



Water relationships of castor bean (*Ricinus communis* L.) seeds related to final seed dry weight and physiological maturity

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ARTICLE INFO

Article history:

Received 30 December 2010

Received in revised form 14 April 2011

Accepted 16 April 2011

Keywords:

Ricinus communis

Castor bean

Physiological maturity

Grain moisture

Grain growth

Seed size

ABSTRACT

Castor bean (*Ricinus communis* L.) seeds develop inside capsules from sequential racemes. The indeterminate growth habit causes seeds to have different developmental stages among different orders of racemes. Determining the moment of physiological maturity (PM) for the whole plant and at the crop level presents a challenge. Also, it is unclear if a relationship exists between the final seed size of different orders of racemes and the seed-filling rate, seed-filling duration, or maximum seed water content (MWC_{mg}, absolute amount of water per seed). The aim of the present work was to study the dynamics of seed dry weight (SDW) and seed water content (absolute WC_{mg}) with the objectives of (i) associating final SDW to its seed-filling rate, seed-filling duration and maximum WC_{mg}; (ii) identifying the timing of PM on the basis of seed water concentration (WC_%); and (iii) predicting PM at the crop level using simulation models based on plant WC_% and SDW relationships. Two genotypes (FAUBA 1 and AL GUARANI 2002), contrasting in seed size and branching degree, were studied under field conditions in Buenos Aires in 2006–2007. The evolution of SDW and WC_{mg} was followed in racemes of first (R1), second (R2) and third (R3) order, from the beginning of flowering to harvest maturity. Bi-linear functions were fitted to SDW/WC_% relationships, and the model was validated. Across both genotypes and all orders of racemes, final SDW (FSDW) was associated with seed-filling duration (expressed in both days and thermal time) to a greater extent than with seed-filling rate. Also, a clear positive association between FSDW and MWC_{mg} was observed for a broad range of seed sizes (250–600 mg seed⁻¹). Physiological maturity was attained when seeds exhibited a WC_% of $21.8 \pm 2.4\%$ ($n = 232$; $R^2 = 0.94$; $P < 0.001$). The model was successfully validated with independent data ($R^2 = 0.96$). Simulations were performed to explore the effects of variations in the timing of flowering between racemes at the plant and crop level on fruit WC_% at maximum yield. At the crop level, 22% WC_% corresponded to 90–92% of the maximum attainable seed yields when harvest was delayed until the last growing seeds reached PM. We conclude that a relationship exists between FSDW and MWC_{mg}, and ca. 22% of seed WC_% is a good predictor of PM in castor bean at seed and crop level.

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1. Introduction

Ricinus communis L. is a perennial oilseed crop, from the Euphorbiaceae family, cultivated for its high industrial oil value (Zimmerman, 1958). Castor bean is cultivated in warm temperate areas (Hemerly, 1981; Koutrobas et al., 1999) and in northern Argentina it has a large potential for expansion (Wassner, 2006). Racemes are produced in an acropetal direction in the axillary buds of the branches, defining an indeterminate growth habit.

According to the order of appearance, the racemes are called primary, secondary, tertiary, and their numbers increase geometrically associated with the number of branches. In the racemes, there are spherical capsules with three locules containing one seed per locule. The duration of the fruiting period increases significantly in the branchy genotypes, and can last between 30 and 80 days (Moshkin, 1986). At maturity, the capsules are dried and may have dehiscence, depending on the genotype (Hocking, 1982). The successive generation of racemes in plants causes the coexistence of mature racemes, immature racemes and racemes at flowering (Baldanzi et al., 2002), making it difficult to determine the appropriate harvest time. This moment should be determined as the moment when seeds cease their growth (i.e. PM) in the whole plant, so the final yield of the crop is defined.

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Table 1
Genotypes and details of the experiments used to model (M) or validate (V1 and V2) the dynamics of seed dry weight (SDW) as a function of seed water concentration ($WC_{\%}$) in castor bean.

Experiment	Genotypes	Sampled racemes	Season of seed-filling	Age of sampled plants	Management
M	FAUBA 1 and AL GUARANÍ 2002	R1, R2 and R3	Summer (December to February)	1/2 year	Normal sowing (October), with spacing of 1 m × 1.2 m; with borders.
V 1	FAUBA 1	R2	Spring (November to December)	2 year	Plant generated by the re-growth of the main stem, pruned the previous year.
V 2	AL GUARANÍ 2002	R3	Autumn (March to May)	1/2 year	Late sowing (February), with spacing of 1 m × 1 m; no borders.

Until now, PM determination has been performed by visual methods (capsule color, dehiscence starting at primary racemes) that are subjective and may vary with environmental conditions and crop health status. Patterns of seed dry weight and water content have been shown to be closely related during seed filling in several grain crops (Egli, 1990), being therefore good indicators of developmental stages. Grain moisture values coincident with PM have been determined for soybean (Fraser et al., 1982), maize (Egli and TeKrony, 1997), wheat (Dodds et al., 1979; Schnyder and Baum, 1992; Calderini et al., 2000; Pepler et al., 2006) and sunflower (Rondanini et al., 2007). In castor bean, qualitative descriptions of seed filling divide it into four phases (formation of capsules, milky, waxy and maturity; Moshkin, 1986), and there are also more detailed descriptions of seed morphological development in ten stages (Greenwood and Bewley, 1981). However, there are no studies linking these qualitative stages of seed development with seed water content (WC_{mg}) and seed water concentration ($WC_{\%}$), which would lead to step from a qualitative description (scores) to the generation of numerical functional relations between biomass accumulation and water dynamics in castor bean seeds.

The FSDW is determined by its growth rate ($mg\ day^{-1}$) and the seed-filling duration (days) (Egli, 1990). In castor bean, it is not known if the FSDW is most often associated with the rate or with the duration of filling, and whether these associations are modified with the raceme order. The influence of the raceme order on seed weight is also unclear, since seed weight reductions (Kittock and Williams, 1967) and increases (Lins, 1976; Dos Santos Souza and Fernandes Távora, 2006) have been observed with the raceme order. In several species, positive associations have been observed between seed maximum water volumes and FSDW (Schnyder and Baum, 1992; Millet and Pinthus, 1984; Egli, 1998; Borrás et al., 2004; Borrás and Westgate, 2006; Sala et al., 2006; Rondanini et al., 2007) that can be used as predictors of the potential seed weight. The association between the FSDW and the MWC_{mg} reached during seed development has not yet been established for castor bean.

Extrapolation of individual seed–water relations to the whole plant level involves more complexity due to the temporal differences between the growth of different reproductive structures. The integration of water and dry matter dynamics involving seeds from different orders of racemes in a single model at the whole plant level has been possible in sunflower for seeds in different positions of the capitulum (Rondanini et al., 2007), while in sorghum, such integration was not possible for basal and apical grains of the panicle (Gambín and Borrás, 2005). So far, these issues have not been studied in modern genotypes of castor bean, despite the significant temporal divergence between the growth of the reproductive structures in different orders of racemes, which influences the dynamics of seed–water relations at the plant and crop level.

The aim of the present study was to assess the dynamics of seed dry weight (SDW) and seed water content (absolute WC_{mg}) in two castor bean genotypes contrasting in seed size and branch-

ing degree, with the objectives of (i) associating final SDW to its seed-filling rate, seed-filling duration and maximum WC_{mg} ; (ii) identifying the timing of physiological maturity (PM) on the basis of seed water concentration ($WC_{\%}$); and (iii) predicting PM at the crop level using simulation models based on plant WC and SDW relationships.

2. Materials and methods

2.1. Field experiment

Three experiments were carried out in the experimental field of the Chair of Industrial Crops, Faculty of Agronomy, University of Buenos Aires (34°35'S 58°29'W). The modeling experiment (M, Table 1) was planted on October 9th, 2006, on a silty clay loam soil (Typic Argiudoll). Two genotypes were used: FAUBA 1, an experimental variety, obtained in the Department of Industrial Crops (FAUBA) after three selection cycles (one year of selection between genotypes and two years of mass selection); and AL GUARANI 2002, a commercial variety originated in Brazil (CATI, 2002). These genotypes are contrasting in seed weight and branching degrees, FAUBA 1 having a lower seed weight and a higher branching degree than AL GUARANÍ 2002. In addition, genotypes differ in the inflorescence color, being red in FAUBA 1 and green in AL GUARANI 2002. The experimental layout was a complete randomized block design (CRBD) with 3 replications, and 16 plants were sub-sampled from each plot. Plants were arranged at 1 × 1.2 m (plant density of 8300 plants ha^{-1}) with a border row. Weeds, pests and diseases were controlled chemically. Temperature recordings during the experiment were obtained from the Davis Station Weather Monitor II (Davis Instruments, Hayward, CA) located 200 m from the experimental plot. The average monthly mean temperature during the crop cycle (October 2006–March 2007) was $22 \pm 2.4^{\circ}C$, and the accumulated rainfall in the same period was 847 mm, sufficient for castor bean development. Seed-filling duration and seed-filling rate were calculated in both days (d) and thermal time ($^{\circ}Cd$), using a base temperature of $10^{\circ}C$ (Canvin, 1965; Vijaya Kumar et al., 1997). Data from two independent experiments (V1 and V2, Table 1), with their seed filling periods occurring in summer 2006 (V1) and autumn 2007 (V2), were used to validate the relationships observed between seed growth and $WC_{\%}$. In Exp V1, secondary racemes of FAUBA 1 generated by the regrowth of two year-old plants (pruned in April 2006) were used, whereas primary racemes of AL GUARANI 2002, generated in late sowing dates (February 2007), were used in V2 (Table 1).

2.2. Measurements

The flowering dynamics at the crop level was recorded from 48 plants of each genotype in Exp M (Table 1). The occurrence of full flowering in each raceme was listed, considering full flowering as the moment when anthesis occurred in 50% of the female flowers

Table 2

Mean values \pm SE of the filling duration (days), filling rate (mg day^{-1}) and final seed dry weight (FSDW, mg) obtained by adjusting bi-linear functions of SDW as a function of time from the beginning of seed-filling of primary (R1), secondary (R2) and tertiary (R3) racemes for FAUBA 1 and AL GUARANI 2002 genotypes. Results of a two-way ANOVA, regression coefficients of the adjustments (R^2) and the number of samples (n) are included.

Genotype	Order of raceme	Seed-filling duration (days)	Seed-filling rate (mg day^{-1})	FSDW (mg)	R^2	n
FAUBA 1	R1	39.12 \pm 5.65	10.14 \pm 2.38	343.68 \pm 38.20	0.81	38
	R2	30.14 \pm 4.08	11.06 \pm 2.01	343.32 \pm 24.95	0.88	42
	R3	29.29 \pm 5.12	10.42 \pm 2.56	331.27 \pm 35.58	0.79	49
AL GUARANI 2002	R1	51.18 \pm 5.33	10.81 \pm 1.24	470.69 \pm 30.41	0.93	38
	R2	44.99 \pm 5.70	10.35 \pm 1.27	464.15 \pm 32.14	0.94	30
	R3	35.84 \pm 4.50	11.65 \pm 1.88	387.40 \pm 50.93	0.88	44
Genotype		*	ns	**		
Raceme order		ns	ns	ns		
Interaction G \times R		ns	ns	ns		

Level of significance: $P < 0.05$ (*), $P < 0.001$ (**) and not significant (ns).

in each raceme. Periodic samples of capsules were conducted in the first three orders of racemes (R1, R2 and R3) on 5 plants per block from the beginning of flowering up to about 20–30 days after raceme PM. One capsule was extracted weekly from the middle portion of the raceme to follow the evolution of seed filling. Individual capsules were collected in sealed plastic bags to prevent drying, and were taken immediately to the laboratory, situated 200 m from the plot. Seeds were extracted manually from the capsules and were weighed in ceramic containers for fresh weight determination. They were subsequently dried in an oven at 105 °C for 48 h until constant weight and were then weighed for SDW determination. The seed WC_{mg} (mg of water per seed) was calculated as the difference between fresh and dry seed weight. The seed $\text{WC}_{\%}$ (% moisture content on a wet basis) was calculated as the ratio between WC_{mg} and fresh weight, expressed as a percentage.

2.3. Data analysis

The nonlinear statistical routine of Prism (GraphPad Software Inc., 1999) was used to fit bi-linear regression models with unknown cutoff to the SDW function across time. The adjusted model was: $y = a + bx$ (for $x < c$) and $y = a + bc$ (for $x \geq c$) where y is the SDW (mg), x is the time from flowering (days), a is the SDW (mg) at the time of flowering, b is the seed-filling rate (mg day^{-1}) and c is the moment of PM (days). Significant differences ($P < 0.05$) in seed-filling rate, filling duration and FSDW for genotype and order of raceme were determined by two-way ANOVA and the multiple comparison test SNK (Student–Neuman–Keuls). In addition, one-way ANOVAs and SNK tests were performed to check means differences among the different orders of racemes within the same genotype. Maximum WC_{mg} was estimated individually for each replicate by fitting second-order polynomial functions to WC_{mg} as a function of the days from flowering (DFF). To determine the moment of maximum WC_{mg} , the value of x that makes the first derivative of y as a function of x (maximum optimal function) equal to zero was estimated. To standardize the differences between genotypes with different final seed dry weights (FSDW), the SDW was expressed as a percentage of the final value attained at PM, obtaining the relative SDW (RSDW). To determine PM as a function of $\text{WC}_{\%}$, bi-linear functions with unknown cutoff were fitted to the relationship between RSDW and the $\text{WC}_{\%}$ for all genotypes and orders of racemes, according to the following model: $y = a - bx$ (for $x > c$) and $y = a - bc$ (for $x \leq c$), where y is the RSDW (%), x is the seed $\text{WC}_{\%}$, a is the intercept, b is the slope of the linear regression (indicates how much seed water concentration is reduced per unit of reduction of RSDW), and c is the cutoff point of the function, which coincides with the value of $\text{WC}_{\%}$ at PM. The proposed model to determine PM with the seed $\text{WC}_{\%}$ was tested with data from two independent experiments (V1 and V2, Table 2), by performing

adjustments with bi-linear functions between the RSDW and seed $\text{WC}_{\%}$. Mean values \pm 1 SE are given in the text.

2.4. Simulation of seed growth and water dynamics at the crop level

Simulations based on the functions fitted to seed $\text{WC}_{\%}$ and RSDW dynamics for R1, R2 and R3 raceme orders of both genotypes (Exp M, Table 1) were used to estimate the evolution of seed $\text{WC}_{\%}$ at the crop level to determine the maximum relative seed yield. In order to upscale from seeds at a particular order of raceme to the whole plant level, the functions were assumed to be representative for the three corresponding sub-populations assuming a relationship between racemes from different orders of 1:2.6:4 in FAUBA 1 and 1:1.6:2.3 (see below Section 3.1), and assuming a fixed total number of seeds per raceme, equivalent to the observed proportions of seeds for each order of raceme in both genotypes. To upscale from an individual plant to the whole crop, a normal distribution of the full flowering observed within the population was used, assuming the observed dispersions (Fig. 1) between the first and

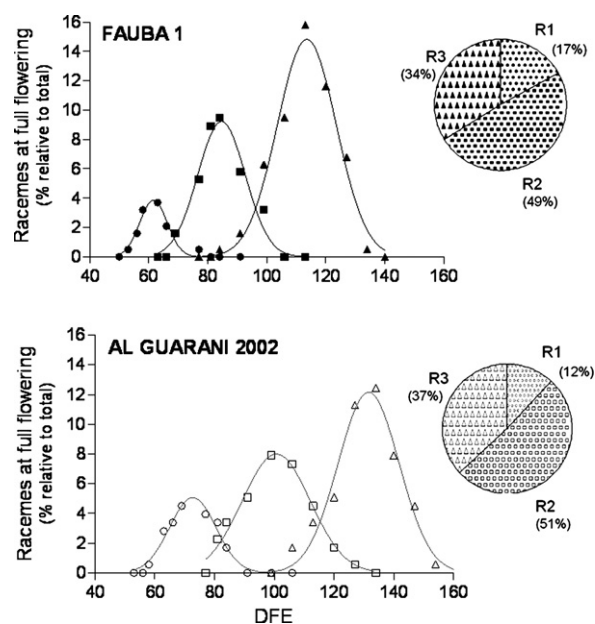


Fig. 1. Temporal dynamics of full flowering (expressed as a % of racemes at flowering as a function of the days from emergence, DFE) for the three orders of racemes, R1 (●/○), R2 (■/□) and R3 (▲/△) from genotypes FAUBA 1 (solid symbols) and AL GUARANI 2002 (open symbols) in the sampled plants ($n = 48$ for each genotype) from experiment M. Gauss curve adjustments for each raceme order are shown. The percentage contribution of the total number of seeds from each order of raceme for both genotypes is also shown (insert).

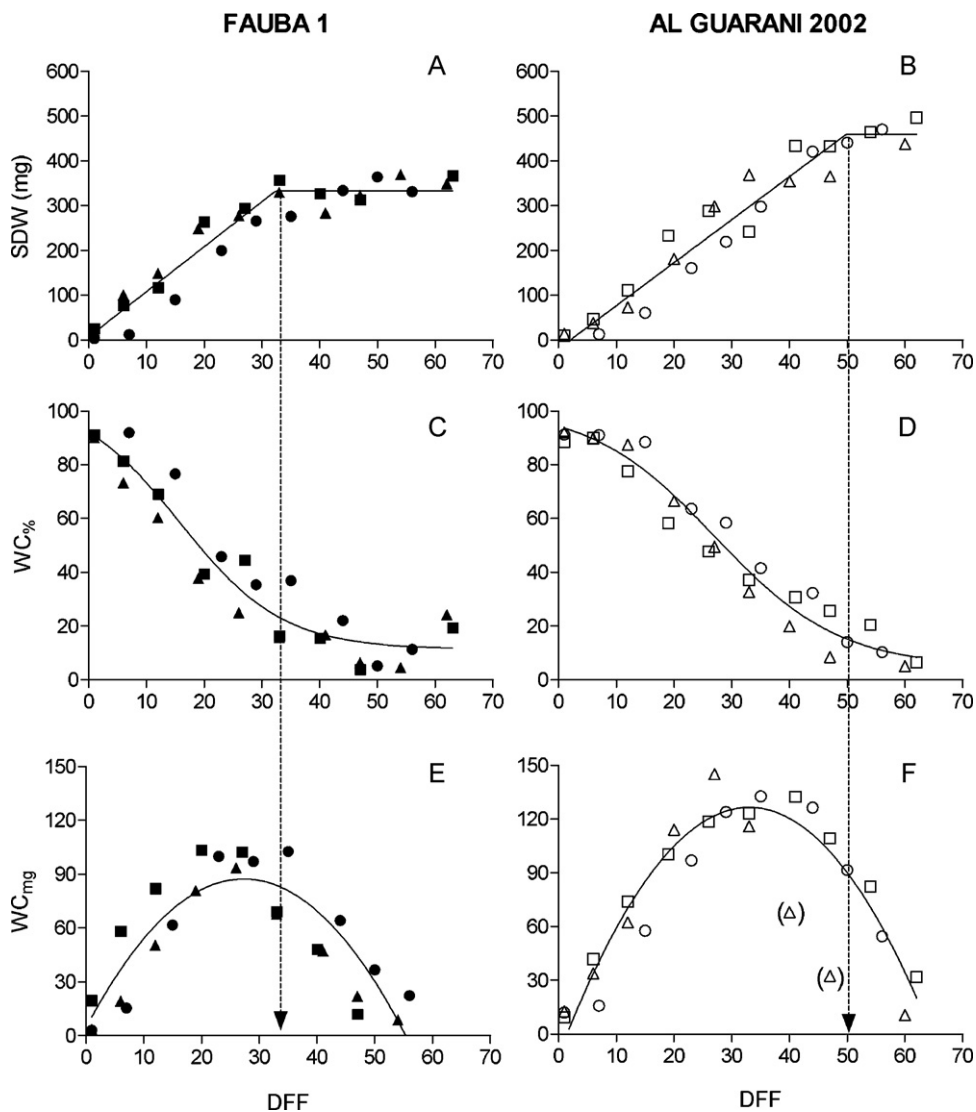


Fig. 2. Evolution in seed dry weight (SDW, mg) [A and B], seed water concentration (WC%) [C and D] and seed water content (WC_{mg}) [E and F] as a function of the days from full flowering (DFF) for the three orders of racemes, R1 (●/○), R2 (■/□) and R3 (▲/△) from genotypes FAUBA 1 (solid symbols) and AL GUARANI 2002 (open symbols). Each point represents an average of 3 repetitions. Bi-linear adjustments are shown for A and B, third-order polynomial adjustments for C and D and second-order polynomial adjustments for E and F. Values between parentheses were not included in the adjustment. The dotted arrow indicates the time of physiological maturity for each genotype.

last plants reaching full flowering. Also, other simulations included changes in the contribution of the seeds from R3 (increases and decreases of about 50% for the number of seeds observed in R3, situations that may be observed in high-branching genotypes or in late summers with a low source of photoassimilates, respectively). Simulated relative (to maximum) crop seed yield during seed filling was estimated as the weighted sum of SDW from all the orders of racemes and all plants in the crop relative to the final maximum crop yield.

3. Results

3.1. Raceme flowering dynamics at the crop level and raceme contribution to seed yield

The length of the vegetative period (from emergence to the first appearance of floral buds) in Exp M was similar in both genotypes, totalling 37 and 39 days in FAUBA 1 and AL GUARANI 2002, respectively. The beginning of flowering for each raceme (i.e. opening

of the first female flower in the raceme) occurred approximately from 7 to 10 days before full flowering (i.e. opening at the 50% female flowers in the raceme). The full flowering period was 20 days longer in AL GUARANI 2002 than in FAUBA 1, being more dispersed in each raceme order in AL GUARANI 2002, especially in R2 (Fig. 1). Thus, the total period of flower opening at the crop level (from the opening of the first raceme R1 to the last one in R3 in the plant population) took 103 and 121 days, respectively. FAUBA 1 was more branched than AL GUARANI 2002, generating more second and third order racemes, having no racemes R4 for both genotypes. The relationship between the number of R1:R2:R3 racemes per plant was, on average, 1:2.6:4 in FAUBA 1 and 1:1.6:2.3 in AL GUARANI 2002. The percentage contribution of total number of seeds per order of raceme was similar between genotypes (Fig. 1, insert) due to the lower number of seeds per raceme in FAUBA 1 that counterbalanced the larger number of racemes. In both genotypes, R2 contributed more to total seed number per plant (Fig. 1, insert). The average seed yield per plant was 216.6 ± 18.4 and 164.9 ± 8.9 g plant⁻¹ in FAUBA 1 and AL GUARANI 2002, respectively.

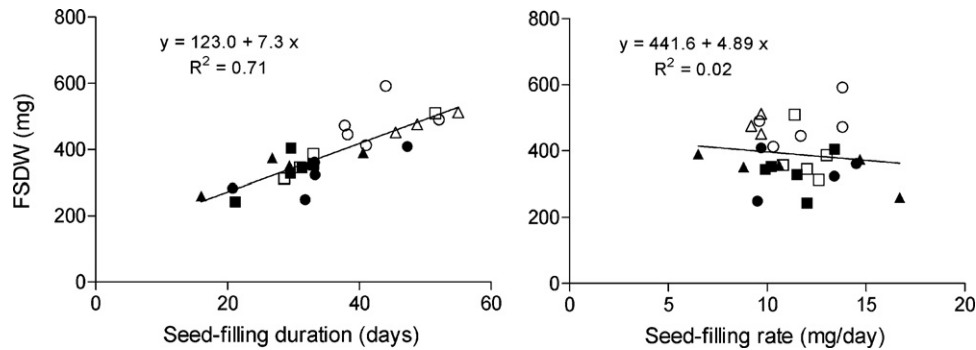


Fig. 3. Relationship between final seed dry weight (FSDW, mg) and [left panel] seed-filling duration (days), and [right panel] seed-filling rate (mg day^{-1}) for the three orders of racemes, R1 (\bullet/\circ), R2 (\blacksquare/\square) and R3 (\blacktriangle/\triangle) from genotypes FAUBA 1 (solid symbols) and AL GUARANI 2002 (open symbols). Fitted linear regressions are shown.

3.2. Seed growth dynamics

The different genotypes and racemes orders used in the experiment generated a wide range of FSDW (331.3–470.7 mg), seed-filling rates ($10.35\text{--}11.65 \text{ mg day}^{-1}$; $0.5\text{--}1.1 \text{ mg } ^\circ\text{Cd}$) and seed-filling durations (29.3–51.2 days; $240\text{--}750 ^\circ\text{Cd}$) (Table 2, Fig. 2A and B). As expected, there were significant differences between genotypes ($P < 0.001$) for FSDW (FSDW was 37% higher in AL GUARANI 2002 than FAUBA 1), while no significant differences were found for the raceme order or the interaction genotype–raceme order ($P > 0.05$; Table 2). Seed-filling duration was statistically different ($P < 0.05$) between genotypes, but not within raceme orders of each genotype (Table 2), lasting about 44 and 32 days (equivalent to 470 and $600 ^\circ\text{Cd}$) in AL GUARANI 2002 and FAUBA 1, respectively. Seed-filling rates showed no significant variations ($P > 0.05$) for raceme orders and genotypes (Table 2). Thus, the seed-filling duration expressed in both days and thermal time was the most important factor in determining the FSDW, with a significant slope ($P < 0.0001$) of $7.3 \pm 0.9 \text{ mg}$, representing the increase in the FSDW for each extra day of seed-filling duration (Fig. 3, right), equivalent to $0.4 \pm \text{mg}$ of FSDW for each extra $^\circ\text{Cd}$ of seed-filling duration. The seed-filling rate was not associated with FSDW when expressed in $\text{mg } (^\circ\text{Cd})^{-1}$ ($P > 0.1$), nor when expressed in mg day^{-1} ($P > 0.2$; Fig. 3, left).

3.3. Seed water content dynamics

During seed development, the increase in SDW (Fig. 2A and B) was accompanied by a sigmoidal decrease in seed $\text{WC}_\%$ (Fig. 2C and D). The absolute WC_{mg} (mg seed^{-1}) showed a curvilinear optimum-type function, rising to a maximum value and subsequently declining before the seed reaches PM (Fig. 2E and F). The maximum value of WC_{mg} (MWC_{mg}) differed significantly ($P < 0.01$) between genotypes and raceme orders, with an average across orders of racemes of 92 mg seed^{-1} in FAUBA 1 and 114 mg seed^{-1} in AL GUARANI 2002, with lowest MWC_{mg} values for R3 (Table 3). The full range of values found for MWC_{mg} was wide, from 50 to 170 mg seed^{-1} (Fig. 4). The moment when MWC_{mg} was reached differed significantly between genotypes and orders of racemes, and their interaction was also significant (Table 3). When the moment of MWC_{mg} was expressed relative to the filling duration, MWC_{mg} was reached at 80 and 70% of the total filling duration for FAUBA 1 and AL GUARANI 2002, respectively (Table 3). When the moment of MWC_{mg} occurred, seeds from FAUBA 1 and AL GUARANI 2002 had a $\text{WC}_\%$ between 38 and 40%, having accumulated 83 and 67% of the FSDW, respectively (Fig. 2). A positive linear relationship ($R^2 = 0.57$) was found between MWC_{mg} and the corresponding seed FSDW, with a slope of 2.3 mg of increase in SDW per mg of increase in MWC_{mg} (Fig. 4, upper panel). This indicates an association between the MWC_{mg} and the potential SDW. In addition,

the MWC_{mg} showed a positive association with the seed-filling duration (Fig. 4, lower panel).

3.4. Seed water concentration at physiological maturity

The dynamics of SDW and $\text{WC}_\%$ as a function of days from flowering (DFF) showed similar patterns for both genotypes and all racemes levels (Fig. 2), showing a sigmoidal decrease in $\text{WC}_\%$ and a constant increase in SDW up to PM. When the accumulation of SDW was related to the decrease in $\text{WC}_\%$, bi-linear functions were observed for both genotypes and raceme orders, with cut-off values in the range of 20.3–22.4%, indicating the seed moisture concentration at PM (Fig. 2). To standardize the effects of different FSDW between genotypes, values were expressed relative to their respective FSDW, called relative seed dry weight (RSDW). Adjusting bi-linear functions to the relationships of RSDW (%) as a function of

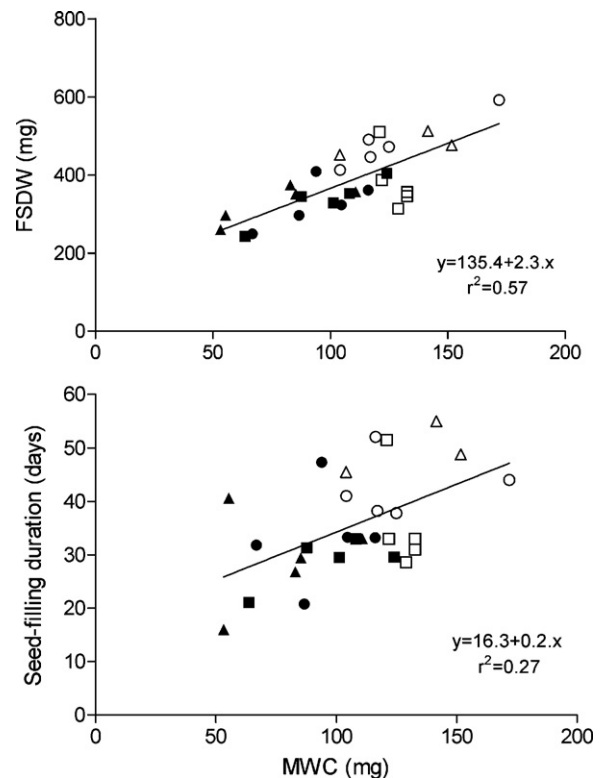


Fig. 4. Relationship between maximum seed water content (MWC, mg) and [upper panel] final seed dry weight (FSDW, mg), and [lower panel] seed-filling duration (days) for the three orders of racemes, R1 (\bullet/\circ), R2 (\blacksquare/\square) and R3 (\blacktriangle/\triangle) from genotypes FAUBA 1 (solid symbols) and AL GUARANI 2002 (open symbols). Fitted linear regressions are shown.

Table 3
Mean values \pm SE of maximum water content (MWC, mg seed⁻¹) moment of MWC (DFF) and moment of MWC relative to seed-filling duration (%) of primary (R1), secondary (R2) and tertiary (R3) racemes for FAUBA 1 and AL GUARANI 2002 genotypes. Results of a two-way ANOVA, regression coefficients of the adjustments (R^2) and the number of samples (n) are included.

		MWC (mg seed ⁻¹)	Moment of MWC (DFF)	Moment of MWC relative to seed-filling duration (%)	R^2	n
FAUBA 1	R1	96.40 \pm 19.11	30.27 \pm 1.73	77.39 \pm 12.92	0.66	36
	R2	98.96 \pm 18.79	22.58 \pm 2.20	74.93 \pm 4.35	0.63	35
	R3	82.83 \pm 20.89	25.78 \pm 6.14	88.02 \pm 22.03	0.40	42
AL GUARANÍ 2002	R1	123.38 \pm 5.39	34.71 \pm 3.60	67.83 \pm 14.08	0.62	35
	R2	128.25 \pm 25.09	33.61 \pm 2.68	66.85 \pm 1.21	0.74	30
	R3	91.24 \pm 26.21	33.07 \pm 3.73	81.06 \pm 13.36	0.64	39
Genotype		**	**	*	–	–
Raceme order		**	**	**	–	–
Interaction G \times R		ns	*	ns	–	–

Level of significance: $P < 0.05$ (*), $P < 0.001$ (**) and not significant (ns).

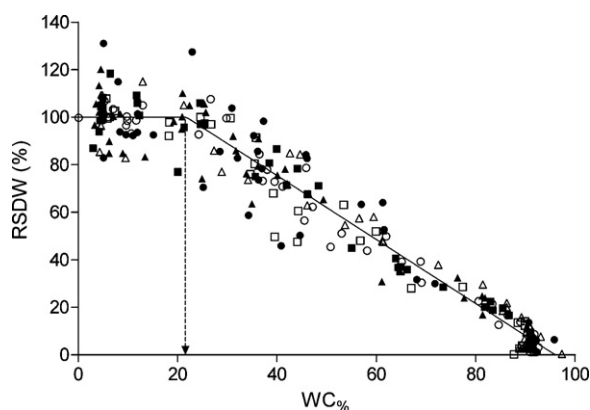


Fig. 5. Relationship between seed water concentration (WC, %) and relative seed dry weight (RSDW, % relative to final seed weight; absolute values in Table 3) for the three orders of racemes, R1 (●/○), R2 (■/□) and R3 (▲/△) from genotypes FAUBA 1 (solid symbols) and AL GUARANI 2002 (open symbols). Each point represents an average of 3 repetitions. The bi-linear adjustment [Eqs. (1) and (2)] to the dataset is shown ($n = 232$; $R^2 = 0.94$). The dotted arrow indicates the water concentration value corresponding to physiological maturity ($21.8 \pm 2.4\%$).

seed WC% (Table 4), a single model for all data was obtained (Fig. 5) with a seed WC% value at PM that showed no significant differences ($P < 0.01$) between genotypes and raceme orders (Table 4). The bi-linear model fitted to all the data set (Fig. 5) throughout the whole WC% range showed that PM was reached with an average seed WC% of $21.8 \pm 2.4\%$ ($n = 232$, $R^2 = 0.94$). The horizontal portion of the adjustment function shows the loss of moisture after

Table 4
Mean values \pm SE of the parameters in the bi-linear adjustment to the relationships of RSDW (%) as a function of the WC% in seeds of primary (R1), secondary (R2) and tertiary (R3) racemes for FAUBA 1 and AL GUARANI 2002 genotypes. The parameter a represents the intercept, b is the slope (indicates the reduction of water concentration in seeds per unit of increase in the RSDW), and c is the cutoff point (seed WC% at PM). Results of a two-way ANOVA, regression coefficients of the adjustments (R^2) and the number of samples (n) are included.

		a	b	c	R^2	n
FAUBA 1	R1	129.8 \pm 6.27	-1.335 \pm 0.22	22.60 \pm 9.32	0.87	39
	R2	131.4 \pm 6.43	-1.361 \pm 0.10	21.03 \pm 4.31	0.96	39
	R3	128.7 \pm 12.04	-1.342 \pm 0.20	22.14 \pm 7.49	0.92	45
AL GUARANÍ 2002	R1	127.2 \pm 5.98	-1.339 \pm 0.87	20.56 \pm 4.5	0.78	40
	R2	130.5 \pm 7.58	-1.400 \pm 0.13	21.79 \pm 5.03	0.95	41
	R3	126.8 \pm 8.87	-1.293 \pm 0.12	22.45 \pm 5.95	0.98	40
Genotype		ns	ns	ns	–	–
Raceme order		ns	ns	ns	–	–
Interaction G \times R		ns	ns	ns	–	–

Level of significance: $P < 0.05$ (*), $P < 0.001$ (**) and not significant (ns).

PM (RSDW equal to 100%). The equations based on the regression model (Fig. 5) were:

$$\text{RSDW}_i (\%) = 129.4 - 1.35 \text{WC}_{\%i} (\%) \text{ for } \text{WC}_{\%} > 21.8\% \quad (1)$$

$$\text{RSDW}_i (\%) = 100\% \text{ for } \text{WC}_{\%} > 21.8\% \quad (2)$$

where $\text{RSDW}_i (\%)$ is the SDW relative to FSDW, and $\text{WC}_{\%i}$ is the seed water concentration (%) at time i . The value of 21.8% is equal to the cutoff function and represents the seed WC% at PM. The model equations [Eqs. (1) and (2)] provide estimates of the RSDW (as a proportion of the FSDW) at any time of seed filling depending on its WC%.

3.5. Model validation

The model proposed to determine RSDW as a function of the WC% [Eqs. (1) and (2)] was tested with data from two independent experiments with contrasting sowing dates and management conditions (V1 and V2, Table 1) that generated different seed-filling durations (38.7 ± 3.3 d for V1 and 70.2 ± 9.4 d for V2), filling rates (13.1 ± 1.5 mg day⁻¹ for V1 and 5.1 ± 0.8 mg day⁻¹ for V2), and FSDW (460.3 ± 76.4 mg for V1 and 350.4 ± 89.7 mg for V2). Despite the important differences in the seed-filling dynamics, there were no significant differences ($P < 0.05$) for seed WC% values at PM between V1 and V2, averaging $20.5 \pm 4.8\%$ and $22.5 \pm 5.8\%$, respectively (Fig. 6). In addition, no significant differences ($P < 0.05$) were found between the seed WC% values at PM for both experiments and the value obtained with the model [Eqs. (1) and (2)]. The relationship between RSDW estimated vs. observed in V1 and V2 ($R^2 = 0.96$) had a slope that was not statistically different from 1 and an intercept not statistically different from 0 ($P < 0.05$). Thus, the model

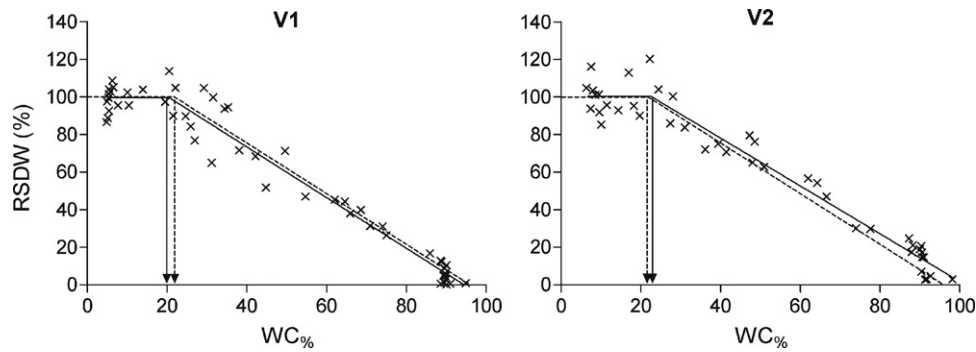


Fig. 6. Relationship between seed water concentration ($WC_{\%}$) and relative seed dry weight (RSDW, % relative to FSDW; absolute values in Table 3) for two independent validation experiments (V1 and V2, Table 1). Solid lines represent fits with observed data from V1 and V2, and dotted lines represent fits to the general model [Eqs. (1) and (2)]. Solid arrows indicate $WC_{\%}$ at PM in V1 (20.5%) and V2 (22.5%), and dotted arrows indicate the $WC_{\%}$ at PM according to the general model (21.8%).

generated a good estimate of RSDW along the seed-filling phase in both experiments.

3.6. Simulated seed water concentration at crop maximum seed yield

To scale data from a single seed to the crop level, simulations were performed using the observed dynamics of SDW and $WC_{\%}$ from Exp M (Fig. 2), considering the delay of beginning of flowering among different racemes in a plant and among different plants within the crop (Fig. 1). Percentage contributions to the total number of seeds per order of raceme observed in Exp M were also considered (Fig. 1, insert). Simulated PM at the crop level was reached at 164 and 189 days after the beginning of flowering of R1, in FAUBA 1 and AL GUARANI 2002, respectively (Fig. 7, upper panel). Simulated maximum crop grain yields were reached with crop level-seed $WC_{\%}$ values of 11 and 4% for FAUBA 1 and AL GUARANI 2002, respectively (Fig. 7). Such values were below the 21.8% observed in Eqs. (1) and (2). However, when the seed population reached 21.8% $WC_{\%}$ at the crop level, the 92 and 90% of maximum relative crop seed yield was achieved in FAUBA 1 and AL GUARANI 2002, respectively (Table 5, Fig. 7, lower panel). When a different seed contribution to the total crop number of seeds of R3 was considered ($\pm 50\%$), values of $WC_{\%}$ at PM at the crop level were only slightly modified (Table 5) and 21.8% $WC_{\%}$ at the crop level coincided with relative yields of 90 and 92.5% for FAUBA 1 and AL GUARANI 2002, respectively (Table 5).

4. Discussion

This study analyzes seed–water relations in castor bean during the seed-filling period, using two genotypes, contrasting in FSDW, and three different orders of racemes. In both genotypes, FSDW was not affected by the raceme order (Table 2), as observed by Kittock and Williams (1967), but contrary to the findings of Lins (1976) and Dos Santos Souza and Fernandes Távora (2006). In many crops, source limitation can reduce the FSDW at lower priority sinks (Borrás et al., 2004). In this study, the lack of differences observed in the FSDW between different orders of racemes within the same genotype (Table 2) suggest that there is no competition for photoassimilates between seeds from different orders of racemes. Crop factors, such as low harvest indices (10–20%, according to Moshkin, 1986) and/or high photosynthetic rates during seed filling (Dai et al., 1992), support this idea. Analyzed data showed that seed-filling duration was positively associated to FSDW, while the latter was not associated to the seed-filling rate (Fig. 3). This result indicates that the selection of genotypes with longer filling durations (expressed both in days and thermal time) may be a valid

strategy to increase FSDW, as used in other oily crops (Velasco and Fernandez-Martinez, 2002).

Water relations in developing seeds play a regulatory role during filling and are useful to explain differences in FSDW in many grain crops (Egli, 1990). The dynamics of the absolute seed WC_{mg} for all orders of racemes and genotypes of castor bean showed an optimum-type function, with maximum MWC_{mg} average values of 92 and 114 mg of water per seed, depending on the genotype

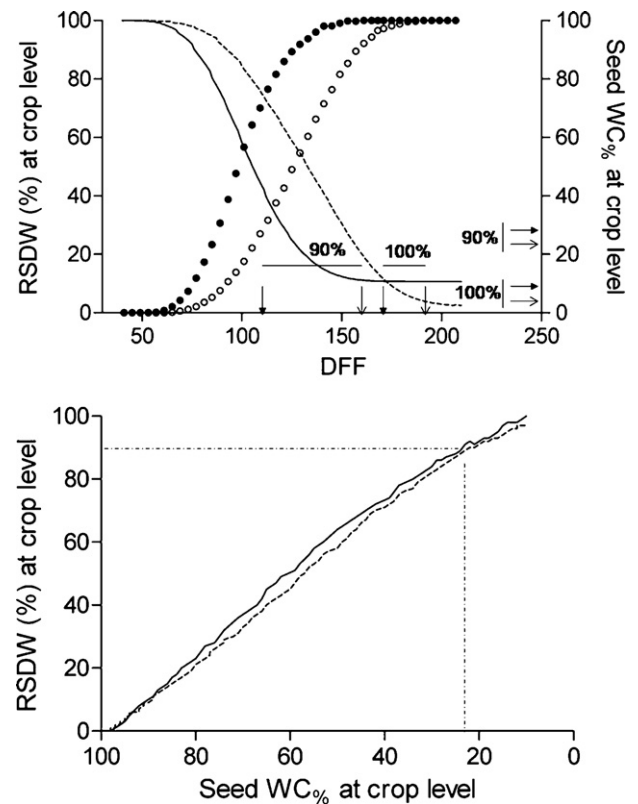


Fig. 7. Simulated dynamics of relative seed yield at crop level (increasing functions, 100% = sum of final weights of all seeds in the population) and simulated seed $WC_{\%}$ dynamics (decreasing functions) during seed filling, considering the observed normal distributions of the beginning of flowering in the population and the contributions of seeds from each order of raceme for FAUBA 1 (solid line, solid symbols) and AL GUARANI 2002 (dotted line, open symbols) [upper panel]. Arrows on the x-axis indicate timings of 90 and 100% of relative grain yield and arrows on the right y-axis indicate crop fruit WC at 90 and 100% relative grain yield (closed arrows for FAUBA 1 and open arrows for AL GUARANI 2002). Simulated relative seed yield/seed $WC_{\%}$ relationships at crop level for FAUBA 1 (solid line) and AL GUARANI 2002 (dotted line) [lower panel]. Vertical dotted line indicates a crop fruit WC around 21.8%, and horizontal dotted line shows the corresponding relative grain yield = 90%.

Table 5
Simulated values of seed WC_% at the crop level for different values of relative seed yield (100% = maximum grain yield). The growth of a population of seeds was simulated using observed SDW and WC_% dynamics during the seed-filling period [Eqs. (1) and (2)]. Values between parentheses represent the days after flowering of the first raceme in the population.

Relative seed yield (%)	FAUBA 1			AL GUARANI 2002		
	Control population	+50% of seeds in R3	–50% of seeds in R3	Control population	+50% of seeds in R3	–50% of seeds in R3
90	24.0 (126)	25.0 (120)	22.5 (133)	22.0 (158)	21.9 (153)	21.4 (163)
95	20.2 (131)	22.5 (123)	20.7 (135)	19.2 (161)	19.4 (156)	19.3 (165)
97	17.1 (136)	18.2 (129)	18.0 (138)	15.5 (166)	15.5 (161)	16.4 (168)
99	15.7 (139)	15.2 (135)	15.0 (144)	12.2 (170)	11.7 (161)	11.7 (174)
100	11.0 (164)	10.6 (164)	11.4 (164)	4.4 (189)	3.9 (189)	5.0 (189)

(Table 3, Fig. 4). This is the first quantitative report of castor bean seed water dynamics comprising different genotypes and positions in the plant, representing an advancement in the comprehension of the general qualitative descriptions available to the moment (Moshkin, 1986). Furthermore, this study revealed a positive linear relationship ($R^2 = 0.57$) between the MWC_{mg} and the FSDW (Fig. 4). Since the MWC_{mg} has a regulatory role in defining the potential FSDW, the time during seed-filling when MWC_{mg} is reached can affect the degree of adjustment of FSDW to changes in seed growth conditions (Borrás et al., 2004; Gambín and Borrás, 2007). In castor bean, MWC_{mg} is reached late in the seed development phase (about 70–80% of the seed-filling period, Table 3), when seeds have accumulated 67–83% of the FSDW (Fig. 4). This value results much higher than those cited for wheat (40%, Borrás et al., 2004) and sunflower (30%, Rondanini et al., 2007), and is similar to soybean (80%, Borrás et al., 2004).

This study showed that seed WC_% in castor bean is consistently associated with the SDW dynamics for the two genotypes and three raceme orders studied (Fig. 1). When differences between genotypes were expressed relative to their FSDW (RSDW), a single relationship was found between RSDW and the WC_% in seeds, adjusted by a bi-linear function with a cutoff value of $21.8 \pm 2.4\%$, which indicates the WC_% at PM (Fig. 5). The validation of the generated model [Eqs. (1) and (2)] showed a good fit to the observed data for the same genotypes at different environmental conditions and managements (Exp V1 and V2, Fig. 6). Compared with other crops, the value of seed WC_% at PM in castor bean (21.8%) is below the values found in soybean (60%, Fraser et al., 1982), sunflower (38%; Rondanini et al., 2007), maize (35%; Egli and TeKrony, 1997, 36%; Borrás and Westgate, 2006) and wheat (37%, Calderini et al., 2000). Also, the model, which adjusts a bi-linear function, resembles the models developed for wheat (Calderini et al., 2000), soybean (Fraser et al., 1982) and maize (Egli and TeKrony, 1997) and differs from tri-linear models found in maize (Borrás and Westgate, 2006) and sunflower (Rondanini et al., 2007). Unlike the case with sorghum (Gambín and Borrás, 2005), where obtaining a single model for grains located at different positions of the panicle was not possible, a unique model for three different raceme orders was possible in castor bean.

While the definition of a fixed WC_% at PM for a single seed is important, this criterion does not translate to the crop level because of differences in the timing of PM within the orders of racemes on a single plant and among plants within a crop. The simulations conducted in this study (Fig. 7) indicate that considering PM when random samples of seeds from the whole population have a WC_% of about 22% would involve only a small yield penalty (<10%) with respect to maximum possible crop yields in both genotypes (Table 5), which is slightly higher than the yield penalty simulated by sunflower crops (Rondanini et al., 2007) but is acceptable for castor bean if there is a risk of harvest loss. Also, these simulations were consistent even when considering different contributions to total seed numbers in the third raceme order (Table 5). The potentially

important technological outcome of this study needs direct verification with a larger number of genotypes growing under different environmental conditions.

5. Conclusions

This study has shown that there is a robust relationship between FSDW and WC_% for castor bean that holds for both studied genotypes, under different management conditions and raceme orders. The results indicate the importance of considering the seed-filling duration as a main physiological variable associated with increases in SDW, and the existence of a positive relationship between the FSDW and its MWC_{mg}, achieved at an advanced stage, when 70–80% of the seed-filling duration has elapsed. Physiological maturity is reached when seed WC_% approaches 22%. Simulations at the crop level indicate that using 22% WC_% as a predictor of the potential crop yield would involve only a small yield penalty compared with the yield obtained after all the grains have reached PM. In this study, a model for predicting PM in castor bean on the basis of seed WC_% was generated, representing an advance in the comprehension of castor bean–water relations, and a useful tool to assist in deciding the appropriate harvest time. Determining the time of PM quickly and easily using moisture meters could be helpful for deciding the moment of applying desiccants for an early crop harvest. This aspect may be of greater importance against possible damage of biotic (diseases, pests) or abiotic (flooding) scenarios during the grain-filling period and at harvest maturity.

Acknowledgements

We thank D Fernandez Abeijón, R Gimenez, MB Agosti, C Morvillo, and M Teixeira for their technical assistance. A Gil, JA Curá and I C Daverede granted us useful comments. This work was supported by the ANPCyT (PICT 1617) competitive research grant.

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