Varietal Difference in Early Vegetative Growth during Seedling Stage in Soybean

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Abstract: Rapid development after emergence is important for seedling establishment and early vegetative growth, especially at a low planting density or inferior environmental conditions. This study was conducted to understand the varietal difference in the growth parameters during the seedling stage in soybean. Twenty-seven soybean varieties originating from six countries were examined in 2009 and 2010. The pots were arranged in a completely random block design with 5 replications (10 pots per variety), and the seedlings were sampled at 14 and 28 days after sowing (DAS). The shoot dry weight at 14 and 28 DAS was highly correlated with seed size, cotyledon digestion, and leaf area. However, no positive correlation was found between shoot dry weight and photosynthetic rate at 28 DAS. Chamame, a Japanese cultivar, with the largest seed size grew rapidly, and showed the heaviest shoot dry weight, greatest cotyledon digestion, fast leaf expansion and high photosynthetic rate. However, Moyashimame, a medium-seed-size cultivar, also grew rapidly with a high photosynthetic rate. Some varieties such as Tachinagaha (Japan), Hefeng (China), Parana and Pérola (Brazil), had a large or medium seed size, and high photosynthetic rate but showed a relatively small leaf area and light shoot dry weight. These results suggested that big seeds with rapid cotyledon digestion developed a wider leaf area and therefore large dry matter production, indicating that the conversion of stored energy was more important than the leaf photosynthetic activity for early growth.

Key words: Cotyledon digestion, Photosynthetic rate, Seedling development, Soybean.

Early development after emergence is important for seedling establishment and early vegetative growth of soybean. The ability of seedlings to become autotrophic rapidly and to develop a large plant in a short period is ecologically advantageous (Nelson and Larson, 1984). Furthermore, rapid seedling growth results in extensive growth of root and shoot systems.

Seedling vigor and rapid early vegetative growth may be more important under a low planting density and inferior environmental conditions such as a limited plant-growing season, and low temperatures in early spring. The advantage of seedling vigor is to enhance plant survival and develop the leaf area to intercept radiation, and also to enhance weed-suppressive ability as has been documented in rice (Kawano et al., 1974; Garrity et al., 1992; Johnson et al., 1998; Zhao et al., 2006) as well as in soybean (Jannink et al., 2000).

There are many reports on seed vigor and seedling emergence (Hamman et al., 2002; Colete et al., 2004; Trimble and Fehr, 2010; Sadeghi et al., 2011) but less information is available on soybean seedling development. Longer et al. (1986) reported that the seedlings emerged from large seeds face greater impedance from the soil crust, but a large seed size could accelerate seedling growth and early vegetative growth of soybean (Buris et al., 1973) due to the energy stored in the cotyledon.

In soybean, the green cotyledons play an important role after emergence because the cotyledons not only have a storage-mobilizing function but also assimilate energy through photosynthesis (Harris et al., 1986; Brown and Huber, 1987; Marek and Stewart, 1992; Ramana et al., 2003; Kitajima, 2003; Hanley and May, 2006; Zhang et al., 2008). Zheng et al. (2011) insisted that the cotyledons provide a major proportion of the assimilation needed for seedling growth until the first true leaf becomes a significant exporter of photosynthate. Therefore, the dry matter accumulation during seedling growth is faster in soybean compared with some leguminous crops with small seeds (mung bean) or those that keep the cotyledons underground (Azuki bean, pigeon pea, etc.).

To our knowledge, there have been no detailed reports
on the relationship between growth parameters such as stored energy used, primary leaf area, photosynthetic rate and early vegetative growth rate in soybean seedlings. This study was conducted to understand the magnitude of early vegetative growth and varietal differences in these early-stage growth parameters in soybean.

Materials and Methods

1. Plant material

Twenty-seven soybean varieties from six countries were used in experiments conducted in 2009 and 2010. These varieties have various characteristics including seed size (0.10–0.38 g), and maturity group (0–IX, plus tropical varieties). The soybean seeds used in the experiment were harvested in the year before the experiment was conducted.

2. Growth condition

The experiments were conducted from May 19 to June 15, 2009 and from June 10 to July 7, 2010 at the Coastal Bioenvironment Center, Saga University, Karatsu, Japan (33°27’N and 129°58’E). The daily mean temperature during plant growth was 16.3°C to 20.9°C during the 2009 experiment and 20.9°C to 26.9°C during the 2010 experiment (Fig. 1).

Plants were grown in the plastic pots containing 500 g of top soil from the experimental field of the Coastal Bioenvironment Center. The soil characteristics were as follows: total nitrogen, soluble phosphate and exchangeable potassium were 130 mg, 19.5 mg and 17.3 mg per 100 g dry soil, respectively, and pH (H₂O) was 5.7. No fertilizer was added to the soil. Four seeds were sown in each pot, and the seedlings were thinned at 7 days after sowing (DAS) to allow one plant per pot.

The pots were arranged in a completely randomized block design with 5 replications (10 pots per variety). The plants were irrigated by water spray to prevent water stress. Weed and insect were removed manually. Although fertilizer was not added, the leaf was normally green based on the SPAD value (26.6–43.9 in 2009 and 30.8–39.2 in 2010), showing no nutrient deficiency symptoms. Five plants of each variety were sampled at 14 and 28 DAS after the measurement of photosynthetic rate. At the time of second sampling, the cotyledons in most varieties were abscised in 2010 but not in 2009. Nodules were found on the roots at the end of the experiment.

3. Measurement of leaf apparent photosynthetic rate and related parameters

The leaf apparent photosynthetic rate was measured with a portable gas exchange system LI-6400 (LI-COR Bioscience, Lincoln NE, USA) in 2009, and with by the ADCBioScientific LIpro+ System (ADCBioScientific Ltd., England, UK) in 2010. Photosynthetic photon flux density (PPFD) was fixed at 1500 μmol m⁻² s⁻¹ using a red blue LED light source, but other environmental factors were not controlled. Air was induced through a rubber hose from 10 m far from the plants outside the green house.

In both experiments, the measurements were taken from 1000 to 1500 on the primary leaf only at 14 DAS and the second younger fully expanded leaves at 28 DAS. The plants were well watered in the evening of the day before measurement. Three plants were used for photosynthetic rate measurement in each sampling period in both years.

4. Growth analysis

Five plants of each cultivar were sampled at 14 and 28 DAS for growth analysis. The shoot parts of the plant were separated into cotyledon, leaf blades, and stem including leaf petioles. Primary leaf and total leaf area were measured using the automation area meter (AAM-9, Hayashi Denco Co. Ltd., Tokyo, Japan). The dry weight of each part was measured after oven-drying at 80°C for 48 hr. Cotyledon digestion was shown by the difference (g) between the cotyledon dry weight at 14 DAS and the seed weight.

5. Statistical analysis

Duncan’s multiple range test was used for comparisons of the growth parameters measured. Correlation analyses were used to estimate the relationship among growth variables.

Results

The soybean seedling growth performance in 2010 was better than that in 2009 (Fig. 2), because of the higher air temperature during the growth period in 2010 due to later seeding time (Fig. 1). Shoot dry weight at 28 DAS varied from 0.93 to 2.13 g with an average of 1.35 g in 2009 and from 1.90 to 3.32 g with an average of 2.54 g in 2010. Chamame, an early-maturing cultivar with the largest seed size (0.37 g), had the heaviest shoot dry weight at 28 DAS in both experiments. Moyashimame, a Japanese cultivar with a medium seed size (0.19 g), also had a heavy shoot dry weight in 2010. However, Fukuyutaka and Tachinagaha,
Fig. 2. Shoot dry weight at 28 DAS of 27 soybean varieties in two experiments. Within each year’s experiment, bars followed by different letters are significantly different according to Duncan’s multiple range test at the 5% level.

Table 1a. Correlation coefficients among soybean growth characteristics in 2009.

| rxy | CD  | RCD | PR1  | LA1  | SPAD1 | DW1  | PR2  | LA2  | SPAD2 | DW2  |
|-----|-----|-----|------|------|-------|------|------|------|-------|------|
| SS  | 0.93** | −0.24 | 0.02 | 0.45* | 0.21  | 0.59** | 0.04 | 0.39* | 0.07  | 0.61** |
| CD  | 0.12  | 0.14 | 0.52** | 0.27 | 0.75** | 0.05 | 0.48* | 0.14 | 0.71** |
| RCD | 0.37  | 0.25 | 0.23  | 0.34  | 0.13  | 0.14  | 0.20  | 0.19  |
| PR1 | 0.45* | −0.14 | 0.42* | 0.003 | 0.06  | 0.37  | 0.03  |
| LA1 | 0.07  | 0.86** | −0.20 | 0.57** | 0.26  | 0.54** |
| SPAD1| 0.30 | 0.04  | 0.16  | 0.31  | 0.32  |
| DW1 | −0.08 | 0.57** | 0.31  | 0.68** |
| PR2 | 0.34  | 0.49* | −0.40* | 0.31  | 0.41* |
| LA2 | 0.28  | 0.75** | −0.34 | 0.41* |
| SPAD2| −0.41* | 0.48** | −0.16 | 0.60** |

* significant at the 0.05 probability level; ** significant at the 0.01 probability level.

SS, seed size; CD, cotyledon digestion; RCD, ratio of cotyledon digestion to seed size; PR1, apparent photosynthetic rate at 14 DAS; LA1, primary leaf area at 14 DAS; SPAD1, SPAD at 14 DAS; DW1, shoot dry weight at 14 DAS; PR2, apparent photosynthetic rate at 28 DAS; LA2, total leaf area at 28 DAS; SPAD2, SPAD at 28 DAS; DW2, shoot dry weight at 28 DAS.

Table 1b. Correlation coefficients among soybean growth characteristics in 2010.

| rxy | CD  | RCD | PR1  | LA1  | SPAD1 | DW1  | PR2  | LA2  | SPAD2 | DW2  |
|-----|-----|-----|------|------|-------|------|------|------|-------|------|
| SS  | 0.99** | −0.01 | 0.05 | 0.48* | 0.54** | 0.77** | 0.15 | 0.28 | −0.10 | 0.56** |
| CD  | 0.02  | 0.07 | 0.50** | 0.52** | 0.79** | 0.17 | 0.32 | −0.12 | 0.58** |
| RCD | 0.14  | 0.13 | 0.12  | 0.24  | 0.02  | 0.35 | 0.01 | 0.10  |
| PR1 | 0.70** | 0.49* | 0.46* | 0.08  | 0.40* | 0.28 | 0.20  |
| LA1 | 0.25  | 0.71** | −0.01 | 0.51* | −0.34 | 0.41* |
| SPAD1| 0.14 | 0.16  | −0.20 | 0.45* | 0.10  |
| DW1 | 0.16  | 0.48** | −0.16 | 0.16  |
| PR2 | 0.17  | 0.28  | 0.16  |
| LA2 | 0.41* | 0.75** |
| SPAD2| −0.18 |
with a large seed size, 0.36 g and 0.34 g, respectively, had a light shoot dry weight (1.32 and 1.13 g) at 28 DAS. The lightest shoot dry weight at 28 DAS was observed in the cultivars with a medium seed size, Ripley from USA and Kenjian from China in 2009 and Pérola and Parana from Brazil in 2010 (Fig. 2).

The shoot dry weight was correlated significantly with seed size, cotyledon digestion, and total leaf area consistently in the experiments in both 2009 and 2010, but not clearly with the ratio of cotyledon digestion to seed size and leaf photosynthetic rate (Table 1). During seedling development, the seed size and cotyledon digestion correlated with primary leaf expansion significantly at 14 DAS (Fig. 3A and B). Furthermore, primary leaf expansion highly correlated with shoot dry weight at 28 DAS (Fig. 3C). The contribution of seed size, cotyledon digestion and leaf area expansion to the early seedling growth, was evident for shoot dry weight at 28 DAS (Fig. 3D-F).

Fig. 3D shows the positive correlation between seed size and shoot dry weight at 28 DAS. Like seed size, cotyledon digestion also had a positive correlation with shoot dry weight at 28 DAS in both years (Fig. 3E). Chamame (2009, 2010) and Tamahomare (2010) showed the greatest cotyledon digestion (0.31 and 0.30 g) and the heaviest shoot dry weight (3.32 and 2.91 g).

Fig. 3F shows the correlation between the total leaf area and shoot dry weight at 28 DAS and its varietal difference in both experiments. The total leaf area at 14 and 28 DAS...
As compared with seed germination and seedling emergence, the process of seedling development after emergence is less understood. This study showed that seed size, cotyledon digestion and early leaf expansion are the most contributive factors to the early vegetative growth after emergence.

Soybean seed size is representative of food or nutrient storage (McAlister and Krober, 1951). Larger seeds have more nutrients stored in the cotyledons. The factors affecting early seedling growth could be the energy converted from the nutrients stored in the cotyledon and the growth activity by the plant itself. Our results showed that large seeds produced seedlings with a large leaf area, and ultimately a large amount of dry matter (Fig. 3). This result coincided with previous reports that in species with large seeds, young seedling growth depends on the nutrient support from the cotyledons (Leishman and Westoby, 1994; Milberg and Lamont, 1997). In our experiment, Cotyledon digestion (CD) correlated had a positive correlation with shoot dry weight at 28 DAS.

Another important aspect of seedling growth is photosynthesis. Table 1 and Fig. 4A and C show that the photosynthetic rate had no positive correlation with shoot dry weight, except at 14 DAS in the low-temperature conditions of 2009. However, significant negative correlations were found between photosynthetic rate and leaf area at 28 DAS in 2009 and at 14 DAS in 2010 (Fig. 4B and D).

In 2009, soybean varieties that had a high photosynthetic rate at 28 DAS, such as Kenjian (21.3 µmol CO₂ m⁻² s⁻¹) and Tachinagaha (20.73 µmol CO₂ m⁻² s⁻¹) had a small total leaf area at 28 DAS (111.61 cm² and 129.22 cm², respectively). In 2010, Chamame (24.31 µmol CO₂ m⁻² s⁻¹) and Moyashimame (23.25 µmol CO₂ m⁻² s⁻¹) had the highest photosynthetic rate with a large leaf area at 28 DAS (603.51 cm², 613.52 cm², respectively), as well as heavier shoot dry weight while Stressland (23.17 µmol CO₂ m⁻² s⁻¹ and 369.84 cm²) and Hefeng (21.83 µmol CO₂ m⁻² s⁻¹ and 395.36 cm²) had a high photosynthetic rate with a small leaf area (Fig. 4D).

Discussion

As compared with seed germination and seedling emergence, the process of seedling development after emergence is less understood. This study showed that seed size, cotyledon digestion and early leaf expansion are the most contributive factors to the early vegetative growth after emergence.

Soybean seed size is representative of food or nutrient storage (McAlister and Krober, 1951). Larger seeds have more nutrients stored in the cotyledons. The factors affecting early seedling growth could be the energy converted from the nutrients stored in the cotyledon and the growth activity by the plant itself. Our results showed that large seeds produced seedlings with a large leaf area, and ultimately a large amount of dry matter (Fig. 3). This result coincided with previous reports that in species with large seeds, young seedling growth depends on the nutrient support from the cotyledons (Leishman and Westoby, 1994; Milberg and Lamont, 1997). In our experiment, Cotyledon digestion (CD) correlated
significantly with shoot dry weight at 14 and 28 DAS, however, the ratio of cotyledon digestion (RCD) did not (Table 1), indicating that the higher ratio of cotyledon digestion to seed size does not mean higher nutrient conversion to the shoot growth.  

There was a high positive correlation between primary leaf area (leaf area at 14 DAS) and shoot dry weight at 14 and 28 DAS. The primary leaf area was associated with cotyledon digestion rather than seed size (Table 1). This indicated that converted energy from the cotyledons contributes more to the leaf expansion, causing high photosynthetic activities leading to more dry matter production. It was supported by the positive correlation between leaf area at 14 and 28 DAS (Table 1).

Of interest is the lack of correlation between cotyledon digestion and photosynthetic rate found in this study. This indicated that the energy converted from the cotyledons was expressed more in the leaf expansion than in photosynthetic functions. An exception was that the photosynthetic rate at 14 DAS in 2009 showed a significant positive correlation with shoot dry weight and primary leaf area. During the season with a low temperature, digestion of the cotyledons would proceed slowly, such that the photosynthetic rate could contribute actively to the shoot dry weight at the early stage.

Ojima and Kawashima (1968) reported that the mean variation of photosynthetic rate in 38 varieties was around 20%. Our results showed a larger variation in 2009 (11.5–21.3 μmol CO$_2$ m$^{-2}$s$^{-1}$) than in 2010 (18.1–24.3 μmol CO$_2$ m$^{-2}$s$^{-1}$). The big variation in 2009 might be caused by the low air temperature, since the most cultivars with low photosynthetic rate were from the tropical area. However, we also found that the photosynthetic rate tended to correlate negatively with leaf area (Table 1), as reported by Kokubun et al. (1988).

After seedling emergence, the green cotyledons and early developed leaves start photosynthesis to support seedling growth. When the green cotyledons were cut partially, plant growth was suppressed in proportion to the degree of the cut compared with the intact plants (Ikeda and Kiso, 1981). It is certain that cotyledons play an important role in seedling development (Kitajima, 2003; Hanley and May, 2006; Zhang et al., 2008).

Although several studies have shown that leaf photosynthesis affects the plant growth (Hogan, 1988; Makino et al., 1997), our results showed no positive correlation between photosynthetic rate and shoot dry matter except at 14 DAS in 2009 (Table 1 and Fig. 4), suggesting that the photosynthetic rate is not a major factor in the seedling development. Harris et al. (1986) argued that energy storage is a more important role of soybean cotyledons, and that their photosynthetic capacity is sufficient only to compensate for the respiratory losses of the seedling.

Photosynthesis in true leaves totally support the plant growth when cotyledons abscise. It is reported that soybean cotyledons senesce and fall off when the third trifoliate leaf unfolds (Peterman and Siedow, 1985; Harris et al., 1986; Marek and Stewart, 1992). There may be varietal differences in this characteristic.

In both experiments in 2009 and 2010, the photosynthetic rate at 28 DAS showed no positive correlation with shoot dry weight (Table 1). Moreover, the photosynthetic rate at 28 DAS had a lower (2010) or a negative (2009) correlation with leaf area (Table 1 and Fig. 4D). It was hypothesized that soybean varieties with wider leaf area have a higher growth potential than those with smaller leaf area, even if smaller leaf area companied with a higher photosynthetic rate. This phenomenon would be represented by a compensatory effect between leaf area and photosynthetic rate.

Genotypic differences in seedling growth were also found in the experiments. Against our speculation, the cultivars from subtropical and tropical areas did not show vigorous growth even under the high temperature conditions of 2010. Compared with the tropical cultivars, the Japanese cultivars, such as Chamame, Moyashimame and Tamahomare, showed high growth activity in the higher temperature condition of 2010. High temperature could stimulate the cotyledon digestion, resulting in rapid leaf expansion and active seedling growth thereafter. However, under the lower temperature condition in 2009 with slow cotyledon digestion, the plant growth was more dependent on the photosynthetic function. That might be the reason why a positive correlation between the photosynthetic rate and seedling dry weight was found only at 14 DAS in 2009.

The cultivars from Japan commonly have a larger seed size and more rapid growth at the early vegetative stage than those from other countries (Fig. 2). Typically, Chamame, with the largest seed size, was superior in almost all growth parameters in both experiments (2009 and 2010). The rapid early vegetative growth evidenced by shoot dry weight at 28 DAS in Chamame was supported by its large seed size, high cotyledon digestion, faster leaf expansion and high photosynthetic rate. On the other hand, Moyashimame, which has a medium seed size and relatively low cotyledon digestion value, also had a large total leaf area and shoot dry weight at 28 DAS. In Moyashimame, the growth performance in 2010, including photosynthetic rate, total leaf area and shoot dry weight at 28 DAS, was absolutely higher than that in 2009. It is supposed that higher temperatures stimulated shoot growth in Moyashimame through its large leaf area and high photosynthetic rate. Therefore, although there is no significant correlation between photosynthetic rate and shoot dry weight, photosynthetic rate could still be important in determining seedling growth variation.
among varieties as shown in Moyashimame and Chamame.

The results of both experiments suggest that seed size, cotyledon digestion and rapid leaf expansion contribute more to early vegetative growth than photosynthetic rate. High temperature stimulates cotyledon digestion, by which stored energy is converted to leaf expansion; therefore, it enhances dry matter production. Thus, although a high photosynthetic rate is helpful, cultivars with a large leaf area have the best performance in the early vegetative growth stage.

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