Epicuticular wax content in the pericarp of sunflower fruits (*Helianthus annuus* L.) grown under moderate water deficit

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ABSTRACT

The effect of a moderate water deficit (MWD), imposed on field grown plants in two sunflower hybrids from early anthesis (reproductive stage 6 or R6) to harvest maturity (HM), on the development of epicuticular waxes (epw; mg/g) of the fruit's pericarp, was studied in the present work. The experiment was repeated during two consecutive years. In both hybrids and experiments, plants grown under MWD showed an epw content higher than the 'controls. A decrease in the epw from stage R6 to HM was observed. This could be attributed to the erosive action on the surface of the pericarp by particulate solids carried by wind or rain. These results constitute valuable information for sunflower breeders to further investigate about the mechanisms that regulate wax content in the fruit's pericarp.

Keys words: epicuticular wax - *Helianthus annuus* - pericarp - sunflower - water deficit.

INTRODUCTION

After the sunflower oil has been industrially obtained and cooled, a crystalline sediment can be observed which affects its commercial quality (Rivarola et al., 1988). This sediment is mainly composed of waxes of epicuticular origin (epicuticular waxes or epw). They come from the fruit's pericarp (hull; 83%) (Martin and Juniper, 1970; Morrison, 1983), from the seed teguments (16%) and the embryo (1%) (Morrison et al., 1994).

The amount of waxes passing to the oil during the extraction process depends on the relative hull content of the fruit and the amount of wax it carries. In modern hybrids with high oil content, a thin pericarp is strongly adhered to the seed increasing epw transfer to the oil (Morrison et al., 1984). In these hybrids, fruit's hull content is inversely correlated with oil wax content (Morrison, 1983).

Although waxes constitute a problem for the oil industry, no studies on the development of epw in the sunflower hull are available to date. So, there is no information about the variability in the epw content among hybrids or the effect that different environmental factors and agronomical practices could produce on the epw genesis.

It is known that thermal and water stress can trigger and enhance epicuticular wax synthesis in several plant organs (Premachandra et al., 1992) and that the level of response is phenotipically sensitive and genetically controlled (Koornneef et al., 1989; Jenks et al., 2002). So in this work we have analyzed the evolution of epw content in the pericarp through different developmental stages of two sunflower hybrids grown under two water regimes.

MATERIALS AND METHODS

Plant material

Two sunflower hybrids, Dekasol (DK) 3900 and DK4030, were sown at the Department of Agronomy, UNS, experimental field (Bahía Blanca. Argentina, Lat. S., 38° 45'; Long. W, 62°11') during two consecutive growing seasons (Experiment I: 2003/2004; Experiment II: 2004/2005). The crop was grown under drip irrigation and managed according to recommended conventional agronomical practices (Pereyra and Farizo, 1981). Plant density was adjusted at 5.6 plants/m^2. Fruit samples taken from the capitulum's periphery during reproductive stages R6, R9 and harvest maturity (HM) (Schneiter and Miller, 1981) were analyzed (Table 1).

Treatments
During the reproductive stages R4 to R6 a moderate water deficit (MWD) was generated by interrupting irrigation. It was monitored by measuring the relative water content of plant leaves (RWC_{leaf}) in each treatment at different crop developmental stages.

Determination of epw content
Epw content was measured in the pericarp of the fruits at each sampling stage, for each hybrid and experiment, following the technique described by Franchini and Hernández (2006) using carbon tetrachloride as extracting agent. The epw content was expressed in mass of epw by mass of pericarp dry weight (mg/g).

Experimental design and statistical analysis
Both experiments consisted of complete randomized split plots, with water status assigned to main plots and hybrids to subplots. To determine differences between treatments and hybrids, experimental results were processed by ANOVA and differences between means were evaluated with LSD test.

Table 1. Days from first anthesis to attain reproductive stages R6, R9 and HM (Schneiter and Miller, 1981) in each of the hybrids and experiments HM: harvest maturity

	Experiment I		Experiment II	
	Hybrid		Hybrid	
Stage	DK3900	DK4030	DK3900	DK4030
R6	8	12	13	12
R9	58	48	48	44
HM	71	68	60	56

RESULTS

Plant water status
In both experiments and at different sampling times, an overall decrease of RWC_{leaf} was observed in plants under MWD comparing to control plants (Figs. 1A and 1B). Nevertheless a significant reduction (Fig. 1B; $p<0.05$) in the RWC_{leaf} was only observed 79 days after crop emergence in Experiment II accompanied by a temporary leaf wilting. After irrigation was reestablished, leaves recovered their normal turgor.

Epw content in the pericarp.
In both hybrids and treatments a reduction in epw content was observed from R6 to HM (Figs. 2A and 2B). In fruits of DK3900, during Experiment I, the observed reduction was 28 % ($p<0,05$) from stage R6 to HM (Fig. 2A), while during the Experiment II, the observed reduction was not significant ($p = 0,09$; Fig. 2B).

Although a continuous reduction in the epw content of DK4030 fruits was observed from stage R6 to HM, this was not significant in Experiment I ($p>0,05$; Fig. 2A). In Experiment II, epw content was significantly reduced by 14% ($p<0,05$) from R6 to R9, with no significant differences detected between the latter stage and HM (Fig. 2B).

MWD and epw content
Since there was no hybrid x water regime interaction ($p>0,05$) for the variable epw content, only the average results for both hybrids (Table 2) in each experiment are presented. In both experiments and in each reproductive stage studied, epw of fruits from plants under MWD showed a 33% epw increase compared to control plants (Table 2). Nevertheless, it must be mentioned that during Experiment I water deficit was not as high as expected so the differences between treatments might not be so evident.

Table 2. Average content of epw (mg/g) of the pericarp of the sunflower hybrids DK3900 and DK4030 both in control and under moderate water deficit (MWD). R6, R9: Reproductive stages as described by Schneiter and Miller (1981). HM: harvest maturity.

Stage	Experiment I			Experiment II		
	Control	MWD	S.E.	Control	MWD	S.E.
R6	5,08 a*	6,24 a	0,3	5,25 a	7,08 b	0,3
R9	4,72 a	5,59 b	0,4	4,46 a	6,53 b	0,3
HM	3,64 a	4,44 a	0,4	4,24 a	6,22 b	0,3

* In a row, within each assay, means followed by the same letter are not significantly different at p>0,05. MDW: Moderate Water Deficit. S.E.:Standard error.

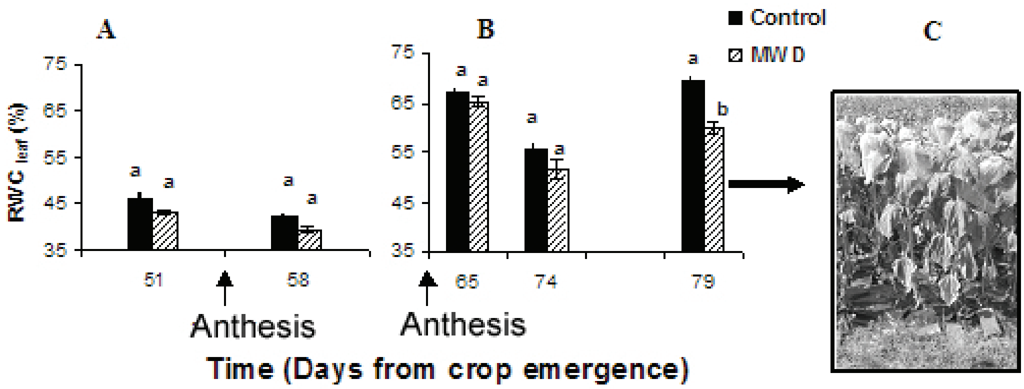

Fig. 1. Leaf relative water content (RWC_{leaf}%) in the sunflower hybrids DK3900 and DK4030 during experiment I (A) and II (B). C. Temporary wilting of leaves of plants under MWD during experiment II, 79 days after crop emergence (24 days after anthesis). Leaves became turgent once irrigation was reestablished. MDW: Moderate Water Deficit. Within each set, bars topped by the same letter are not significantly different at p>0,05.

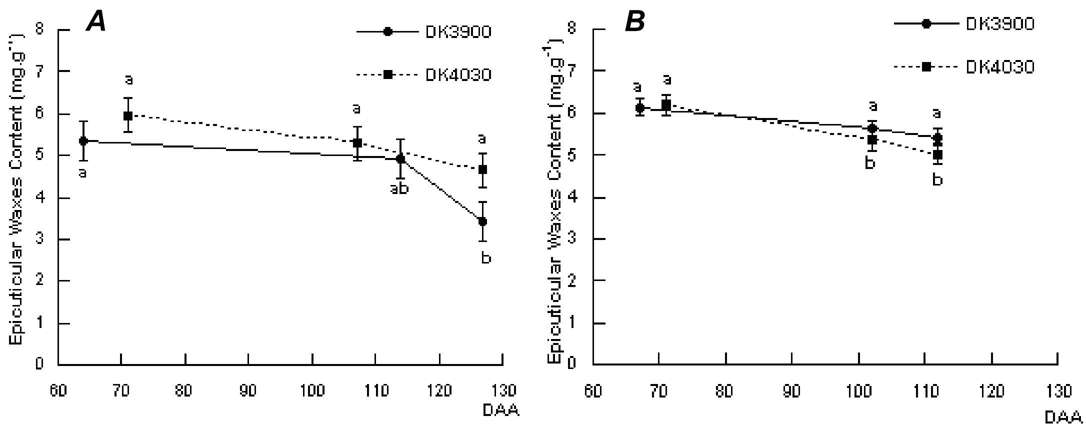

Fig. 2. Changes with time of epw (mg/g) in the pericarp of fruits of the sunflower hybrids DK3900 and DK4030, averaged across water treatments, from R6 to HM. A) Experiment I. B) Experiment II. DAA: Days after anthesis. For each hybrid, values followed by different letters indicate significant differences between sampling dates (p<0,05).

DISCUSSION

Plant water status

The observed RWC_{leaf} magnitudes (Fig. 1) show that, in both experiments, the procedure of irrigation shortage was sufficient to generate a suboptimal water status in the critical developmental stages of the formation of pericarp (stages R5 and R6; Lindström et al., 2000).

Epw content in the pericarp

The observed reduction in epw content from R6 to HM in both hybrids and experiments, could be attributed to the erosive action produced by several environmental factors, among which rainfall and wind are particularly common. They can transport abrasive particulate material removing wax crystals from the pericarp surface. The same effect has been observed in leaves of *Eucalyptus* sp. (Baker and Hunt, 1986), *Brassica* sp. and *Fraggaria* sp. (Neinhuis and Barthlott, 1997). Also, in both hybrids and experiments, the highest content of epw measured in R6, when the pericarp is still young and contains high water concentration (Rondanini et al., 2007), agrees with the phenomenon observed by Neinhuis et al. (2001). These authors demonstrated that cuticular transpiration allows the waxes attached to water molecules to move from the inner regions of the leaf to its outer surface. So, in young epidermis with a thin cuticle, such as that present in undeveloped fruits, with a lesser resistance for the passage of waxes through it compared with mature ones, a higher epw content can be expected.

MWD and epw content

In both experiments and in the three fruit developmental stages (Table 2), the imposed leaf water deficit induced a comparatively higher epw than in the controls. Similar results can be found in leaves of weeping lovegrass (*Eragrostis curvula* Schrad) (Echenique et al., 1986) and sorghum (*Sorghum bicolor* L.) (Premachandra et al., 1992), where a constant water stress led to an increase in the content of epw and a reduction in the cuticular transpiration rate.

CONCLUSIONS

A moderate plant water deficit during fruit development led to an increase of 33 % in the epw content in the pericarp, compared with that of the control plants.

From R6 to HM, epw content decreased, possibly due to the erosive action produced by wind and rain on the fruit surface.

The results shown here can be used as a physiological tool to define the dynamics of wax accumulation in the sunflower fruit pericarp, a variable that can be genetically modified (Jenks et al., 2002). Thus, breeders would be able to manipulate two characters, which are currently antagonists in the sunflower fruit: seed oil and pericarp wax content.

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