Effect of Lag Phase on Soybean Seed Development

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Summary:

In 2018 alone, the United States produced 4.54 billion bushels of soybeans (*Glycine* max), an increase from the 4.41 billion bushels produced in 2017 (Service 2019). Each year, the demand for soybean and other staple crops increases. The human population is growing at an alarming rate and the pressure is being put on farmers, breeders, and biologists to increase their output to meet demand. However, even with perfect growing conditions, there is still genetic control over yield that limits the maximum yield. Yield is determined by the number of seeds and the size. The goal of most breeding programs is to produce larger seeds. In order to understand how plants control seed size and numbers, the Kawashima lab utilizes soybean as the crop model to identify genetic and environmental factors controlling these agronomical seed features as the first step.

Soybean seed development is separated into three phases: lag phase, seed filling phase, and maturation phase. The lag phase in soybean is characterized by the cellular division within the seed while the seed is not physically growing. The seed filling phase is when the seed is physically growing. The maturation phase is when the seed dries to harvestable moisture. Altering environmental conditions during the lag phase causes a negative effect on the final seed size and number. Stressing the plant during the lag phase leads to a decrease in the number of seeds while extending the photoperiod during the lag phase showed an increase in the number of seeds (Nico, Miralles et al. 2015). By extending the lag phase, the soybeans have more time to produce flowers and allow those flowers to reach the critical point. Once the critical point is reached, the flowers can no longer get aborted and will be able to produce pods (Kantolic and Slafer 2007). There is currently no research on how the lag phase of seed development impacts final seed yield.

The goal of this research is to analyze developmental patterns of four cultivars with different seed sizes from the same maturity group and yields to see if there is a correlation between the length of the lag phase and the final seed size and number.

Rationale

This research was conducted because soybean yields will need to increase to meet the needs of the growing population. Even in optimum growing conditions, soybean seeds cannot exceed a size threshold. This is due to a reproductive strategy used by plants: if more seeds are grown, they will be smaller, as a way to allocate resources efficiently (and vice versa). This leads us to believe that the final seed size is under predetermined genetic control. However, in modern-day, soybeans

are given everything they need to be successful and this strategy is now hampering increased yield. If the genetic controls can be identified, there may be a way that they can be modified to increase yield. As there is no research on how the lag phase controls yield, this area of study will be key for the future of agricultural crop improvement.

Methodology

Four different cultivars were grown in 1-gallon pots with a 12:2 ratio of Premier Tech Pro-Mix Bx Growing Medium with Mycorrhizae and topsoil under the same greenhouse conditions at the University of Kentucky in Lexington, Kentucky. Two cultivars were chosen with small seeds (Cultivar 1 and Cultivar 2) and two cultivars were chosen with large seeds (Cultivar 3 and Cultivar 4) (Table 1).

A total of 35 plants of each cultivar was planted with 20 plants being utilized for the non de-podded treatment and 15 plants being utilized for the de-podded treatment. For each cultivar non de-podded treatment, 5 plants were utilized for the "Whole Plant Assays"/phenotypic data collection; 10 plants were utilized for Microscopic Assays, and 5 plants were utilized for *In Vitro* Cotyledon Growth Assays. For each cultivar de-podded treatment, 5 plants were utilized for the "Whole Plant Assays"/phenotypic data collection; 5 plants were utilized for Microscopic Assays, and 5 plants were utilized for *In Vitro* Cotyledon Growth Assays. For the de-podded experiment, the plants were de-podded at each node so that one pod remains to eliminate competition between pods. As the plants grew and started flowering, the duration of the lag phase, seed filling phase, and maturation phase were recorded, along with final seed size and number.

Cultivar	**Seed Weight (g)**	**Yield (megagram/ha)**
607835 (Cultivar 1)	6	1.21
593655 (Cultivar 2)	8.3	1.88
603322 (Cultivar 3)	21.9	1.70
594245 (Cultivar 4)	24	1.37

Table 1: Cultivar information for the cultivars used in this experiment. Data was retrieved from the USDA Germplasm Resources Information Network.

Whole Plant Assays

After 6-7 days of flowering, open flowers were marked using yellow acrylic paint and the the number of marked flowers was recorded. These marked flowers at different nodes for each plant were then used for data collection throughout the experiment. The length or pod elongation rate of marked pods was measured 3 times a week until the start of maturation, which ranged from 19-21 days. As the plants grew, dates of R1, R3, R5, R6, and R7 were recorded. At harvest, pod number, number of seeds, pod weight, seed size, and seed weight were recorded. Flower abortion rate and seed abortion rate were also recorded.

***In Vitro* Cotyledon Growth Rate Assays**

The *In-vitro* cotyledon growth rate was measured by following (Egli and Wardlaw 1980) protocols. After 6-7 days of flowering, open flowers were marked using yellow acrylic paint. Once the marked pods reached the R5.5 growth stage, pods were collected for cotyledon growth rate assay. The collected pods were cleaned thoroughly with soap and water, then isopropanol, bleach, and water twice again in the fume hood. Cotyledons were extracted from the pods and one cotyledon was placed into growth medium and the other cotyledon was dried. A total of 8 cotyledons per plant was grown in the growth medium, 8 cotyledons per plant dried, and then four cotyledons stored in liquid nitrogen.

Results & Discussion

Cultivar	R1 to R2	R2 to R3	R3 to R5	R5 to R6
Cultivar 1	4 days	5 days	5 days	18 days
Cultivar 2	4 days	4 days	6 days	16 days
Cultivar 3	5 days	7 days	8 days	26 days
Cultivar 4	5 days	7 days	8 days	26 days

Table 2: Days for each cultivar to reach different growth stages. Growth staging was done on a marked pod basis instead of a whole plant basis.

The small seed cultivars, Cultivar 1 and Cultivar 2, took less time to reach R6 than the large seed cultivars, Cultivar 3 and Cultivar 4. It takes more time for the pods to grow for the large seed cultivars in order to fit the larger seeds. As aforementioned, within the small seed and large seed cultivars, one is high yielding and the other is low yielding. However, there is still not a difference in the maturation time between them, suggesting that the duration of maturation is controlled by genetics and not by final yield.

Mean Pod Elongation Rate for all Cultivars

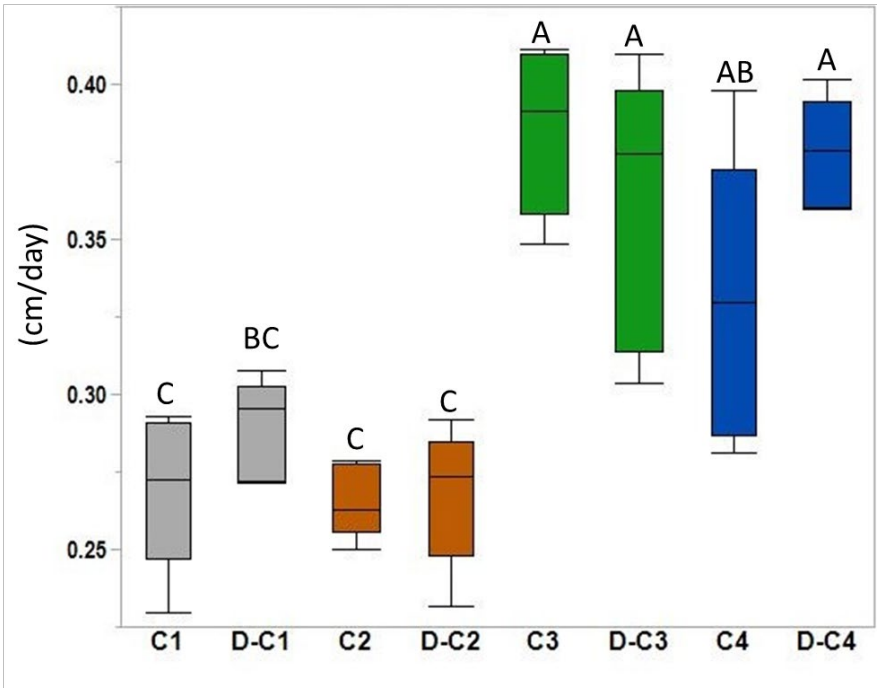

Figure 1: Mean of pod elongation rate for all cultivars under both de-podded and non de-podded conditions. Data collected from 0.5 cm pod to 4 cm pod. Levels not connected by the same letter (A, B, C) are significantly different ($p < 0.05$, Tukey-Kramer HSD test). The box spans first and third quartiles, and the line inside the box shows the median. Bars on the top and bottom represent the maximum and minimum value.

All cultivars used were maturity group 0. Maturity group 0 was used to ensure data collection could be completed in a few months by having the plants mature as early as possible. For each type of seed size (small and large), the two cultivars chosen had a high and low yield.

The pod elongation rates were very similar between de-podded and the non de-podded conditions. In C1 and D-C1, the pod elongation rates are not significantly different from each other. This is also the similar trend seen in C2 and D-C2. Between C1, C2, C1-D, and C2-D, there is no significant difference in pod elongation rates. C3 and D-C3 are also not significantly different from each other, along with C4 and C4-D. Between C3, C4, C3-D, and C4-D, there is also no significant difference in pod elongation rates. However, there is a significant difference between the large and small seed cultivars. Furthermore, these results negate the effect of assimilate supply in pod development. If assimilate supply was what is controlling seed size, the de-podded plants pods should have grown larger than the non de-podded plants pods with significance. There should also have been differences between the higher and lower yielding cultivars, which we did not see. These results further suggest that there is pre-determined genetic control over pod development and seed size.

***In Vitro* Cotyledon Growth Rates**

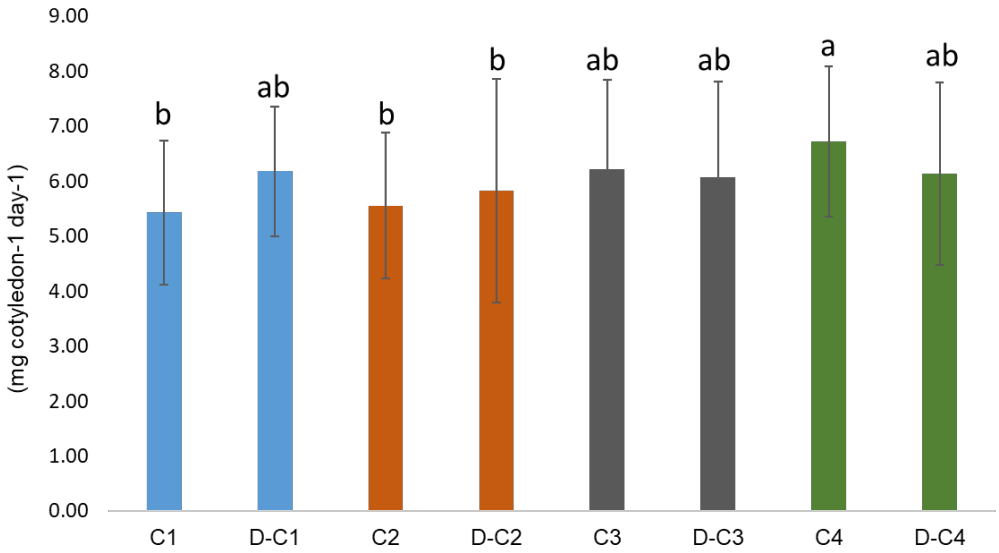

Figure 2: *In vitro* cotyledon growth rates (mg cotyledon-1day-1) of all cultivars, de-podded and non de-podded. Conditions. Cotyledons were grown in nutrient supply for 7 days. Levels not connected by the same letter (a,b,c) are significantly different ($p < 0.05$, Tukey-Kramer HSD test).

The *in vitro* cotyledon growth rate analysis is based on the hypothesis that cell number is what controls the final seed size. If this was the case, C3 and C4 should have grown much larger than C1 and C2 because they have larger seeds, and hypothetically, more cells. The *in vitro* cotyledon growth rate was only significantly different between C4 and C1, C2, and D-C2. The rest of the growth rates were not significantly different from each other. These results suggest that regardless of projected yield and seed size when grown *in vitro*, all the cultivars grew to almost the same size and were not significantly different from each other.

Future Implications

As aforementioned, there is very little research into how the lag phase controls yield, which leaves many questions unanswered. In the Whole Plant Assay, the results showed that regardless of yield (i.e., the number of total seeds), cultivars with similar seed sizes took the same amount of time to mature. In addition, cultivars with larger seeds took more time to mature (Table 2). The pod elongation rates showed that the large seed cultivars had larger elongation rates to account for larger seeds (Figure 1). It also showed that assimilate supply is not controlling seed size. Based on this, we can hypothesize that maybe the pod size is controlling yield. One way to test this would be to allow pods to develop *in planta*, cut open the pods, allow the seeds to develop and see if when they have more room to grow, will they grow larger? If so, then the pod size might be what is controlling the final seed size. Furthermore, one could ask, what genetic mechanisms are controlling pod size?

In the *in vitro* cotyledon growth rate assay, we did not see any significant differences between the cultivars' growth rates (Figure 2). This was unexpected since it was hypothesized that there are more cells in large seeded cultivars and they should have grown faster than the small seeded cultivars. The next step is to analyze the cell count in the cultivars and see whether there is differences or not. If cell count is determining yield, what is determining the cell count?

This research is vital for a number of reasons. First, it will shed light on soybean seed development on the molecular level, as this area has very little research. Second, it will identify differences in cultivar development that might be related to genetic mechanisms. If we could identify the mechanisms controlling cell number and pod size, we will be able to shed light on what controls yield. Once genetic mechanisms are identified, new cultivars can be developed that are modified and new management techniques can be employed to overcome said mechanisms. Finally, these genetic mechanisms might apply to other agronomic crops. To keep up with the growing population and uncertain climate changes; agronomic crops will need to adapt and improve on the physiological and molecular levels for the next frontier challenges.

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