

Kernel water relations and duration of grain filling in maize temperate hybrids

Brenda L. Gambín*, Lucas Borrás¹, María E. Otegui

Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, Capital Federal (C1417DSE), Argentina

Received 31 May 2006; received in revised form 18 August 2006; accepted 1 September 2006

Abstract

Kernel water relations play a key role in controlling the duration of grain filling. This duration is controlled by the relationship between kernel water and biomass development, as it determines the timing kernels reach a critical percent moisture content (MC, measured on a fresh weight basis) at which biomass accumulation stops. The time in which this critical percent MC is attained can be affected by the timing kernel net water uptake stops (i.e. maximum water content is reached), or by the relationship between water loss and biomass deposition after maximum water content is attained. Which of the two mechanisms could be behind genotypic differences in maize (*Zea mays* L.) grain-filling duration was unknown. We also studied the relationship between kernel water and volume development, as it was unknown in this species. Thirteen commercial hybrids were evaluated under different growing environments, and weight, water content and volume of their kernels were measured throughout grain filling.

There were no differences among hybrids in their kernel percent MC at physiological maturity ($p > 0.05$), showing that hybrid differences in grain-filling duration (from 1117 to 1470 °C day) were related to variations in the accumulated thermal time from flowering to this critical percent MC. There were no differences in the accumulated thermal time from silking to kernel maximum water content, and this stage was always reached at the same kernel percent MC (ca. 540 g kg⁻¹). Differences in grain-filling duration were explained by the pattern of percent MC decline after maximum water content was reached. This percent MC decline was dependent upon the relationship between water loss and biomass deposition; the higher the water loss rate and the higher the kernel growth rate the shorter the duration ($r^2 = 0.60$; $p < 0.001$). Maximum kernel volume was achieved after maximum water content, and close to physiological maturity. Hybrids differed ($p < 0.05$) in the kernel volume generated after maximum water content, and this was also related to the relationship between biomass and water development late in grain filling. Results showed the importance of understanding and predicting percent MC development throughout grain filling, as there were no differences between hybrids and environments in their kernel percent MC at specific developmental stages (i.e. maximum water content or physiological maturity). Our results highlighted the importance of the relationship between water loss and biomass deposition during late kernel development in the duration of maize grain filling.

© 2006 Elsevier B.V. All rights reserved.

Keywords: *Zea mays* L.; Maize; Grain-filling duration; Kernel desiccation; Kernel volume; Kernel development

1. Introduction

Kernel water relations are a good indicator of kernel developmental progress during grain filling (Swank et al., 1987; Schnyder and Baum, 1992; Borrás and Westgate, 2006). Developing kernels accumulate more water than reserves early

in development, and both kernel water content and kernel dry matter patterns have shown to be closely related. As such, understanding kernel water relations is a powerful tool for describing and predicting differences in kernel development due to the genotype or the environment.

Percent kernel moisture content (MC, measured on a fresh weight basis) declines throughout grain filling. This decline is most obvious after kernels reach physiological maturity, but also occurs during the effective grain-filling period, and during the early stages of development. Within each species, kernels from different genotypes and environments reach maximum weight (also known as physiological

Abbreviations: MC, moisture content; Exp, experiment

* Corresponding author. Tel.: +54 11 45248039; fax: +54 11 45148739.

E-mail address: bgambin@agro.uba.ar (B.L. Gambín).

¹ Present address: Agronomy Department, Iowa State University, 1301 Agronomy Hall, Ames, IA 50011-1010, USA.

maturity) at a relatively consistent percent MC value (Egli and TeKrony, 1997; Saini and Westgate, 2000). Kernel percent MC has further been used with good success for estimating the percent of maximum weight kernels have achieved at any grain-filling stage in different species (Swank et al., 1987; Schnyder and Baum, 1992; Borrás et al., 2003). Also, kernels reach their maximum water content at a particular percent MC in maize (Borrás and Westgate, 2006) and soybean (Swank et al., 1987). These evidences show that biomass and water development are closely related in developing kernels.

The concept that biomass accumulation stops when kernels reach a critical percent MC value shows the importance of maintaining a kernel water status above this critical one for increasing the duration of grain filling. This could be achieved by increasing the time seeds have a positive net water uptake that delays the achievement of the maximum water content, like occurs in soybean seeds (*Glycine max* L.) (Egli, 1990). It could also be achieved through variations in the relation between water loss and biomass deposition after maximum water content is achieved, as shown by developing sorghum kernels (*Sorghum bicolor* L. Moench) (Gambín and Borrás, 2005). These results illustrate that the achievement of the critical percent MC can be affected not only by changes in the timing net water uptake stops, but also by the rate percent MC declines after maximum water content is achieved.

Kernel final weight and volume have shown to be closely related (Millet and Pinthus, 1984), probably because seeds adjust their volume rather than their density (Gambín and Borrás, 2005). The moment maximum kernel volume is achieved has important implications, as it defines the potential individual kernel weight. Maximum kernel volume was always estimated to be achieved at the same developmental stage kernels reach their maximum water content (Martinez-Carrasco and Thorne, 1979; Saini and Westgate, 2000; Borrás et al., 2003). However, research with sorghum kernels has shown that maximum volume might not always be reached at this stage, but closer to physiological maturity (Gambín and Borrás, 2005). At present, there is no information on the pattern of kernel volume development in maize.

Direct selection for duration of grain filling may increase grain yield (reviewed by Egli, 2004), especially in temperate environments where the length of the growing season is not entirely utilized. In maize, important genotypic differences exist in grain-filling duration (Daynard et al., 1971; Poneleit and Egli, 1979; Cavalieri and Smith, 1985), and differences can be observed among current commercial temperate hybrids (Gambín et al., 2006). In the present article, we evaluated a number of hybrids differing in the duration of grain filling to test: (1) if genotypic differences in total duration of grain filling were related to the timing maximum water content was reached or to the relationship between water loss and biomass deposition after maximum water content was attained, and (2) if maximum kernel volume is reached at the same developmental stage as maximum water content.

2. Materials and methods

Experiments were conducted at the experimental field of the Department of Plant Production at the University of Buenos Aires (35°35'S and 59°29'W) during the 2002–2003 (Exp I), 2003–2004 (Exp II) and 2004–2005 (Exp III) growing seasons. Soils were of the silty clay loam type (Vertic Argiudoll). In Exp I, hybrid DK682 (Monsanto Argentina, 2002) was sown on 23 September at a plant density of 10 plants m⁻². In Exp II, 12 single-cross maize hybrids (Ax610 MG, Xa0675 MG, Ax800 MG, Ax820 MG, Ax832 CL-MG, Ax842 MG, Ax877 MG, Ax878 MG, Ax882 MG, Ax888 CL-MG, Ax889 MG and Ax890 MG) from Nidera Argentina (2003) were sown on 1 October at a plant density of 9 plants m⁻². Details of Exp II have been previously described in Gambín et al. (2006). In Exp III, two hybrids tested in Exp II (Ax842 MG and Ax888 CL-MG) were sown on 8 October at a plant density of 9 plants m⁻². Hybrids for Exp III were selected based on their contrasting duration of the grain-filling period.

In all experiments, treatments were arranged in a randomized complete block design with three replicates. Each individual plot involved 10 (Exp I) or 5 (Exps II and III) rows 0.5 m apart, and 5 m (Exps I and II) or 6.5 m long (Exp III). Plots were over sown and thinned at the three-leaf stage (ligulated leaves) to the desired stand density. Nitrogen was applied twice: at the four-leaf stage and on ca. 15–20 days before flowering in all experiments. The application rate was 100 kg ha⁻¹, except for the second application in Exp I, where 50 kg ha⁻¹ was applied. Experiments were conducted under no visible water stress, and pests and weeds were adequately controlled throughout the growing cycle. Water stress was prevented by means of furrow irrigation, maintaining the soil near field capacity throughout the growing season.

In each experiment, 15 plants were randomly tagged 15 days before male flowering (i.e. anthesis) in each individual plot. Silking date (i.e. first silk visible) of the apical ear was registered for all tagged plants. Beginning at silking in Exps I and III and on 7 days after silking in Exp II, the apical ear shoot of one plant per replicate was harvested every 4–6 days. The ear was rapidly enclosed in an airtight plastic bag and transported to the lab 150 m away. Kernel sampling was made in a humidified box to prevent water loss (Westgate and Boyer, 1986; Borrás et al., 2003). Ten to 15 kernels from the same ear position (between spikelets 10 and 15, from the bottom of the apical ear) were sampled for fresh and dry weight determination. Fresh weight was measured immediately, and dry weight was determined after drying the kernels in a forced air oven at 70 °C for at least 96 h. Fresh and dry weight data were used to calculate kernel water content (mg kernel⁻¹) and percent kernel moisture content (g kg⁻¹) throughout grain filling.

Total duration of grain filling was determined for each hybrid × replicate combination by fitting a bilinear model (see Fig. 1; Eqs. (1) and (2)):

$$KW = a + bTT \quad \text{for } TT \leq c \quad (1)$$

$$KW = a + bc \quad \text{for } TT > c \quad (2)$$

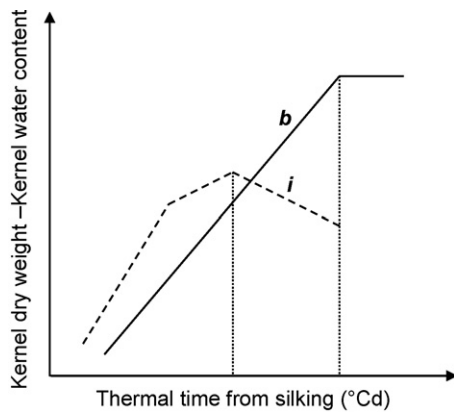


Fig. 1. Schematic diagram illustrating the models used to describe the kernel dry weight (full line) and kernel water content (dotted line) development during grain filling. Letters *i* and *b* show specific model parameters used to detect changes in the relationship between kernel water loss and kernel biomass deposition during late grain filling: *i* represents the rate of kernel water loss from maximum water content to physiological maturity, and *b* represents the kernel growth rate during the effective grain-filling period.

where KW is the kernel dry weight, TT the thermal time after silking ($^{\circ}\text{C day}$), *a* the *Y*-intercept ($^{\circ}\text{C day}$), *b* the kernel growth rate during the effective grain-filling period ($\text{mg } ^{\circ}\text{C day}^{-1}$), and *c* is the total duration of grain filling ($^{\circ}\text{C day}$). The bilinear model was fitted to the kernel dry weight data using the iterative optimization technique of Table Curve V 3.0 (Jandel Scientific, 1991). The adjusted r^2 -values ranged from 0.95 to 0.99 ($p < 0.001$). Daily TT values were calculated using 0°C as base temperature (Muchow, 1990). Mean daily air temperature was calculated as the average of hourly air temperatures registered at a weather station located at approximately 50 m from the experimental plots. The duration of the lag phase for each hybrid \times replicate combination was estimated from the parameters of the model by calculating the TT when kernel dry weight is equal to 0.

Kernel water content in maize follows a parabolic curve (Westgate and Boyer, 1986; Borrás et al., 2003) and we were interested in describing how water content develops after maximum water content was reached. For this, a trilinear model was fitted to the water content data for each hybrid \times replicate combination, similarly to Pepler et al. (2006) (see Fig. 1). The model was fitted using the iterative optimization technique of Table Curve V 3.0 (Jandel Scientific, 1991) (Fig. 1; Eqs. (3)–(5)):

$$\text{WC} = d + e\text{TT} \quad \text{for } \text{TT} \leq f \quad (3)$$

$$\text{WC} = d + ef + g(\text{TT} - f) \quad \text{for } \text{TT} > f \text{ and } \text{TT} < h \quad (4)$$

$$\text{WC} = d + ef + g(h - f) - i(\text{TT} - h) \quad \text{for } \text{TT} \geq h \quad (5)$$

where WC is the water content, TT the thermal time after silking, *d* the *Y*-intercept (mg), *e* the initial rate of kernel water accumulation ($\text{mg } ^{\circ}\text{C day}^{-1}$), *f* the thermal time at which a shift in the rate of water content increase is detected ($^{\circ}\text{C day}$), *g* the rate of kernel water accumulation during the second phase ($\text{mg } ^{\circ}\text{C day}^{-1}$), *h* the thermal time at maximum water content ($^{\circ}\text{C day}$), and *i* is the rate of water loss from maximum water

content to physiological maturity ($\text{mg } ^{\circ}\text{C day}^{-1}$). For each hybrid, water content data after physiological maturity were excluded when fitting the model. The adjusted r^2 -values ranged from 0.75 to 0.99 ($p < 0.005$).

The relationship between water loss and biomass deposition after maximum water content was reached was evaluated for each hybrid \times replicate combination by calculating the difference between the kernel growth rate from models (1) and (2) (parameter *b*, see Fig. 1) and the rate of kernel water loss from the timing maximum water content was attained until physiological maturity from models (3) to (5) (parameter *i*, see Fig. 1).

Percent kernel MC values at physiological maturity were determined using a bilinear model relating kernel dry weight and percent kernel MC data (Eqs. (6) and (7)) (Jandel Scientific, 1991):

$$\text{KW} = i - k\text{MC} \quad \text{for } \text{MC} \geq l \quad (6)$$

$$\text{KW} = i - kl \quad \text{for } \text{MC} < l \quad (7)$$

where KW is the kernel weight, MC the percent moisture content (g kg^{-1}), *j* the *Y*-intercept (mg), *k* the rate of kernel MC decline during grain filling [$\text{mg } (\text{g kg}^{-1})^{-1}$], and *l* is the critical percent MC at physiological maturity (g kg^{-1}). This model was fitted independently for each hybrid \times replicate combination. The adjusted r^2 -values were higher than 0.90 ($p < 0.001$).

Starting on 20 days after silking, 10 additional kernels were taken from the same ear harvested for kernel weight and water determination. These kernels were also removed from positions 10 to 15 along the ear and were used for kernel volume measurements by water volumetric displacement (Martinez-Carrasco and Thorne, 1979; Kiniry, 1988). Volume measurements were done in Exps II and III. In order to address differences in kernel volume determination after kernel maximum water content was reached, we calculated the difference between the maximum kernel volume and the kernel volume at maximum water content registered for each hybrid \times replicate combination.

The thermal time and percent MC at maximum water content were considered as the thermal time and percent MC values when kernels reached the maximum water content in each hybrid \times replicate combination. The same was done for accumulated thermal time and percent MC at maximum kernel volume.

Analysis of variance was used to evaluate the effects of hybrids on the response variables within each experiment, except for the Exp I where only one hybrid was evaluated. Linear regression analysis was applied to the relationships between variables.

3. Results

3.1. Kernel water relations and genotypic differences in grain-filling duration

The total duration of grain filling showed significant differences among hybrids in Exp II (Fig. 2; $p < 0.001$;

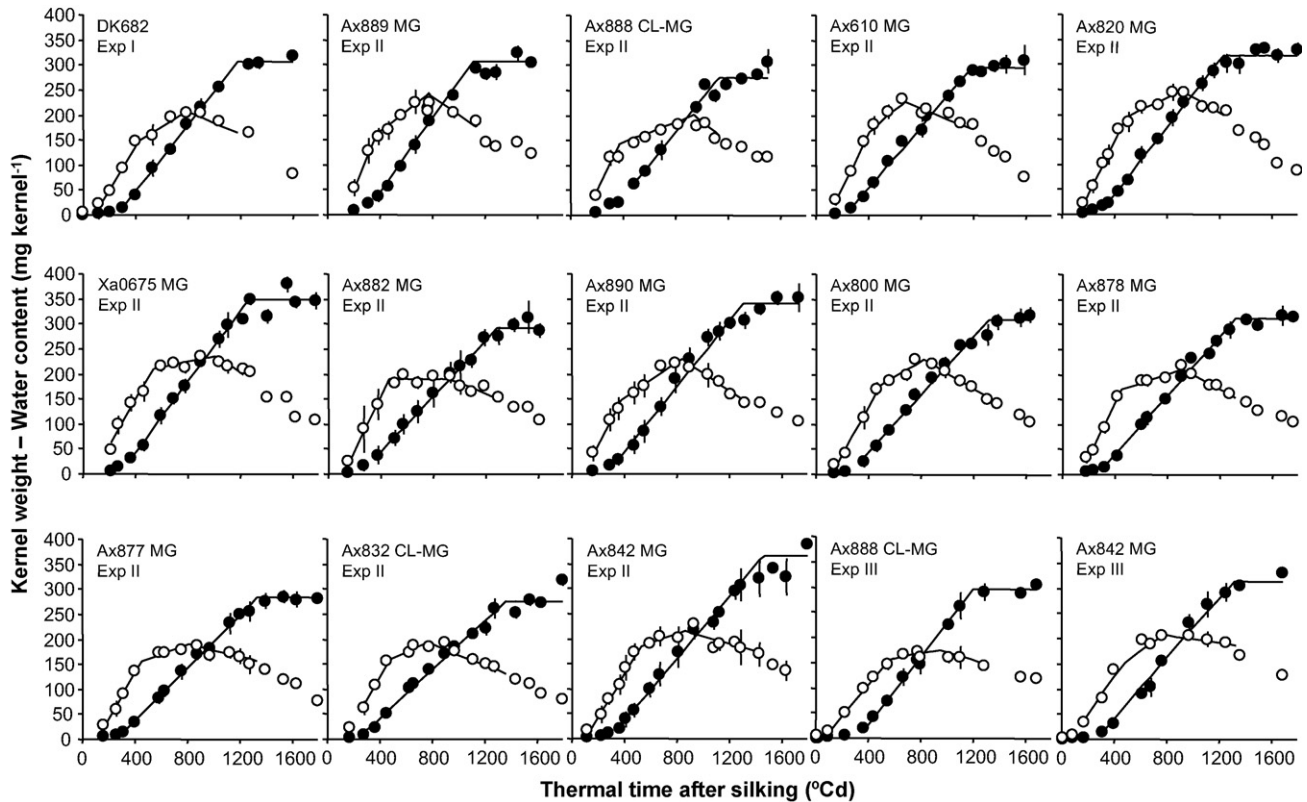


Fig. 2. Kernel dry weight (closed symbols) and kernel water content (open symbols) development during grain filling for all tested hybrids and environments (Exp I: 2002–2003; Exp II: 2003–2004; Exp III: 2004–2005). Lines represent the adjusted models for kernel dry weight and water content data (see Fig. 1). The water content development of hybrid Ax882 MG did not show a clear parabolic curve. However, the slope of the second kernel phase was positive, and maximum water content was attained at 908 °C day (see Table 1).

Table 1; Gambín et al., 2006) and Exp III (Fig. 2; $p < 0.05$; Table 1). When all experiments were considered, variability for total grain-filling duration ranged from 1117 to 1470 °C day (from 44 to 60 days; Fig. 2). In Exp I, the grain-filling duration of hybrid DK682 was 1187 °C day, close to the lowest values of the explored range (Table 1). In the experimental years where hybrids Ax842 MG and Ax888 CL-MG were evaluated, the former always had a significantly longer grain-filling duration than the latter ($p < 0.05$; Table 1), although the magnitude of the difference was much smaller in Exp III than Exp II (Table 1).

There were no differences in the duration of the lag phase across hybrids in Exp II ($p > 0.05$; Fig. 2), indicating that variations in grain-filling duration were related to variations in the duration of the effective grain-filling period. The duration of the lag phase was different between hybrids in Exp III ($p < 0.05$; 328 and 265 °C day for hybrids Ax888 CL-MG and Ax842 MG, respectively). This, however, was not responsible for the difference in the total duration of grain filling. The hybrid with the longest grain-filling duration had the shortest lag phase (Table 1).

The accumulated thermal time from silking to maximum water content showed no significant differences across hybrids in Exps II and III (Table 1). Averaging hybrids within each experiment, the maximum water content was reached at 870 °C day in Exp II and at 780 °C day in Exp III (Fig. 2). In Exp I, kernels from hybrid DK682 reached maximum water

content at an intermediate accumulated thermal time when compared to the explored range from the other two experiments (Table 1). When all data were pooled together, there was no relation between the total duration of grain filling and the time from silking to maximum water content (Fig. 3A). Differences in duration of grain filling were related to variations in the accumulated thermal time from the moment kernels reached maximum water content until physiological maturity ($r^2 = 0.64$; $p < 0.001$; Fig. 3B).

Percent kernel MC at maximum water content showed no difference among hybrids (Table 1). Averaging hybrids within each experiment, kernel maximum water content was reached at 529 g kg⁻¹ in Exp II and at 557 g kg⁻¹ in Exp III. Hybrid DK682 from Exp I showed a similar value (535 g kg⁻¹; Table 1). There were also no genotypic differences in the percent MC of kernels at physiological maturity (Table 1). Averaging hybrids within each experiment, physiological maturity was reached when kernels had 326 and 343 g kg⁻¹ in Exps II and III, respectively. In Exp I, kernels from DK682 reached physiological maturity with a percent MC value of 323 g kg⁻¹, similar to the other hybrids (Table 1). As such, percent kernel MC at specific developmental stages during grain filling (maximum water content and physiological maturity) showed no differences for the hybrids we studied. Differences in the duration of grain filling, measured in accumulated thermal time, were related to the period kernels maintained a percent MC above the critical one at which

Table 1
Accumulated thermal time (TT) from silking to physiological maturity (shown as total grain-filling duration), from silking to maximum water content, and from silking to maximum kernel volume, percent kernel moisture content at maximum water content, at physiological maturity and at maximum kernel volume, kernel growth rate during the effective grain-filling period, rate of kernel water loss from maximum water content to physiological maturity and kernel volume increase from maximum water content to physiological maturity for all hybrids and environments tested (Exp I: 2002–2003; Exp II: 2003–2004; Exp III: 2004–2005)

Experiment	Hybrid	Total grain-filling duration (°C day)	TT at maximum water content ^a (°C day)	TT at maximum kernel volume ^{a,b} (°C day)	Percent moisture content			Kernel growth rate (mg C day ⁻¹ kernel ⁻¹)	Kernel water loss rate (mg C day ⁻¹ kernel ⁻¹)	Volume increase from maximum water content to physiological maturity ^b (μL kernel ⁻¹)
					At maximum water content ^a (g kg ⁻¹)	At physiological maturity (g kg ⁻¹)	At maximum kernel volume ^{a,b} (g kg ⁻¹)			
I	DK682	1187	808	–	535	323	–	0.33	–0.21	–
II	Ax889 MG	1117	938	1010	499	302	463	0.36	–0.19	3
	Ax888 CL-MG	1138	969	1251	447	355	352	0.33	–0.21	17
	Ax610 MG	1189	712	1146	587	366	402	0.30	–0.11	40
	Ax820 MG	1234	906	1173	532	355	423	0.33	–0.16	19
	Xa0675 MG	1266	914	1567	510	335	290	0.34	–0.17	23
	Ax882 MG	1295	908	1294	516	317	363	0.28	–0.11	53
	Ax890 MG	1314	891	1201	499	298	378	0.33	–0.17	51
	Ax800 MG	1323	801	1476	575	315	310	0.30	–0.16	75
	Ax878 MG	1325	908	1364	525	331	342	0.29	–0.14	61
	Ax877 MG	1337	839	1295	537	321	368	0.26	–0.06	73
	Ax832 CL-MG	1366	794	1250	574	302	374	0.24	–0.10	63
	Ax842 MG	1470	862	1750	543	309	289	0.30	–0.07	114
		(140) ^{****,c}	NS	(244) ^{****}	NS	NS	(8) ^{**}	(0.05) ^{**}	(0.08) ^{**}	(57) [*]
III	Ax888 CL-MG	1203	804	1225	538	348	348	0.34	–0.08	80
	Ax842 MG	1320	756	1332	576	338	347	0.30	–0.04	114
	(54) [*]	NS	(28) ^{**}	NS	NS	NS	(0.03) [*]	NS	NS	

*, **, *** Significant at $p = 0.05$, 0.01 and 0.001 , respectively; NS: not significant.

^a TT at maximum water content and at maximum volume might not perfectly match data shown in Figs. 2 and 5 because figures describe the mean of the three replicates for each day, while this table shows the mean of the maximum for each of the three replicates independently of the date. The same for percent kernel moisture content at maximum water content and maximum volume.

^b Kernel volume was not measured in Exp I.

^c LSD value for $p \leq 0.05$.

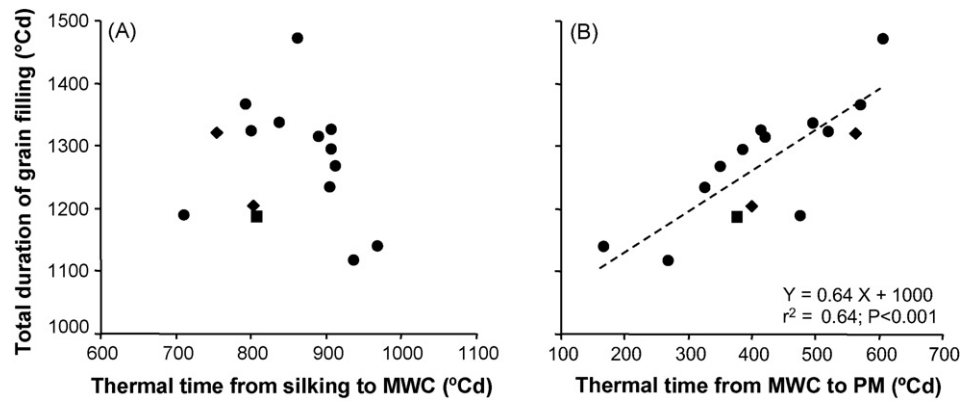


Fig. 3. Relationships between total duration of grain filling and accumulated thermal time from silking to maximum water content (MWC) (A), and between total duration of grain filling and thermal time from MWC to physiological maturity (PM) (B), for the hybrid evaluated in Exp I (square), the 12 hybrids evaluated in Exp II (circles) and the 2 hybrids tested in Exp III (rhombus).

physiological maturity was achieved. As there were no differences in accumulated thermal time from silking to the kernel maximum water content stage, the rate of percent kernel MC decline from maximum water content to physiological maturity explained the observed differences in the duration of grain filling for these hybrids.

As the achievement of a particular percent MC involves variations in the relationship between water development and biomass deposition, we studied the rate of water loss from maximum water content to physiological maturity and the rate of kernel growth (rates illustrated as *i* and *b* in Fig. 1). For a similar kernel growth rate, a steeper decline in water content would reduce the grain-filling duration by making the critical percent MC to be achieved earlier. Similarly, for the same water loss rate after maximum water content, a higher kernel growth rate would imply reaching the critical percent kernel MC earlier and reducing the grain-filling duration. There was a wide range in the rate of water loss after maximum water content across the three experiments (Table 1; Fig. 2). In Exp II significant differences were observed ($p < 0.01$) among hybrids. When the two hybrids from Exp II were repeated the following season in Exp III,

AX888 CL-MG also showed a steeper decline in water loss after maximum water content when compared to AX 842 MG ($p < 0.35$). In relation to the rate of dry matter accumulation, significant differences were observed among hybrids in Exp II ($p < 0.01$) and Exp III ($p < 0.05$; Table 1). Both rates were independently correlated to the duration of grain filling from maximum water content to physiological maturity ($r^2 = 0.53$ and 0.35 ; Fig. 4A and B). It is important to note that the explored range in the rate of water loss was much larger (from -0.04 to -0.21 $\text{mg } ^\circ\text{C day}^{-1} \text{ kernel}^{-1}$) than the explored range in kernel growth rate (from 0.24 to 0.36 $\text{mg } ^\circ\text{C day}^{-1} \text{ kernel}^{-1}$; Fig. 4A and B) across hybrids and environments.

In order to understand the relationship between the rate of water loss and the rate of biomass accumulation, both having an impact on how percent MC declines during late grain filling (Fig. 4A and B), we calculated the difference between them. An increased kernel growth rate or a steeper water loss rate would both increase the difference among them, and would imply a steeper decline in the kernel percent MC. When calculated, the difference between rates showed a considerable range across experiments, and was significantly correlated to the

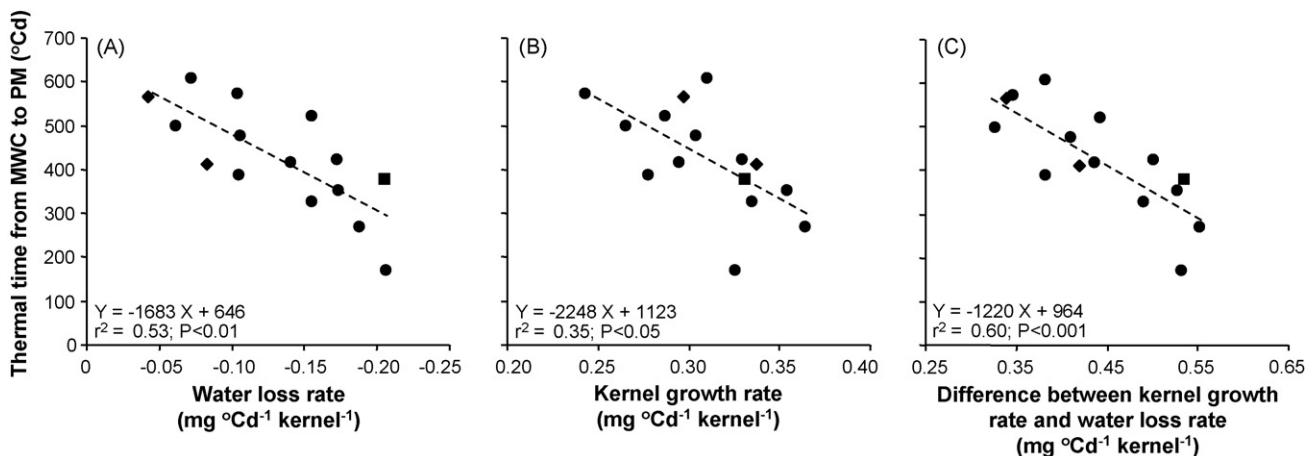


Fig. 4. Relationships between the grain-filling duration from maximum water content (MWC) to physiological maturity (PM) with the rate of kernel water loss during late grain filling (A), with the rate of kernel growth (B), and with the difference between the kernel growth rate and the water loss rate (C). The three figures show the hybrid evaluated in Exp I (square), the 12 hybrids evaluated in Exp II (circles) and the 2 hybrids tested in Exp III (rhombus).

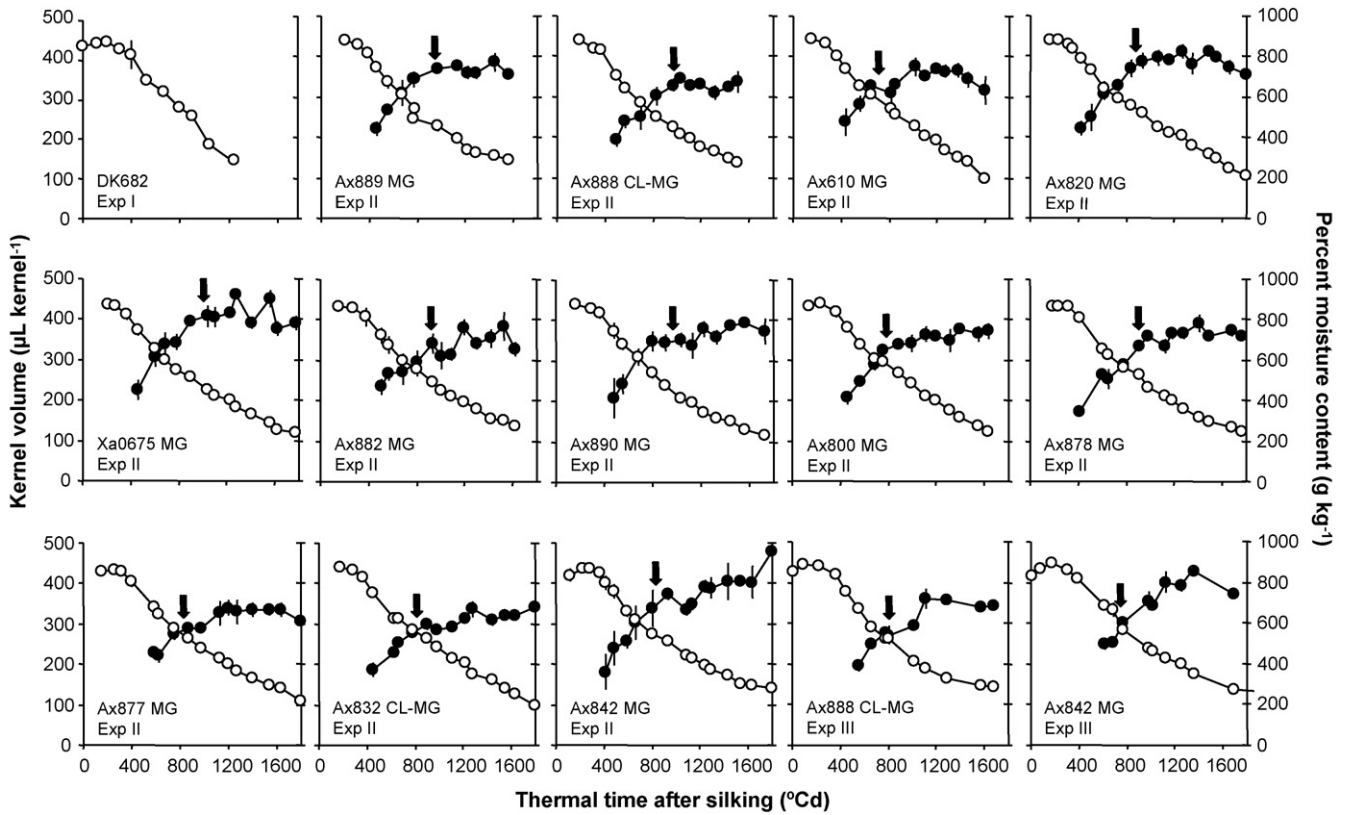


Fig. 5. Development of kernel volume (closed symbols) and percent kernel moisture content (open symbols) during grain filling for all the hybrids and environments tested (Exp I: 2002–2003; Exp II: 2003–2004; Exp III: 2004–2005). Arrows indicate the timing maximum water content was attained in each particular hybrid × experiment combination; maximum water content was determined as the maximum value registered for each replicate × hybrid combination within each experiment, as shown in Table 1. Kernel volume was not measured in hybrid DK682 (Exp I).

accumulated thermal time from maximum water content to physiological maturity ($r^2 = 0.60$; $p < 0.001$; Fig. 4C). Hybrids from Exp II differed in the difference between rates ($p < 0.01$), and the two hybrids repeated in Exp III showed the same trend as in the previous year ($p < 0.18$). Calculating the difference between both rates helped explain with higher confidence the accumulated thermal time from maximum water content to physiological maturity than with any one single rate.

3.2. Kernel water relations and kernel volume

Hybrids differed in the accumulated thermal time from silking to the timing maximum kernel volume was attained (Fig. 5; $p < 0.001$ and < 0.01 in Exp II and III, respectively; Table 1), and kernels achieved their maximum volume later in development than they reached maximum water content (Fig. 6). Averaging across hybrids and experiments, maximum

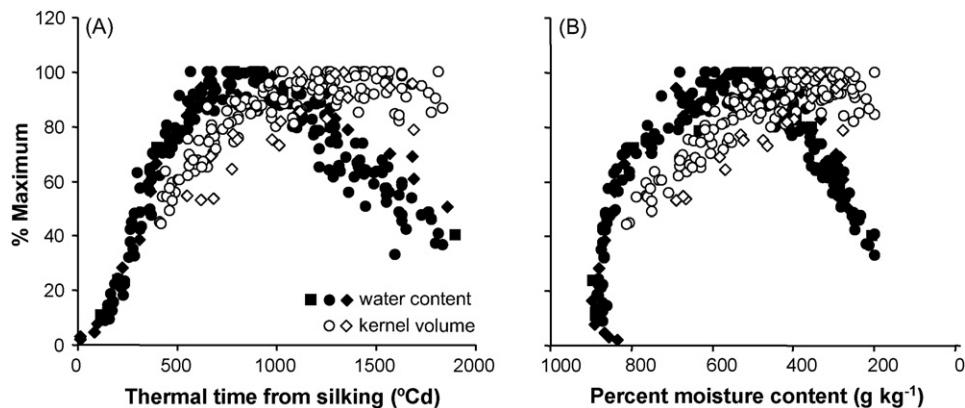


Fig. 6. Kernel water (closed symbols) and volume (open symbols) development throughout grain filling is shown as a relative value to the maximum achieved at any stage during grain filling. Development is expressed as accumulated thermal time (A) or percent kernel moisture content (B). Data show the hybrid evaluated in Exp I (squares), the 12 hybrids from Exp II (circles) and the 2 hybrids from Exp III (rhombus). Maximum water content and maximum kernel volume were considered as the maximum value registered in each hybrid × experiment combination as shown in Figs. 2 and 5.

water content was reached at 854 °C day after silking, while maximum volume was attained at 1310 °C day (Fig. 6; Table 1). Differences among hybrids in the accumulated thermal time to maximum kernel volume were consistent with differences in grain-filling duration. The longer the grain-filling duration, the higher the accumulated thermal time at which kernels reached their maximum volume ($r^2 = 0.48$; $p < 0.01$; $n = 14$).

The kernel percent MC at maximum kernel volume showed differences among hybrids in Exp II ($p < 0.01$; Table 1). The explored range varied between 463 and 289 g kg⁻¹ (Table 1). When comparing the percent MC at maximum water content and at maximum volume, kernels achieved their maximum volume at lower MC values (Fig. 6). Averaging across hybrids and experiments, kernels achieved their maximum water content when their percent MC was ca. 540 g kg⁻¹, while maximum volume was reached when having ca. 330 g kg⁻¹ (Fig. 6; Table 1). The percent MC at maximum kernel volume decreased with longer grain-filling durations ($r^2 = 0.30$; $p < 0.05$; $n = 14$).

When kernels reached maximum water content, the percent of their maximum kernel volume was not always the same across hybrids and experiments, and showed a range between 73 and 99% of their maximum. This volume increase from maximum water content to physiological maturity was significantly different among hybrids in Exp II ($p < 0.05$; Table 1). In Exp III, hybrids did not differ in their volume increase during late grain filling ($p > 0.05$), but Ax842 MG showed a higher value than Ax888 CL-MG, in accordance to Exp II (Table 1). Volume increases during late grain filling were related to the difference between the rate of kernel growth and the rate of water loss during late grain filling (Fig. 7; $r^2 = 0.57$; $p < 0.001$). The larger the difference between the two rates the lower the volume increase. This indicated that variations in the pattern of water and biomass development during late grain filling affected kernel volume determination. A faster desicca-

tion was correlated to smaller, and in some hybrids almost null, kernel volume increases during late grain filling (Fig. 7).

4. Discussion

As expected from previous research (Saini and Westgate, 2000; Borrás and Westgate, 2006), hybrids did not differ in the kernel percent MC at specific developmental stages, like maximum water content and physiological maturity. As such, the period of time from silking to these two specific kernel developmental stages was a consequence of how the percent MC developed during grain filling. This percent MC development is dependent upon the relationship between kernel water and biomass development. For the set of hybrids we studied, differences in the duration of grain filling were a consequence of variations in the relationship between water loss and biomass deposition after maximum water content was reached, and not of the timing kernel maximum water content was attained (Figs. 3 and 4). This result expands what is known from soybean seeds, where the moment maximum water content was reached controlled the duration of grain filling (Egli, 1990), and are in general agreement with previous observations in sorghum. In sorghum, differences in grain-filling duration among kernels from contrasting positions within the panicle were related to differences in the percent MC decline after kernels reached maximum water content (Gambín and Borrás, 2005).

We have shown that maize kernel volume depends not only on water uptake during early grain filling, but also on the kernel water and biomass development during late grain filling. Water uptake during early grain filling was responsible for most of the volume kernels achieved (Figs. 5 and 6), but volume could continue to increase after kernels reached maximum water content. In sorghum, we previously reported that kernel volume could increase after kernels achieved their maximum water content, and that variations in the relation between water and biomass during late grain filling were related to these changes (Gambín and Borrás, 2005). When kernel volume development was measured in wheat (*Triticum aestivum*), kernels reached their maximum volume at the same time as maximum water content (Martinez-Carrasco and Thorne, 1979), but volume increases did not parallel the pattern of water accumulation. As such, findings in several species are showing that kernel volume determination is not only related to positive net water uptake, but to the relation between water and biomass accumulation also.

Changes in assimilate availability regulate kernel water uptake and expansion (Martinez-Carrasco and Thorne, 1979; Egli, 1990; Borrás et al., 2003). Source reductions during the effective grain-filling period accelerate the rate of water loss from kernels while not affecting their biomass accumulation rate, accelerating desiccation and reducing the grain-filling duration (Egharevba et al., 1976; Barlow et al., 1980; Brooks et al., 1982; Jones and Simmons, 1983; Westgate, 1994; Pepler et al., 2006). Our idea of integrating the rate of biomass increase and the rate of water loss, by calculating the difference between both rates, helped understand percent MC development after kernels reached maximum water content. This would allow predicting time to physiological maturity.

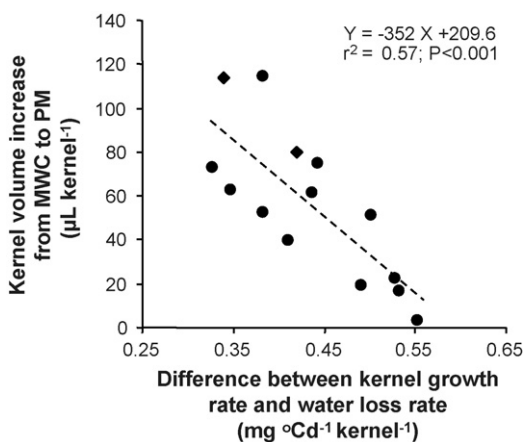


Fig. 7. Kernel volume increase during late grain filling (from kernel maximum water content, MWC, to physiological maturity, PM) and the difference between the kernel growth rate and the kernel water loss rate during the same period. Data depict the 12 hybrids from Exp II (circles) and the 2 hybrids from Exp III (rhombus). A higher difference between rates indicates a faster decline in the kernel percent moisture content during late grain filling.

We have recently shown that genotypic differences in grain-filling duration in most of the hybrids presented in this article were partially explained by the ratio between plant growth rate per kernel during the effective grain-filling period and their kernel growth rate (Gambín et al., 2006). This could suggest, at least partially, that a differential post-flowering source–sink ratio is behind the observed genotypic differences in kernel water relations and grain-filling duration during late grain filling. In sorghum, the post-flowering assimilate availability per kernel helped explain differences in kernel water relations of contrasting panicle positions during late grain filling (Gambín and Borrás, 2007). However, manipulative treatments increasing the assimilate availability per developing kernel never increased the duration of grain filling in maize (Schooper et al., 1982; Andrade and Ferreiro, 1996; Cirilo and Andrade, 1996; Borrás et al., 2003), showing this process not to be source limited. Together with other recent evidences (Borrás et al., 2004; Borrás and Westgate, 2006; Gambín et al., 2006) results indicate that, during early kernel growth, each maize hybrid adjusts a number of kernels in relation to a source strength around flowering which places further kernel growth close to a saturated assimilate availability condition for biomass accumulation. As such, this condition may differ when hybrids are compared (Gambín et al., 2006), but it does not indicate that source limitations are common during late grain-filling stages in maize.

5. Conclusions

Hybrids did not differ in the kernel percent MC at maximum water content and maximum weight. This illustrates the importance of understanding how kernel percent MC develops throughout grain filling to predict genotypic and environmental differences in grain-filling duration. Differences in the rate percent MC declined from maximum water content to physiological maturity showed to be more important than the rate from silking to maximum water content in determining the duration of grain filling for the set of hybrids we studied. Differences in the rate of percent MC decline during late grain filling were related to differences between the water loss and biomass accumulation rates. Also, the relationship between water and biomass development affected kernel volume determination during late grain filling. As such, the volume maize kernels achieve is not only related to the pattern of water development.

Acknowledgements

The present work was partially supported by Nidera Argentina. B.L. Gambín held a graduate fellowship from, and M.E. Otegui is a member of CONICET, the Scientific Research Council from Argentina. We appreciate the comments and suggestions done by an anonymous reviewer.

References

Andrade, F.H., Ferreiro, M.A., 1996. Reproductive growth of maize, sunflower and soybean at different source levels during grain filling. *Field Crops Res.* 48, 155–165.

Barlow, E.W.R., Lee, J.W., Munns, R., Smart, M.G., 1980. Water relations of the developing wheat grain. *Aust. J. Plant Physiol.* 7, 519–525.

Borrás, L., Westgate, M.E., 2006. Predicting maize kernel sink capacity early in development. *Field Crops Res.* 95, 223–233.

Borrás, L., Westgate, M.E., Otegui, M.E., 2003. Control of kernel weight and kernel water relations by post-flowering source–sink ratio in maize. *Ann. Bot.* 91, 857–867.

Borrás, L., Slafer, G.A., Otegui, M.E., 2004. Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Res.* 86, 131–146.

Brooks, A., Jenner, C.F., Aspinall, D., 1982. Effects of water deficit on endosperm starch granules and on grain physiology in wheat and barley. *Aust. J. Plant Physiol.* 9, 423–436.

Cavaliere, A.J., Smith, O.S., 1985. Grain filling and field drying of a set of maize hybrids released from 1930 to 1982. *Crop Sci.* 25, 856–860.

Cirilo, A.G., Andrade, F.H., 1996. Sowing date and kernel weight in maize. *Crop Sci.* 36, 325–331.

Daynard, T.B., Tanner, J.W., Duncan, W.G., 1971. Duration of the grain filling period and its relation to grain yield in corn, *Zea mays* L. *Crop Sci.* 11, 45–48.

Egharevba, P.N., Horrocks, R.D., Zuber, M.S., 1976. Dry matter accumulation in maize in response to defoliation. *Crop Sci.* 7, 151–156.

Egli, D.B., 1990. Seed water relations and the regulation of the duration of seed growth in soybean. *J. Exp. Bot.* 41, 243–248.

Egli, D.B., 2004. Seed-fill duration and yield of grain crops. *Adv. Agron.* 83, 243–279.

Egli, D.B., TeKrony, D.M., 1997. Species differences in seed water status during seed maturation and germination. *Seed Sci. Res.* 21, 289–294.

Gambín, B.L., Borrás, L., 2005. Sorghum kernel weight: growth patterns from different positions within the panicle. *Crop Sci.* 45, 553–561.

Gambín, B.L., Borrás, L., 2007. Plasticity of sorghum kernel weight to increased assimilate availability. *Field Crops Res.* 100, 272–284.

Gambín, B.L., Borrás, L., Otegui, M.E., 2006. Source–sink relations and kernel weight differences in maize temperate hybrids. *Field Crops Res.* 95, 316–326.

Jandel Scientific, 1991. Table Curve V 3.0. User's Manual Version 3.0 AISN Software. Jandel Scientific, Corte Madera, CA.

Jones, R.J., Simmons, S.R., 1983. Effect of altered source–sink ratio on growth of maize kernels. *Crop Sci.* 23, 129–134.

Kiniry, J.R., 1988. Kernel weight increase in response to decreased kernel number in sorghum. *Agron. J.* 80, 221–226.

Martinez-Carrasco, R., Thorne, G.N., 1979. Physiological factors limiting grain size in wheat. *J. Exp. Bot.* 30, 669–679.

Millet, E., Pinthus, M.J., 1984. The association between grain volume and grain weight in wheat. *J. Cereal Sci.* 2, 31–35.

Monsanto Argentina, 2002. Maíz: Híbridos Dekalb. Catálogo de Productos 2003. Monsanto, Buenos Aires, Argentina, pp. 5–6.

Muchow, R.C., 1990. Effect of high temperature on grain-growth in field-grown maize. *Field Crops Res.* 23, 145–158.

Nidera Argentina, 2003. Maíz: Híbridos Nidera. Catálogo de Productos 2003. Nidera, Buenos Aires, Argentina, pp. 3–10.

Pepler, S., Gooding, M.J., Ellis, R.H., 2006. Modeling simultaneously water content and dry matter dynamics of wheat grains. *Field Crops Res.* 95, 49–63.

Poneleit, C.G., Egli, D.B., 1979. Kernel growth rate and duration in maize as affected by plant density and genotype. *Crop Sci.* 19, 385–388.

Saini, H.S., Westgate, M.E., 2000. Reproductive development in grain crops during drought. *Adv. Agron.* 68, 59–96.

Schnyder, H., Baum, U., 1992. Growth of the grain of wheat (*Triticum aestivum* L.). The relationship between water content and dry matter accumulation. *Eur. J. Agron.* 2, 51–57.

Schooper, J.B., Johnson, R.R., Lambert, R.J., 1982. Maize yield response to increased assimilate supply. *Crop Sci.* 22, 1184–1189.

Swank, J.C., Egli, D.B., Pfeiffer, T.W., 1987. Seed growth characteristics of soybean genotypes differing in duration of seed fill. *Crop Sci.* 27, 85–89.

Westgate, M.E., 1994. Water status and development of the maize endosperm and embryo during drought. *Crop Sci.* 34, 76–83.

Westgate, M.E., Boyer, J.S., 1986. Water status and the developing grain of maize. *Agron. J.* 78, 714–719.