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Investigation of Soy Protein–based Biostimulant Seed Coating for Broccoli Seedling and Plant Growth Enhancement

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***Abstract*. This research presents a novel method of using plant-derived protein hydrolysates as seed coating materials. The objective of this study was to develop seed coating formulations using soy flour, a sustainable, inexpensive, and green source, as a biostimulant using broccoli as the model system. A 10% suspension of soy flour was used as the seed treatment binder in all coatings. The solid particulate filler was composed of mixtures of soy flour, cellulose, and diatomaceous earth, together termed as SCD. All SCD components were homogenized in water, then dried and ground to a fine particle size <106 μm. The SCD coatings were applied with rotary pan seed coating equipment at 25% of the seed weight. Increasing the proportion of soy flour increased the seed coating strength and also the time for the coating to disintegrate after soaking in water. As a result, the seed coatings reduced the percentage germination and the germination rate compared with the nontreated control. However, the 10-day-old seedling root and shoot growth showed significant improvement for all SCD coating treatments compared with controls. Plant growth and development was also measured after 30 days in the greenhouse. Fresh weight (FW) and dry weight (DW), leaf area, plant height, leaf development, Soil-Plant Analyses Development (SPAD) index (chlorophyll measurement), and nitrogen (N) per plant were all greater from coatings with 30%, 40%, and 50% soy flour than the noncoated control. Nitrogen, from the soy flour applied in the seed coatings, ranged from 0.024 to 0.073 mg per seed, while the enhanced N per plant ranged from 1.7 to 8.5 mg. The coating treatment with 0.063 mg N per seed resulted in the greatest plant leaf area and highest N content. Nitrogen applied in the seed coating only accounted for 1% to 2% of the enhanced N in the plants, indicating the soy flour acted as a biostimulant rather than a fertilizer.**

Several techniques have been used to facilitate sowing, and to improve seedling establishment and growth under a range of environmental conditions. These techniques, generally described as seed enhancements, are performed to seeds before sowing (Halmer, 2004). Seed enhancements may be defined as postharvest treatments that improve germination or seedling growth, or facilitate the delivery of seeds and other materials required at the time of sowing (Taylor et al., 1998). Enhancements include seed priming, seed conditioning, and seed coating. Seed coating technology is concerned with uniform application of materials onto the seed surface and is expressed as the percent build-up or percent weight increase. Thus the seed coating provides delivery of desired quantities of materials, and an opportunity for reduced application rate per hectare by reducing the need to treat the seed furrow or bulk soil.

Three coating techniques have been routinely used for vegetable crop seeds: film coating, encrusting, and pelleting (Taylor, 2003). Film coating is a liquid application method containing a binder and other components dispersed in water resulting in a continuous deposition of materials and minimizing product dust-off without obscuring the seed size and shape. The increase in weight or build-up is relatively small and ranges from 0.5% to 10%. Encrusting and pelleting involve the application of solid particles as fillers in addition to the binder resulting in a larger weight increase than film coating. Pelleting was originally developed to allow for the precision planting of irregularly shaped seeds by mechanical means. Pelleted seed weight varies by crop species and application rates of materials. Seed weight from pelleting can increase from 200% to ≥5000%. By contrast, seed coating or encrusting is a minimal application of inert materials resulting in a smaller seed size increase of just 20% to 200% (Taylor, 2003).

Seed coatings and seed treatments may be grouped into different classes based on their mode of action or properties including: plant protection, environmental stress reduction, or plant growth enhancement. Selective treatments may be used for early season pest management to protect the seeds and seedlings from fungal and insect attacks (Taylor et al., 2001) or act as an herbicide safener (Rushing et al., 2013). Coating materials can influence the microenvironment during germination by holding water around the seed (Scott, 1989) or providing a source of oxygen. For instance, calcium peroxide may be applied as a dry powder during pelleting that produces hydrogen peroxide after sowing to release oxygen to the germinating seeds (Hill, 1999). Seed coatings to enhance seedling and plant growth can provide micro- and macronutrients (Farooq et al., 2012), growth regulators (Halmer, 2004), or other biostimulants.

A plant biostimulant is any substance and/or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance, and/or crop quality traits (Du Jardin, 2015). Biostimulants are a broad group of mostly natural ingredients that contain one or more components such as microbial inoculants, humic acids, fulvic acids, protein hydrolysates, and seaweed extracts (Calvo et al., 2014). Of particular interest to the present research are protein hydrolysates and amino acids that can act as biostimulants. Foliar applications of amino acids have increased plant height, FW and DW, and N content (Shehata et al., 2011), as well as, reduced salinity damage (Sadak et al., 2015). Biostimulants are generally applied to plants at low dosages to enhance plant growth and development. However, biostimulants are not classified as fertilizers and are thus not applied to provide plant nutrients. Biostimulants have no direct action against pests, and therefore do not fall within the regulatory framework of pesticides (Biostimulant Coalition, 2016; Calvo et al., 2014; EBIC, 2014). Most of the research on biostimulants has been conducted using foliar applications while little work has been conducted on biostimulants as seed treatments. Combining the attributes of biostimulants and applying them to seeds via coating may have tremendous potential to enhance early plant growth and development.

The specific objective of this research was to determine the effect of a plant-derived biostimulant, soy flour, on coated broccoli seed. Characteristics measured were seed germination, seedling and plant growth, and N content. An added advantage of soy flour is that it is inexpensive and commercially available in most parts of the world.

Materials and Methods

Seed and coating materials

A seed lot of broccoli [*Brassica oleracea* (var. Italica 'Centura')] was provided by Rogers USA Inc., in Boise, ID, and was used for all studies. A seed coating composition based on a mixture composed of a plant-based protein product and a plant-based cellulose fiber product was adapted for this project (Hoffmann et al., 2010). Three solid filler materials were used to make the seed coating formulations, including defatted soy flour, provided by Archer Daniels Midland Co., in Decatur, IL, cellulose fibers extracted from a local newspaper (*Cornell Daily Sun, newspaper*) by grinding to a fine powder, and diatomaceous earth (DE) obtained from Perma-Guard, Inc., Albuquerque, NM. Coating treatments designated as "SCD" consisting of different proportions by weight of soy flour, cellulose fibers, and DE (00:00:100, 20:00:80, 30:00:70, 40:00:60, 50:00:50, 20:20:60, 30:20:50, 40:20:40, 50:20:30). After blending 100 g SCD of known proportions in 300 mL distilled water, thin films were prepared from the SCD suspension by pouring it into Teflon® trays and drying in a forced air oven (Gallenkamp, Loughbrough, UK) for 6 h at 60 °C. Dried films were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and sieved to a fine particle size <106 µm. A liquid binder was prepared with a 10% aqueous suspension of soy flour (same material used as a filler) and adjusted to pH 10 to help solubilize the proteins in the soy flour. The binder liquid was prepared and used on the same day.

Seed coating (encrustment) method

A laboratory-scale rotary pan coater, R-6 (Universal Coating Systems, Independence, OR) was used to coat seeds. The R-6 has a 15-cm diameter rotary pan able to coat 25 g seed samples. Seed coating treatments consisted of blends of three components and were applied as dry powder to seeds. The powder was dusted onto seeds followed by alternate application of the liquid soy flour binder. Coated seeds were then dried under ambient conditions. The coating treatments were controlled to result in a 25% build-up in seed weight.

Seed coating properties

Seed coating integrity. Integrity of coated seeds is important to prevent breakage of coatings during handling, transporting, and sowing. The binder used must ensure the integrity of the coating during handling and planting. Although different methods can be employed to test the coating's integrity, in the present research, a Ro-Tap Testing Sieve Shaker (Ro-Tap Testing Sieve Shaker No. 1506; The W.S. Tyler Co., Cleveland, OH) method was used.

Four replications of 1.5 g of coated broccoli seeds from each SCD formulation were tested to assure integrity and reproducibility. Samples were weighed and shaked for 1 min using a standard Ro-Tap Testing Sieve Shaker with a U.S. Standard Testing Sieve No. 14 (1.41 mm opening) and a solid catch pan, and then weighed again. The percentage loss of coating from the coated seeds was calculated from the weight before and after Ro-tap procedure. Coating material that passed through the sieve considered to be poorly adhered to the seed.

The weight of coating material, which passed through a No. 14 sieve, was reported as % weight loss (WL%) of material. Percent WL was calculated by Eq. (1).

$$\text{Weight loss \%} = \frac{\text{Weight before Ro Tap} - \text{Weight after Ro Tap}}{\text{Weight before Ro Tap}} \times 100 \qquad (1)$$

Seed coating hydration test. The wet coating strength relies primarily on the adhesive properties of the binder and filler after immersion in water. A hydration test was conducted to assess the time necessary for the coating to disintegrate during soaking and to determine if the coating impedes germination. The longer the period of time for the coating to disintegrate when immersed in water, the greater the probability that the coating will retard germination. Hydration tests were performed by placing four replicates of 1 g coated seeds into 5 mL water while recording the time for the coatings to disintegrate. Observations were made at 5-min intervals.

Nitrogen content of seed coatings. The percent N content of soy flour used in the study was tested at the Cornell University Stable Isotope Laboratory (Ithaca, NY). The total amount of soy flour from the filler and binder was calculated for each SCD treatment on a single seed basis, and then expressed as mg of N applied per seed.

Germination and plant growth studies

Laboratory germination test. A germination test was conducted by placing four replicates of 50 coated seeds between paper towels (30.48 × 45.72 cm, Regular Germination Paper, Anchor Paper Company, St. Paul, MN) premoistened with tap water with two paper towels beneath and one above the seeds. The paper towels were then rolled up and placed upright in small plastic containers. Ten milliliters of tap water was added to each container. After preparation, all roll towels were transferred to a seed germinator (Percival germinator, Model I-36LL, Perry, IA) maintained at 20/30 °C 16/8 h, respectively, with a 16/8-h photoperiod (AOSA, 2014a). Germination (radical >2 mm) was recorded every 12 h for 7 d. All germinated seeds were counted and removed every 12 h to avoid the double counting of seeds. The number of germinated seeds after 7 d was recorded as the maximum percent germination (Gmax) and the germination rate was expressed as the time in hours for 50% total germination (T50). Ten days after sowing four replicates of 50 seeds of each treatments in the roll towel, 40 seedlings were randomly selected and the shoot and root length were measured in millimeters. The sum of the shoot and root length was recorded as the seedling length (SL). The seed vigor index (SVI) was calculated as the product of the Gmax and SL.

Greenhouse plant growth study. Study on plant growth and development from coated broccoli seeds was conducted in a controlled greenhouse maintained at 24/21 °C temperature with 14/10 h photoperiod at New York State Agricultural Experiment Station in Geneva, NY. Based on SVI results from coated seeds in Table 1, five SCD treatments were selected for the greenhouse study: 00:00:100, 20:20:60, 30:20:50, 40:20:40, and 50:00:50 along with a noncoated control. Three seeds of each experimental unit were sown in a 10 × 10 cm plastic pot that contained Fafard PV-1 (Conrad Fafard Inc, Agawam, MA), a commercial greenhouse medium. The PV-1 medium was composed of 70% peat, 15% vermiculite, and 15% perlite. Each pot was thinned to one plant 7 d after sowing. There were 90 plants for each treatment (6 treatments × 6 replicates × 15 samples per replicate = 540 plants) placed in a completely randomized design. All plants were used for nondestructive and destructive measurements 30 d after sowing.

Chlorophyll measurements were made using a Minolta SPAD-502 chlorophyll meter (Spectrum Technologies, Plainfield, IL). The SPAD unit of Minolta Camera Co. developed the SPAD-502 chlorophyll meter (Minolta Camera Co., Japan), a handheld, self-calibrating, convenient, and nondestructive lightweight device used to calculate the amount of chlorophyll present in plant leaves (Minolta, 1989; Yadava, 1985). This device records optical density measurements at two wavelengths, converts these measurements into digital signals, and then into numerical values (Minolta, 1989). Three SPAD meter measurements were recorded per plant on the most recently expanded leaf. After recording SPAD data, plant height, and stage of leaf development (the percentage of plants with five and six true leaves, L_5D and L_6D, respectively) were recorded 4 weeks after sowing. Total leaf area was measured using a CI-202 Leaf Area Meter (CID Bio-Science,

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Table 1. Seed coating tests: weight loss (WL) after mechanical impact test, disintegrating time (DT) after soaking coated seeds in water, nitrogen applied per seed (N/seed). Laboratory germination test results: maximum percent germination (Gmax), germination rate (T50), shoot, root, and seedling length (SL), and seed vigor index (SVI = Gmax × SL) from coated and noncoated (control) broccoli seeds.

SCD treatment^z	WL (%)	DT (min)	N/seed (mg)	Gmax (%)	T50 (h)	Root (cm)	Shoot (cm)	SL (cm)	SVI
Control	–	–	–	92 a	28 a	5.7 f	4.7 c	10.4 c	9.5 c
00:00:100	6.0 c	35 a	0.024	89 a	32 b	8.0 a–c	6.1 a	14.1 a	12.5 a
20:00:80	3.5 b	60 b	0.044	86 b	33 b	8.0 a–c	6.4 a	14.4 a	12.3 a
30:00:70	2.0 ab	90 c	0.053	78 d	34 b	6.7 e	6.1 a	12.8 b	9.9 bc
40:00:60	1.5 ab	120 e	0.063	78 d	37 c	7.3 b–e	6.0 ab	13.3 a	10.3 b
50:00:50	1.0 a	180 g	0.073	85 b	40 cd	7.5 a–d	6.3 a	13.8 a	11.7 a
20:20:60	1.8 ab	90 c	0.044	81 c	38 cd	7.0 de	6.5 a	13.5 a	10.9 ab
30:20:50	1.5 ab	105 d	0.053	83 bc	40 cd	7.2 c–e	6.3 a	13.6 a	11.2 a
40:20:40	1.2 a	120 e	0.063	79 c	40 cd	8.1 ab	6.2 a	14.3 a	11.3 a
50:20:30	0.5 a	150 f	0.073	80 c	41 d	7.2 de	5.7 b	12.9 b	10.3 b

Means followed by the same letter are not significantly different from each other (least significant difference test, $P > 0.05$).

^{z}SCD = Proportion by weight of soy flour, cellulose fiber, and diatomaceous earth in each coating formulation.

Camas, WA). Plant leaf surface area was also recorded for the first three true leaves. FW was recorded and then the plants were oven-dried at 105 °C for 3 d to obtain the DW. The dry tissue was ground to a particle size of 2 mm using a Wiley mill (Thomas Scientific). Six subsamples (100 mg) of ground leaf tissues of each treatment were sent to the Cornell University Stable Isotope Laboratory (Ithaca, NY) for N analysis.

Statistical analysis. In all experiments, analysis of variance (ANOVA) ($\alpha = 0.05$) and Fisher's least significant difference test was performed on each of the significant variables measured. Data of the Gmax, WL, and percentage of leaf number five and six development (L_5D and L_6D) were arcsine transformed and analyzed by one-way ANOVA.

Results

Seed coating properties

Seed coating integrity. Seed coating using the laboratory-scale rotary pan equipment resulted in a uniform build-up over the seed surface. The SCD formulation 40:20:40 treatment had a smooth surface finish, whereas the 50:00:50 treatment exhibited an uneven surface and some cracking occurred during drying (Fig. 1B and C). The physical integrity of the seed coatings was tested using a mechanical shaking method with Ro-Tap equipment as described earlier. The 00:00:100 coating treatment had the greatest WL (6.0%), compared with the other treatments tested (Table 1). All treatments that contained soy flour as a component of the SCD formulation had less than a 4.0% weight loss. Increasing the proportion of soy flour in the filler from 20% to 50% with or without 20% cellulose fibers resulted in decreased WL. The addition of cellulose to the coating increased the compression strength of the coated seed before breakage (Amirkhani and Huang, unpublished data).

Seed coating hydration test. All tested seed coatings softened when placed in water. The time required for the coatings to disintegrate (DT) ranged from 35 min for the 00:00:100 treatment to 180 min for the 50:00:50 treatment (Table 1). Similar to the mechanical shaking results, increasing the proportion of soy flour in the filler from 20% to 50% with or without 20% cellulose increased the time for the coating to disintegrate. A significant negative correlation was measured between WL% and DT ($r = -0.85$) (Table 2).

Nitrogen content of seed coatings. The percent N of the dry soy flour powder provided by the manufacturer was 8.5% as determined by the Cornell University Stable Isotope Laboratory. A 10% solution of soy flour was applied in all the coating treatments as a liquid binder, resulting in 0.024 mg applied N per seed (Table 1). Soy flour in the SCD formulation contributed additional N and the sum of N in the coating from the binder and SCD filler. Coatings with 20%, 30%, 40%, and 50% soy flour resulted in 0.044, 0.053, 0.063, and 0.073 mg applied N per seed, respectively (Table 1). There was a significant negative correlation between WL% and N per seed ($r = -0.90$), and a significant positive correlation between DT and N per seed ($r = 0.94$) (Table 2).

Germination and plant growth studies

Laboratory germination test results. The Gmax and rate of germination (T50) were determined for all treatments in the laboratory (Table 1). The control had a greater Gmax and faster germination rate (lower T50) than all the SCD formulation treatments containing soy flour. The coating treatment without soy flour (00:00:100 SCD) had >10% point higher germination than the 30:00:70 and 40:00:60 SCD treatments. Coating formulations 50:00:50, 30:20:50, 40:20:40, and 50:20:30 had a ≥12-h delay in germination compared with the control.

In spite of the detrimental effect that the coatings had on both the percent and rate of germination, seedling growth was enhanced with the SCD coating formulations (Fig. 1). Further, the control had more short and abnormal seedlings than the coated treatments as defined by seed testing criteria (AOSA, 2014b) (Fig. 1D–F). All coated treatments had significantly greater root and shoot length, and total SL compared with the control (Table 1). The optimal SCD formulation determined based on the SVI were the 00:00:100, 20:00:80, 50:00:50, 40:20:40, 30:20:50, and 20:20:60 formulations.

Greenhouse plant growth study. The seed coatings enhanced plant growth and development. All coated seed treatments had a greater DW compared with the noncoated control (Fig. 2). The SCD treatments 30:20:50 and 40:20:40 resulted in a greater dry mass than the other treatments (Table 3). SCD treatments with ≥30% soy flour in the coating formulation had greater FW, plant height, and leaf area compared with the control. All the coated treatments had enhanced plant development than the control, based on the percentage of plants with five or six leaves (L_5D, L_6D) and SPAD readings. There was a general increase in N content per plant as the proportion of soy flour increased. The 40:20:40 treatment had the greatest N content. The N content then declined as the proportion of soy flour further increased to 50% in the 50:00:50 treatment (Table 3).

The plant N content of the noncoated control was 13.0 mg (Table 3), and the enhanced N per plant was calculated by the difference between the control and each coating treatment. All coating treatments enhanced N uptake, and the enhanced N content ranged from 1.7 to 8.5 mg N per plant (Fig. 2). The amount of N applied per seed presented in Table 1 ranged from 0.024 to 0.073 mg, and these data are shown in relation to enhanced N uptake per plant (Fig. 2). The greatest enhancement in plant N uptake was recorded at 0.063 mg N applied per seed, which corresponded to the 40:20:40 coating treatment.

Discussion

Soy flour was investigated as a biostimulant and broccoli was the model system used to study germination, seedling, and plant growth. In this study, soy flour was applied as a seed coating binder at a concentration of 10% and as a component of the SCD coating formulation. The soy flour coated seeds had greater seedling root and shoot growth compared with the noncoated control (Table 1; Fig. 1D–F). Colla et al. (2014) conducted laboratory bioassays using a plant-derived protein hydrolysate by soaking corn seedlings and tomato cuttings to the treatments (Colla et al., 2014). In their study, the protein hydrolysate increased corn coleoptile elongation in a dose-dependent trend, and increased tomato root dry weight, root length, and root area compared with the control. Collectively, the plant-derived protein

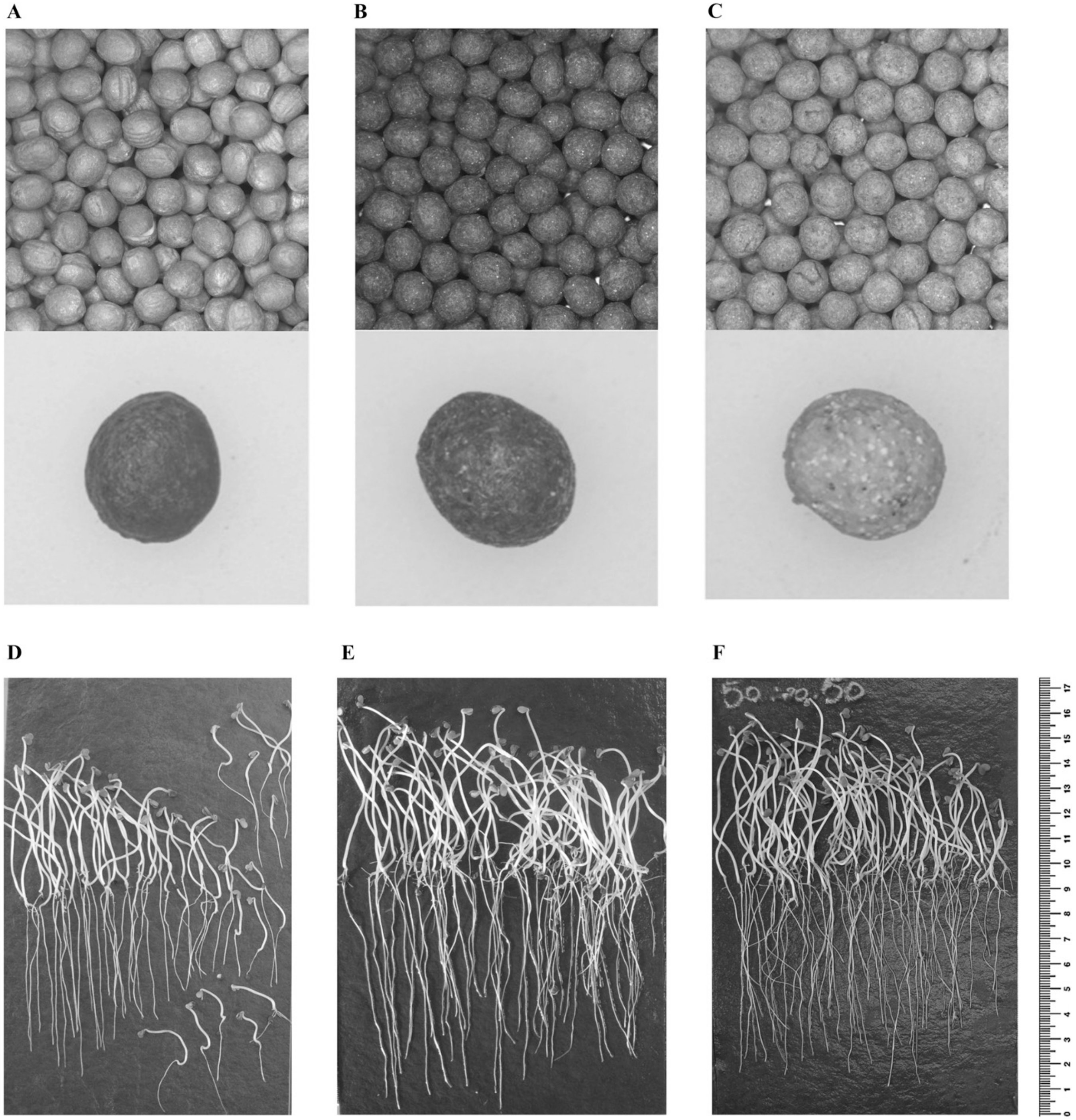

Fig. 1. Broccoli seeds (**A**) noncoated, encrusted with 25% weight buildup of coating formulation SCD 40:20:40, (**B**) and SCD 50:00:50, (**C**), comparison of seedling growth from noncoated and coated broccoli seeds, (**D**) noncoated, (**E**) 40:20:40, and (**F**) 50:00:50. SCD is the proportion by weight of soy flour, cellulose fiber, and Diatomaceous earth in each coating formulation.

hydrolysate containing amino acids and small peptides elicited auxin-like activity (Colla et al., 2014).

The soy flour treated seeds were sown in a peat–perlite–vermiculite medium in this greenhouse study. Additional research studies have also documented enhanced plant growth with the application of protein hydrolysates or amino acids (Liu and Lee, 2012). Gelatin, an animal-derived protein, applied as gelatin capsules was placed adjacent to seeds and acted as a biostimulant in greenhouse studies (Wilson, 2015). Gelatin capsule treatments increased cucumber (*Cucumis sativus* L.) plant FW and DW and leaf area compared with seeds sown without gelatin capsules. An amino acid mixture was sprayed twice onto bean plants at 3 and 6 weeks after sowing (Abdel-Mawgoud et al., 2011). The amino acid treatment increased plant height, number of leaves, and FW and DW compared with the nontreated control.

It has been stated that seed coating should be neutral with respect to its influence on germination speed, uniformity, and total percentage (Kaufman, 1991). However, the seed coating may have a detrimental effect on these germination parameters acting as a barrier for water uptake (Mucke, 1988), gas exchange, (Hill, 1999) and radicle emergence (Sachs et al., 1981). The 10% soy flour solution served well as a binder in the seed coating process. In addition, the soy flour added as a component of the dry SCD formulation became hydrated during the seed coating operation and also acted as a seed coating binder that contributed to seed coating strength. Increasing the proportion of soy flour in the coating increased the disintegration time after soaking in water (Table 1). A longer period of time for the coating disintegration, as expected, was accompanied by a slower germination rate, and there was a significant positive relationship between coating disintegration time and T50 (r = 0.81) (Table 2). Though the coatings negatively affected the Gmax and germination rate (T50) (Table 1), seedling growth recorded after 10 d was enhanced. Moreover, short seedlings observed in the control treatment (Fig. 1D) were not observed from the coated treatments (Fig. 1E–F). The soy flour biostimulant "invigorated" seeds as measured by increased seedling growth. Uniformity of lettuce plant growth was improved at harvest after a soil application of an amino acid formulation (Tsouvaltzis et al., 2014).

Soy flour applied at 30%, 40%, and 50% in the SCD coating formulation increased the total N per plant compared with the noncoated control. The maximum enhanced N per plant resulted from the 40:20:40 SCD coating treatment (Table 3). One possibility to explain these results was that the N in soy flour served as a fertilizer. However, the N applied in the seed coating only accounted for 1% to 2% of the enhanced total N in the plant (Tables 1 and 3). The enhanced N per plant increased as coating N content increased from 0.044 to 0.063 mg per seed, and then decreased at coating N content of 0.073 mg per seed (Fig. 2). The data suggest that there might be an optimum application rate of N from soy flour on N uptake by plants and DW. In corroboration, gelatin was studied as a biostimulant on several crop seeds, and gelatin capsules were placed adjacent to seeds in the greenhouse studies (Wilson, 2015). Increasing the number of capsules from zero to three resulted in a quadratic growth response for leaf area, FW and DW for tomato, pepper, and corn (Wilson, 2015). An alternative explanation for the measured optimum application rate is that the highest N application rate had the greatest seed coating strength, which impaired germination rate and subsequent plant growth. Reduced plant growth was measured from the 50:00:50 treatment compared with the 40:20:40 treatment as a lower SPAD reading and a lower percentage of plants with six leaves were measured (Table 3). Further research is needed to understand the limiting effects of biostimulant application rate on plant growth.

Table 2. Correlation coefficients of disintegrating time (DT), nitrogen applied per seed (N/seed), and germination rate (T50) with Nseed, DT, and weight loss (WL).

	DT (min)	N/seed (mg)	T50
WL (%)	–0.85**	–0.90**	–0.85**
DT (min)	1.00	+0.94***	+0.81*
N/seed (mg)		1.00	+0.78*

*, **, ***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

The SCD coatings improved plant growth and development at the whole plant and plant nutrition levels in this study, which was attributed to enhancing the physiological processes in the germinating seed and plant. The biostimulant activity of a plant-derived protein hydrolysate was examined on tomato (*Solanum lycopersicum* L.) (Colla et al., 2014). Soaking tomato cuttings in 5 or 10 mL/L protein hydrolysate increased the SPAD index and the leaf N content by 15.0% and 21.5%, respectively. By contrast, 40:20:40 SCD coating treatment increased the SPAD index and the leaf N content by 12.1% and 65.4%, respectively, compared with the control (Table 3). Colla et al. (2014) proposed that the plant-derived protein hydrolysate increased plant growth parameters through stimulation of N uptake and assimilation by enhancing key enzymes involved in N metabolism. In support, root applications of protein hydrolysates increased N assimilation through increased nitrate reductase and glutamine synthetase activities (Ertani et al., 2009). Schiavon et al. (2008) applied alfalfa protein hydrolysate to hydroponically grown maize and reported an increase in the activity of three enzymes in the tricarboxylic acid cycle (malate dehydrogenase, isocitrate dehydrogenase, and citrate synthase) and five enzymes involved in N reduction and assimilation (nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase, and aspartate aminotransferase). In the same study, increased gene expression of the three tricarboxylic acid cycle enzymes, nitrate reductase, and asparagine synthetase was confirmed by reverse transcriptase-polymerase chain reaction in the roots following application of alfalfa hydrolysate. Wilson et al. (2015) suggested that gelatin, an animal protein, increased expression of amino acid and N transporter genes, which may be responsible for enhanced N uptake by roots.

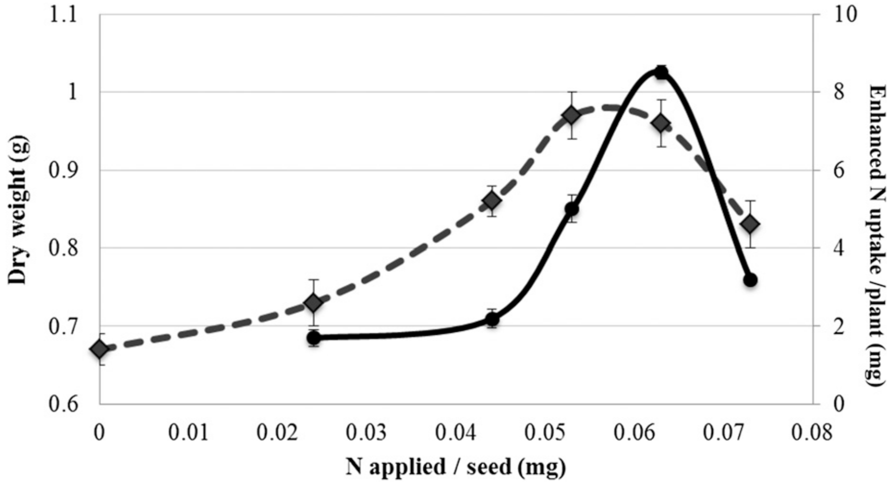

Fig. 2. Dry weight (g, dash line), enhanced nitrogen uptake (mg) per plant (30 d seedlings), and applied nitrogen (mg) per coated seed from coating formulations investigated in Table 2. Standard error bars are shown.

In summary, seed coatings provide an excellent delivery system for materials to be applied at the time of sowing. These materials have the potential to enhance seedling and plant growth. The present study clearly demonstrated that soy flour, an inexpensive source of plant-derived protein, acted as a biostimulant to enhance plant growth and uptake of N in broccoli plants. Enhanced plant growth and development were observed as increased biomass, plant height, leaf area, leaf development, and chlorophyll content were measured. Seed coating technology is widely used in agriculture and the biostimulant material could be applied as a component of a seed coating blend. This biostimulant is a natural plant material and could be adopted

Table 3. Greenhouse data: fresh weight (g), dry weight (g), plant height (H), leaf area (LA), percent plants with five and six leaves (L_5D, L_6D), SPAD unit, and tissue total nitrogen (mg) per plant from coated and noncoated (control) broccoli seeds.

SCD treatment[z]	FW (g)	DW (g)	H (cm)	LA (cm^2)	L_5D (%)	L_6D (%)	SPAD	N per plant (mg)
Control	3.95 b	0.67 d	14.2 c	143 b	36.0 d	0.0 d	47.0 c	13.0 d
00:00:100	4.16 b	0.73 c	14.4 bc	151 b	58.0 c	3.0 c	50.8 b	14.7 cd
20:20:60	4.22 b	0.86 b	14.3 bc	150 b	71.0 b	2.0 c	51.3 b	15.2 cd
30:20:50	4.96 a	0.97 a	14.8 ab	164 a	85.0 a	13.0 a	51.7 ab	18.0 b
40:20:40	5.26 a	0.96 a	15.2 a	165 a	90.0 a	13.0 a	52.7 a	21.5 a
50:00:50	5.37 a	0.83 b	15.7 a	162 a	78.0 ab	9.0 b	51.0 b	16.2 c

Means followed by the same letter are not significantly different from each other (least significant difference test, $P > 0.05$).

[z]SCD = Proportion by weight of soy flour, cellulose fiber, and diatomaceous earth in each coating formulation.

for organic crop production, and may also reduce the need for high levels of N fertilizer as the biostimulant can enhance N uptake efficiency. Biostimulants provide the seed industry with a tool that may reduce environmental stress and have minimal regulatory requirements compared with pesticides.

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