

Growth and Allocation of Dry Matter in Bean Seedlings Developed up to the Senescence of the Cotyledons

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Summary

The allocation of dry matter in seedling of the common bean (*Phaseolus vulgaris* L.) depends on the nutrient content in the cotyledons on principle of germination. The importance of these structures for the growth of the seedling ranges from the time of germination to the emergence of the seedling and the time when the simple leaves can realize photosynthesis. The objective of this research was to study and quantify the distribution of dry matter from the stage of germination until senescence of cotyledons during the seedling stage. Two experiments were conducted using the common bean variety Cacahuatate-72, one in a greenhouse and another in growth chamber with a constant temperature of 25°C. To determine the dry weight of the structures sampling was carried out at different ages of the seedling. The stem accumulated more dry matter than the root, 56% and 44% respectively. The distribution of dry matter in the shoot structures was in the following order: simple leaves 60%, hypocotyl 25%, epicotyle 8%, petioles 5% and first trifoliolate leaf 2%. Root dry matter was distributed in the following order: secondary roots 46%, adventitious roots 42%, taproot 10% and tertiary roots 2%. The cotyledons exhausted its reserves of nutrients by the 18th day and they senesced at the same time. At the time of senescence of the cotyledons the hypocotyl structure was longer than the stem. Considering the root, secondary roots presented more length and were the most abundant in number. The input of fertilizer to the soil is necessary when cotyledons are beginning the process of senescence.

Key words

Phaseolus vulgaris L., dry matter, senescence of cotyledons, seedling

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Introduction

Factors affecting development during the development phases are different. These phases are: embryo, seedling, juvenile state, reproductive state and senescence (Salisbury and Ross, 1994). In each phase occurs nutrition and translocation of nutrients among structures of the plant. Thus, during plant growth accumulates and assigns to their structures different proportions of dry matter. The supply of nutrients from imbibition until it triggers photosynthesis depends on the nutrient content stored in the cotyledons. These structures are the first ones that come to the senescence in the state of seedling which coincides with the depletion of its stored reserves.

The translocation of assimilate starts from seed germination and continues during all the development stages of the plant (Ho et al., 1989). In this regard, there are sources of photosynthate conceived as the regions of net export of nutrients and sink of photosynthate, which are places of nutrients import. Thus a nutrient concentration gradient is established between source and sink and the nutrients are moved through the vascular system (Ho et al., 1989). During the process of germination begins mobilization of nutrients from seed cotyledons to the embryonic axis. This process is maintained until seedling state, in which the first sources (cotyledons) are exhausted and seedling becomes autotrophic. The translocation of reserves is reflected in dry weight, which decreases in the cotyledons, yet increases in hypocotyl and, subsequently increases in epicotyl, plumule and roots (Mayer and Poljakoff-Mayer, 1989; Diaz et al., 1999).

After germination, the plantule begins the establishment, ranging from the time the seedling emerges from the ground until it becomes autotrophic. There are epigeal species, whose cotyledons emerge and entails above the soil surface and hypogeal species whose cotyledons remain below the soil (Bewley and Black, 1985; Bell, 1991). Under normal conditions, seedlings (epigeal or hipogeal) rapidly initiate the synthesis of photosynthates passing into the autotrophic phase, thus ensuring its establishment (Mayer and Poljakoff-Mayer, 1989). At this stage the cotyledons are indispensable for supplying of nutrients that are used for the growth of the different structures of the seedling. The cotyledons are the source and other structures are the demand. To emerge from the soil the seedling starts photosynthetic activity and primary leaves complement the supply of nutrients to the seedling as it requires because the nutrients reserves of cotyledons are virtually exhausted.

The objective of this study was to determine the dynamics of growth and the dry matter allocation among the structures of the seedling developed in light conditions until the senescence of cotyledons. Knowing the time when the cotyledons contribute dry matter or nutrients to the growth

of shoot structures is important in determining the most appropriate step to provide nutrients to the soil.

Materials and methods

Experiment conditions and planting

Two experiments were conducted under light conditions: one in the greenhouse and another in a growth chamber at a constant temperature of 25°C.

The planting of beans "Cacahuete-72" with growth habit of type I (Debouck and Hidalgo, 1985) was carried out in transparent polyethylene cylindrical tubes 14 cm in diameter and 70 cm depth. The tubes were filled with sand washed with water. The seed was placed at depth of 2.5 cm after a field capacity irrigation. The seed was placed in an upright position with the micropyle up and the lens down in order to facilitate the growth of the radicle. The seeds sown in the greenhouse weighed between 290 and 300 mg per seed and in the growth chamber they weighed between 270 and 280 mg.

Handling seedlings

In the greenhouse experiment five samples were taken: eight, 13, 18, 21 and 25 days after planting (DAP) and in the growth chamber two, eight, 13 and 18 DAP. In the greenhouse the sample size was two seedlings in the first and second sampling, and three seedlings in the other samples. In the growth chamber were three seedlings in all samples.

The seedlings were removed from the tubes after the roots being complete. The tubes were cut longitudinally and the roots freed of sand using water. The seedlings were placed on wet newspaper to prevent drying. From each seedling different structures were separated. The fresh weight of structures was determined and then dried to a constant weight at temperature of 80°C for 48 hours to get the dry weight on analytical scale. The length of hypocotyl, epicotyl, petioles and leaves was measured with a ruler. The roots were separated by category: taproot, adventitious, secondary and tertiary. The number of roots in each category was counted and the length was determined with a curvemeter.

Shoot structures

The structure of stem include cotyledons, epicotyl, hypocotyl and radicle. The last three structures form the embryonic axis. The plumule encloses all parts located above the cotyledons. Epicotyl is the portion of the stem between the cotyledons and the simple leaves. Hypocotyl is located between the cotyledons and the neck of the root differentiated by a concave line at the root. The simple leaves occur at the second node. They are opposite and are divided into blade and petioles. The compound leaf is formed at the third node and is divided into leaflets and petiole.

Category of roots

The root system of beans is characterized by a taproot. The roots arising from taproot are secondary roots and the roots formed from secondary roots are tertiary roots. The adventitious roots are formed at the base of hypocotyl.

Roots impression

The impression of the root system on Ozalid paper is based on vapors of ammonium hydroxide (NH₄OH). The roots were carefully spread out on a glass plate and the Ozalid paper was exposed to the sun long enough (no more than half a minute) for the yellow color to change to white. The impression was used for counting the roots and for the measuring of their length.

Using reserves

The dry matter contained in the cotyledons represents metabolizable dry matter (which is the reserve material) and non-metabolizable (which is what constitutes the cell walls). The amount of cotyledons reserve was seen as its initial dry weight minus the dry weight when they reach their steady weight after drying representing the zone of structural cotyledons (non-metabolizable dry matter).

Statistical analysis

The information obtained was analyzed by calculating the mean and standard deviation.

The average was obtained by the formula:

$$X = \sum \frac{Xi}{n}$$

where:

- X = The average
- Xi = Value of the observation
- i = Observation 1, 2, ..., n
- n = Number of observations

The standard deviation is calculated using the formula:

$$\sigma = \sqrt{\frac{(Xi - X)^2}{n - 1}}$$

where:

- σ = Standard deviation
- X = Value of the observation
- i = Observation 1, 2, ..., n
- n = Number of observations

Results

Experiment I: Greenhouse

Allocation of dry matter

Shoot and root. The dry matter content decreased in the cotyledons and increased in the shoot and the root whose growth presented by sigmoid curve (Fig. 1A). The dry weight of cotyledons was stabilized after 21 days and the

shoot and root dry weight increased linearly between the eighth and 21st day, after which there was a breakthrough in dry weight. The weight of the shoot, root and cotyledons obtained until the stabilization of the weight in the cotyledons was 325, 250.6 and 18.3 mg, respectively. The cotyledons were senesced completely on the 21st day.

The allocation of dry matter between the shoot and root presented bigger differences in the first samples (Fig. 2.A). On the eight day 71% of dry matter was allocated to the shoot and the rest to the root. On the 18th day the allocation of dry matter tended to be balanced, the same amount was sent toward the shoot (56%) and to the root (44%), which was maintained until the last sampling.

Structures of shoot. The growth dynamics of the structures of the shoot was different in the weight of dry matter showing sigmoid growth (Fig. 3A). In sampling performed on the eighth day, hypocotyl accumulated more dry matter than other structures. On the 13th day simple leaves accumulated more dry matter than other structures. The epicotyl accumulated more dry matter than petioles. Upon stabilization of the weight of the cotyledons, the allocation of dry matter in the blades, hypocotyl and epicotyl were 197.6, 83 and 25 mg respectively.

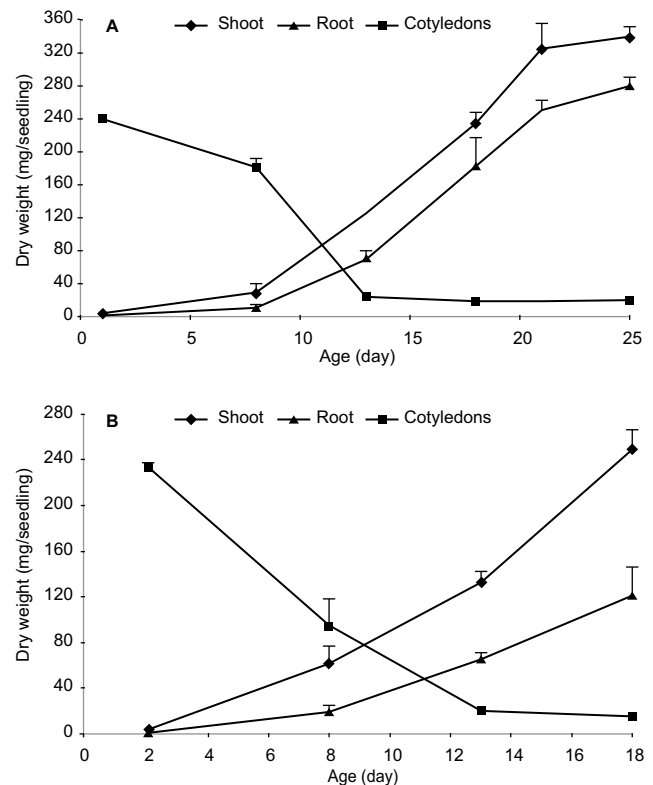


Figure 1. Accumulation of dry matter in bean seedling variety Cacahuete 72 developed in greenhouse (A) and chamber (B). Bars represent half of the standard deviation.

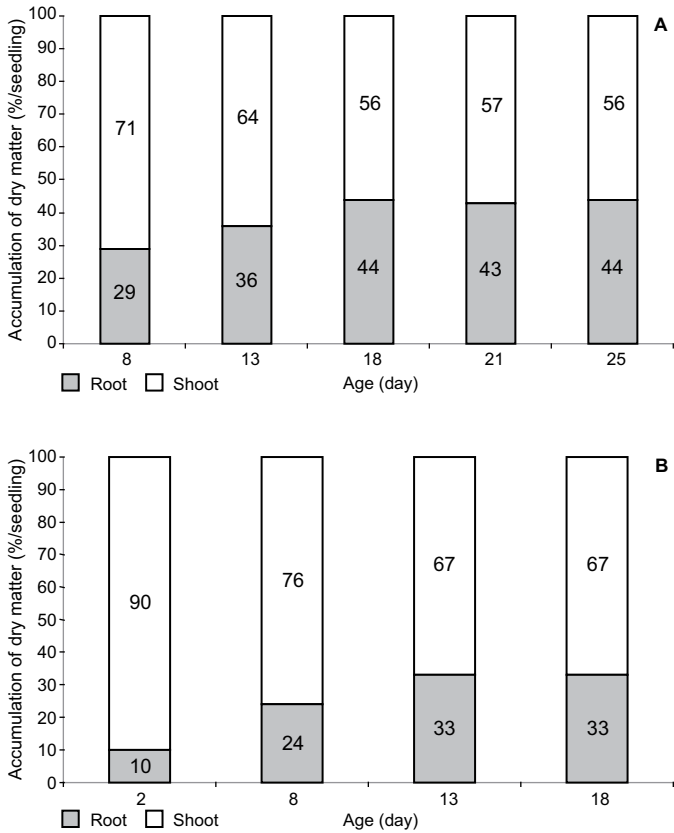


Figure 2. Allocation of dry matter in bean seedling variety Cacahuete 72 developed in greenhouse (A) and growth chamber (B).

Roots. After eight days the taproot had more dry matter than adventitious roots. After 13 days adventitious roots had the most weight. After 18 and 21 days secondary roots had the most weight and after 25 days the adventitious roots (Fig. 4A). After 13 days the taproot had less weight than the adventitious and the secondary roots, but more than the tertiary roots. The secondary roots had higher dry matter than the main root and tertiary roots on the 13th and 18th day. On the 21st day the allocation of dry matter was: in the secondary roots was 115 mg, in the adventitious root 106.3 mg and in the taproot 25 mg.

Growth of the shoot and roots structures

Length of shoot structures. Growth in length of the shoot structures tended to be sigmoid (Fig. 5A). The hypocotyl was longer than the other structures in all samples and had the maximum length (6.2 cm) after 18 days. The epicotyl was longer than the petioles on the 13th, 18th and 25th day, but on the 21st day the petioles were the longest. On the 21st day the length of: the hypocotyl was 2 cm, the epicotyl was 2.5 cm and the petioles were 3.7 cm.

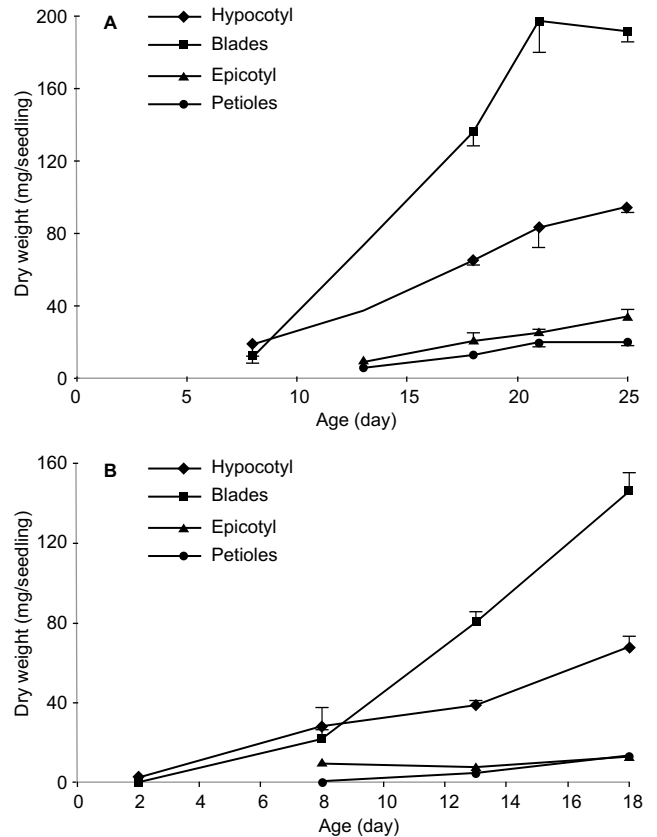


Figure 3. Accumulation of dry matter in the structures of shoot in bean seedlings developed in the greenhouse (A) and growth chamber (B). Bars represent half of the standard deviation.

Length of the roots. The secondary roots were longer than other roots in all samples except on the eighth day (Fig. 6 A). The adventitious roots were longer than other roots on the eighth day, while they were longer than the tertiary roots and the taproot, they were shorter than the secondary roots in all other samples. The tertiary roots were the longest on the 21st day and were shorter than other roots on the 18th and 25th day. The taproot maintained a steady growth in length in all samples with an increase on the 25th day. On the 21st day the length: of the secondary roots was 787.2 cm, of the adventitious roots was 213.8 cm, of the taproot was 25 cm and of the tertiary roots was 173 cm.

Number of roots. The secondary roots were abundant (Fig. 7A) with the exception of the eight day sample when they were overtaken by the adventitious roots. The number of tertiary roots was greater than the number of adventitious roots in all samples. The number of adventitious roots was stable after eight days. In the sample with stabilized dry weight of cotyledons (21st day) 490 secondary roots were quantified, 188 tertiary roots and nine adventitious roots.

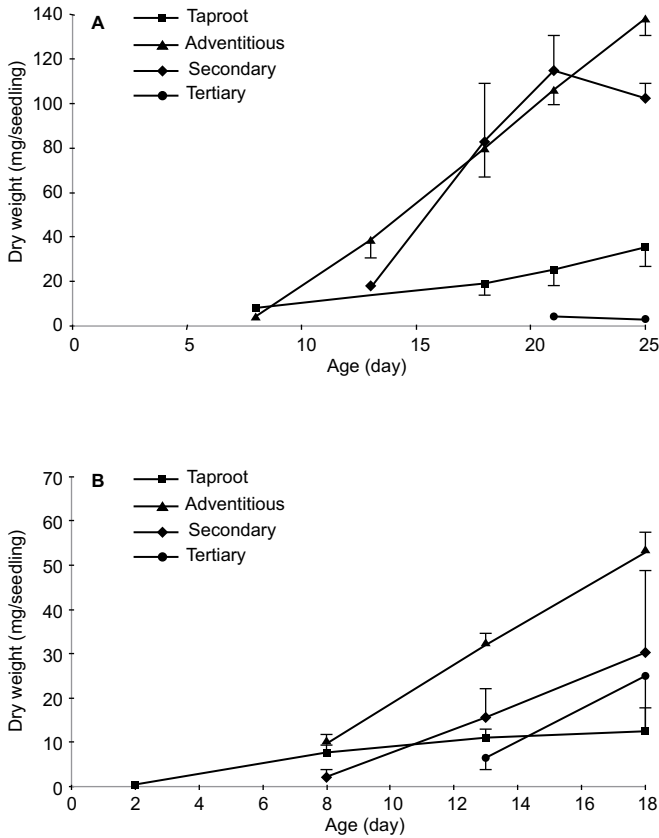


Figure 4. Accumulation of dry matter in roots of bean seedling variety Cacahuete 72 developed in greenhouse (A) and growth chamber (B). Bars represent half of the standard deviation.

Experiment II: Growth chamber

Allocation of dry matter

Shoot and root. The dry matter content decreased in the cotyledons and increased in the shoot and root (Fig. 1B). Between the eighth and 18th days, the increase in dry weight in the shoot and root was linear. The shoot had more dry weight than the root in all samples. The cotyledons had the lowest dry weight on the 18th day and that was stability weight of the cotyledons. The weight of shoot, root and cotyledons at the time when the weight of cotyledons was stabilized was 241.6, 120.7 and 15.6 mg respectively.

The allocation of dry matter between the shoot and root showed significant difference in the first sampling. For the first two days 90% of dry matter was allocated to the shoot, then the allocation tended to be balanced in proportion and mobilized the same amount to both sides (Fig. 2B). In all samples higher dry matter was assigned to the shoot than to the root. Stability in the proportion of dry matter allocated to the shoot and root was reached on the 13th day estimating 67 and 33% respectively.

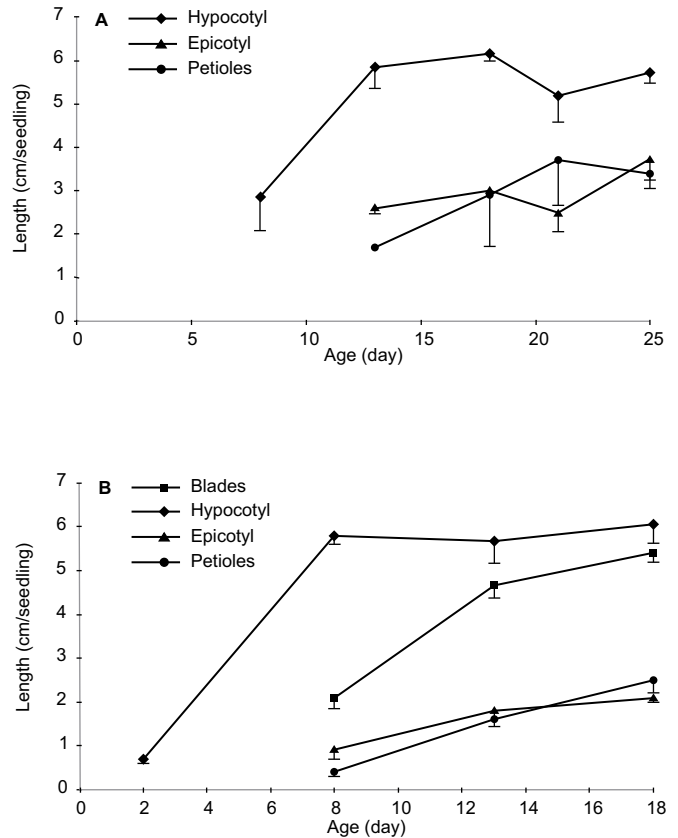


Figure 5. Length of the structures of shoot of bean seedling variety Cacahuete 72 developed in greenhouse (A) and growth chamber (B). Bars represent half of the standard deviation.

Structures of shoot. The growth dynamics of the shoot structures presented in the weight of dry matter showed sigmoid curve (Fig. 3B). On the second and eighth day hypocotyl accumulated more dry matter than other structures. On the 13 day simple leaves accumulated more dry matter. The epicotyl accumulated higher dry matter than the petioles except for the 18th day sample. Upon stabilization in dry weight of cotyledons (18th day) the amount of dry matter allocated to the blades, hypocotyl, petioles and epicotyl was 146, 68.3, 14 and 13.3 mg respectively.

Roots. The roots formed in the bean seedlings showed growth with sigmoid trend. The seedlings did not form roots from the taproot in the first two days. Between eighth and 18th day adventitious roots showed bigger accumulation of dry matter than the other roots (Fig. 4B). The secondary roots extracted higher dry matter than the main root and tertiary roots on 13th and 18th day. The tertiary roots had a higher amount of dry matter than the main root on the 18th day. When cotyledons stabilized their dry weight (18th day), adventitious roots accumulated 52.9 mg, secondary roots 30 mg, tertiary roots 25 mg and taproot 12 mg of dry weight.

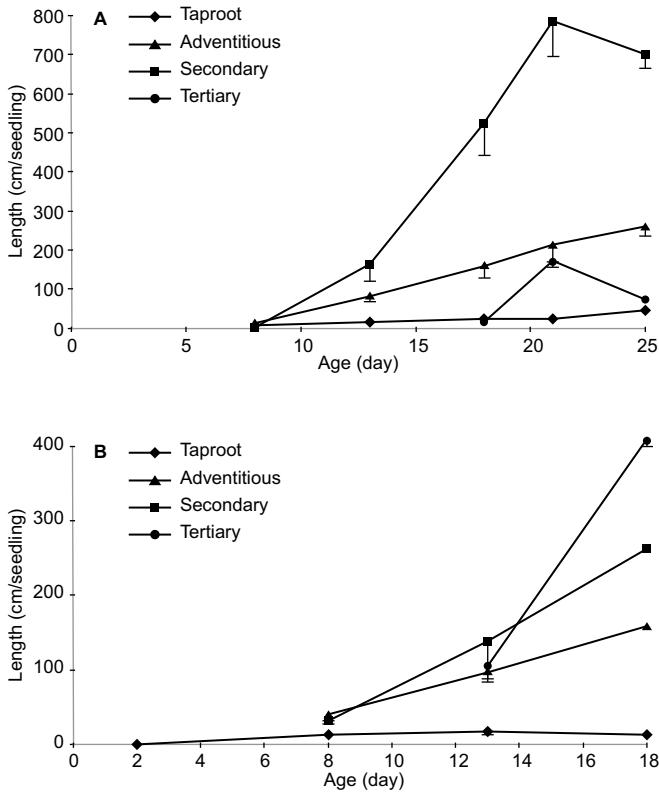


Figure 6. Length of the different category of roots of bean seedling variety Cacahuete 72 developed in greenhouse (A) and growth chamber (B). Bars represent half of the standard deviation.

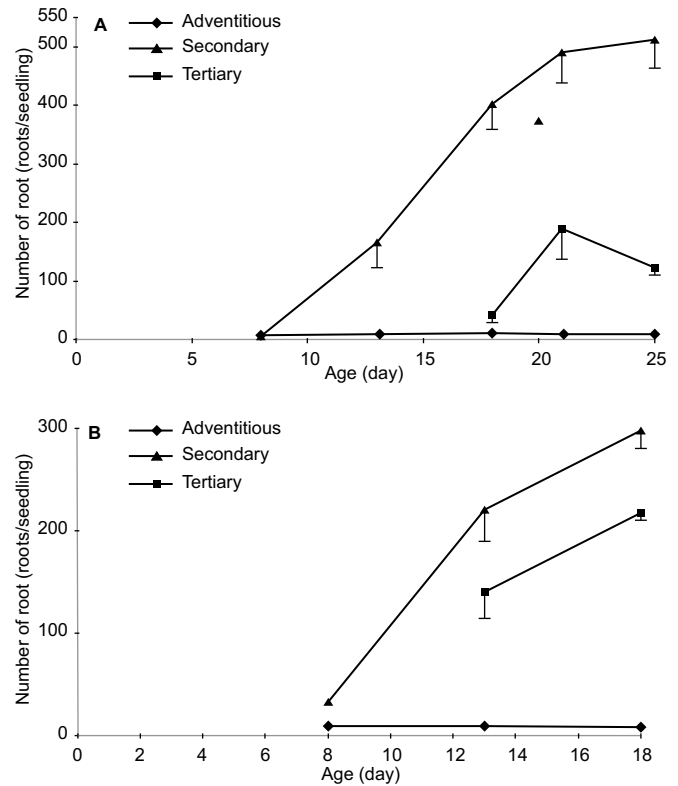


Figure 7. Number of roots of different category formed in bean seedling variety Cacahuete 72 developed in greenhouse (A) and growth chamber (B). Bars represent half of the standard deviation.

Growth of the structures of shoot and roots

Length of shoot structures. The growth trends in the length of the structures were sigmoid (Fig. 5B). The hypocotyl showed higher length in all samples. The petioles were longer than the epicotyl on the 18th day. The blades were longer than the epicotyl and the petioles and shorter than the hypocotyl in all samples. By the date of the cotyledons dry weight stabilization (18th day), the hypocotyl grew 6.1 cm, simple leaves blades 5.4 cm, petioles 2.5 cm and epicotyle 2.1 cm.

Length of the roots. The growth trends in length were linear except for the root that showed sigmoid trend (Fig. 6B). On the eighth day adventitious roots were the longest and in the following samples grew more than the taproot. The secondary roots were longer than the taproot but shorter than the adventitious roots on the eighth day. Upon stabilization of the dry weight of the cotyledons (18th day) the length of: the tertiary roots, the secondary and the adventitious root was 409.1, 262, 159.1 and 12.7 cm respectively.

Number of roots. All types of roots showed sigmoid tendency in increase of numbers except tertiary roots, which

showed linear phase only (Fig. 7B). The tertiary roots had more branches than the adventitious roots in all samples. The amount of adventitious roots was stable after eight days. On the 18th day, when the cotyledons senesced, the number of secondary roots was 298, tertiary roots 217 and adventitious roots eight.

Discussion

Allocation of dry matter

The cotyledons are indispensable during germination until the emergence of the seedling, at this stage, the increase in dry weight of the shoot and root is attributed to the input of nutrients from the cotyledons, which is reflected in the decline of its dry weight and the weight gained in of shoot and root (Fig. 1A and 1B). The increase in dry matter of seedlings between emergency and the time of depletion of the reserves in the cotyledons is due to nutritional reserves in the cotyledons and the photosynthesis carried out by shoot structures formed. According to Barloy (1984) the seedling during its establishment passes through three stages: the heterotrophic, transition and au-

trophic, in this case, we could say that the cotyledons are exhausted in the transition phase. The loss of dry weight in the cotyledons due to the use of its reserves led to a decline of 92.7% of their total dry weight, that could correspond to the mobilization of the reserves and more nutritious consumption by respiration. The stability of the dry weight of cotyledons recorded on the 21st day (experiment in greenhouse) and 18th day (experiment in growth chamber) indicates that the seedling uses the nutrients contained in the cotyledons as a source of food in the early days of its development, the rest belongs to the cell walls of cotyledons that consist of pectins soluble in alkali solution and hot water (Shiga and Lajolo, 2006). The shoot accumulated more dry matter than the root (Fig. 1A and 1B), which is typical for annual plants (De Souza and Da Silva, 1987). In the seedling stage the shoot is the most developed and it requires an increase of nutrients, however roots are starting their development so demand is lower.

The blades of the simple leaves become the main source of nutrients from the moment that the cotyledons do not provide nutrients. This indicates that in the seedling the flow of photosynthate from structures that manufactured to the structures that demand, begins in the cotyledons, continues in the simple leaves and ends in the compound leaves. In this way, the seedling demand the highest proportion of nutrients from the cotyledons during the early days of germination until the simple leaves manufactured enough nutrients for export to the structures that are sink. The transition of the simple leaves as sink organ to source organ is associated with their ability to manufacture nutrients and maintain a balance between their syntheses and use (Loescher et al., 1982). Later, the contribution of the cotyledons (reflected in the decline of its dry weight) is smaller than the contribution of the leaves. After the death of cotyledons, simple leaves begin the senescence as a first organ with its maximum growth and are the main organ sources of photosynthate. However, when this occurs the first trifoliolate leaf is capable of exporting nutrients. Under these conditions, the dry matter production of the seedling is a function of the nutrients generated in the simple leaves, so if there is any damage in them, the growth of the seedling is affected. Hodgkinson and Baas Becking (1977) indicate that the defoliation of seedlings cause roots death and they decreased in number, however, the roots have resource of carbohydrate that lets them survive until the seedling is restored (Buwai and Trlica, 1977).

The dynamics of dry matter accumulation in the different types of roots formed was similar. Upon senescence of cotyledons (21st day) the secondary and tertiary roots decreased in dry weight, however, the taproot and adventitious roots increased their weight. The decrease in the weight of roots indicates that the new roots are most affected when the source of nutrients is damaged; in this case

it was reflected in the decrease in the number and length of the roots. The adventitious roots always remained in a stage of high growth and only in the third and fourth sampling were overtaken by the secondary roots. These roots apparently reached their maximum dry matter accumulation on 21st day, coinciding with the maximum accumulation of dry matter in the blades. It is likely that such a coincidence resulted from a decrease in the supply of nutrients from the simple leaves to the roots. The taproot and adventitious roots continued growing in both parameters, possibly due to the increased demand of nutrients by the root. Apparent stability can be seen in the adventitious roots on the 18th day since their accumulation of dry matter and nutrients reduces the demand that is used in maintenance. Seedling adventitious roots are responsible for providing support and secondary root are responsible for exploring and absorption of nutrients from the soil. According to Fitter (1991), the roots of smaller diameter have greater ability to obtain nutrients and water from the environment in which the plant is developed. In our case, the secondary roots were the most important. Due to their specific radical length (data not shown) they were able to explore a greater volume of the substrate. The replacement rate of secondary roots was higher than the replacement rate of the adventitious roots (not quantified but if it was noticeable). In general the roots that perform this function strongly were the secondary and adventitious roots.

Growth in length and number

The hypocotyl reached the highest growth in length but continued to increase its dry weight. It is probably due to nutrients demand from the simple leaves and continuation of growth in diameter. The elongation can be attributed to the accumulation of dry matter. On the other hand, among the vegetative organs, the stem contains a higher amount of carbohydrates, hence it can act as temporary storage organ (Tanaka and Fujita, 1979) and carries out smaller scale photosynthesis due to chlorophyll contain.

The behavior presented by secondary and tertiary roots can be caused by the heterogeneity in the rhizosphere. Where there is more moisture in the soil profile there are more roots formed and they fell in the driest horizons of soil (Smucker, 1993). For its part, Smart (1994) indicates that the senescence of roots and leaves is determined by internal and external factors, particularly by interactions among some of them and control genetically during its development.

The number of secondary roots does not stabilize, which indicates the existence of two events: new roots formed and dead roots which are being replaced by new roots (Vogt and Bloomfield, 1991) and can be attributed to an indirect effect caused by the loss of secondary roots, some of them shed tertiary roots attached to them.

Fertilization of seedlings is required at the time when the cotyledons begin senescence (18th day), which helps the growth and development of new organs that demand nutrients, thus avoiding stress due to lack of nutrients in the seedling and the significant loss of structures of stem and roots.

Conclusions

The shoot accumulated more dry matter (56%) than the roots (44%). The allocation dynamic of dry matter in the stem was in descending order: blades of simple leaves (60%), hypocotyl (25%), epicotyl (8%), petioles (5%) and first compound leaf (2%). In the roots the allocation was in following order: secondary root (46%), adventitious roots (42%), taproot (10%) and tertiary root (2%).

The senescence happened only in some structures. In the stem, the cotyledons senesced. In the root system, the senescence was observed at individual roots, which were replaced by new roots. The senescence presented should not be viewed as a complete process, but as part of the development of the seedling. This phenomenon occurred when the blades of the simple leaves reached its maximum accumulation of dry matter and the first compound leaf began its growth.

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