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RESEARCH PAPER

Relationship of source and sink in determining kernel composition of maize

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Abstract

The relative role of the maternal source and the filial sink in controlling the composition of maize (*Zea mays* L.) kernels is unclear and may be influenced by the genotype and the N supply. The objective of this study was to determine the influence of assimilate supply from the vegetative source and utilization of assimilates by the grain sink on the final composition of maize kernels. Intermated B73×Mo17 recombinant inbred lines (IBM RILs) which displayed contrasting concentrations of endosperm starch were grown in the field with deficient or sufficient N, and the source supply altered by ear truncation (45% reduction) at 15 d after pollination (DAP). The assimilate supply into the kernels was determined at 19 DAP using the agar trap technique, and the final kernel composition was measured. The influence of N supply and kernel ear position on final kernel composition was also determined for a commercial hybrid. Concentrations of kernel protein and starch could be altered by genotype or the N supply, but remained fairly constant along the length of the ear. Ear truncation also produced a range of variation in endosperm starch and protein concentrations. The C/N ratio of the assimilate supply at 19 DAP was directly related to the final kernel composition, with an inverse relationship between the concentrations of starch and protein in the mature endosperm. The accumulation of kernel starch and protein in maize is uniform along the ear, yet adaptable within genotypic limits, suggesting that kernel composition is source limited in maize.

Key words: C/N, carbon, grain, maternal, nitrogen, protein, sink, source, starch, transport.

Introduction

There is considerable debate as to whether the supply of assimilates from the maternal plant (the source) or the ability of the reproductive sink to accumulate these assimilates limits the growth and composition of maize kernels (Borras *et al.*, 2002; Gambin *et al.*, 2006). What is clear is that the assimilate supply can be altered by N availability and by genotype, and that both can affect yield and grain composition (Uribelarrea *et al.*, 2004). Although studies altering the source supply by shading, plant population, or altered leaf number indicate that current assimilates play a large role in kernel growth, under normal growing conditions yield appears to be mostly sink limited (for a review, see Borras *et al.*, 2004). Deficiencies in N supply usually decrease grain yield by lowering kernel number per plant (Carcova *et al.*, 2000; Paponov *et al.*, 2005) as a result of less synchronous pollination (Uribelarrea *et al.*, 2002), and/or greater kernel abortion (Uhart and Andrade, 1995). Synchronous pollination of ears often results in more kernels than natural pollination (Carcova *et al.*, 2000), and aborting kernels have lower levels of polyamines (Liang and Lur, 2002) and equal, or greater, concentrations of carbohydrates (Reed and Singletary, 1989) and amino acids (Reed *et al.*, 1988), all indicative of a sink limitation. However, individual kernel weight can also be reduced when plant N uptake or sugar supply is limited (Cazetta *et al.*, 1999; Paponov *et al.*, 2005) which suggests a source limitation.

Grain composition is a result of the genetic make-up of the endosperm sink, the maternal plant, and the environment

(Lopes and Larkins, 1991; Uribelarrea *et al.*, 2004), but it is not clear how much the amount and form of assimilates provided from the mother plant (source supply) influences grain composition (Paponov *et al.*, 2005; Gambin *et al.*, 2006). Nitrogen assimilate level directly influences the activity of enzymes, as well as zein proteins in the kernel (Singletary *et al.*, 1990; Cazetta *et al.*, 1999), and indirectly affects the assimilate supply by altering plant biomass, photosynthesis, and N metabolites (Seebauer *et al.*, 2004; Fageria and Baligar, 2005). Maize kernels grown *in vitro* with defined levels of N and sucrose adjust both their weight and composition according to the media supply (Cazetta *et al.*, 1999), indicating a source influence on grain composition. However, the genotype also plays a large role in determining the kernel's ability to utilize the available assimilates for storage product deposition, as providing high levels of N cannot overcome a genetic tendency towards low kernel protein (Wyss *et al.*, 1991; Uribelarrea *et al.*, 2004).

The process of transferring assimilates from the maternal source into the developing seed sink further complicates the source versus sink question, as it is also affected by the environment, time, and the genotype (for a review, see Lalonde *et al.*, 2003). In maize, N assimilates, which are supplied as varying proportions of amino acids, have different compositions in the cob than in the developing kernel tissues as a result of preconditioning by the cob prior to use by the kernels (Seebauer *et al.*, 2004). In the same way, phloem unloading of sugars may be dependent upon enzyme levels within the pedicel area of the cob, which facilitates the osmotic gradient for continued unloading (Andersen *et al.*, 2002). Studies using ^{14}C as a tracer further show the multitude of interconversions of assimilates between the maternal plant and the developing ovule (Balconi *et al.*, 2004). Determining the assimilate supply available to developing maize kernels, however, is difficult, as the husks and developing ovules prevent utilizing the aphid stylet technique that is common for leaves (Weiner *et al.*, 1991). The agar trap method is perhaps the best way to determine the actual assimilate level and composition available to the developing maize kernels, because it removes progeny cellular components that can modify or utilize the assimilates for the production of storage products (Porter *et al.*, 1985, 1987).

Kernel growth and storage compounds may be influenced by the level and composition of assimilates transported by the mother plant. To investigate this hypothesis further, a range of source-to-sink supplies was obtained by differing genotypes, N supplies, and ear truncation, then the composition of the source supply and the kernels were examined. It is shown that kernel protein and starch concentrations are relatively consistent throughout an entire ear, and that the final endosperm composition resembles the assimilate source supply at 19 DAP.

Materials and methods

Ear profile of kernel traits

Plants of two intermated B73×Mo17 recombinant inbred lines (IBM RILs) of maize (*Zea mays* L.) (Lee *et al.*, 2002), which contained contrasting levels of starch and protein (M0116 and M0288) were grown at the Pioneer field nursery in Johnson, IA in the summer of 2005 with an adequate supply of fertilizer N. At physiological maturity, three fully self-pollinated ears were selected per genotype, from which three adjacent kernel rows were sampled lengthwise along the ear. Ears were allowed to air dry to constant weight, and individual kernels collected beginning at the base of the ear, weighed, and then analysed for protein and starch concentrations by single seed-near infrared (SS-NIR). Ears of M0116, on average, contained 29 kernels row^{-1}, while M0288 had 34, resulting in decile classes consisting of approximately 3 kernels row^{-1}. To determine the influence of N assimilate supply on individual kernel composition, the commercial hybrid Pioneer 31N27 was grown in the field at the Crop Sciences Research and Education Center at Champaign, IL in 2005 with three rates of fertilizer applied N ranging from deficient to excessive (0, 67, and 269 kg N ha^{-1}). Kernels from well-pollinated ears harvested from the centre of four row plots were sampled from three adjacent rows within an ear and analysed for weight and protein and starch concentrations by SS-NIR. The composition of single seeds was predicted with NIR using a novel near-infrared sampling apparatus as described by Haefele *et al.* (2007) and Janni *et al.* (2008). In principle, individual kernels are tumbled with an air-stream in an integrating sphere and reflectance spectra (400–1700 nm) measured for 12 s with a spectrometer via a fibre-optic probe. Calibrations for predicting kernel starch and protein concentrations were based, respectively, on the starch reference method described herein and N determination by combustion.

Plant culture for truncation and agar trap study

Six intermated IBM RILs which display contrasting concentrations of endosperm starch were grown in the field at Champaign, IL in 2004 with either a low or a high availability of fertilizer N (0 or 168 kg N ha^{-1}). The three high starch concentration lines were M0008, M0015, and M0195, while the low starch lines were M0116, M0241, and M0380. The inbreds were planted in four row plots, and plots were arranged as a randomized complete block experimental design with four replications. Ear-shoots were covered with bags before silking and self- or sib-pollinated 4 d after initial silk emergence, to maximize the number of silks exposed for pollination (Carcova *et al.*, 2000).

Truncation and agar traps

At 15 d after pollination (DAP), the source-to-sink ratio was altered by partial ear truncation in a subset of the plants in each plot in 2004. A sterilized knife was inserted through the husks at a point approximately half-way along the ear, and the cob plus distal kernels severed and removed, with the husk and proximal ear-shoot remaining intact. This truncation procedure resulted in the top 45% of the ear being removed. The instantaneous assimilate supply into the kernels (for two high and two low starch lines, at both N availabilities) was determined at 19 DAP using the agar trap technique (Porter *et al.*, 1985) in duplicate ears per treatment. Husks were carefully cut lengthwise and pulled back to expose the developing kernels. Two sets of five contiguous kernels were chosen at similar regions in full and truncated ears, and the exposed, distal portion of those kernels excised, then the endosperm and embryo removed while retaining the basal endosperm transfer cells. Empty pericarp cups were rinsed with a buffer solution of 20 mM KH_2PO_4 (pH 7) and 15 mM EGTA, then a second buffer solution was allowed to remain in the cups for 5 min. This pre-incubation solution was then removed and replaced with approximately 80 μl of warmed buffer plus 0.75% (w/v) agar solution (A-7002; Sigma-Aldrich, St Louis, MO). After 10 min, a baseline measurement was obtained by removing and combining five agar traps from each ear, with an equivalent

sampling after 4 h. Agar traps were immediately frozen using dry ice, and stored at –80 °C prior to analyses. Duplicate plants were used per ear treatment (full versus truncated ear) for each experimental unit.

Assimilate determination in agar traps

Soluble sugars and amino-N were extracted from each pool of five agar traps in 1.0 ml of 80% (v/v) ethanol at 21 °C for 1 h. Supernatants were collected following centrifugation at 14 000 rpm for 5 min and the extraction repeated for 30 min. Combined supernatants were taken to dryness in a Speedvac (Savant Model SC250DDA) and resuspended in 400 µl of water. Following dilution (76–152-fold), soluble sugars were measured according to Viola and Davies (1992) using a 100 µl aliquot. Measured glucose, fructose, and sucrose amounts were summed to represent total soluble sugar. The resuspended supernatant was diluted 26-fold before using a 40 µl aliquot and leucine standards in a microplate format to measure amino-N according to Moore (1968). Assimilate levels determined for baseline (10 min) agar traps were subtracted from values obtained for the 4 h traps and the difference divided by the exact collection time to establish transport rates. The C/N ratio of assimilates transported into agar traps was the quotient of soluble sugar and leucine masses.

Mature kernel analyses of truncation experiment

At physiological maturity, the relative ear truncation was determined by comparing estimates of kernel numbers (rows×kernels per row) between truncated and full ears. For determining seed growth and endosperm composition, five representative kernels were removed from each of three ears per plot and pooled. Embryos were manually separated from endosperms and the endosperm ground into a fine meal for starch and protein analysis. Endosperm tissue was analysed for N concentration using a combustion analyser (NA2000, CE Elantech, Lakewood, NJ) and protein was determined by multiplying the N concentration by 6.25. Starch was determined by digesting 100 mg of tissue in 0.9 ml of MOPS buffer (50 mM MOPS, pH 7.0, 5 mM $CaCl_2$, 0.02% Na-azide) containing 100 units of heat stable α-amylase (A-4551; Sigma-Aldrich, St Louis, MO). Following incubation at 90 °C for 75 min, 0.6 ml of acetate buffer (285 mM Na-acetate, pH 4.5, 0.02% Na-azide) containing 5 units of amyloglucosidase (catalogue number 11202367001; Roche Applied Science, Indianapolis, IN) was added. Reactions were held at 55 °C overnight, stopped by boiling, and centrifuged (14 000 *g*) for 5 min. Glucose concentration was determined using a Skalar San^{++} instrument (Skalar, The Netherlands) and a method (catalogue number 353) based on the principles described in Jones *et al.* (1977). A minimum of duplicate digests were processed for all samples and the results were corrected for moisture content. The composition of mature whole kernels was measured by SS-NIR using 30 kernels per ear.

Statistical analysis

Statistical analyses of phenotypic traits measured were conducted with JMP version 7 (SAS Institute, Inc., Cary, NC). Results from individual RILs were pooled and averages for each starch class, high or low, presented. Field trials were analysed separately by year, location, and physiological parameter. Responses were analysed by analysis of variance as randomized complete blocks with starch class, RIL, ear position, nitrogen level, and ear treatments as fixed effects. All trait means, standard errors, and least significant differences (P=0.05) were calculated using the Least Squares Fit model. Trend lines are displayed and the measure of linear association is designated by the Pearson's correlation coefficient where appropriate.

Results

Effect of genotype and N supply on ear profile of kernel traits

Two RILs with contrasting starch levels differed in individual kernel weight, but not in how weight was affected by kernel decile position (Fig. 1A). Even though MO228 had slightly aberrant responses at the very base and tip of the ear, individual kernel weights tended to be highest at the base and decreased steadily towards the tip of the ear. The RILs differed as expected in starch and protein concentrations, exhibiting almost a 30 g kg^{-1} difference in starch, and a corresponding but opposite difference in protein (Fig. 1B, C). Except for some minor irregularities in starch, the concentrations of starch and protein were remarkably constant along the ear for both RILs. These results show that genotype plays a much larger role than kernel decile

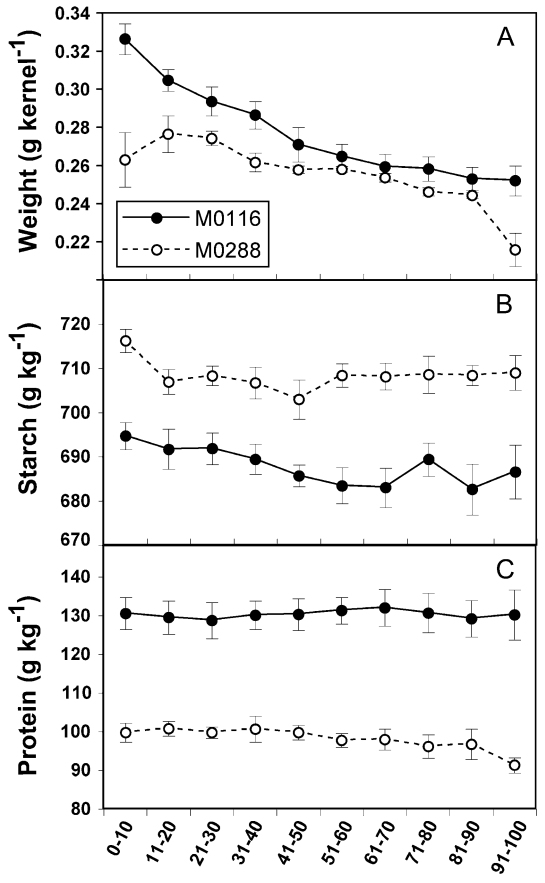

Fig. 1. The effect of ear decile position on individual kernel dry weight (A), and the concentrations of starch (B) and protein (C) along the ear for two IBM RILs with contrasting concentrations of starch and protein. The RIL M0116 is shown as closed circles, and M0288 as open circles. Data are the means ±1 SE (n=9). As determined by two-sample t test, RILs were significant (P <0.001) for all comparisons, and the percentile class difference was significant (P <0.001) for weight, starch, and protein concentration.

position in determining kernel composition, but not in final kernel weight.

In examining the influence of N supply on F_2 kernels developing along the ear of a commercial hybrid, the highest N supply (269 kg N ha^{-1}) increased individual kernel weight compared to the two lower N supplies (0 and 67 kg N ha^{-1}) but did not overcome the decline due to decile position (Fig. 2A). Regardless of the N supply, individual kernels were heaviest in the second decile from the base and steadily decreased as kernel position progressed toward the tip of the ear. In contrast to kernel weight which was similar for 0 and 67 kg N ha^{-1}, the concentration of starch incrementally decreased, and protein concentration increased, with increases in N supply (Fig. 2B, C). Kernels grown with 269 kg N ha^{-1} increased grain protein concentration by an average of 17% compared to kernels grown without N, and 12% for kernels grown with moderate N (67 kg N ha^{-1}). By contrast, growth without fertilizer N promoted the greatest concentration of kernel starch, which was, on average, 16 g kg^{-1} greater than kernels grown with the maximum N supply. Similar to the RILs with contrasting starch and protein, the concentrations of starch and protein in kernels of the commercial hybrid were relatively similar along the length of the ear for each N supply. Most of the variation in starch or protein composition due to kernel position occurred near the tip of the ear. Regardless of this apical variability, N fertility influences the final kernel composition to a greater extent than does kernel position along the earshoot.

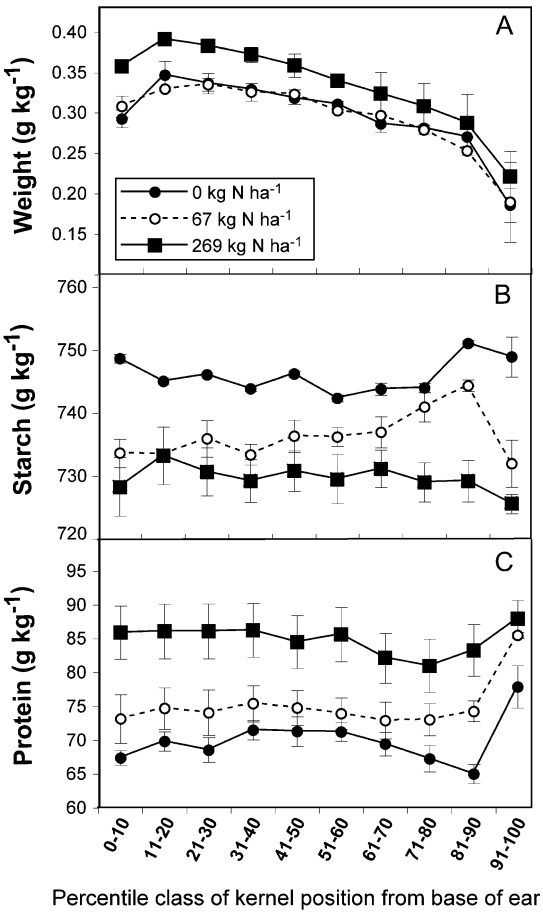

Fig. 2. The effect of ear decile position on individual kernel dry weight (A), and the concentrations of kernel starch (B) and protein (C) along the ear for a commercial hybrid grown with either 0 kg N ha^{-1} (closed circles), 67 kg N ha^{-1} (open circles) or 269 kg N ha^{-1} (closed squares). Data are the means ±1 SE (n=6). For kernel weight (A), N supply was significant (P <0.001), and the percentile class difference was significant (P <0.001). For starch concentration (B), N supply was significant (P <0.001) and the percentile class difference was non-significant. Kernel protein concentration (C), N supply was significant (P <0.001), and the percentile class difference was significant (P <0.001).

Besides N, the environment can play a role in kernel set and grain fill. The RIL experiments were hand pollinated to maximize kernel set (Carcova *et al.*, 2000), while for the commercial hybrid, open pollinated ears were selected that were well filled. In 2005, the RILs in Fig. 1 were grown in a slightly warmer mean temperature (+1 °C) and about 10% greater rainfall than the 30 year average during the grain-filling period. This environment did not alter the genetic tendency for low or high starch concentration in the grain. The inbreds grown in 2004 for the agar trap experiment experienced 1.5 °C cooler mean temperatures and 108% of the mean rainfall than the 30 year average during the grain-filling period. The commercial hybrid was grown in 2005, with a slightly warmer mean temperature (+1 °C) and approximately 10% less rainfall than the 30 year average. This hybrid yielded 7.1, 9.0, and 10.8 Mg ha^{-1}, respectively, when grown with 0, 67, and 269 kg N ha^{-1}. The difference in yield between the fertilizer levels was due to a difference in kernel weight when supplied with N, and approximately a 20% decrease in kernel number when grown without N (data not shown).

Influence of ear size on seed growth and composition

Nearly all of the starch and approximately 75% of the protein found in kernels occurs in the endosperm (Earle *et al.*, 1946). In order to study the influence of assimilate provision on mature kernel composition, ear truncation was performed on three inbreds from each of the two class types, namely those high or low for endosperm starch concentration. Ear truncation of 45% increased endosperm dry weight by an average of 27% for the high starch class and a lesser amount (19%) for the low starch class (Table 1). Nitrogen fertilizer supply did not affect endosperm dry weight, starch, or the endosperm-to-embryo ratio. Regardless of starch class, growth of the embryo responded to ear truncation to the same degree as the endosperm, as shown in the endosperm-to-embryo ratio, even though the kernel fractions are distinctly different in composition. Ear truncation increased both endosperm starch and protein contents (Table 1). When grown at the same N level, the low starch class exhibited a greater amount of endosperm protein (Table 1).

Influence of sink size on endosperm composition

In full ears, starch concentration was 36 g kg^{-1} greater, on average, in the high starch class compared to the low starch class (Table 2). The response in starch concentration to N or truncation was dependent upon the starch class. The low starch class displayed its characteristic lower concentration of starch than the high starch class RILs. Ear truncation furthermore decreased endosperm starch concentration of the low starch class. Supplementing with N fertilizer also reduced endosperm starch concentrations to a greater degree in the low, compared to high, starch RILs.

Endosperm protein concentration in full ears was 34 g kg^{-1} greater, on average, in the low starch class than in the high starch class (Table 2). Source-to-sink ratio alteration by ear truncation increased endosperm protein concentration, with the greater change occurring in the high starch compared to the low starch class. Nitrogen fertilizer increased endosperm protein concentration, regardless of the ear treatment or genotype (Table 2).

For plants grown without N, truncation decreased the starch concentration to a greater extent than the protein concentration increased when innate protein levels were already high, as in the low starch genotype class (Table 2). Conversely, in truncated ears grown without N fertilizer, the starch concentration remained the same, while the protein concentration increased in inbreds innately high in starch (i.e. the high starch lines). By selecting RILs divergent for starch, and altering ear size and fertilizer N supply, a range of starch and protein concentrations of 84 g kg^{-1} and 76 g kg^{-1}, respectively, was obtained (Fig. 3). Regardless of starch class, N level, or truncation treatment,

Table 1. The influence of sink size (full versus truncated ear) and N supply on endosperm dry weight, endosperm:embryo ratio, endosperm starch, and endosperm protein content in mature kernels of IBM RILs divergent for endosperm starch concentration

Data are means (n=36) of ears from three inbreds for each starch class.

Starch class	N supply (kg ha^{-1})	Endosperm dry weight (mg)		Endosperm:embryo ratio (wt wt^{-1})		Endosperm starch (mg)		Endosperm protein (mg)	
		Full	Truncated	Full	Truncated	Full	Truncated	Full	Truncated
High	0	176	218	9.3	9.6	147	180	11	17
	168	178	233	9.4	9.8	146	182	14	19
Low	0	175	210	7.7	8.2	140	165	16	22
	168	178	211	7.8	7.8	139	164	18	24
LSD ($P \leq 0.05$)		30		1.1		17		1	
ANOVA[a]									
Source of variation									
Starch class		NS		***		**		***	
Nitrogen		NS		NS		NS		*	
Ear truncation		***		NS		***		***	

[a]*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; NS, not significant.

Table 2. The influence of sink size (full versus truncated ear) and N supply on the concentration of starch and protein in mature endosperm of IBM RILs divergent for endosperm starch concentration

Data are means (n=36) of ears from three inbreds for each starch class.

Starch class	N supply (kg ha^{-1})	Starch (g kg^{-1})			Protein (g kg^{-1})		
		Full	Truncated	Difference	Full	Truncated	Difference
High	0	846	837	−9	70	87	17
	168	835	825	−10	84	96	12
Low	0	813	798	−15	108	116	8
	168	796	780	−16	116	134	18
LSD ($P \leq 0.05$)		14			18		
ANOVA[a]							
Source of variation							
Starch class		***			***		
Nitrogen		***			**		
Ear truncation		***			**		

[a]*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. NS = Not significant.

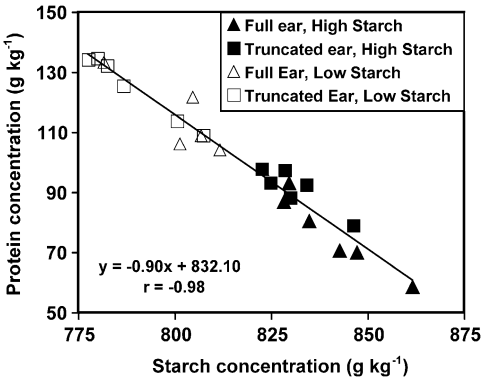

Fig. 3. Relationship between the concentrations of starch and protein in mature endosperm for six IBM RILs divergent for endosperm starch concentration, grown at two nitrogen rates, either with or without alteration in ear sink size from truncation. Full ears are represented by triangles, truncated ears as squares, high starch class types are filled symbols, and low starch class types are open symbols. Values are the mean of four replications (n=36).

there was a strong inverse relationship (r=–0.98) between the concentrations of starch and protein in the endosperm (Fig. 3).

Assimilate supply

The agar trap technique was used to determine the instantaneous assimilate supply to the developing kernels from the maternal source at 19 DAP. The RILs divergent in endosperm starch concentration exhibited more than 2-fold differences in the C/N ratio of the transport assimilate supply in response to changes in ear size and fertilizer supply (Fig. 4). The differences in assimilate supply during kernel growth reflected the final starch/protein composition of the kernel at maturity (r=0.87). Accordingly, low starch lines generally had an assimilate transport C/N ratio of 6–10 compared to 9–15 for high starch lines.

Although the C/N ratios of transported assimilates segregated into two discrete class types relating to the high or low starch inbreds (genotypes), the same was not true for the absolute rates of total sugar or amino-N transport (Fig. 5). Discerning this difference was made possible by the large range of sugar transport rates, especially for high starch inbreds, and amino acid transport rates, especially for low starch inbreds, produced by employing ear reduction and N fertilizer treatments. More specifically, across the ear and N treatments it was observed that some high and low starch inbreds had similar transport rates of total sugar, while similar comparisons showed that other high and low starch inbreds had comparable rates of transported amino-N. Even though inbreds could not be classified as high or low starch based on absolute rates of sugar or amino-N transported, in all cases the parental genotype controlled the proportion of sugar and amino acid transported such that the inbreds could be discerned as high or low starch types regardless of ear and N treatments (Fig. 5).

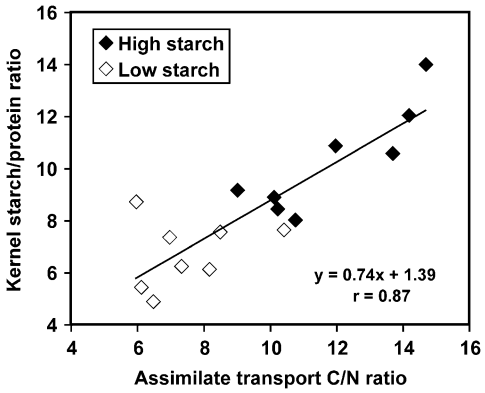

Fig. 4. Relationship between the C/N ratio of the incoming assimilate transport stream at 19 DAP and the final starch/protein ratio in the kernel at maturity. Starch class types, either high or low based on endosperm starch concentration, were each represented by two IBM RILs. Transported assimilate was collected in agar traps, while mature kernel composition was determined by SS-NIR. Data are the mean of two replications grown at two N supplies and including two ear sink size treatments (full versus truncated) (n=8). High starch class types are represented by filled diamonds, and low starch class types are open diamonds.

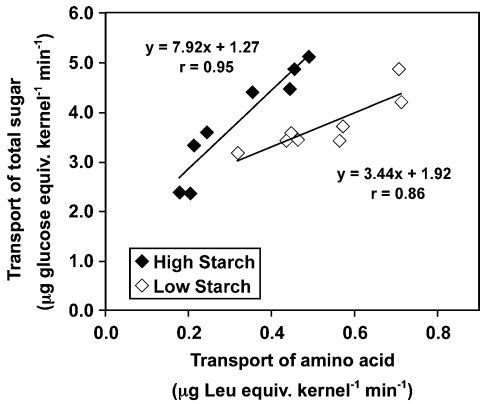

Fig. 5. Relationship between the transport rates of total sugars and amino acids into agar traps situated for 4 h in place of developing endosperm at 19 DAP. Starch class types, either high or low based on endosperm starch concentration, were each represented by two IBM RILs. Data are the mean of two replications, grown at two N supplies and including two ear sink size treatments (full versus truncated) (n=8). High starch class types are represented by filled diamonds, and low starch class types are open diamonds.

Discussion

Maize kernels have three phases of filling: a lag phase, a linear phase, and a phase of slowing down of dry weight accumulation approaching physiological maturity (Johnson and Tanner, 1972). The lag phase generally lasts 15–18 DAP, during which time very little dry weight is accumulated. The linear phase begins around 18 DAP during which the kernel develops and increases in weight at a constant rate.

Within the developing ear-shoot, starch and protein are accumulated in fairly equal proportions by all kernels, but the proportions can be modulated by the available N supply and by the maternal genotype (Figs 1, 2). Early in reproductive development, the N supply helps to establish sink size through setting the number of kernels to be filled (Paponov *et al.*, 2005). How the maize ear senses the available N supply is unclear, but may be related to the concentration or the ratio of key amino acids (Seebauer *et al.*, 2004; Miller *et al.*, 2007). Once kernel number is established, the N supply clearly plays a role maintaining consistent kernel N concentration along the length of the ear, despite a decrease in kernel size from base to tip (Figs 1, 2). Enzymes within the maternal cob and pedicel–placento-chalazal tissues may also control and alter the source composition supplied to the kernels (Thompson *et al.*, 2001; Balconi *et al.*, 2004; Seebauer *et al.*, 2004). Thus, the maize ear is sensitive to the level of N available, resulting in all grains on the ear-shoot having fairly consistent protein and starch compositions.

Previous work has suggested a stronger genetic than environmental control on grain composition, which is supported by our study where the high and low starch lines exhibited their characteristic phenotype in the mature grain regardless of the N supply or the source-to-sink ratio (Fig. 1; Table 2). Similarly the Illinois Protein Strains, which have an even wider range in grain composition, retain their characteristic grain phenotypes when the N supply is altered in either the field (Uribelarrea *et al.*, 2004) or in culture (Wyss *et al.*, 1991). Other *in vitro* studies have also shown that the maternal genotype dictates the final kernel composition within limits that can be modulated by the N supply (Czyzewicz and Below, 1994). Maize varieties clearly differ in how efficiently they utilize N for storage product formation, and as a result yield (Uribelarrea *et al.*, 2004; Fageria and Baligar, 2005; Paponov *et al.*, 2005), and much of this variation appears to be due to genetically determined processes within the maternal plant.

Several lines of evidence from the present study point to a source limitation in supplying assimilates for starch and protein accumulation in maize kernels. These include the ability to increase endosperm dry weight, starch, and protein in the remaining kernels following ear truncation (Table 1), and that supplying N fertilizer promoted an increase in endosperm protein concentration (Fig. 2; Table 2). The alterations in kernel starch and protein by additions of N fertilizer or truncation supports the idea that the source-to-sink ratio was not optimal at low N (Borras *et al.*, 2002). Similar evidence that source supply limits maximum storage product accumulation comes from the greater weights obtained when apical maize kernels are grown in culture (Hanft *et al.*, 1986), and when the inflorescence of wheat is partially degrained (Triboi *et al.*, 2006). In maize, final kernel weight is influenced by the source-to-sink ratio and the plant growth rate per kernel in the early post-pollination period (Gambin *et al.*, 2006), and plants with a greater source-to-sink ratio are able to produce more weight per grain. On average, inbreds produce less plant mass and require less N fertilizer than hybrids, which may account for the lack of response to N supply in endosperm dry weight or the endosperm-to-embryo ratio (Table 1). Within a starch class, changes in the source-to-sink ratio resulted in similar endosperm-to-embryo ratios, even though they are distinctly different in composition, suggesting that the kernel assimilates are utilized proportionally to make kernel components (Table 1).

A negative relationship between grain yield and grain protein is typically observed among cereal crops (Tsai *et al.*, 1992; Simmonds, 1995), and a strong inverse relationship was observed between the concentrations of endosperm starch and protein in our study (Fig. 3). Most of the earlier evidence for a negative starch/protein relationship in maize grain comes from evaluations of the Illinois Protein Strains, which have been selected for extremes in grain protein concentration (Dudley and Lambert, 1992; Uribelarrea *et al.*, 2004). It is shown here that this negative relationship also exists among maize materials that have not been selected for extremes in grain composition. Although the concentrations of grain starch and protein can be altered by the N supply, or by the source-to-sink ratio, these grains still adhere to the negative relationship between starch and protein (Fig. 3). Similar results have been reported for wheat (Triboi *et al.*, 2006), suggesting that the accumulation of C and N by cereal grains is primarily dependent upon the composition of assimilates received from the mother plant.

The close relationship between the assimilate transport composition at 19 DAP and the C/N ratio in mature kernels (Fig. 4) shows that source supply at the onset of linear grain fill determines the final grain composition. The starch class also differed in transport composition, as endosperm of high concentration lines received more sugar per amino acid from the maternal plant, than the low starch lines. Conversely, the low starch endosperms were supplied more amino acids per sugar in the assimilate stream than the high starch lines (Fig. 5). Although some of the low starch lines transported an above-average level of sugars to the kernels, those low starch lines also concurrently transported a higher level of amino acids than the high starch lines, leading to the lower starch concentration classification of the endosperm (Fig. 5). This balance of the sugar to amino acid concentrations in the assimilate supply affects the final endosperm composition. Similar findings have been reported for Illinois High Protein which has a low concentration of grain starch and transports a higher proportion of amino acids than sucrose to the grain, and Illinois Low Protein which has a high concentration of grain starch and transports more sucrose per amino N (Lohaus *et al.*, 1998). Sucrose and N power kernel growth by interdependent means, with sugars regulating endosperm C metabolites, while N promotes kernel sink capacity, protein, and starch accumulation (Cazetta *et al.*, 1999). The assimilate C/N ratio measured at 19 DAP differs from the final endosperm C/N (Fig. 4) in part because maize plants continue to take up and remobilize N throughout the grain-filling stage, with the most recent N accumulated preferentially allocated to the kernels (Subedi and Ma, 2005). Thus, the composition

of the source supply early in the grain-filling period sets the stage for the final sink composition.

In conclusion, by using a commercial maize hybrid and IBM RILs which display extremes in endosperm starch concentration, it has been shown that kernel starch and protein concentrations are inversely related, consistent along the ear, and dependent upon the source assimilate supply composition at the beginning of the linear fill period.

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