

Visual definition of physiological maturity in sunflower (*Helianthus annuus* L.) is associated with receptacle quantitative color parameters

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Abstract

Identifying physiological maturity (PM) in sunflower (*Helianthus annuus* L.) by visual methods is subjective. The present study was conducted during two years in two short season sunflower hybrids (Macón and MG60) to determine the relationship between quantitative color parameters in the receptacle and physiological markers such as fruit dry weight (FDW) and fruit water content (FWC) from flowering to PM. Fruits from the external 25% of the capitulum radius were sampled at 3-5-day intervals from first anthesis until harvest maturity. Fruit and receptacle fresh and dry weight were calculated, and color changes of the receptacle base were followed over time using a spectrophotometer. Comparison of colorimetric coordinates a^* and b^* , defined by the CIELAB color space enabled quantitative correlation of color changes in the receptacle with the maturation stage of the fruits and their moisture content. In both hybrids and years, fruits attained maximum dry weight when the receptacle color turned from dark green to buttery-yellow. Strong correlations were found between FWC and a^* for Macón the first ($r=-0.877$) and second year ($r=-0.934$) and for MG60, ($r=-0.912$ and $r=-0.891$) the first and second year, respectively. The same results were found for b^* for Macón ($r=-0.901$ and $r=-0.829$) and for MG60 ($r=-0.898$ and $r=-0.863$) for the first and second year respectively. Maximum b^* at FWC between 40 to 41% had the highest correlation with maximum fruit dry weight for both hybrids and years, and was a good indicator for identifying the attainment of PM. This work represents an original contribution and a first step towards the development of a model for predicting PM in sunflower by using colorimetric measurements.

Additional key words: CIELAB; color correlation; fruit growth; yield.

Introduction

When conducting field observations an accurate description of crop phenology is a necessary tool for efficient crop management. During the last 50 years morphological scales of developmental stages or external changes of some reproductive organs in crop plants have been developed. For example, phenological scales have been developed for maize (Hanway, 1963; Hunter *et al.*, 1991; Ritchie *et al.*, 1992), soybean (Fehr *et al.*, 1971; Gbikpi & Crookston, 1981), and rapeseed (Elías & Copeland, 2001; Sabry & Copeland, 2001).

For sunflower, phenological scales have been elaborated by Siddiqui *et al.* (1975), Robinson (1983), and

Merrien (1992). However, one of the most widely used scales is the decimal notation developed by Schneiter & Miller (1981). This scale clearly discriminates the vegetative phase (V), determined by the number of true leaves, from the reproductive phase (R), starting with the appearance of the "star stage" (R1) and ending with harvest maturity (HM) (Aguirrezábal *et al.*, 1996; Connor & Hall, 1997).

Fruit moisture content has to be low enough to allow safe harvest and storage. In the field, sunflower maximum fruit dry weight (FDW) is usually attained when the fruit moisture content is about 38% and the receptacle's tissue moisture content is about 70% (Anderson, 1975; Unger & Thompson, 1982; Rondanini *et al.*, 2007;

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Gesch & Johnson, 2012). In the decimal notation by Schneiter & Miller (1981), the attainment of maximum fruit dry weight, also defined as phenological stage R9 or physiological maturity (PM), is externally observed when the phyllaries become brown and brittle and the receptacle base turns buttery yellow.

The time elapsed to attain PM in sunflower varies among genotypes and with management conditions such as nitrogen and soil water availability (Connor & Hall, 1997) and the same genotype can differ up to 10 days to reach PM in response to different environmental conditions (Kaya *et al.*, 2004). Changes in color of the sunflower receptacle when approaching PM are observed with the naked eye leading to at least some subjectivity. Therefore, the scale by Schneiter & Miller (1981) has been questioned when comparing PM in genotypes with delayed leaf senescence, also defined as “stay green” genotypes (SG) (Cukadar-Olmedo & Miller, 1997). In these genotypes the base of the receptacle at PM is green or yellowish green and only the phyllaries become slightly brown (Cukadar-Olmedo *et al.*, 1997; Cukadar-Olmedo & Miller, 1997). Rondanini *et al.* (2007) defined PM in sunflower quantitatively, assessing variations in percentage of fruit moisture content. A predictive model was developed indicating PM is attained when fruit moisture is 38%. Recently, Gesch & Johnson (2012) defined in two oilseed hybrids that, at PM, average moisture content in fruits from middle and outer concentric thirds of the capitulum was about 40% regardless of environment.

The present study was designed to determine the relationship between quantitative parameters of receptacle color after flowering and the attainment of fruit PM. The aim was to 1) determine, using a handheld spectrophotometer, how chromaticity factors in the receptacle base are related to receptacle color changes from first anthesis (FA) until harvest maturity (HM), and 2) determine if the visual scale developed by Schneiter & Miller (1981) gives a consistently reliable indication of PM based on fruit measurements.

Material and methods

Plant material

The short season sunflower hybrids Macón (Syngenta, Argentina) and MG60 (Dow-Agrosciences, Argentina) were used in the study. The cycle of MG60 was shorter

by 3-4 days from emergence to flowering and has a higher leaf green retention than Macón without being considered a fully “stay green hybrid” (De la Vega & Hall, 2002).

The experiments were conducted on a deep coarse sandy soil (Typic Ustipsamment; Soil Survey Staff, 1999) at the Agronomy Department-UNSur, Bahía Blanca, Argentina (38° 45' S; 62° 11' W) during the spring-summer seasons of 2008/09 (planting date October 22, 2008; Exp. 1) and 2009/10 (planting date September 5, 2009; Exp. 2).

For both years, hybrids were grown at a crop population density at flowering of 6 plants m⁻². The experimental design was a randomized complete block design with three replicates. Plot size was 4 rows × 6 m with an inter-row spacing of 0.70 m. Daily incident radiation (MJ m⁻² d⁻¹), temperature and rainfall were recorded at a meteorological station located about 1,200 m from the experimental plots.

The crop was managed according to the recommended conventional agronomical practices (Díaz Zorita *et al.*, 2003). Environmental conditions during crop growth kept soil water content above 50% of maximum soil available water. When necessary, soil water was supplemented by drip irrigation. Nutrient deficiencies were prevented with pre-planting and pre-flowering fertilization with nitrogen (60 kg N ha⁻¹) applied as potassium nitrate. Weeds were controlled manually. Insect pests were not an important factor in either year.

Fruit and receptacle water content determinations

Crop phenology was followed every two days from emergence to HM on 60 tagged plants of each hybrid. Vegetative and reproductive development observations followed the Schneiter & Miller (1981) scale. When plants reached first anthesis (FA; phenological stage R5.1; Schneiter & Miller, 1981) 30 plants for each hybrid with three replications were labeled for fruit sampling. PM was calculated from the dynamics of fruit growth (Ploschuk & Hall, 1995). For convenience of fruit size and weight stability, fruits from the external 25% of the capitulum radius were harvested every 3 to 5 days from FA to HM. At each harvest, 5 fruits per plant were taken from three randomly selected plants per plot that had not been sampled previously. Fruits were kept in a cooler with ice and taken to the laboratory within 90 min of sampling. Fruit fresh weight was

determined to calculate the fruit water content (FWC%, on a dry weight basis). The fruits were dried at 70°C for at least 48 h before weighing. Fruit dry weight (FDW%) was then calculated. To estimate receptacle water concentration (RecWC%), at 7-10 day interval three equally spaced tissue samples of approximately 1 cm³ each were taken using a 1 cm diameter cork borer, following a transect of the receptacle radius from the outward facing (lower) portion of the receptacle. That receptacle region is mainly composed by a mix of epidermis-collenchyma-small cell parenchyma-vascular tissues. The receptacle central portion that often contains a large proportion of pith (large parenchyma cells) of lower water concentration was not included for analysis. Samples were kept cold until analyzed. This measurement was made on 30 tagged plants of each hybrid. The plants sampled were not the same as those used for fruit sampling or color measurements. Receptacle fresh and dry weight were determined and RecWC% calculated.

Determination of quantitative color parameters

For each hybrid 15 of the tagged plants described before were selected when they reached FA. From then on, capitula color measurements *in vivo*, were taken. Thus, simultaneously with fruit sampling, in each plant, three color readings were taken across a transect of the receptacle radius from the outward facing (lower) portion of the receptacle. The readings were taken from 800 to 900 hours using a portable spectrophotometer X-Rite, model i1 (X-Rite Inc. MI, USA) with a 5.3 mm aperture connected to a portable computer. Each reading was taken placing the measuring instrument against the receptacle surface. The equipment was calibrated against a standard white tile ($L^* = 97.03$; $a^* = -0.05$; $b^* = 1.96$) with the CIE Standard Illuminant C as reference (CIE, 2001). Each reading taken with the software ColorShopX vers.1.5 (X-Rite Inc. MI, USA), provided the values of the parameters L^* , a^* and b^* within the CIELAB color space, (CIE, 2001). Within the CIELAB uniform space a psychometric index of lightness, L^* , and two color coordinates, a^* and b^* , are defined. The coordinate a^* takes positive values for red colors and negative values for green ones, whereas b^* is positive for yellow colors and negative for the blue ones. L^* is an approximate measure of lightness, allowing each color to be considered as equivalent to a member of the gray scale, and

thus taking on values ranging from 0 (black) to 100 (white) (HunterLab., 2001). For convenience, colorimetric data was plotted against time in calendar days rather than thermal time.

Statistical analysis

A biphasic fit of FDW vs. time (days from FA) of pooled data of two years and hybrids was performed with the nonlinear routine of the software SigmaPlot v. 12.0 (Systat Software Inc., San Jose, CA, USA) using the model: $y = a + bX$ (for $X < c$); $y = bc$ (for $X > c$), where y is FDW (g), X is time (days from FA) and a and b are the intercept and the slope, respectively, of the linear regression. The constant c corresponds to the unknown break point of the two linear functions, and indicates the timing of achievement of maximum weight, equivalent to PM (Ploschuk & Hall, 1995).

Differences between the maximum fruit dry weight and between their water concentration (FWC%) and RecWC% in both genotypes at PM were compared by one way ANOVA.

Once L^* , a^* and b^* parameters were obtained for each genotype, a Pearson correlation analysis was conducted among color parameters and fruit parameters to define which of them best fit the color, maximal dry weight of the fruits, and maximum FWC relationship. Infostat Professional statistical software was used in these procedures (Di Rienzo *et al.*, 2008).

To determine whether the defined FWC for PM was within the range of measured a^* and b^* , the correlation between those variables was calculated. Thus, a piecewise regression with a break point was developed to predict the range of FWC with color change (magnitude of a^* and b^*). The break point reflected the value at which the dependent variable changed as a function of the FWC, thereby establishing a regression with two equations.

Results and discussion

Table 1 summarizes the seasonal patterns shown by the environmental variables registered for the two experimental periods. Both years presented a similarity in the environmental conditions during three phases of crop development. Maximum and minimum temperatures during the reproductive period as well as average daily incident radiation were between the range of optimum

Table 1. Mean environmental data for three crop developmental phases in each year. Anthesis (A) and physiological maturity (PM) dates used for defining the mean crop stages represent the average for both hybrids at each experimental year. Crop phase A represents the 15-d interval centered on the mean date of full anthesis. Crop interval S-A is the time elapsed between sowing and 7 days before the mean date of full anthesis. A-PM is the interval between 7 days after full anthesis and the mean date of PM for both hybrids. Maximum and minimum temperature mean values with 1 SD in parentheses

Environmental factor	Year	Crop development phase		
		S-A	A	A-PM
Maximum temperature (°C)	1	22.1 (3.3)	23.9 (4.1)	29.0 (4.8)
	2	23.3 (3.9)	29.8 (3.7)	29.8 (4.0)
Minimum temperature (°C)	1	12.4 (1.3)	14.0 (2.5)	14.0 (1.8)
	2	13.3 (1.9)	17.4 (2.1)	15.3 (1.1)
Daily incident radiation (MJ m ⁻² d ⁻¹)	1	19.8	20.6	21.4
	2	18.6	20.5	21.9
Total growing season rainfall (mm)	1	282.3	20.3	0.3
	2	439.3	16.3	1.2

values for sunflower (Connor & Hall, 1997; Table 1). Rainfall was better distributed in the first year than in the second year (Table 1), when a period of heavier rainfall concentrated between 15 and 22 days from sowing to anthesis. However, in both years no rainfall was observed at the moment of attaining PM.

For MG60, maximum FDW was observed 29 and 30 days after FA and did not differ ($p < 0.01$) between years, ranging between 0.0437 to 0.0446 g fruit⁻¹ (Fig. 1a). Maximum FDW in both years was attained between 39.8% and 39.5% FWC (Fig. 1b). The moisture content of the receptacle at the time of physiological maturity was approximately 77% both years (Fig. 1c).

For Macón maximum FDW did not differ ($p < 0.01$) between years, ranging between 0.0452 to 0.0466 g fruit⁻¹ attained at 27 and 29 days after FA respectively (Fig. 1e). Maximum FDW was attained at a FWC of 41.2% the first year and 40.3% in the second year (Fig. 1f). Even though FWC between both hybrids at the time of PM did not show significant differences ($p < 0.05$) (Figs. 1b and 1f), the receptacle moisture content, perhaps as a consequence of a green mass retention trait, diminished more slowly in MG60 than in Macón (Figs. 1c and 1g respectively). In both genotypes for both years the maximum FDW was associated with the observed visual PM according to the morphological characteristics defined by Schneiter & Miller (1981).

Magnitudes of a^* and b^* moved over time from $-a^*$ (green component; HunterLab., 2001) to $+a^*$ (yellow-red component; HunterLab., 2001) (Figs. 1d and 1h).

The parameter b^* (yellow-blue component; HunterLab., 2001) always had positive values (Figs. 1d and 1h).

The magnitudes of the CIELAB values at the time of PM for MG60 were: L^* : 72.1 (1st year) and 77.6 (2nd year); a^* : -11.3 (1st year) and -8.2 (2nd year); b^* : 57.2 (1st year) and 58.3 (2nd year) (Fig. 1d). For Macón the magnitudes of these values at the time of PM were: L^* : 81.1 (1st year) and 77.6 (2nd year); a^* : -13.3 (1st year) and -12.9 (2nd year); b^* : 62.1 (1st year) and 58.4 (2nd year) (Fig. 1h). The differences observed in L^* , a^* and b^* across years and hybrids were not significant ($p > 0.05$).

Perhaps because of the variability observed in the pubescence of the receptacle base between plants, hybrids and years (results not shown here), the L^* magnitude for both hybrids showed fluctuations in both experimental periods averaging 73.5 in MG60 (Fig. 1d) and 78.3 in Macón (Fig. 1h) during the reproductive period from R5.1 to R10. However, in both years and hybrids, L^* decreased after capitulum maturity (Figs. 1d-1h) in response to increasing opacity and darkening of receptacle tissue as observed by Shewfelt *et al.* (1988).

For the whole of the experimental period a strong correlation between the FWC% and a^* occurred the first ($r = -0.877$) and second year ($r = -0.934$) for Macón. For MG60 the FWC% and a^* correlations were $r = -0.912$ the first year and $r = -0.891$ the second (Table 2). The same results were found for b^* ($r = -0.901$ and $r = -0.829$) for Macón and for MG60 ($r = -0.898$ and $r = -0.863$) for the first and second year,

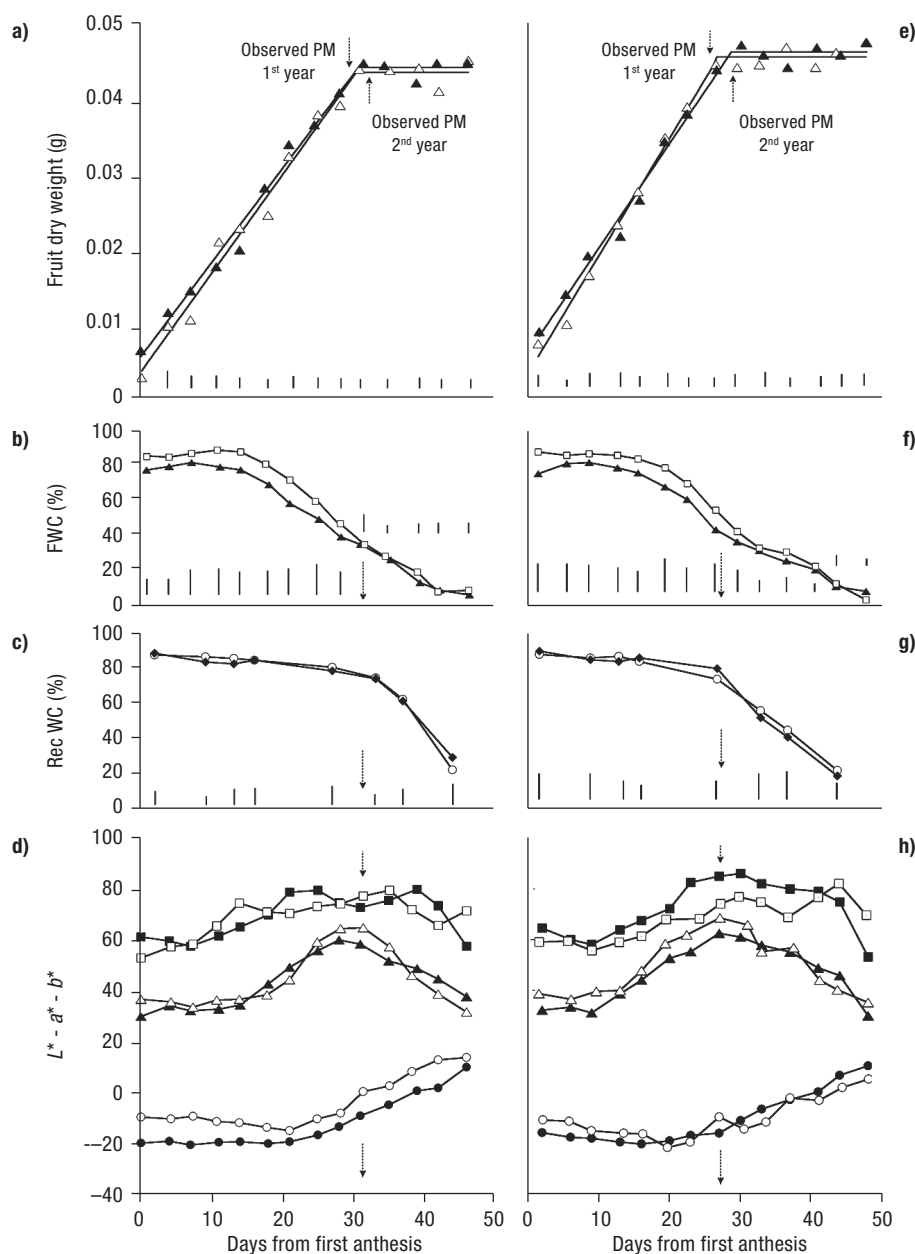


Figure 1. Evolution of fruit dry weight (FDW) fruit water content (FWC), receptacle water content (RecWC), and changes in the CIELAB parameters in the base of capitula from first anthesis up to harvest maturity in MG60 (a-d) and Macón (e-h) for the first year (closed triangles) and the second year (open triangles) respectively. In Figs. 1d and 1h, colorimetric coordinates are noted as L^* : (■; □), a^* : (●; ○) and b^* : (▲; △) for the first year (closed symbols) and the second year (open symbols) respectively. Vertical bars, inside each plot, indicate LSD between years at each sampling date at $p < 0.05$. Arrows indicate the time (days from first anthesis) when visual physiological maturity following Schneiter & Miller (1981) was noted.

respectively (Table 2). This lead us to choose a^* and b^* as the best color parameters to quantify capitulum color changes with time up to PM.

Piecewise regressions with a break point fitted to the relationships between FWC and a^* or b^* as a function of FWC, generated a pair of equations with

statistical significance and high determination coefficients R^2 (Fig. 2). The models fitted the data well between FWC of 10% to 80% ($p < 0.001$) (Fig. 2).

After changes in color parameters a^* and b^* that occurred in relation with FWC as crop maturity advanced and fruits became drier (Fig. 2), a color shift was

Table 2. Correlation coefficients (Pearson) between the variables considered in the analysis. L^* , a^* , b^* , maximum fruit dry weight at physiological maturity (FDW; g) and fruit water content (FWC%) for each genotype in the two experimental years were evaluated

	L^*	a^*	b^*	FDW (g)	FWC (%)
<i>MACON</i>					
<i>First year</i>					
L^*	1.000				
a^*	0.170 ^b	1.000			
b^*	0.762 ^a	0.011 ^b	1.000		
FDW (g)	0.601 ^a	0.598 ^b	0.624 ^a	1.000	
FWC (%)	-0.431 ^a	-0.877 ^a	-0.901 ^a	-0.877 ^a	1.000
<i>Second year</i>					
L^*	1.000				
a^*	0.234 ^b	1.000			
b^*	0.320 ^b	0.013 ^b	1.000		
FDW (g)	0.652 ^a	0.778 ^a	0.745 ^a	1.000	
FWC (%)	-0.239 ^b	-0.934 ^a	-0.829 ^a	-0.845 ^a	1.000
<i>MG60</i>					
<i>First year</i>					
L^*	1.000				
a^*	0.165 ^b	1.000			
b^*	0.667 ^a	0.239 ^b	1.000		
FDW (g)	0.734 ^a	0.733 ^a	0.762 ^a	1.000	
FWC (%)	-0.336 ^b	-0.912 ^a	-0.898 ^a	-0.913 ^a	1.000
<i>Second year</i>					
L^*	1.000				
a^*	0.179 ^b	1.000			
b^*	0.598 ^b	0.318 ^b	1.000		
FDW (g)	0.791 ^a	0.844 ^a	0.829 ^a	1.000	
FWC (%)	-0.403 ^b	-0.891 ^a	-0.863 ^a	-0.865 ^a	1.000

^a Correlation is significant at 0.05 level (two-tailed). ^b Correlation is non-significant.

visually detected on the receptacle base in both genotypes. Then a^* moved slowly toward less green ($-a^*$) until a break point (FWC = 39.7 %), becoming more strongly positive thereafter as fruits continue to dry.

For both hybrids, the results showed the magnitude of b^* increased up to the moment of maximum FDW and then decreased, following the decrease in FWC with, plant senescence (Fig. 2). The a^* value increases as capitulum maturity advances (Fig. 2), allowing the b^* component (yellow) to stand out. Yellowing of the receptacle was characterized, as expected, by a constant increase in the value a^* (less green) and a maximum magnitude of b^* (more yellow) (Fig. 2).

The sharpest changes were observed in b^* . The b^* value increases in the first part of the model (Fig. 2) indicating a shift towards the yellow color until rea-

ching the break point (40.8 % FWC), and then sharply decreasing to reach similar values to those attained at the beginning of measurements. At harvest maturity this was expressed as an intense brown color at the receptacle base.

Yellowing of the receptacle base was characterized by an increase in a^* (less green) and a maximum value of b^* (more yellow). It is well known that in green organs as maturity advances, chlorophyll degradation (Sexton & Woolhouse, 1985) and the predominance of xanthophylls and other carotenoid pigments causes the color change from green to yellow ($+a^*$) (Sinecker *et al.*, 2002).

Although higher leaf green retention in MG60 was observed at the moment of attaining PM, this was not associated with changes in the dynamics of FWC from

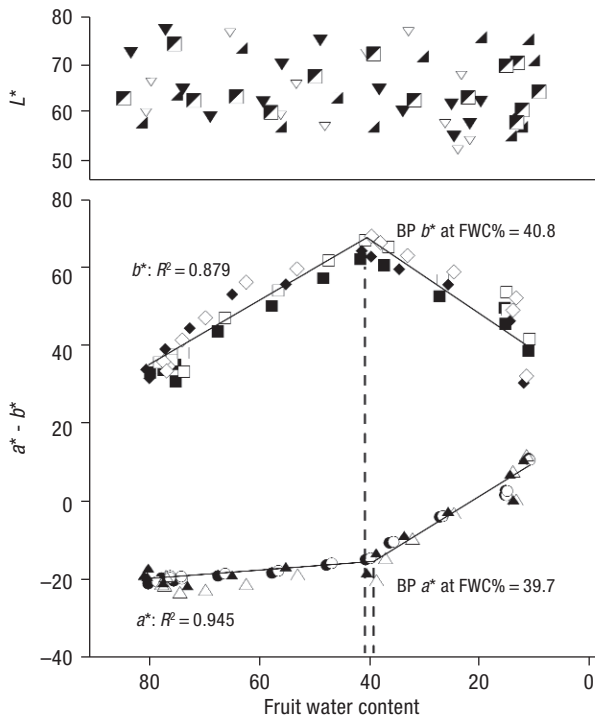


Figure 2. Relationship between the fruit water content (%) and the values of the CIE parameters L^* , a^* and b^* from pooled data taken for both experimental years and hybrids (first year: closed symbols; second year: open symbols). MG60 a^* : (■; □); Macón a^* : (◆; ◇); MG60 b^* : (▲; △); Macón b^* : (●; ○). The lines were fitted by piecewise linear regression. The relationship between FWC (%) and a^* or b^* is defined by two linear regressions ($a + bX$) with different slopes (b_1 and b_2) and the breakpoint (BP) where the two lines intersect.

Because its high variability, values of L^* obtained for the two hybrids and experiments (Macón L^* : [▼; ▽], MG60 L^* : [▲; ■]; first year and second year respectively), are depicted at the top of the figure but were not fitted. Each point is the mean of 45 readings taken in receptacle base of each hybrid. Breakpoints (BP) and R^2 values are shown on the plot. The dashed lines indicate for each parameter a^* and b^* the magnitude of FWC at the breaking point. For X = fruit water content (%), bilinear equations arising from the adjustment of the colorimetric parameters a^* (Eq. [1]) and b^* (Eq. [2]) are:

$$a^* = 13.882 - 0.6303 * X, \text{ (for } X \leq 39.7); \quad [1]$$

$$-18.51 - 0.0006784 * X, \text{ (for } X > 39.7); \quad R^2 = 0.945$$

$$b^* = 32.634 + 0.7112 * X, \text{ (for } X \leq 40.8); \quad [2]$$

$$102.69 - 0.8582 * X, \text{ (for } X > 40.8); \quad R^2 = 0.879$$

anthesis to maturity. In fact, both genotypes showed that FWC% did not significantly differ ($p < 0.05$) at the moment of their minimum a^* value (FWC% = 39.7) and their maximum b^* (FWC% = 40.8; Fig. 2).

The relationship between FWC and a^* or b^* from 5 and 40 days after FA in pooled data of both hybrids and years (Fig. 2) showed how receptacle color change and fruit water content were related. The dynamics of the

color parameter b^* was closely associated with the attainment of the visual PM (Fig. 1a, 1e, 1d and 1h), corresponding to phenological stage R9, and this was similar for both hybrids.

Maximum b^* at a FWC between 40% and 41% was strongly associated with the maximum dry weight of fruits in both genotypes (Figs. 1a and 1e, Fig. 2, Table 2). The initial slope of b^* was twice as steep as that of a^* (Fig. 2), indicating that although the fruits were losing water the receptacle color rapidly shifted from predominantly green to yellow. After the breakpoint, the abrupt decrease in b^* and the increase in a^* was associated with the browning of the receptacle.

The values of L^* (lightness; CIE, 2001) showed great fluctuation (Figs. 1d and 1h; Fig. 2), which made it not useful in describing FWC changes. Thus, no consistent changes were observed in lightness when green and red colors predominated. However, maximum L^* values attained when reaching PM both during maturation (Figs. 1d and 1h) and in the bilinear model (Fig. 2) ranged between $L^* = 57$ and 80.

The study described here, comparing colorimetric coordinates a^* and b^* , enabled quantitative correlation of the magnitude and type of color changes occurring with maturation of the sunflower fruits and their moisture content. The colour-based model proposed in the present work overestimates by between 2 and 3% the fruit moisture content of the fruits obtained by Rondanini *et al.* (2007). Nevertheless it is still valid and practical for definition of PM in the field. Maximum b^* at FWC between 40 to 41% had the highest correlation with maximum fruit dry weight for both genotypes and years, and was a good indicator for precise quantitative estimates of PM.

Even though this work deals with the results obtained in only two sunflower genotypes in a narrow range of environmental and management conditions, it represents an original contribution and a first step towards the development of a model for predicting PM in sunflower by using colorimetric measurements. Nevertheless, further research is needed to ensure the use of this approach to a wide range of genotypes with different cycle duration.

So to increase the applicability of our findings, and to validate the proposed model, it would be desirable to extend the range of environmental and management conditions, including treatments with different levels of N fertilization and a degree of drought, both of which strongly modulate tissues color, and therefore can alter the dynamic of color described here.

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