altering the localization patterns of trichoblasts and atrichoblasts. The root hair surface area was increased by about 16.6 times. The origin protein for RHPP was KTI, and RHPP was purified using KTI and the alkaline protease from *B. circulans* HA12. The structure of RHPP was analyzed by MALDI-TOF MS, and the amino acid sequence was identified (GGIRAAPTGNER; M.W. 1198.2 Da).
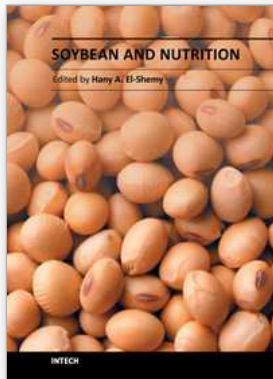
Section 3

Peptides in DSP were absorbed from the root system of B. rapa, but the uptake ratio was different for each peptide. RHPP was also absorbed into the plant and accumulated in both trichoblasts and atrichoblasts of the plant root. RHPP seemed to stimulate specific gene(s) that increase of number and length of root hair.

6. References

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Soybean and Nutrition

Edited by Prof. Hany El-Shemy

Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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