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Timing of morphological and histological development of sunflower fruit. A description model with practical implicances.

Lilia I, Lindström[1] and Luis F. Hernández[1,2]
[1] Lab. de Morfología Vegetal.Dpto. de Agronomía-UNSur, Bahía Blanca, 8000 y [2]CIC-Provincia de Buenos Aires, La Plata, 1900. Argentina. ivlind@criba.edu.ar

ABSTRACT

- *Background and aims.* Until now, studies related to the sunflower fruit histogenesis have been focused on some developmental stages of the pericarp or embryo separately. The preanthesis period when the ovary structure is formed has not been considered. Moreover, the events described so far have not been referenced to a phenological scale, a key aspect to take into account when phenology and ecophysiology results are to be cross referenced. This paper describes the development of microspores and the full histological evolution of the sunflower fruit in time and in a phenological scale, from the ovary into the pericarp and from the ovule into the embryo, in a period comprised between an early reproductive stage (R2) and physiological maturity (Schneiter and Miller, 1981).
- *Methods.* Two commercial hybrids, Dekasol 3900 and Dekasol 4030 were grown in the experimental field of the Agronomy Department of the Universidad Nacional del Sur - Bahía Blanca (38°45' S; 62°11' W) in a fully randomized design with three replications. Histological studies were made on flowers and fruits taken from the mid position on the capitulum. Progress in ovary, ovule, pericarp and embryo dimensions, pericarp and embryo dry weight accumulation and embryo cell division were also determined.
- *Key results.* The fruit developmental model was similar between hybrids. Sporogenesis and gametogenesis took place between R2 and R4. The number of cell layers in the carpel was fixed at R4. Anthesis in the mid position of the capitulum occurred at R5.7. Pericarp final volume and weight were respectively reached 8 and 13 days after anthesis (DAA). Embryo cell division lasted 18 DAA. The linear phase of the embryo growth took place between 11 DAA and 31 DAA.
- *Conclusions.* The potential volume and weight of the pericarp were set during the preanthesis period (R4) while its final volume and weight were reached at early postanthesis (11-13DAA) The potential weight of the embryo was fixed 18 DAA, while its final dry weight was reached 31 DAA.
- *Nature of the contribution to current knowledge achieved by the research.* The sunflower fruit developmental model described in this paper will allow the assessment of the effects of genotype and environmental conditions at different moments of the fruit ontogeny on fruit yield and quality.

Key words*:* Fruit anatomy; fruit development; *Helianthus annuus* L.; hull; seed; crop yield.

INTRODUCTION

The weight and structure of the sunflower fruit affects yield and the efficiency of the industrial process for oil extraction. The fruit weight is the last yield component to be fixed, while other fruit parameters such as the relative pericarp development with respect to the embryo and the anatomy of the pericarp affect fruit hullability. The quality and costs of the byproducts obtained from the oil extraction process depend on the latter parameter (Denis *et al.*, 1994).

The final fruit structure is the result of a coordinated development of the pericarp and the seed (Roth, 1977). However, studies on the sunflower fruit only describes the pericarp (Roth, 1977, Mantese *et al.*, 2006) or embryo developmental stages separately (Newcomb, 1973a, b; Cantagallo *et al.,* 2004; Gotelli *et al.*, 2008). Moreover, the events described in there are not referenced to a phenological scale, a key aspect to be considered when comparing the results of ecophyisiological investigations. Furthermore the preanthesis period when the ovary develops has not been analyzed. After fertilization the ovary is the organ that will become the pericarp. In barley, wheat and sunflower ovary weight at anthesis correlates well with fruit weight at physiological maturity (Scott *et al.*, 1983; Calderini and Reynolds, 2000, Cantagallo *et al.*, 2004). For this reason, the early ovary development could play a critical role in the control of the fruit potential size.

In maize and soybean the number of cells established in the endosperm or embryo can limit their capacity to store assimilates during later stages of kernel growth (Egli, 1998). However, references about the sunflower embryo cell division process are scarce. The dynamics of embryo dry matter accumulation allows us to speculate that the active cell division of the sunflower embryo would be located in the lag phase of growth (the first ten days of embryo growth), so the embryo potential size would be established upon the completion of this period.

In this paper, followings the events are referred to the reproductive stages defined by Schneiter and Miller (1981), a sunflower developmental model based on the analysis of cell division activity, volume increase and differentiation of the ovary tissues, the ovule, the pericarp and the embryo from reproductive stages R2 to PM is proposed.

MATERIALS AND METHODS

Two commercial sunflower hybrids, Dekasol (DK) 3900 and DK4030 (Monsanto®, Argentina) were grown at the experimental field of the Agronomy Department-UNSur, Bahía Blanca, Argentina (38°45' S; 62°11' W) in a fully randomized design with three replications. At seedling emergence plant density was adjusted to 5.6 plants/m^2.

In order to describe ovary, ovule, anther, pericarp and embryo histogenesis five ovaries and, after fecundation, fruits from the mid position of the capitulum of 2 plants per plot at R2, R3, R4, R5.1, R5.7 (anthesis) R5.7 + 3 days (three days after anthesis; DAA), 8DAA, 13DAA and at physiological maturity (PM) stages (Schneiter and Miller, 1981) were preserved in fixative solution. Fixed samples were embedded in paraffin, cut at 12µm in a tranverse or longitudinal direction and stained according to conventional techniques. Observations of sections were done using a Nikon Labophot-2 microscope and measurements were taken with an ocular micrometer.

To evaluate pericarp length and width and the length, width and maximum cross section of the embryo, five flowers and fruits of the mid position of the capitulum of 2 plants per plot were sampled every 3 to 5 days from anthesis (R5.7) up to physiological maturity (PM). Pericarp and embryo dry weight of the same samples was determined after drying at 60° C for 72 h.

Duration and rates of pericarp mass increase were estimated using a non-linear fitting algorithm included in the software Kaleidagraph v. 4.04 (Synergy Software, USA).

Cotyledon cell number, embryo growth rate (EGR, mg/embryo/day) and the duration of the lag phase and effective filling period (EFP, days) were determined following Lindström *et al.* (2006).

The experimental results of each variable were processed by analysis of variance and differences between treatment means were evaluated with LSD test.

RESULTS

Ovary and fruit histological development was similar between hybrids. Microsporogenesis and megasporogenesis took place between reproductive stages R2 and R3, around 15-12 days before anthesis (DBA), respectively. Microgametogenesis and megagametogenesis occurred between reproductive stages R3 and R4, between 12 and 6 DBA, respectively (Fig. 1). Cell division rate in the ovary wall (future pericarp) decreased as from R2 with no dividing cells observed at R4. At R5.1 (3DBA) the vascular bundles of the ovary wall had differentiated. The ovary was almost twice as long but the cross section

thickness of its wall was similar to that in the previous stage (Fig. 2). Pericarp length, width and cross section thickness became stable 8 DAA, while the most important changes in the embryo dimensions took place between 8 and 13 DAA (Fig. 2). Pericarp maximum dry weight and sclerification were attained between 11 and 13 DAA (Fig. 2).

Pollination and fertilization were followed by 10 to 11 days of rapid cell division during which embryo structures were defined. Nevertheless, final cell divisions were registered 18DAA (Fig. 2). The lag phase (11.2 ± 0.17 days) and EFP duration (19.1 ± 0.98 days) did not differ between hybrids ($P > 0.05$). The most important changes in the embryo dimensions took place between 8 and 15 DAA. The difference ($P<0.05$) in the weight reached by the embryos of the two hybrids at PM, was associated with the highest EGR of DK4030 (2.2 mg/day) with respect to DK3900 (1.8 mg/day).

DISCUSSION

In sunflower, effects of genotype, environment or crop management practices on fruit weight have been studied mostly during the fruit filling period. So changes occurred were mostly explained as a consequence of variations in growth rate or duration, or both. However, some authors have suggested that some fruit features are fixed before that stage, and so it is inferred that the number of cells fixed in the ovary and the embryo are involved in fruit weight determination (Aguirrezábal *et al*., 2003; Cantagallo *et al.* 2004*;* Mantese *et al.,* 2006; Rondanini *et al.,* 2009).

The sunflower fruit development model presented here provides novel information about the starting moment and duration of the different anatomical and physiological processes that are involved in the determination of the potential and final weight of the sunflower fruit (Figs. 1 and 2). These parameters are strongly related to crop yield. Moreover, unlike other cases, the descriptions presented in this work were referred to a well known phenological scale (Schneiter and Miller, 1981), thus making it practical to compare results among equivalent research works. The pericarp potential volume was set in R4. (Fig. 1). The development of a fruit starts with a cell division process at the reproductive meristem level of the receptacle, which originates the carpels, stamens and seminal primordia. This is followed by volume increase and cell differentiation. Thus, the generation of the potential volume of the outer fruit would start at EF5, at the same time when the fruit potential number starts to be fixed, which ends at EF8 (Marc and Palmer, 1980). This indicates that part of the period when the fruit potential number is set, overlaps with the period when the fruit potential volume is determined. A decrease of the intercepted solar radiation in shaded plants during this period is able to reduce the number and cross section of differentiated floret primordia (Cantagallo *et al.*, 2002). However, the differences in the primordia cross section progressively diluted up to its disappearance at anthesis. This resulted in a dramatic decrease in the number and similar or slightly higher dry weight of the fruits than in control treatment at harvest, thus showing a certain kind of compensatory effect between both variables. The compensation between the fruit number and the final fruit weight was attributed to the coexistence of the processes involved in establishing the potential value of these two parameters in different sorghum [(*Sorghum bicolor* (L.) Moench] genotypes (Yang *et al.*, 2009).

After fertilization, the ovule generates the seed, and the maternal tissue derived from the ovary wall will generate, after a series of morphological changes, the mature pericarp (Fig. 2). In the case of the two sunflower hybrids under analysis, the maximum pericarp volume was reached 8DAA, while maximum dry weight was recorded 5 days later, the time when lignification of the secondary cell walls were completed. (Figs 1 and 2). Rondanini *et al.* (2009) determined that the maximum water content in the pericarp was reached between 5 and 7 days before its maximum dry weight.

The embryo volume and potential weight were set 18DAA (Fig. 2). This confirms that the latest cell duplications overlapped with the initial part of the dry matter fast accumulation stage of the embryo, which took place between 11 and 31 DAA. At that moment, the maximum embryo weight and the PM of the fruit were recorded.

The timing of morphological and histological events of the sunflower fruit development presented here sets a non-existent conceptual framework of analysis for this species. It can be used to evaluate and understand how the genotype, environment (temperature, solar radiation, water availability, etc.) or agricultural management practices (crop density and sowing date) could affect the sunflower fruit weight and shape as well as pericarp anatomy, parameters directly associated with both crop yield and fruit hullability.

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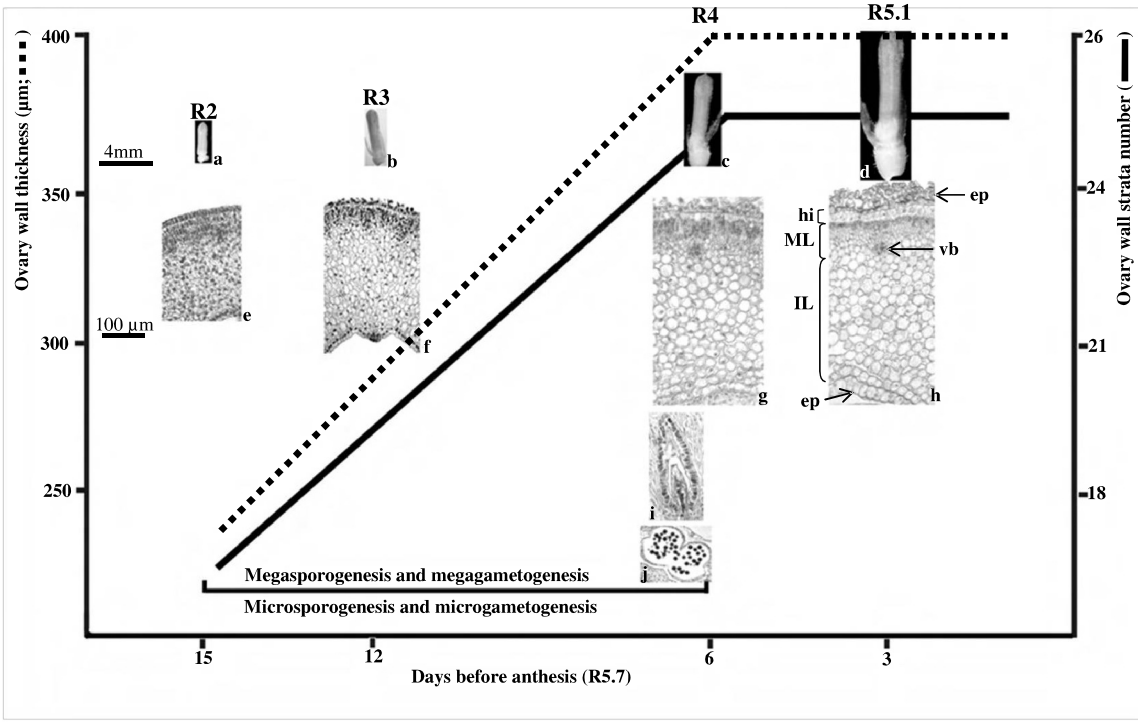

Figure 1. Development of female and male reproductive structure at the middle position of the capitulum at reproductive stages R2, R3, R4 y R5.1 (Schneiter and Miller, 1981). **a-d** flowers. **e-f.** ovary wall cross section. **i**. longitudinal section of the embryo sac**. j**. anther cross section. **ep**.: epidermis. **hi**.: hypodermis. **IL**.: inner layer. **ML**.: middle layer. **vb**.: vascular bundle. Scale bar: **a-d** = 4mm; **e-h** = 100 µm.

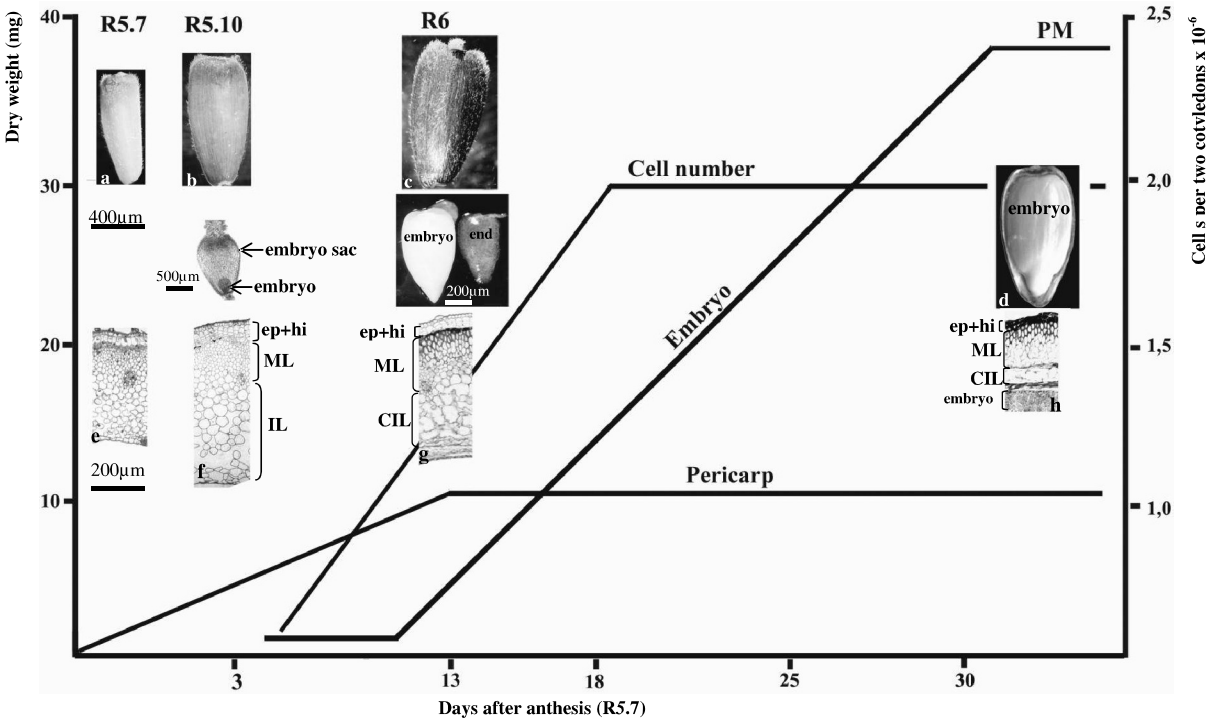

Figure 2. Changes with time in pericarp weight and embryo cell number and weight of fruit at the middle position of the capitulum. R5.7, R5.10 and R6 reproductive stages of Schneiter and Miller (1981). **a-d** fruits. **e-f.** pericarp cross section. **ep**.: epidermis. **CIL**: compressed internal layers. **end**: remains of endosperm. **hi**.: hypodermis. **IL**.: inner layer. **ML**.: middle layer. **vb**.: vascular bundle. Scale bar: **a-d** = 400 µm; **e-h**: 200µm.