

Quantitative determination of the contribution of photosynthetic cotyledons on growth of sunflower seedlings

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ABSTRACT

- ^ Sunflower cotyledons are storage organs and play a photosynthetic role in the seedling initial growth. It is generally believed that the photosynthetic ability of cotyledons can be crucial in vigor and other seedling characteristics. There are a few studies about the photosynthetic contribution to seedling growth. In previous work it was determined that 37% and 80% of weight of sunflower and squash seedlings respectively were supply by own cotyledons photosynthesis. In both species the distribution of the photo assimilates, and the elongation of the hypocotyls in seedling with covered cotyledons were different to the seedlings with discovered cotyledons. The aim of this work was to quantify the photosynthetic contribution of sunflower cotyledons to the seedling growth.
- ^ We sowed an experimental cultivar in controlled conditions of light and temperature. Ten seedlings were randomly selected for each one of the following treatments: control, uncovered cotyledons (T1); one covered cotyledon (T2); two covered cotyledons (T3). At eighteen days, length of root and hypocotyls; cotyledons area; and root, hypocotyls, leaves and cotyledons dry weight were evaluated. With dry weight data was calculated the root/aerial organs index (RAI) and absolute growth index (ACI). Ten seedlings of T1 and T3 were selected to determinate SPAD units and chlorophyll content by absorbance. Also, the number of stomata, and chloroplast in occlusive cells in epidermis was recorded. The information was analyzed by ANOVA and averages comparison.
- ^ Roots length differed significantly ($p < 0,05$) between T2 and T3; while T1 showed an intermediate value. Hypocotyls length was significantly higher in T3 than T1, T2 had an intermediate value. The area of cotyledons, SPAD units and chlorophyll content of the cotyledons in T3 were significantly smaller that in T1. The number of stomata in the epidermis was significantly different between treatments and it was higher in T3, while there were no differences in the number of chloroplast in occlusive cells. Dry weight of roots, leaves and cotyledons were different between T1 and T3, T2 showed an intermediate value, while the weight of the hypocotyls did not show significant differences in any treatments. Dry weight of complete seedlings was significantly higher in T1 than T3, T2 showed intermediate values. Root/aerial organs index (RAI) showed that the growth of the radicular fraction decreased more than the growth of the aerial fraction in covered cotyledons seedlings, and the absolute growth index (AGI) was smaller in T3. If we consider 100% (maximum possible growth), the total dry weight of seedlings with their uncovered cotyledons (T1) and that T3 only grew for the contribution of the reserves tissues, we determinate that the contribution for photosynthesis of cotyledons in these conditions was 63%.
- ^ Our data shows that the contribution of the cotyledon by photosynthesis to initial growth of sunflower plants is essential. However this topic deserves further studies, to generalize such influence in sunflower and other species with epigeal cotyledons.
- ^ This work provides a quantification of the photosynthetic contribution of the cotyledons, which could confirm that the photosynthetic ability of cotyledons can be relevant in vigor and plant establishment.

KEY WORDS: contribution- cotyledons- photosynthesis- seedling growth

INTRODUCTION

Epigeous cotyledons are storage organs for example in *Phaseolus vulgaris* (Lovell y Moore, 1970), they have a little photosynthetic contribution (Raven, *et al.* 1992). In *Cucurbita máxima*, *Lotus glaber*, *Helianthus annuus* L. cotyledons play an important photosynthetic role in the seedling initial growth. It is generally believed that the photosynthetic ability of cotyledons can be crucial in vigor and other seedling characteristics.

In *Trifolium pratense*, *Cucurbita pepo*, *Sinappis alba* y *Cucumis sativus* the cotyledon expansion and dry weight increase during the initial growth (Lovell and Moore, 1970). Changes in photosynthetic cotyledons were studied during early germination and seedling growth until senescence on different aspects in: *Glycine max*, (Treffry, *et al.*, 1967); *Paulownia tomentosa* (Rickson ,1968); *Cucurbita maxima* (Lott, 1970); *Robinia pseudoacacia*, *Ulmus americana*, *Betula alleghaniensis*, *Acer sacharum* and *Ahlanthus altissima* (Marshal and Kozlowsqui, 1974) *Robinia pseudoacacia*, *Fraxinus pensilvanica*, *Ailanthus altissima* (Marshal and Kozlowsqui, 1976). Raven, *et al.* (1992) found a significant contribution the cotyledon of *Allium cepa*.

Mujica and Rumi (1998) was evaluated the photosynthetic ability of cotyledons. These authors concluded that this ability may be determining the seedling vigor and other characteristics related with field establishment like number and size of leaf, stem, and root length. Those aspects improve the speed and uniformity of emergence and decrease the opportunity for plagues and diseases to attack. That affects consequently the yield per unit area (Cardinali, 2004). However, in sunflower, high plasticity of leaf and root growth make it possible to decrease the negative effect of non uniformity of the resulting culture (Satorre, *et al.*, 2003).

Very little is known about the quantitative contribution to seedling growth by photosynthetic cotyledons activity. Mujica and Rumi (1998) found that by shading the cotyledons in *Lotus glaber* seedlings, the root dry weight decreased significantly, because, possibly, the root is the main target of the cotyledon contribution. Also leaf dry weight was significantly decreased in covered cotyledons. Scorziello, *et al* (2010) determined that 80% dry weight of squash were supply by own cotyledons photosynthesis, and Murcia, *et al* (2011) was determined that in sunflower 37% of dry weigh were supplied by cotyledons. In both species the distribution of the photo assimilates, and the elongations of the hypocotyls in seedling with covered cotyledons were different to the seedlings with discovered cotyledons.

The aim of this work was to quantify the photosynthetic contribution of sunflower cotyledons to the seedling growth.

MATERIALS AND METHODS

We sowed an experimental cultivar in controlled conditions of light (16/8 h; 750 lux) and temperature (25°C ± 1). Ten seedlings were randomly selected for each one of the following treatments: control, uncovered cotyledons (T1); one covered cotyledon (T2); two covered cotyledons (T3). At eighteen days, seedlings were collected and root and hypocotyls length, cotyledons area (with LI-Cor. Inc. Lincoln, Nebraska USA) and; root, hypocotyls, leaf and cotyledons dry weight were evaluated. With dry weight data was calculated the root/aerial organs index (RAI) and absolute growth index (AGI), (total dry weight/ and days after sowing Ten seedlings of T1 and T3 were selected to determinate SPAD units (with Minolta® SPAD 502) and chlorophyll content by absorbance (Dere, *et al.*, 1998). Also, epidermis was extracted from 10 cotyledons for stomatal counts in five replications per cotyledon, corresponding to the field of the microscope (100X) and chloroplasts in guard cells was recorded. The information was analyzed by ANOVA and averages comparison by R DEVELOPMENT CORE TEAM.

RESULTS AND DISCUSION

Table 1 shows significant differences ($p < 0,05$) between T2 and T3 in root length; T1 show an intermediate value.

Table 1. Root and hypocotyl length and area of cotyledons

Treatment	Root length (cm)	Hypocotyl length (cm)	Cotyledon area (cm ²)
1	24.78 AB	2.53 B	8.30 A
2	26.67 A	4.10 AB	-----
3	18.02 B	5.84 A	5.71 B

Means followed by the same letter in columns are not significantly different at $p \leq 0,05$.

While these results do not match with the work of Scorziello, *et al* (2010) in *Cucurbita maxima* var. Zapallito, in both cases the root length in seedlings with covered cotyledons were lower, and this result could be an effect of the shading, as in *Lotus glaber* (Mujica and Rumi, 1998). According to these authors, the root could be the main target of the photo assimilates supplied by the cotyledons. Hypocotyls length was significantly higher in T3 than in T1, T2 showed an intermediate value. These results are consistent with previous in sunflower (Murcia, *et al.* 2011) and squash under greenhouse by Scorziello, *et al.* (2010). According to Black y Shuttleworth, (1976) the hypocotyl elongation is stopped because the cotyledons are involved in hypocotyl growth inhibition by perceiving the light stimulus; Mujica y Rumi (1998) shows that function of photoreceptors of the cotyledons in *Lotus glaber* was important in determining of the hypocotyl enlargement.

Cotyledons area, SPAD units and chlorophyll content of cotyledons were significantly lower in T3 than in T1 (Table 2).

Table 2. Chlorophyll a and b content and SPAD units of cotyledons

Treatment	Chlorophyll a (mg/g of tissue)	Chlorophyll b (mg/g of tissue)	SPAD (units)
1	37.76	36.94	47.79
3	22.07	15.26	15.26

These variables may be related to their photosynthetic ability in every treatment, and the incidence of light (Salisbury and Ross, 1994); the results match with the previous work under greenhouse in squash and sunflower (Scorziello, *et al.* 2010).

Figure 1 shows the number of stomata in epidermis and figure 2 shows the number of chloroplast by guard cell. Such variables do not appear to be related to the growth, development and photosynthetic activity of cotyledons. To Lovell and Moore (1970) the frequency of mature stomata generally decreases during cotyledon expansion, suggesting that the production of stomata did not keep up with the increase in cotyledon area. That could explain the major frequency observed in covered cotyledons.

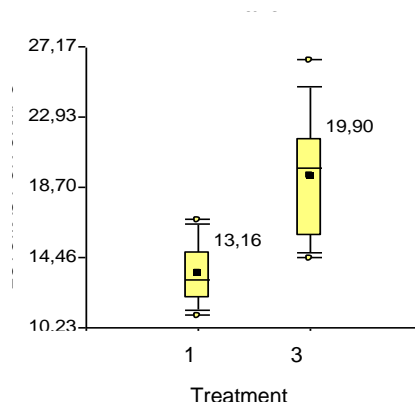


Figure 2. Number of stomata in epidermis

About the number of chloroplasts per guard cell, that is not associated with photosynthetic activity neither the chlorophyll content. In spite of the differences between T1 and T3 are not significant absolute value is high in T3 (Figure 2). Which is possible because the chloroplast can formed in darkness. Some development in internal membranes occurred at 3 days in soybean, when cotyledons were green, and continued until 7 days after imbibitions. Chlorophyll is accumulated rapidly in coincidence with the development of internal membranes in the plastids (Teffry, *et al*, 1967) and because they differ in dark and light; probably the difference is at ultrastructural level.

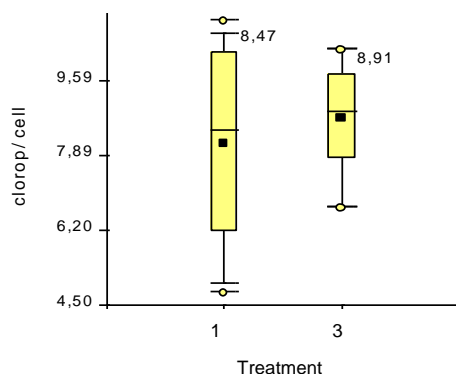


Figure 2. Number of chloroplasts per guard cell

Nevertheless a set of anatomical studies will be needed to analyze the characteristics of mesophyll, which is mainly parenchyma, specific tissue for photosynthesis

Dry weight of roots and leaves in agreement with results in *Lotus glaber* by Mujica and Rumi (1998) decreased significantly, in seedlings with covered cotyledons (T3). Also, cotyledons in T1 were significantly higher than T3, T2 showed an intermediate value, while the weight of the hypocotyls did not show significant differences in any treatments (Table 3)

Dry weight of complete seedlings was significantly higher in T1 than T3, T2 showed intermediate values. These results match with previous studies in greenhouse for sunflower and squash (Scorziello, *et al*, 2010); and confirm that cotyledons play an important photosynthetic role in the initial growth of sunflower seedling, like in *Lotus glaber* (Mujica and Rumi, 1998).

Table 3. Dry weight of different seedling organs and total weight

Treatment	Hypocotyl	Root	Cotyledon	Leaf	Total
1	32.05 A	228.28 A	42.79 A	444.79 A	747,91 A
2	28.23 A	127.79 AB	30.23 B	302.27 AB	488,52 AB
3	24.82 A	56.67 B	26.11 B	173.75 B	281,35 B

Means followed by the same letter in columns are not significantly different at $p \leq 0,05$.

Root/aerial organs index (RAI) showed that the growth of the radicular fraction decreased more than the growth of the aerial fraction in covered cotyledons seedlings (T3), and the absolute growth index (AGI) was smaller in T3 seedlings (Figure 3). These variables confirm the effect of photosynthetic contribution of sunflower cotyledons to the seedling growth. Also shows that the distribution of the photo

assimilates in seedling with covered and uncovered cotyledons were different, in agreement with previous results in sunflower and squash (Scorziello, 2010) .

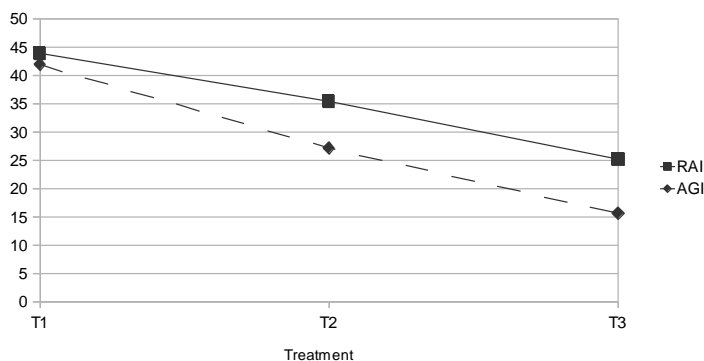


Figure 3. Root/aerial organs index (RAI) and the absolute growth index (AGI)

If we consider 100% (maximum possible growth), the total dry weight of seedlings with their uncovered cotyledons (T1) and that T3 only grew for the contribution of the reserve tissues, we determinate that the contribution of cotyledons for photosynthesis in these conditions was 63% (Figure 4).

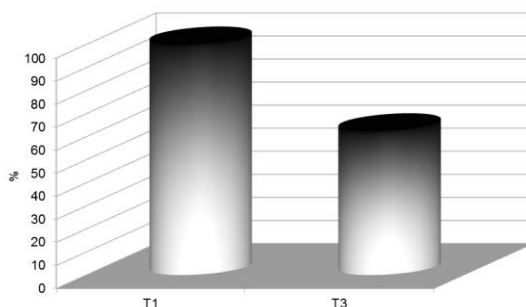


Figure 4. Total dry weight of seedlings with their uncovered cotyledons (T1) and that T3 only grew for the contribution of the reserve tissues

This work provides a quantification of the photosynthetic contribution of the cotyledons, which could confirm that the photosynthetic ability of cotyledons can be relevant in vigor and plant establishment. However deserves further studies, to generalize such influence in sunflower and other species with epigeal cotyledons.

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