Cotyledon Cells and Seed Growth Relationships In CO_2-enriched Peanut[1]

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ABSTRACT

Seed size is a dynamic component of seed yield. Factors affecting seed size in peanut (*Arachis hypogaea* L.) are not well defined. The objectives of this study were to investigate the effects of CO_2 enrichment and timing of pod formation on cotyledon cell and seed growth in virginia-type peanut. The results indicated that the number of cotyledon cells was relatively constant across all the treatments. However, size of cotyledon cells and seed growth rate (SGR) increased in the pods developed in high CO_2 conditions. Striking differences in both cell size and SGR also existed between early and late formed pods. Our data indicate that assimilate supplies strongly limit cotyledon cell size, and accordingly affect SGR and final seed size

Key Words: CO_2 enrichment, peanut, cotyledon cell, seed growth.

The contribution of seed size to final yield has been studied in many grain crops. In peanut (*Arachis hypogaea* L.), seed size is mainly under genetic control (9), but assimilate supply which determines seed growth rate (SGR) may also influence seed size (11). Nimbkar *et al.* (9) pointed out that SGR of peanut was constant per cotyledon cell, and they concluded that genetic differences in SGR were mainly related to the number of cells in the cotyledons. Similar findings were also reported in other crop species (4,6,7). Egli *et al.* (5) reported that alterations in assimilate supplies by changing source-sink ratios would also affect the number of cells in soybean (*Glycine max* L. Merr.) cotyledons and accordingly resulted in a concomitant change in SGR. These results seem to suggest that the physiological status of the soybean plant during the cell division phase of seed growth is also crucial in determining cotyledon cell number.

Previously we have found that CO_2 enrichment with subsequent depegging tended to increase seed size in peanut (3). The present study was designed to examine whether changing the assimilate supply to the developing seeds, by using CO_2 enrichment, influenced the size and number of cotyledon cells and subsequent growth of the seeds.

Materials and Methods

Four seeds of a virginia-type peanut (cv. Li-Chih-Taze) were planted in plastic pots (60x45x35 cm) in a mixture of soil and perlite (1:1, v:v) and placed outdoors under natural sunlight. Starting from growth stage R_3 (1), CO_2 enrichment (1,000 mL/L) was provided to half of the plants. The plants were subjected to high CO_2 conditions from R_3 stage (pod formation) to R_8 stage (final harvest). The semi-closed CO_2 enrichment chambers were 5m x 1.5 m and 1.2 m in height. The chamber was enclosed with polyethylene film (2 mm in thickness), with a blower operating at approximately 3,000 L/min. Carbon dioxide was metered into the chamber through a 8-mm in diameter perforated PVC pipe running the length of the chamber. Carbon dioxide level was monitored by taking air samples from each chamber and analyzing the samples with an infrared CO_2 analyzer (ADC model LCA-2). For non-enriched controls, canopy CO_2 concentration averaged 340 ml/L. Carbon dioxide enrichment was only applied during the daytime, from 800 to 1700 h (Central Standard Time).

Flowers at nodes in the lower portions of the canopy were tagged at anthesis (R_1). These flowers were sampled later for cotyledon cell number, cell size, and seed growth rate (SGR) determinations. Because peanut has a somewhat indeterminate growth habit, the time from the first flower to last flower at nodes in the lower portions of the canopy was about 35 days. As a result, some seeds set later at lower nodes were only at anthesis when seeds set earlier at the bottom of the plant were at growth stage R_3 and were ready to receive CO_2 treatment. Thus, to investigate the effect of timing of pod setting, both early- and late-formed pods in the lower position nodes were tagged at flowering (there was 18 days of difference in the date of flowering between early- and late-formed pods) and sampled later for cotyledon cell counting.

Starting from day 42, tagged pods were sampled at approximately five to seven day intervals for SGR determination. After the samples were collected, seeds were dried (70 C) for two days and dry weights measured. Linear regression analysis was used to estimate SGR for each treatment, after eliminating the nonlinear points from the initial and final phase of seed development.

For cotyledon cell number and cell size determinations, seeds sampled at each stage were fixed in glutaldehyde, followed by a graded series of alcohol and tert-butyl alcohol dehydrations with infiltration, and embedded in paraffin. Samples were cut into 10 mm cross sections, stained with safranin and fast green, then examined and measured on a microscope. Fifty sectioned cotyledon samples were measured and an average cell diameter was determined using a micrometer. The number of cells per maximum cross section area per cotyledon pair was determined using the line transect technique (8). In this technique, a straight line of 300 microns was laid in the observation field and the cells intercepted by the line were counted. An average of fifty observations were made, and then converted to the number of cells per maximum cross section area per cotyledon pair using the predetermined area data for each of the sectioned cotyledon pairs.

The statistical significance of treatment differences was evaluated by the least significant differences (LSD) test. Nonlinear regression (sigmoid curve) was also used to characterize seed response over sampling stage for cotyledon cell number and cell size.

Results and Discussion

During the first 15 days after anthesis, cell number per maximum cross section area per cotyledon pair increased slowly (approximately 200 cells per day) in early-formed pods of CO_2-enriched plants and in both early and late-formed pods of control plants (Fig. 1A). The rate of increase was considerably higher in the seeds of late-formed pods from CO_2-enriched plants because they developed under high CO_2 condition from R_1 till R_8. After day 15 (around R_3 stage), cotyledon cells began to divide rapidly at a rate of 460 cells per day per cotyledon pair. Cell divisions eventually terminated around day 50 to day 55 when maximum cotyledon cell numbers were attained. The approximate time at which the maximum cell number was obtained appeared to be treatment and flowering time independent. Carbon dioxide treatment had very little effect on cell division rate and resultant cell number per maximum cross section area per cotyledon pair (Fig. 1A). In fact, only the late-formed pods from control plants showed slightly fewer cotyledon cells than their counterparts (early-formed pods). An explanation for fewer cell numbers of late-formed pods from control plants is not evident, but could result from the reduction of foliar assimilate supply (3) and strong competition between early and late-formed pods (11). Thus, seeds of virginia-type peanut seem to respond to reduction in assimilate supply in a manner similar to soybean (5), but the reduction in cell numbers was less for peanut.

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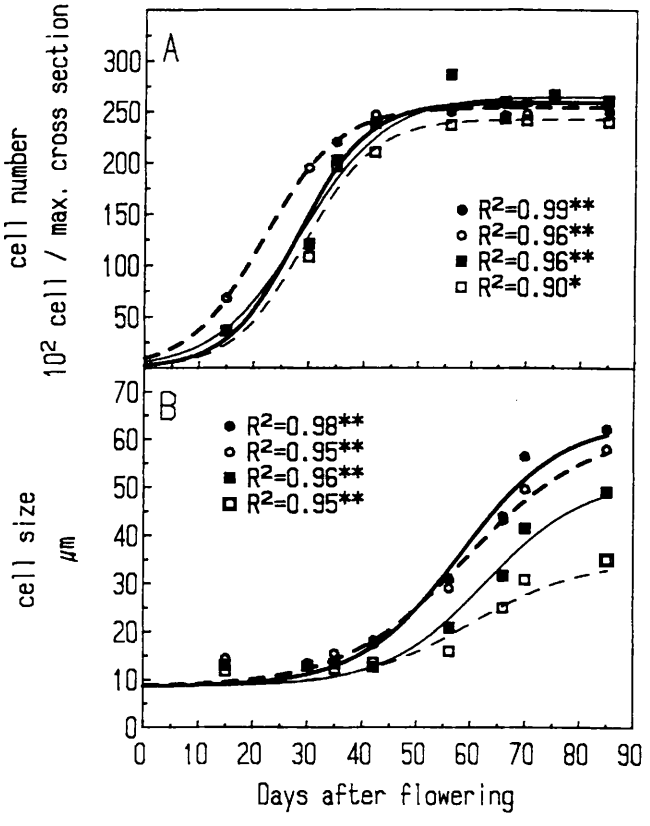

Fig. 1. Cotyledon cell number (A) and size (B) as a function of days after flowering for peanut seeds from early (_____) and late (----) formed-pods of Co_2-enriched (●, ○) and control (■, □) plants (*, ** significant at 0.05 and 0.01 levels, respectively).

The size of cotyledon cells, regardless of CO_2 treatments and flowering timing, changed little during the rapid division phase (Fig. 1B). Nevertheless, they began to enlarge when the number of cotyledon cells approached their maxima. Cell size continued to increase and approach their maxima at day 85 (R_8 stage). The maximal size of cotyledon cells (averages of early-and late-formed pods) ranged from 40 μm for seeds of ambient CO_2-grown plants to 60 μm for seeds of high CO_2-grown plants. Under high CO_2 conditions, cotyledon cells from seeds of late-formed pods were similar in size to those cells from seeds of early-formed pods even though they were different in timing of pod setting. On the other hand, the time of pod formation (early vs late) had a significant effect on cotyledon cell size in control plants. Maximum cell size of seeds from late pods was 30% smaller than that of seeds from early pods (Fig. 1B). We believe that the differences in assimilate supply between CO_2 treatments and timing for pod formation must account for the observed variations in cell size. Similar variations in cell size also have been reported in other crop species (2,4,10).

The patterns of seed dry mass accumulation for early and late-formed pods are shown in Table 1. Previously Egli *et al.* (5) reported cell division in soybean cotyledons was complete when the seed enters the linear phase of seed growth. Our data demonstrate that the same is true for peanut seeds. Seed mass paralleled the cotyledon cell size, and was increased significantly under high CO_2 conditions. As observed for cell size, seed dry mass at each sampling time was reduced in seeds set later compared with those set earlier. When the

Table 1. Dry mass of peanut seeds from early and late formed-pods of CO_2-enriched and control plants sampled at different days

Treatment	Days after flowering (R stage)			
	42(R_6)	55(R_6)	70(R_7)	85(R_8)
	g/seed			
340 μL/L				
early-formed pod	0.025	0.391	0.633	0.680
late-formed pod	0.018*	0.323*	0.532*	0.571**
1,000 μL/L				
early-formed pod	0.026	0.442*	0.721**	0.773**
late-formed pod	0.030*	0.483**	0.690*	0.742**

*,** significantly different from early-formed pods developed under 340 μL/L based on the LSD at α=0.05 and 0.01, respectively.

seed dry mass data were regressed on cotyledon cell size, across all treatments and sampling times, there was a linear relationship between the two parameters (r=0.88**). Thus, the relationship between cell size and seed mass was maintained when the variation in cell size was created by altering assimilate supply. These data clearly demonstrate the importance of cotyledon cell size in determining seed dry mass accumulation.

The seed growth rate (SGR), estimated by fitting a linear regression to seed dry mass between 42 (R_5) and 70 (R_7) days after flowering, ranged from 12.8 mg per seed per day to 17.4 mg per seed per day (Table 2). High- CO_2 treatment that enlarged cell size also tended to increase SGR. In both treatments, there was a trend for the early pods to have a higher SGR than the late pods (Table 1), which was consistent with the argument that assimilate supply controls SGR (11).

Seed size of legume species is a function of the number and size of cells in the cotyledons, and both are influenced by genotype and assimilate supply (2,5,6). Genotypic differences in seed size in peanut are related to the number of cells in the cotyledons (9). Nevertheless, changes in seed size for a given peanut genotype can also result from variations in cotyledon cell enlargement, which is under the strong influence of assimilate supply. The data reported here clearly demonstrate that developing cotyledon cells may not have the capacity for significant enlargement if assimilate supplies

Table 2. Seed growth rate of peanut from early and late-formed pods of CO_2-enriched and control plants.

Treatment	Seed growth rate
	mg/seed/day
340 μL/L	
early-formed pod	15.1
late-formed pod	12.8**
1,000 μL/L	
early-formed pod	17.4**
late-formed pod	16.5*

*,** significantly different from early-formed pods developed under 340 μL/L based on the LSD at α=0.05 and 0.01, respectively.

are restricted. Therefore, assimilate supply is important in determining cotyledon cell size, SGR and the actual seed size formed.

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