Dormancy in sunflower line A-3: the role of the pericarp

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Abstract

Sunflower (*Helianthus annuus* L.) can often display seed dormancy, which causes a delay for immediate sowing. The final degree of "whole-seed" dormancy is determined by the contributions of the tissues that comprise it such as, embryo, seed coat, and/or pericarp. The sunflower dormancy can be reduced during after-ripening and by removing seed constraints. Our objective was to study how the conditions of storage and removal of the pericarp affect the level of dormancy in line A-3. Also we provide insight on the basis of the morphological characteristics of A-3 pericarp-imposed dormancy. A germination test was conducted on dry cypselas with and without pericarp at 30 and 70 days after-harvest. For histological analysis, permanent slides of pericarp cross-sections were obtained. The germination percentage showed significant differences between cypselas with intact pericarp (30 days after-harvest: 26%; 70 days after-harvest: 77%) and cypselas without pericarp (30 days after-harvest: 65%; 70 days after harvest: 96%). This indicates that the pericarp plays an important role in regulating seed physical dormancy of sunflower line A-3 and its relative contribution to the dormancy level is modified during after-ripening.

Key works: after-ripening, dormancy, histology, pericarp, sunflower

Introduction

Sunflower (*Helianthus annuus* L.) is an important agricultural crop, mainly due to quality of its oil, which is useful for the human consumption and for production of biodiesel (Paniego et al. 2007). Thus, the high demand of vegetable oil makes it necessary to increase sunflower seed production. Several factors such as temperature, water stress, and soil salinity affect seed germination, and seedling emergence of sunflower (Andrade et al. 2009; Luan et al. 2014). In addition, the seed dormancy can cause an important delay for immediate sowing and makes commercialization difficult (Bazin et al. 2011). The final degree of "whole-seed" dormancy can be determined by the contributions of different tissues that comprise a seed (Graeber et al. 2012). Sunflower fruit, commonly referred to as seeds, is known to botanists as a cypsela. Two basic parts of the cypsela are the pericarp which represents about 20-25% of its dry weight, and the true seed where lipids are synthesized and accumulated. The seed is formed by an embryo and a seed coat that surrounds it.

In sunflower, the embryo, the seed coat, and/or the pericarp have been previously identified as sources of dormancy (Finch-Savage and Leubner-Metzger 2006; Brunick 2007). Particularly, the pericarp has a complex effect on germination and dormancy as a result of both physical and chemical factors, such as prevention of water absorption, prevention of leakage of chemical inhibitors, inhibition of radicle protrusion, and accumulation of chemical inhibitors (Sari et al. 2006; Rathjen et al. 2009). The thickness of the seed coat also affects sunflower seed dormancy mainly through the supply of oxygen to the developing embryo (Rolletschek et al. 2007). Moreover, Szemruch et al. (2014) associated the dormancy level of sunflower seed with the wall thickness of the outer cell of the endospermatic cell layer of the seed coat.

Our previous study showed that the pericarp contributes partially to the seed dormancy of B123 sunflower inbred line (Andrade et al. 2015). The anatomical analysis showed that the establishment of physical dormancy may be related to the increased cell wall thickness, sclerification of the middle layer, and the absence of parenchyma in the micropylar area of B123 dormant seeds, in comparison

with the B91 non-dormant seeds. In addition, in B123 cypselas, pericarp rays and some cells of the inner side of the middle layer were impregnated with a substance similar to the phytomelanin found in the subepidermal layer (Andrade et al. 2015).

In order to minimize the period of dormancy of sunflower seeds, several treatments have been used such as seed storage under dry conditions (after-ripening) and by removing constraints (i.e. tissue surrounding the embryo) (Bazin et al. 2011; Nasreen et al. 2015). Therefore, to contribute to understanding the dormancy of sunflower seeds, we studied how the conditions of storage and removal of the pericarp affect the level of dormancy in seeds of sunflower inbred line A-3. Also, we provide insight into the morphological characteristics of A-3 pericarp-imposed dormancy. The importance to study the sunflower A-3 inbred line is based that it is used in the production of commercial hybrids in Argentina.

Material and Methods

Plant material

The A-3 *H. annuus* dormant line was grown in an experimental field of Argentine Cooperatives Association (ACA) (Hughes, Santa Fe, Argentina, latitude 33°44'01.99" S; longitude W 61°22'11.73" W). This area has an Argiudol soil type (texture: silt loam, deep and well drained) and warm-wet climate with a mean annual temperature of 14 ºC and mean annual precipitation of 900 mm which occurs from October to April. The A-3 cypselas were stored for 30 days after harvest at room temperature (25 ± 1°C, 50% relative humidity).

Germination assays

Cypselas were harvested and stored during 30 and 70 days at room temperature (25 ± 1°C, relative humidity 50%) in paper packets sealed. Germination tests were conducted on dry cypselas after-ripened for 30 and 70 days after-harvest. Cypselas were divided into two groups. In group 1 (cypselas without pericarp) the pericarps were carefully peeled off by hand to avoid mechanical

damage. In group 2 (cypselas with pericarp) the cypselas were kept intact. Four biological replicates (each 25 cypselas) were sown in 16x12-cm pots between filter papers moistened with 25 ml deionized water, and placed in a GR48 controlled environment walk-in rooms (Conviron, Winnipeg, Canada) programmed with a cycle of 16 h light (10.800 lux/ s) at 28 °C and 70% relative humidity, and 8 h dark, 20 °C and 80% relative humidity. Cypselas with pericarp were considered to have germinated when the radicle protruded through the covering layers (seed coat and pericarp). Germination in cypselas without pericarp was defined as visible growth of the radicle through the seed coat. Germination percentage was recorded at 10 days. The viability of cypselas was determined using a standard tetrazolium test (Moore 1962).

The contribution percentage of different sunflower cypsela tissues to dormancy level at 30 and 70 days after-harvest was determined by considering the germination data of cypselas (with pericarp) and seeds (without pericarp). All data were adjusted to the viable seeds (96% of viability according tetrazolium test). Percentage of dormancy imposed by the embryo and/or seed coat: percentage of fresh seed (dormant) recorded at 10 days of germination test from seed without pericarp. Percentage of dormancy imposed by pericarp: percentage of germinated seed (without pericarp) – percentage of cypselas (with pericarp) divided by percentage of viability * 100.

Histological analysis of the pericarp

Cypselas were fixed in formalin/acetic acid/alcohol (FAA) solution. To obtain cross sections of the middle portion and the lower end of the pericarp, the fixed samples were embedded in paraffin wax and processed using conventional techniques for cutting (10 µm) and staining (safranin–fast green) (Ruzin 1999). The sections were mounted in glycerine/water (1:1). Photographic observations and recordings were made using a Nikon Labophot-2 microscope with a Nikon Coolpix 4500 camera and ocular micrometer attached to it.

Statistical analysis

The experiment was laid out in a randomized complete design and replicated four times. The germination percentage data were arc-sine transformed to satisfy the assumptions of normality (Zar 1996). Differences in germination percentage were analyzed by one-way ANOVA. Data were subjected to Multiple Range Test *a posteriori* (Fisher LSD test). The analysis was conducted using Statgraphics plus v.3 statistical software (Manugistics 1997).

Results

Cypselas germination

The germination of mature dry cypselas of the A-3 sunflower line was monitored at 30 and 70 days after-harvest to determine germination capacity and dormancy. The germination percentage showed significant differences between cypselas with pericarp (30 days after-harvest: 26%; 70 days after-harvest: 77%) and cypselas without pericarp (30 days after-harvest: 65%; 70 days after harvest: 96%) (Fig. 1). At 30 days after-harvest, A-3 seeds showed a level of dormancy of 73%, which was explained on 41% by the pericarp while the remaining 32% could be attributed to the embryo and/or seed coat (Fig. 2A). On the contrary, at 70 days after-harvest the dormancy was fully explained by the pericarp (19%) (Fig. 2B).

Pericarp anatomy

The histological analysis of the pericarp of A-3 cypselas stored during 30 days after harvest showed that the cross section through the central region was 160.9 ± 7.9 µm of thickness. At physiological maturity, the epidermal cells, the compressed hypodermis, and the phytomelanin layer formed a black layer (Lindström et al. 2007). Under the optical microscope, the substance that impregnates the rays and some of the compressed cells of the middle layer has an appearance similar to that of the phytomelanin layer (arrow in Fig. 3A). The cross section (205.7 ± 9.8 µm) and cell wall thickness of the middle layer cells of each carpel was greater in distal than in the central region of the cypsela. Also, at the lower end of the cypsela, the epidermal cells were significantly larger and not

compressed. Partially compressed parenchyma cells were observed at the junction point of the two carpels. The rays and some of the compressed cells of the middle layer (arrows in Fig. 3B) were impregnated with phytomelanin.

Discussion

The transition from dormancy to germination is a critical control stage in the initiation of vegetative growth. Consequently, seed dormancy is an undesirable trait for crops that require rapid and uniform germination after sowing. Seed dormancy is a complex trait that is influenced by both environmental and endogenous factors. Additionally the final level of dormancy is determined by the individual contributions of the different tissues that comprise the seed. However, dormancy can be artificially released by different treatments such as cold stratification, mechanical scarification, light stimulation, chemical addition of potassium nitrate, ethylene or gibberellins (Finch-Savage and Leubner-Metzger 2006; Iglesias-Fernández et al. 2011).

The A-3 cypselas registered low emergence rates in the field which could be attributed, at least in part, to seed dormancy. Therefore, the dormancy of its seeds causes problems for the pre-commercial production of sunflower hybrids. In sunflower, cypsela structures may influence seed dormancy and germination; in fact, this process may be controlled by the embryo, seed coat, and/or pericarp (Brunick, 2007). During dry after-ripening, differences were observed in germination percentage of A-3 cypselas with and without pericarp, indicating that the pericarp was implicated in the dormancy of sunflower line A-3. Indeed, the negative effect of the pericarp on germination has been attributed to the presence of different physical and chemical factors (Xiao et al. 2009).

It is widely accepted that after-ripening is determined by moisture and oil contents, seed covering structures, and temperature (Manz et al. 2005). Particularly in sunflower, it has been demonstrated that the complex relationship between temperature and embryo moisture content governs the nature of the mechanisms involved in sunflower embryo dormancy release during dry storage (Bazin et al.

2011). In our experimental conditions, the percentage of seed germination rose as dry storage time increased. Finch-Savage and Leubner-Metzger (2006) reported that the rise in time of after-ripening is associated with a widening of the conditions required for germination resembling gradual dormancy loss. Moreover, the time required for a complete release of dormancy shows high inter- and intra-species variation (Graeber et al. 2012). In sunflower, Andrade et al. (2015) showed that *H. annuus* line B123 required 33 days at room temperature (25°C±1) to break dormancy while Presotto et al. (2014) observed that a period of 12 months after-ripening at 5°C (cold storage) reduced seed dormancy in the wild genotype of sunflower and its progeny. In *Arabidopsis*, the accessions Landsberg erecta (Ler) and Cape Verde Islands (Cvi) have very different after-ripening requirements (Alonso-Blanco et al. 2003).

On the other hand, Brunick (2007) reported that sunflower seeds that have after-ripened for four or more weeks can be readily germinated by simply removing the seed coat and pericarp. In fact, the combination of treatments such as storage at room temperature (25°C±1) under dry conditions and removal of pericarp was effective to remove the A-3 seed dormancy. In addition, in the A-3 line the embryo and/or seed coat-imposed dormancy is shorter than that imposed by the pericarp because that associated with the embryo and/or seed coat (32%) was observed only up to 30 days after harvest compared to those imposed by the pericarp (41%) which extended up to 70 days after harvest. It was determined that the sunflower embryo dormancy is often relatively short (4-8 weeks), whereas the effect of the seed coat and pericarp may persist for longer periods of time (>32 weeks) (Maiti et al. 2006; Brunick 2007). Thereby, the level of sunflower line A-3 dormancy could be determined by the contributions of the different tissues that comprise the cypsela. It was reported that there is variability among genotypes of sunflowers regarding dormancy caused by the different tissues of the cypsela (Weiss et al. 2013; Presotto et al. 2014). Different studies have reported the effects of seed coat on germination of sunflower seeds (Brunick 2007; Rolletschek et al. 2007; Szemruch et al. 2014). Thus, the anatomy of the sunflower seed coat could explain variations in levels of dormancy, *e.g.*, the outer

cell wall thickness of the endospermatic cell layer of the seed coat (Szemruch et al., 2014). Recently, Andrade et al. (2015) reported that the pericarp seems partly involved in dormancy of B123 sunflower line.

Previous studies have shown that the pericarp can prevent germination through characteristics of their structure and thickness, content of soluble chemicals, prevention of diffusion of gases and/or uptake of water (Bradford and Nonogaki 2007; Sun et al. 2009). The A-3 pericarp showed a similar histological pattern to those observed in the B123 dormant line (Andrade et al. 2015). Both lines presented larger epidermal cells and the absence of parenchyma in the micropylar area. Also, an increase in cell wall thickness, a high sclerification of middle layer, and presence of a substance similar to phytomelanin were observed in rays and cells of middle layer (composed of ten to 15 layers of axially oriented cells) in both lines. In contrast, the pericarp of B91 non-dormant sunflower inbred line did not show these traits (Andrade et al. 2015). We hypothesize that in the A-3 and B123 pericarp, the high sclerification of middle layer and the continuous phytomelanin layer could be inhibiting the emergence of the radicle (germination). In addition, the phytomelanin layer would be a mechanical protection against the invasion of pathogens or to protect the seed embryo from unfavourable environments such as desiccation (De Pandey and Dhakal 2001; Jana and Mukherjee 2014).

The contribution of the pericarp to physical dormancy of A-3 line is determined, at least in part, by the large size of the epidermal cells and the absence of parenchyma cells in the micropylar area, which is a crucial region for radicle emergence. However, we cannot rule out the possibility of physiological dormancy which could be attributed to changes in the endogenous hormonal content (e.g., OPDA, ABA and GAs) during the dry storage period. Thus, further studies are necessary in order to fully understand the physiological sunflower dormancy attributed to hormone tissue-specific role, particularly in pericarp and seed coat.

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Legends

Fig. 1. Germination percentage of A-3 cypselas with and without pericarp at 30 and 70 days after-harvest. Data are means ± SE of four replicates. Values with the same letter are not different at $P \leq 0.05$.

Fig. 2. Contribution percentage of different sunflower cypsela tissues to dormancy level at 30 (A) and 70 (B) days after-harvest. All data were adjusted to the viable seeds.

Fig. 3. Pericarp cross-section through the central region of the cypsela (A) and at the lower end of the cypsela (B). **CIL**: compressed internal layers; **BL**: epidermis plus hypodermis plus phytomelanin layer; **E**: epidermis; **P**: parenchyma; **PML**: parenchymatic middle layer; **SML**: sclerified middle layer; **r**: ray. In (A), the black arrow indicates the cells of the CIL impregnated with phytomelanin. In (B), the black arrows indicate the rays and cells of the SML impregnated with phytomelanin.