



## INTRODUCTION

In maize (*Zea mays* L.), final grain yield is determined by the number of harvestable kernels (KN) and the individual weight of these kernels (KW). Final grain yield is predominantly affected by the variation registered in KN (1, 5), but adverse growing conditions during the active kernel-filling phase can reduce assimilate availability for kernel growth markedly (3). Similarly, a marked decrease in the source-sink ratio during this phase may cause a decrease in the relative protein content and an increase in the relative starch content (4, 7) of hybrids with contrasting endosperm composition (dent, flint and dent × flint). Based on this evidence, the analysis of the genetic control of the physiological determinants of maize KW and kernel quality must include a broad range of growing conditions and genetic background.

**OBJECTIVE:** to expand the analysis of the genetic control of maize KW and its physiological determinants (KGR: kernel growth rate and KFD: kernel-filling duration) to (i) a RILs family of broad genetic background (dent × flint-Caribbean), and (ii) grain composition (protein, starch and oil, in %).

## MATERIALS AND METHODS

**Field experiments:** performed during 2009-2010 (Exp 1) and 2011-2012 (Exp 2) at INTA Pergamino research station, (33°93' S, 60°55' W), Argentina.

**Treatments:** a population of 181 RILs, their parental inbreds (B100: American semident; LP2: Caribbean-Argentine flint) and a tester inbred (LP612 in Exp 1 and LP122 in Exp 2).

**Experimental design:** completely randomized block design with two replicates.

**Crop husbandry:** Stand density: 7 pl m<sup>2</sup>. Plot size between 8.4 and 10.5 m<sup>2</sup>. Sowing dates: (i) 21-Oct-2009 in Exp 1, and (ii) 14, 18 and 23 Nov-2011 in Exp 2, for avoiding the confounded effect of inbreds variation in time to anthesis (tested in Exp 1). Sprinkler irrigation throughout the cycle. Permanent control of pests, weeds and diseases. No nitrogen (N) fertilization (soil N high in Exp 1 and low in Exp 2).

**Measurements and estimated parameters:** A total of 13 plants were tagged in each plot, and the date of silking was registered on all these plants. The apical ears of eight tagged plants were collected each 4 days from 18 days after silking onwards, and 15 grains per ear were sampled from the 10th (bottommost) spikelet position. Kernels were oven dried and weighed for the determination of potential KW (KWp), KGR and KFD by fitting a bi-linear model (2). Mean KW (KWm) and protein, oil and starch concentration were measured at maturity on all kernels set per plant in the remaining five ears. Kernel composition was established by near-infrared transmittance (Infratec 1227, Tecator, Sweden).

**Statistical analyses:** Mixed models analysis, Pearson's regression analysis, coefficient of variation (CV), Normality test (D'Agostino Pearson) and broad sense heritability (H<sup>2</sup>) (6).

**Genotypic evaluation and map construction:** 117 SSR and 35 SNP markers were used to characterize the RIL population. Uniformly distributed SSR markers across the ten maize chromosomes were selected. Primer sequences were obtained from Maize Gene Data Base ([www.maizegdb.org](http://www.maizegdb.org)). Phenotypic means (BLUPs) estimated with mixed models were used for QTL mapping. QTL mapping was performed by means of a mixture model following the multi-trait multi-environment approach with WinQTL Cartographer V2.5 program (8).

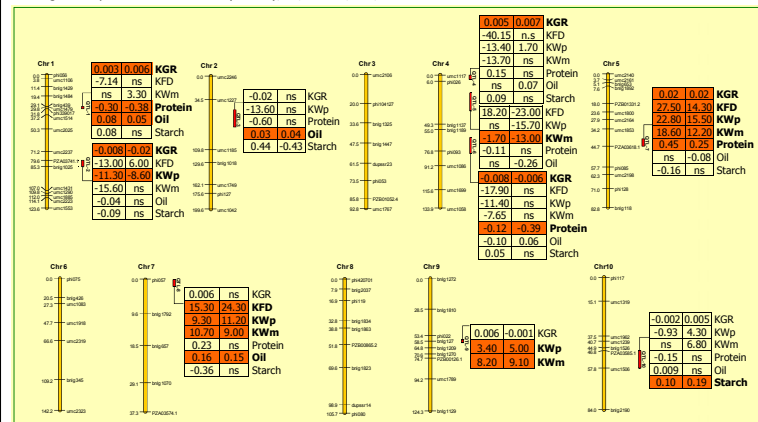
## RESULTS

Significant genotypic, year and genotype × year interaction effects (P<0.01) were detected for all measured traits, except for KFD (no year effect). All traits had a normal distribution (p>0.5), except for KFD in Exp 1 and starch in Exp 2. Heritability was high for KWp and KWm (H<sup>2</sup>=0.74), and intermediate for the other traits (from 0.62 to 0.42, Table 1).

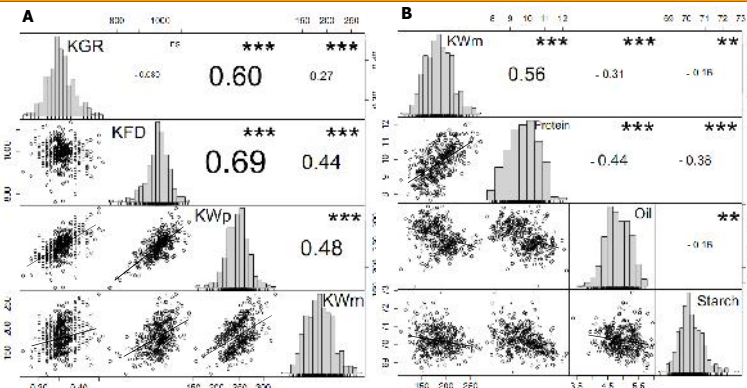
**Table 1:** Descriptive statistic and heritability (H<sup>2</sup>) of potential kernel weight (KWp), mean KW (KWm), kernel growth rate (KGR), kernel filling duration (KFD) and, protein, oil and starch concentration. Data correspond to 181 RILs and its parental inbred lines (B100 and LP2) cropped at two contrasting environments (Exp. 1 and Exp. 2). CV, coefficient of variation.

Trait	Exp. 1				CV	N <sub>ns</sub> <sup>c</sup>	Exp. 2				CV	N <sub>ns</sub> <sup>c</sup>	h <sup>2</sup> ± s.e.
	B100 <sup>a</sup>	LP2	RILs	Range <sup>b</sup>			B100	LP2	RILs	Range			
KWp (mg)	253	231	240	122-355	15.3	ns	228	208	249	170-337	12.2	ns	0.73±0.04
KWm (mg)	186	172	204	106-292	16.9	ns	154	153	172	99-230	16.0	ns	0.74±0.04
KGR (mg °C day <sup>-1</sup> )	0.32	0.39	0.35	0.24-0.47	12.0	ns	0.36	0.38	0.37	0.25-0.51	13.0	ns	0.56±0.07
KFD (°C day <sup>-1</sup> )	1071	896	990	675-1187	8.38	***	969	848	982	795-1161	6.72	ns	0.42±0.09
Protein (%)	10.3	9.67	10.4	8.03-13.2	9.37	ns	9.59	7.44	8.91	6.70-11.8	12.1	ns	0.59±0.06
Oil (%)	4.80	4.13	4.68	3.57-5.64	8.08	ns	5.91	5.47	5.52	4.04-6.49	7.68	ns	0.62±0.06
Starch (%)	70.0	71.1	70.1	68.2-72.5	1.22	ns	66.8	68.2	67.5	65.1-71.0	1.64	**	0.53±0.07

<sup>a</sup> Mean values of B100, LP2 and RILs population. <sup>b</sup> Minimum and maximum values of RILs population. <sup>c</sup> D'Agostino Pearson normality test: ns, not significantly different from normal (P>0.05); \*\*, P<0.01; \*\*\*, P<0.001.



**Figure 2:** Chromosomal location of QTL detected for KwP, KwM, KGR, KFD, and protein and starch concentration. QTL are presented by bars with a connector to the corresponding position on the chromosome. The two columns represent the additive effects of the QTL in each environment (Exp.1 on the left and Exp. 2 on the right column). Consistent effects across experiments are indicated in red. Additive effects correspond to LP2 allele.



**Figure 1:** Correlation analysis and frequency distribution of (A) KGR, KFD, KwP and KwM, and (B) KwM, protein, oil and starch concentration. Frequency distributions are in the diagonal, Pearson correlations values above the diagonal and regression analysis plots below the diagonal. Data correspond to 181 RILs and its parental inbred lines (B100 and LP2) cropped at two contrasting environments. ns, not significant; \*\*, P<0.01; \*\*\*, P<0.001.

Ten joint QTL were detected on seven chromosomes, but with inconsistent effects across years. Despite changes in effect size, most QTL were significant in both environments. Nine QTL were associated with variations in KwP, KwM and KGR, eight with variations in protein concentration and seven with KFD. Two QTL (chromosomes 5 and 7) had a large and consistent effect for KFD, KwP, and KwM across years, the one in chromosome 5 also for KGR and protein concentration (Figure 2).

## CONCLUSIONS

**In this work we characterized the genotypic variability of the physiological determinants of KW and quality traits in a population of RILs. Results showed that KW had the highest heritability, and also a strong correlation with KFD, KGR and protein concentration. Consistent QTL co-localization of KW with KFD and KGR found on chromosome 5 of the evaluated germplasm (dent × flint-Caribbean) did not support a differential genetic control for both determinants as were reported for dent germplasm. These results increase and expand our knowledge on the genetic bases of KW associated with its physiological determinants and quality traits.**

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