Published September 26, 2014

RESEARCH

Seed Water Concentration and Accumulation of Protein and Oil in Soybean Seeds

Florencia B. Poeta, José L. Rotundo,* Lucas Borrás, and Mark E. Westgate

ABSTRACT

Seed development is partitioned into a 'lag' phase, a 'seed filling' phase, and a 'maturation' phase. Transitions between phases correspond to seed water concentration (WC) values that are fairly consistent within species. For soybean (*Glycine max* L.), linear seed filling begins at approximately 85% (WC_L) and maximum dry weight is attained at approximately 60% (WC_M). While such WC values benchmark the progress of seed development, their utility for establishing onset and duration of individual seed chemical component accumulation is not known. Our objectives were (i) to determine WC_L and WC_M for seed protein, oil, and residual (mostly carbohydrates), (ii) to assess stability across genotypes and environments, and (iii) to investigate their relationship with the duration of accumulation. The WC_L and WC_M for oil, protein, and residual were significantly different. Values were higher for residual and lower for oil. Since seeds desiccate throughout their development, residual accumulation was initiated first, followed by protein, then by oil. The parameter WC_L was more stable across genotypes than was WC_M. Genotypes with lower WC_M values had a longer duration of component accumulation. Increasing assimilate supply per seed decreased WC_L for all seed components, but had little impact on WC_M. Our results indicate that a water relations framework can be used to characterize accumulation patterns of individual seed components across genotypes and environments, providing a common basis for modeling the composition of soybean seeds.

F.B. Poeta, J.L. Rotundo, and L. Borrás, Departamento de Producción Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, S2125ZAA, Zavalla, Santa Fe, Argentina; M.E. Westgate, Agronomy Dep., Iowa State Univ., Ames, IA 50011. Received 12 Mar. 2014. *Corresponding author (jrotundo@unr.edu.ar).

Abbreviations: Desrate, dessication rate; WC, seed water concentration; WC_L, water concentration at the beginning of linear filling; WC_M, water concentration at maximum seed biomass accumulation.

Seed development is commonly partitioned into three phases: a 'lag' phase dominated by histo-differentiation and early cell expansion, a 'seed-filling' phase involving rapid storage deposition and continued cell expansion, and a 'maturation' phase associated with maximum dry matter and desiccation tolerance (Bewley and Black, 1994). The lag phase is a period of active cell division characterized by a rapid increase in water content with little dry-weight accumulation. During seed filling, the rate of dry weight accumulation reaches a maximum when storage products are rapidly synthesized and condensed in storage organelles. For convenience, this rate is often approximated as linear to calculate an effective filling period (Egli, 1990). During maturation, seeds attain maximum weight (physiological maturity) and acquire desiccation tolerance entering in a quiescent state.

Seed WC (mg water mg fresh seed^{-1} × 100) typically declines throughout seed development as water is replaced by storage components. Transitions between the above-mentioned phases consistently match specific WC values within species. In soybean, the linear filling period begins at approximately 85% WC (WC_L) and maximum dry-weight (physiological maturity) is attained at approximately

Published in Crop Sci. 54:1–8 (2014).
doi: 10.2135/cropsci2014.03.0204

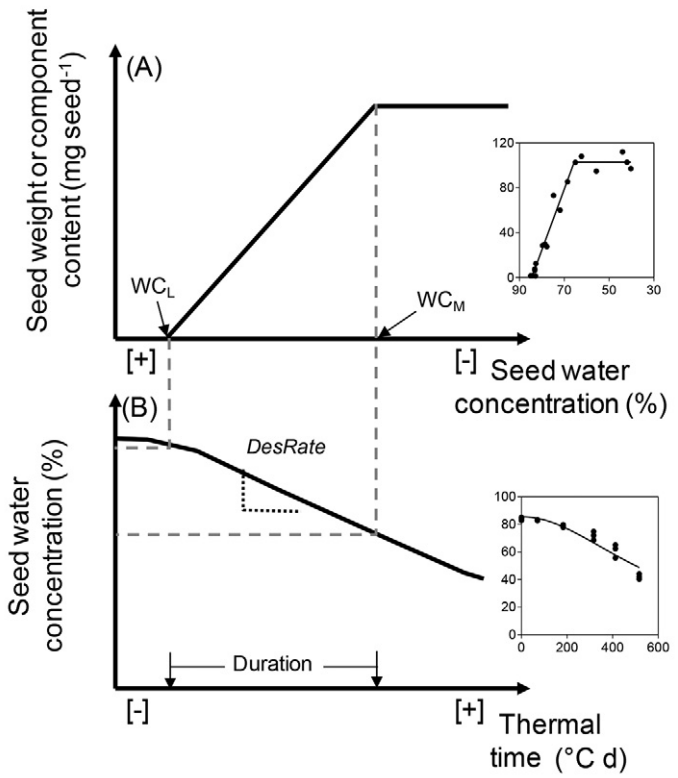

Figure 1. Theoretical relationship between (A) seed weight or component content and seed water concentration and (B) seed water concentration and thermal time. WC_L, water concentration at the beginning of linear phase of seed component accumulation; WC_M, the water concentration at maximum seed component content; DesRate, desiccation rate. Note that (A) panel *x* axis spans from higher to lower concentration. Inserts show actual data for control treatment of the genotype Evans.

60% WC (WC_M; see Fig. 1; Swank et al., 1987). In many species, these values are consistent across genotypes and environments, and seed WC has been used to benchmark the progress of seed development (Borrás and Westgate, 2006; Calderini et al., 2000; Swank et al., 1987). Currently, seed water concentration is frequently used as a benchmark for seed development and not seed water potential. Seed water potential is mostly stable during the entire seed filling until physiological maturity, while WC changes throughout development, helping depict the grain-filling stages (Borrás et al., 2003; Egli and Tekrony, 1997). All previous research on water relations of seed development focused on seed dry-weight accumulation. Here we propose to extend this previous framework to characterize the accumulation of individual seed components.

Final seed component content (mg $seed^{-1}$) depends on accumulation rate and duration (Swank et al., 1987). Rate is usually related to assimilate supply, uptake, and utilization (Jenner et al., 1991; Rotundo et al., 2009), while duration in soybean depends on a developmental program associated with the photo-thermal environment (Grimm et al., 1994). Evidence from several species indicates that longer seed fill duration is associated with a lower WC_M and/or slower desiccation rate. Increasing assimilate per seed slowed soybean seed desiccation rate and increased duration (Egli et al., 1985). In sorghum [*Sorghum bicolor* (L.) Moench], reductions in WC_M associated with different panicle positions increased grain filling duration (Gambín and Borrás, 2005). In maize (*Zea mays* L.), increased duration can be associated with reduced kernel desiccation rate during the linear seed filling period (Gambín et al., 2007) and with genotypic differences in WC_M (Borrás et al., 2009). An assessment of these relationships for duration of protein, oil, and residual accumulation is currently lacking.

An average mature soybean seed has 37% protein, 18% oil, 32% residual, and 13% moisture (Wilson, 2004). Residual is mostly (ca. 95%) carbohydrate, including soluble carbohydrates and structural material such as cellulose (Hanson et al., 1961). These values, however, can vary considerably across genotypes, environments, and source-to-sink ratio scenarios (Rotundo and Westgate, 2009; Rotundo et al., 2009). Efforts to model the influence of these factors on soybean composition have been less than favorable (Piper, 1993). The framework we propose may have implications to provide a common basis for modeling both the development and composition of soybean seeds. Also, evaluating stability of the critical WC values under different source-to-sink conditions and diverse genotypes is critical to understand whether these parameters can be considered species specific. This has implications on the applicability of this concept to help improve seed composition modeling.

We propose these critical WC values as a useful framework to approximate seed component accumulation. Since the different seed components (broadly categorized in protein, oil, and residual carbohydrates) have different metabolic functions during seed development (e.g., enzymes production, storage, and basal metabolism) and different accumulation timings, we hypothesized we can describe this using a water relations framework. The objectives of this study were (i) to assess WC_L and WC_M for protein, oil, and residual accumulation in soybean seeds, (ii) to assess the stability of these indicators by examining genotypes varying in seed composition and by altering assimilate availability per seed during seed filling, and (iii) to evaluate the relationships between seed component accumulation, WC_L, WC_M, and seed desiccation rate. To address these objectives, we investigated the seed water relations and seed component accumulation patterns of closely related soybean lines and commercial cultivars differing in seed composition. If developmental patterns of seed protein, oil, and residual do indeed exhibit different WC benchmarks, we expected this to be evident in genotypes with highly contrasting seed composition.

MATERIALS AND METHODS

Plant Material

Seven soybean genotypes were evaluated: cultivar Evans, three closely related experimental lines (BC_3F_8) derived from Evans (PR142, PR41, and PR84), and three current elite cultivars (IA2034, IA2068, and IA3011). Experimental lines were developed from crosses using Evans as a recurrent parent (PI153296, 36% protein) and either a low-protein donor line (PI453472, 32% protein) or a high-protein donor line (PI153296, 46% protein) (Rotundo et al., 2009). These experimental lines (Isolines) share approximately 94% of their genomes with Evans; PR142 was derived from the low-protein donor, while PR41 and PR84 were derived from the high-protein donor. Evans is included in the experimental lines comparison since the latter are derived from Evans. Commercial lines IA2034, IA2068, and IA3011 were locally adapted germplasm developed by the Iowa State University soybean breeding program. IA3011 is a high-protein cultivar. The average seed composition values and days to maturity for these lines are presented in Table 1. Evans and experimental isolines were tested in 2006 and 2007, while commercial cultivars were tested in 2007.

Plant Culture

Experiments were conducted in 2006 and 2007 at Iowa State University Hinds Research Farm located in Ames, IA. Planting dates were 10 May 2006 and 16 May 2007. Stand density was 31 plants m^{-2}. Soil type was Cumulic Hapludol, Spillville loam series. Plots were four rows, 6-m long, 0.76 m between rows. Measurements were always taken in the two central rows. Weeds were chemically controlled at planting and hand removed during crop growth. Pests were controlled with standard agronomic practices for the region.

Samples Collection

Pods were sampled weekly after R5.5 (Fehr and Caviness, 1977) until harvest maturity from 0.5 m of two central rows (1-m total). Since soybean seeds from different canopy positions differ in size and developmental stages at any time point, samples were taken from two nodes in the upper third of canopy height and two nodes in the lower third (Escalante and Wilcox, 1993a, b). All pods in those nodes were sampled. This sampling strategy allows having an integrated estimate intended to represent the whole plant. After sampling, pods were immediately placed in plastic hermetic bags and stored on ice for transport to the lab. Seeds were excised from pods in a humid box to avoid water loss. Between 15 and 40 seeds were pooled per sample and weighted. All further chemical determinations were done on a sample weight base, allowing the comparison of samples having different seed numbers.

Measured Variables

During seed development, seed fresh weight (mg $seed^{-1}$), seed dry weight (mg $seed^{-1}$) after drying at 65°C for 96 h, seed WC (mg water mg seed fresh $weight^{-1} \times 100$), and chemical composition (protein, oil, and residual) were estimated. Protein concentration (%) was estimated as nitrogen concentration multiplied by 6.25 using the combustion method in a 0.5-g subsample (Jung et al., 2003). Oil concentration (%) was determined gravimetrically after extraction with hexane in another 0.5-g subsample. Protein and oil content (mg $seed^{-1}$) for each seed sample were estimated as the product between individual seed dry weight and component concentration. Residual content (mg $seed^{-1}$) was calculated as the difference between total seed dry weight and protein and oil contents (Hanson et al., 1961). Concentration is reported on a 13% moisture basis and seed component contents (mg $seed^{-1}$) are expressed on a dry matter basis.

Table 1. Average seed protein and oil concentration and cycle duration for experimental soybean isolines and for commercial cultivars evaluated for relationships between seed component accumulation and water concentration at beginning of linear phase accumulation (WC_L) and at maximum content (WC_M). Data is from field grown plants at 13% moisture, from Rotundo et al. (2009). Cycle duration are means of 2 yr for the isolines and 1 yr for commercial cultivars.

Line	Protein	Oil	Cycle duration
	%		days
Experimental isolines			
Evans	36	20	103
PR142	34	20	104
PR41	42	16	108
PR84	42	17	118
Commercial cultivars			
IA2068	34	19	109
IA2034	39	17	112
IA3011	40	18	122

Both WC_L and WC_M of seed dry weight, protein, oil, and residual were estimated using a bi-linear model with plateau (Fig. 1A; Gambín and Borrás, 2011):

$$\text{SW or CC (mg seed}^{-1}) = a + b \star \text{WC for WC} < c \text{ (linear function)}$$

$$\text{SS or CC (mg seed}^{-1}) = a + b \star c \text{ for WC} > c \text{ (plateau function)}$$

where SW is seed weight, CC is the component content, WC is the seed water concentration after crop stage R5, a is the y-intercept (mg $seed^{-1}$), b is the rate of seed weight or component content accumulation, and c is WC_M. The WC_L was calculated as the WC value when seed dry weight or component content equals zero. Figure 1A shows a theoretical figure depicting WC_M and WC_L. The insert shows actual seed weight data for the Evans cultivar.

Desiccation rate was determined by a nonlinear model (Gambín and Borrás, 2011):

$$WC = 1/\left(a + b\,TT^2\right)$$

where WC is the seed water concentration after crop stage R5, TT is thermal time after R5 (°C d), b is the desiccation rate, and a is the y-intercept. Daily TT values were obtained with a base temperature of 8°C (Sinclair et al., 2003). Mean daily air temperatures were calculated from the daily maximum and minimum temperatures provided from a weather station located in the experimental field. Figure 1B shows a theoretical

figure depicting the desiccation rate. The insert shows actual desiccation data for the Evans cultivar.

Duration of accumulation also was determined as a function of thermal time using the bi-linear model (Gambín and Borrás, 2011):

$$\text{SW or CC (mg seed}^{-1}) = a + b \star \text{TT for TT} < c \text{ (linear function)}$$

$$\text{SS or CC (mg seed}^{-1}) = a + b \star c \text{ for TT} > c \text{ (plateau function)}$$

where SW is seed weight, CC is the component content, TT is thermal time after R5 (°Cd), a is the y-intercept (mg seed^{-1}), b is the linear rate of component accumulation (mg seed^{-1} °C d^{-1}), and c is TT at maximum seed weight or component content. Duration of component accumulation (°C d) was calculated as c − (a/b). Daily TT was calculated using 8°C as base temperature (Sinclair et al., 2003). Mean daily air temperature was calculated from daily maximum and minimum of a weather station located approximately 100 m from the experimental plots.

Manipulative Treatments to Modify Assimilate Availability per Seed during Seed Filling

Assimilate supply to the seeds during seed filling was increased by a depodding treatment imposed at R5.5 in the whole plants. This consisted in hand removing half of the pods from each node (main stem and branches) in all plants in the two central rows of the plot. The purpose of this treatment was doubling assimilate availability per seed during seed filling (Rotundo et al., 2009).

Experimental Design and Statistical Analysis

Experiments were conducted using a randomized complete block design with three replicates. Separate analyses were conducted for experimental isolines and for commercial cultivars. The data were analyzed using the SAS software (SAS Institute 1999).

Seed components (protein, oil, and residual) were classified as repeated factors to facilitate comparison of their WC_L and WC_M values. To account for their lack of independence, we followed the approach described by Holland (2006), where measurements on the same experimental units are treated as repeated measures.

Forward stepwise multiple-regression was used to analyze the influence of WC_L, WC_M, and seed desiccation rate on the accumulation duration of each seed component.

RESULTS

WC_L and WC_M for Major Seed Components (Objective 1)

On average, the experimental lines plus Evans began seed dry weight accumulation at 84.1% water concentration (WC_L) (Fig. 2A). The WC_L was 82.5% for the commercial cultivars (Fig. 2B). There was a significant component effect on WC_L for the experimental lines plus Evans ($P < 0.05$; Table 2). Oil synthesis had the lowest, protein intermediate, and residual the highest WC_L, showing that

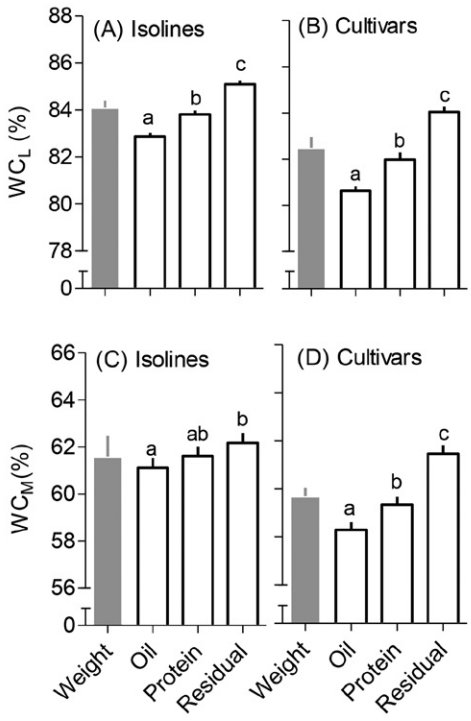

Figure 2. (A, B) Water concentration at beginning of linear phase accumulation (WC_L) and (C, D) at maximum content (WC_M) for (A, C) oil, protein, and residual in experimental soybean isolines and (B, D) commercial cultivars. Grey bars are WC_L and WC_M for total grain weight included for comparison. Different letters compare means at $P < 0.05$ within each panel. Data are mean ±SE, $n = 48$ (2 treatments, 4 genotypes, 2 yr, and 3 blocks) for experimental isolines and $n = 18$ (2 treatments, 3 genotypes, 1 yr, and 3 blocks) for commercial cultivars.

Table 2. Analysis of variance for soybean seed water concentration at beginning linear phase seed component accumulation (WC_L) and at maximum seed component content (WC_M). Since oil, protein, and residual were estimated in the same experimental unit, component type was treated as a repeated factor following (Holland, 2006) to account for the lack of independence among composition samples.

Source	Experimental isolines		Commercial cultivars	
	WC_L	WC_M	WC_L	WC_M
	df			
Component	2***	2***	2***	2***
Component × Genotype (G)	6***	6*	4***	4***
Component × Treatment (T)	2***	2 NS†	2***	2 NS
Component × G × T	6 NS	6 NS	4 NS	4 NS
Error	70	70	20	20

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

† NS, not significant.

residual accumulation started earlier, protein later, and oil was the latest component to start its accumulation (Fig. 2A). In the case of the commercial cultivars as an average, seed chemical components also differed significantly in WC_L (component $P < 0.05$; Table 2); protein WC_L was

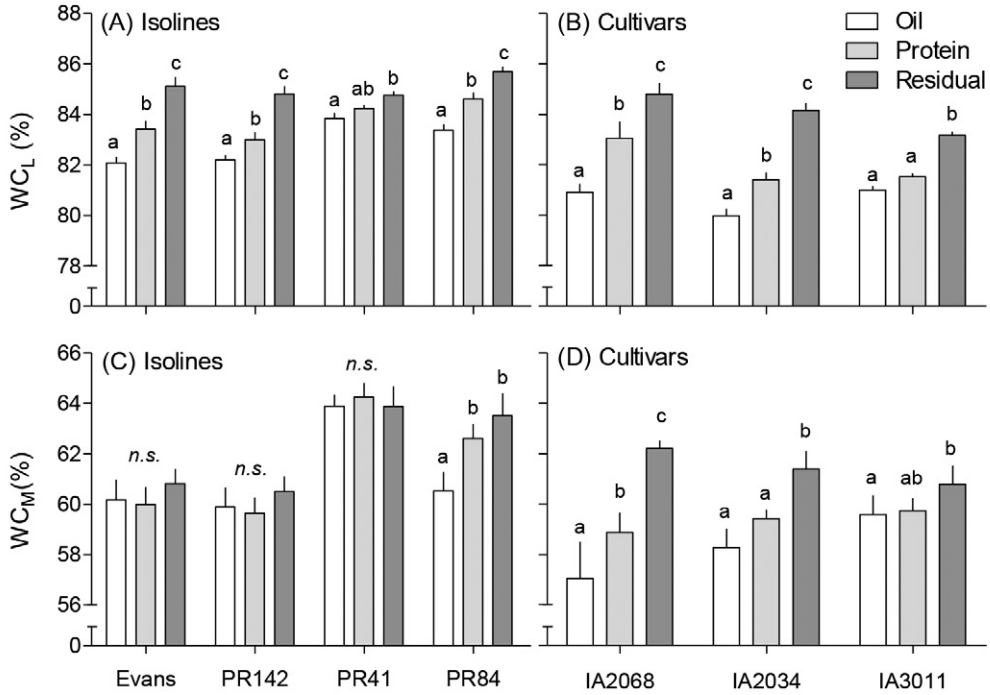

Figure 3. (A, B) Water concentration at beginning of linear phase accumulation (WC_L) and (C, D) at maximum content (WC_M) for (A, C) oil, protein, and residual for soybean cultivar Evans and three different experimental soybean isolines and (B, D) for three different commercial cultivars. PR84, IA2034, and IA3011 are high-protein genotypes (Rotundo et al., 2009). Different letters compare means at $P < 0.05$ within each panel. n.s. indicates nonsignificant differences within each panel. Data are mean ± SE, $n = 12$ (2 treatments, 2 yr, and 3 blocks) for experimental isolines and $n = 6$ (2 treatments, 1 yr, and 3 blocks) for commercial cultivars.

similar to the seed dry weight, while oil and residual WC_L were either lower or higher than protein, respectively (component $P < 0.05$; Fig. 2B).

Water concentration at WC_M was 61.6% for the average of the experimental lines (Fig. 2C). Protein WC_M was similar to dry weight WC_M for the seed and was not significantly different from oil or residual WC_M. The WC_M for seed residual was significantly higher compared with oil (component $P < 0.05$; Table 2). For the commercial cultivars as an average, WC_M for seed dry weight was 59.7% (Fig. 2D). Seed components differed significantly in WC_M (component $P < 0.05$; Fig. 2D). The critical value for protein WC_M was similar to seed dry weight. The WC_M for oil was significantly lower when compared with the protein value, while the residual component was significantly higher when compared with protein (Fig. 2D).

Stability of Component WC_L and WC_M Values across Genotypes and Source Manipulations (Objective 2)

Stability of WC_L and WC_M for seed components was assessed by analyzing component × genotype and component × treatment interactions (Table 2). There were significant component × genotype interactions for WC_L for both experimental lines and commercial cultivars (Table 2). The general tendency of oil < protein < residual WC_L did not vary across individual genotypes or between low and high protein ones (Fig. 3A, D). This significant component × genotype interaction arises because differences between component WC_L values were smaller for some genotypes compared with others. For example, in the experimental line PR41, differences in WC_L between oil and protein accumulation or between residual and protein accumulation were not significant, while in the other genotypes, the WC_L for all the components were different from each other (Fig. 3A). Similarly for the commercial cultivar IA3011, differences in WC_L between oil and protein were not significant while for the other genotypes they were different (Fig. 3B).

There was a strong component × genotype interaction for WC_M among the experimental isolines plus Evans (Table 2; Fig. 3C). The general tendency of WC_M values for oil < protein < residual observed as an average for the experimental lines plus Evans (Fig. 2C) only held for the high protein line PR84. Differences in WC_M for oil, protein, and residual were not significant for Evans, PR142, and PR41 ($P > 0.05$). In contrast, there was a significant component × genotype interaction ($P < 0.01$) for WC_M among the commercial cultivars, but the general trend of oil < protein < residual WC_M was always maintained (Fig. 3D).

Increasing assimilates supply to soybean seeds via depodding treatments increases seed weight and protein concentration (Rotundo et al., 2009, 2011). In terms of water relations, the depodding treatment lowered only the protein WC_L for the experimental lines. The treatment had no apparent impact on the WC_L for the oil and residual seed components (component × treatment $P < 0.05$;

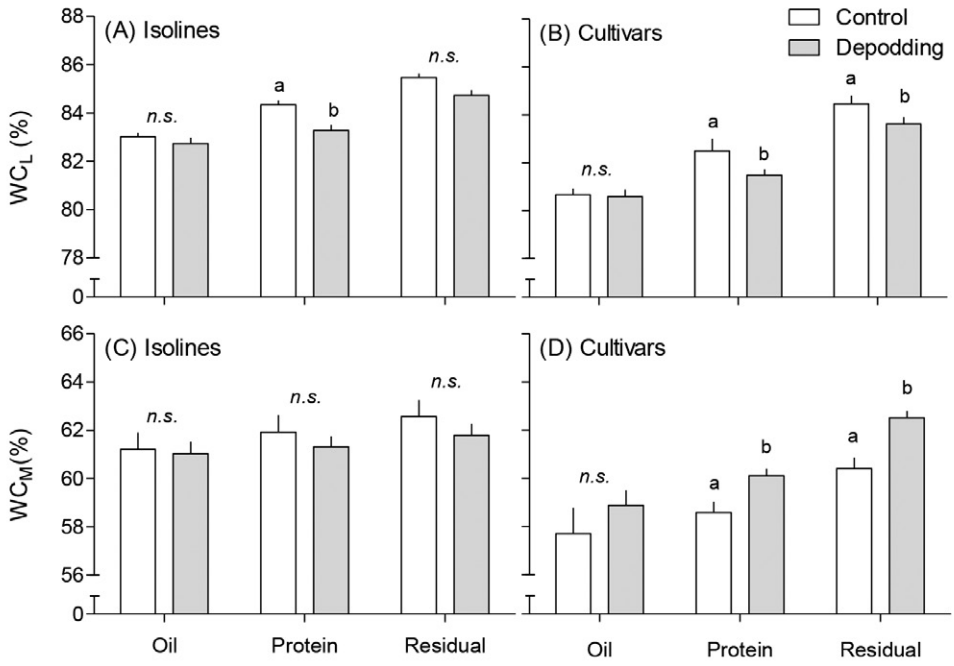

Figure 4. (A, B) Water concentration at beginning of linear phase accumulation (WC_L) and (C, D) at maximum content (WC_M) for (A, C) oil, protein, and residual for experimental soybean isolines and (B, D) for commercial soybean cultivars. Empty bars indicate control treatments while grey bars indicate depodding treatment, which had 50% increase in assimilates supply (Rotundo et al., 2009). Different letters compare means at $P < 0.05$ within each panel. n.s. indicates nonsignificant differences within each panel. Data are mean ± SE, $n = 24$ (4 genotypes, 2 yr, and 3 blocks) for experimental isolines and $n = 9$ (3 genotypes, 1 yr, and 3 blocks) for commercial cultivars.

Table 2; Fig. 4A). Depodding also lowered protein and residual WC_L for the commercial cultivars (Fig. 4B). As such, depodding delayed the initiation of the accumulation of some seed components.

The depodding treatment had no effect on WC_M for any of the seed components in the experimental lines (Fig. 4C). The depodding treatment increased protein and residual WC_M of the commercial lines (Fig. 4C), but the effects were small.

WC_M and the Duration of Seed Component Accumulation (Objective 3)

Changes in the duration of oil, protein, and residual deposition within seeds can be related to WC_L, WC_M, and to a lesser degree to the desiccation rate (Fig. 1). For the experimental lines, forward stepwise multiple-regression showed that duration of seed component accumulation was related primarily to changes in WC_M and to a lesser degree on desiccation rate (Table 3; Fig. 5). The WC_L did not enter in the forward regression (Table 3). Thus, the WC value at which each seed component stops accumulating is fundamental for explaining differences in the duration of each seed component accumulation. Most of the variation in WC_M was associated with genotypes. For the duration of oil and residual accumulation, the seed desiccation rate also explained a significant but small proportion of the total variation. Partial R^2 increase due to inclusion of desiccation rate in the model was <15% (Table 3).

Table 3. Summary of forward selection for stepwise multiple regression relating duration of seed component accumulation with water concentration at the beginning of the linear phase of component accumulation (WC_L, not entered into the model), water concentration at maximum seed component content (WC_M), and seed desiccation rate (DesRate, see Fig. 1) for the experimental soybean isolines. Commercial cultivars did not show any significant association.

Seed Component	Step	Variable	Partial R^2	Model R^2	Pr > F
Oil	1	Oil WC_M	0.53	0.53	*
	2	DesRate	0.13	0.67	*
Protein	1	Protein WC_M	0.83	0.82	***
	2	DesRate	0.01	0.84	NS†
Residual	1	Residual WC_M	0.79	0.79	***
	2	DesRate	0.08	0.87	*

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

† NS, not significant.

None of the seed water relations variables (WC_L, WC_M, or desiccation rate) explained a significant amount of the observed variation in the duration of seed component accumulation in the commercial cultivars. This outcome is probably related to the general lack of variation in the accumulation duration for each seed component in these genotypes (Fig. 5). Interestingly, there was a common linear relationship between duration of seed component accumulation and WC_M when experimental and commercial lines are combined.

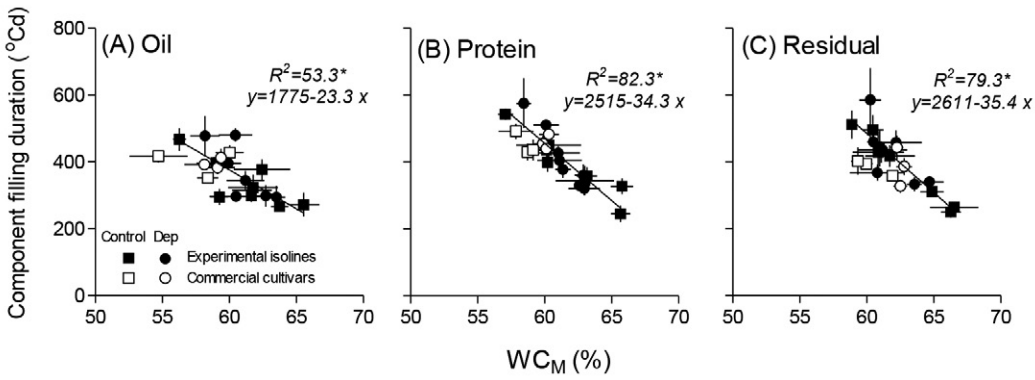

Figure 5. Relationship between seed component filling duration (thermal time) and water concentration at maximum content (WC_M) for (A) oil, (B) protein, and (C) residual. Closed symbols represent experimental soybean isolines (n = 16, 4 genotypes, 2 treatments and 2 yr). Open symbols represent commercial soybean cultivars (n = 6, 3 genotypes, 2 treatments and 1 yr). Squares are control seeds; circles are seeds for the depodding treatments. Regression models are fitted to experimental isolines data. Each data point is mean ± SE (n = 3). Asterisk (*) indicates statistical significance at $P < 0.05$.

DISCUSSION

Seed water relations have been used extensively to describe patterns of seed dry weight accumulation (e.g., Borrás et al., 2003; Egli, 1990; Egli and TeKrony, 1997; Gambín et al., 2007; Rondanini et al., 2007; Swank et al., 1987). These studies demonstrate the importance of defining critical benchmark values such as WC_L and WC_M to normalize developmental patterns across species and experimental treatments. Except for Gesch and Johnson (2013), who reported WC_M values for oil in two sunflower hybrids, there have been no other attempts to establish these parameters for accumulation patterns of valuable seed components such as protein and oil. This is the first study to document seed water concentration benchmarks for seed chemical components (protein, oil, residual) for a set of diverse soybean cultivars and seed filling conditions.

In spite of some interactions associated with genotypes and source-to-sink manipulations, a general pattern of WC_L and WC_M emerged for protein, oil, and residual accumulation. Jenner et al. (1991) presented evidence that accumulation of major seed components are independently controlled. Recent studies identifying distinct genomic regions controlling the rate of protein and oil accumulation also support the hypothesis that these processed are largely independent (Jiang et al., 2010, 2011). This independence is to some degree reflected in the different WC_L and WC_M values detected for each seed component.

There were differences in WC_L and WC_M associated with genotypes. The ranking of WC_L for oil, protein, and residual components held for all genotypes but PR41 and IA3011. For these two, differences in WC_L between oil and protein were not significant. The observed values of WC_L (oil < protein < residual) indicates a progression in the onset of the linear phase of seed component accumulation. During early seed development, high initial levels of sucrose and starch may account for residual higher WC_L (Yazdi-Samadi et al., 1977). Water concentration for maximum component content was more variable than WC_L, at least for the experimental lines. In this case, the ranking observed in WC_M among components only held for PR84. In the other experimental lines (plus Evans), there were no differences in WC_M among the seed components. This genotype-specific response was unexpected since the four lines share approximately 94% their genomes. For the commercial cultivars, however, the tendency of WC_M oil < protein < residual was consistently observed, implicating a genetic component defining these water relation benchmarks.

Treatments that increased assimilate availability per seed accelerate the rate of oil, protein, and residual accumulation. The impact on the accumulation duration of each individual seed component is lower (Rotundo et al., 2011). The depodding treatment lowered WC_L for protein accumulation, delaying the onset of protein accumulation relative to the other seed components. When comparing the response of different components to depodding, evidences indicate that the rate of protein accumulation increases more than the oil and residual accumulation rate (Rotundo et al., 2011). This response explains the higher protein concentration in soybean seeds observed when assimilate supply increases during seed filling (Rotundo et al., 2009). As such, any delay in the onset of protein accumulation is overcompensated by an increase in protein accumulation rate that results in higher seed protein content. In terms of WC_M, source-to-sink manipulations modified protein and residual parameters for the commercial cultivars. Evans and experimental isolines were not affected.

Final seed component content depends on rate and duration of accumulation. It was possible to model duration of all seed constituents as a function of WC_M. This relationship was evident across the experimental lines but not in the commercial lines because there was limited variation among them in duration of component accumulation.

The magnitude of the variation in WC_L and WC_M across components was rather limited in absolute values. It was expected that differences among WC_L and WC_M for protein, oil, and residual to be low. Seeds accumulate all

components concurrently during most of the seed filling period. However, small but consistent differences in the onset and end of accumulation were evident. Modeling efforts describing the determination of seed composition may take advantage of these parameters to precisely characterize the dynamics of component accumulation, following seed weight accumulation (Borrás and Westgate, 2006; Calderini et al., 2000; Swank et al., 1987).

The use of declining seed water concentration as a developmental benchmark during seed development has been critical for studying seed biomass accumulation in many species. In the present manuscript, we have applied this concept to describe the accumulation of protein, oil, and residual in soybean seeds. Developmental benchmarks are different for each specific seed component, showing the accumulation of each seed component starts and ends at different water concentration values.

Acknowledgments

FP holds a graduate scholarship from CONICET, the Argentine National Research Council. JLR and LB are both members of CONICET.

References

Bewley, D.J., and M. Black. 1994. Seeds: Physiology of development and germination. Plenum Press, New York.

Borrás, L., and M.E. Westgate. 2006. Predicting maize kernel sink capacity early in development. Field Crops Res. 95:223–233. doi:10.1016/j.fcr.2005.03.001

Borrás, L., M.E. Westgate, and M.E. Otegui. 2003. Control of kernel weight and kernel water relations by post-flowering source-sink ratio in maize. Ann. Bot. (Lond.) 91:857–867. doi:10.1093/aob/mcg090

Borrás, L., C. Zinselmeier, M.L. Senior, M.E. Westgate, and M.G. Muszynski. 2009. Characterization of grain-filling patterns in diverse maize germplasm. Crop Sci. 49:999–1009. doi:10.2135/cropsci2008.08.0475

Calderini, D.F., L.G. Abeledo, and G.A. Slafer. 2000. Physiological maturity in wheat based on kernel water and dry matter. Agron. J. 92:895–901. doi:10.2134/agronj2000.925895x

Egli, D.B. 1990. Seed water relations and the regulation of the duration of seed growth in soybean. J. Exp. Bot. 41:243–248. doi:10.1093/jxb/41.2.243

Egli, D.B., R.D. Guffy, L.W. Meckel, and J.E. Leggett. 1985. The effect of source-sink alterations on soybean seed growth. Ann. Bot. (Lond.) 55:395–402.

Egli, D.B., and D.M. Tekrony. 1997. Species differences in seed water status during seed maturation and germination. Seed Sci. Res. 7:3–11. doi:10.1017/S0960258500003305

Escalante, E.E., and J.R. Wilcox. 1993a. Variation in seed protein among nodes of determinate and indeterminate soybean near-isolines. Crop Sci. 33:1166–1168. doi:10.2135/cropsci1993.0011183X003300060012x

Escalante, E.E., and J.R. Wilcox. 1993b. Variation in seed protein among nodes of normal-protein and high-protein soybean genotypes. Crop Sci. 33:1164–1166. doi:10.2135/cropsci1993.0011183X003300060011x

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean development. Iowa Agricultural and Home Economics Experiment Station Special Report: 3–11.

Gambín, B.L., and L. Borras. 2005. Sorghum kernel weight: Growth patterns from different positions within the panicle. Crop Sci. 45:553–561. doi:10.2135/cropsci2005.0553

Gambín, B.L., and L. Borrás. 2011. Genotypic diversity in sorghum inbred lines for grain-filling patters and other related agronomic traits. Crop Pasture Sci. 62:1026–1036. doi:10.1071/CP11051

Gambín, B.L., L. Borrás, and M.E. Otegui. 2007. Kernel water relations and duration of grain filling in maize temperate hybrids. Field Crops Res. 101:1–9. doi:10.1016/j.fcr.2006.09.001

Gesch, R.W., and B.L. Johnson. 2013. Postanthesis development of oil content and composition with respect to seed moisture in two high-oleic sunflower hybrids in the northern US. Field Crops Res. 148:1–8. doi:10.1016/j.fcr.2013.03.019

Grimm, S.S., J.W. Jones, K.J. Boote, and D.C. Herzog. 1994. Modeling the occurrence of reproductive stages after flowering for 4 soybean cultivars. Agron. J. 86:31–38. doi:10.2134/agronj1994.00021962008600010007x

Hanson, W.D., R.C. Leffel, and R.W. Howell. 1961. Genetic analysis of energy production in the soybean. Crop Sci. 1:121–126. doi:10.2135/cropsci1961.0011183X000100020011x

Holland, J.B. 2006. Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. Crop Sci. 46:642–654. doi:10.2135/cropsci2005.0191

Jenner, C.F., T.D. Ugalde, and D. Aspinall. 1991. The physiology of starch and protein deposition in the endosperm of wheat. Aust. J. Plant Physiol. 18:211–226. doi:10.1071/PP9910211

Jiang, Z.F., Y.P. Han, W.L. Teng, Z.C. Zhang, D.S. Sun, Y.H. Li, and W.B. Li. 2010. Identification of QTL underlying the filling rate of protein at different developmental stages of soybean seed. Euphytica 175:227–236. doi:10.1007/s10681-010-0172-5

Jiang, Z.F., B.B. Zhang, W.L. Teng, Y.P. Han, X. Zhao, D.S. Sun, Z.C. Zhang, and W.B. Li. 2011. Impact of epistasis and QTL x environmental interaction on the oil filling rate of soybean seed at different developmental stages. Euphytica 177:431–442. doi:10.1007/s10681-010-0290-0

Jung, S., D.A. Rickert, N.A. Deak, E.D. Aldin, J. Recknor, L.A. Johnson, and P.A. Murphy. 2003. Comparison of Kjeldahl and Dumas methods for determining protein contents of soybean products. J. Am. Oil Chem. Soc. 80:1169–1173.

Piper, E. 1993. Adapting the SOYGRO crop growth model to predict oil and protein composition of soybean seed. PhD thesis. University of Florida, Gainesville. p. 536.

Rondanini, D.P., R. Savin, and A.J. Hall. 2007. Estimation of physiological maturity in sunflower as function of fruit water concentration. Eur. J. Agron. 26:295–309. doi:10.1016/j.eja.2006.11.001

Rotundo, J.L., L. Borrás, and M.E. Westgate. 2011. Linking assimilate supply and seed developmental processes that determine soybean seed composition. Eur. J. Agron. 35:184–191. doi:10.1016/j.eja.2011.05.002

Rotundo, J.L., L. Borrás, M.E. Westgate, and M.E. Orf. 2009. Relationship between assimilate supply per seed during seed filling and soybean seed composition. Field Crops Res. 112:90–96. doi:10.1016/j.fcr.2009.02.004

Rotundo, J.L., and M.E. Westgate. 2009. Meta-analysis of environmental effects on soybean seed composition. Field Crops Res. 110:147–156. doi:10.1016/j.fcr.2008.07.012

SAS Institute. 1999. SAS software. SAS Institute, Cary, NC.

Sinclair, T.R., J.R. Farias, N. Neumaier, and A.L. Nepomuceno. 2003. Modeling nitrogen accumulation and use by soybean. Field Crops Res. 81:149–158. doi:10.1016/S0378-4290(02)00221-6

Swank, J.C., D.B. Egli, and T.W. Pfeiffer. 1987. Seed growth-characteristics of soybean genotypes differing in duration of seed fill. Crop Sci. 27:85–89. doi:10.2135/cropsci1987.0011183X002700010022x

Wilson, R.F. 2004. Seed composition. Soybeans: Improvement, production and users. 3rd ed. ASA, CSSA, SSSA, Madison, WI.

Yazdi-Samadi, B., R.W. Rinne, and R.D. Seif. 1977. Components of developing soybean seeds- oil, protein, sugars, starch, organic-acids, and amino-acids. Agron. J. 69:481–486. doi:10.2134/agronj1977.00021962006900030037x