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The contribution of green parts of the ear to grain filling in old and modern cultivars of bread wheat (*Triticum aestivum* L.): Evidence for genetic gains over the past century

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ABSTRACT

Green parts of the ear (e.g. bracts, awns) are sources of photosynthates in C_3 cereals. However, the contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.) is less known compared with other sources of assimilates (e.g. flag leaf photosynthesis, C stem retranslocation). The main objectives of this work were: (i) to analyze if empirical breeding has changed the contribution of ear photosynthesis to grain filling through a retrospective study of cultivars of bread wheat released in Argentina between 1920 and 2008 and (ii) to study if the introgression of dwarfism alleles has changed the contribution of ear photosynthesis to grain filling using near-isogenic lines of a cultivar of bread wheat differing in absence/presence in *Rht* alleles.

In both experiments we covered ears with aluminum foil to reduce photosynthesis, in intact and defoliated plants. Grain weight per ear was measured and the ear contribution to grain filling was calculated as: $[(GW_{ear}$ of non-shaded ear $- GW_{ear}$ of shaded ear$) \times 100/GW_{ear}$ of non-shaded ear], where GW_{ear} is total grain weight per ear.

The contribution of ear photosynthesis increased in modern cultivars, more in defoliated than intact plants. Ear contribution was negatively correlated with plant height ($r^2 = 0.62$ and 0.71 in intact and defoliated plants, respectively) and especially with stem dry weight ($r^2 = 0.75$ and 0.80 in intact and defoliated plants respectively). Dry matter translocation efficiency (DMT_e) did not change consistently with year of release of the cultivars. The contribution of preanthesis stem assimilates to grain yield (CPA, i.e. the ratio of stem mobilized reserves to GW_{ear}) clearly decreased in more modern cultivars. The correlation of ear contribution with awn size was rather low in this retrospective study.

In near-isogenic lines differing in *Rht* alleles, the contribution of the ear was higher when dwarfism alleles were present, mainly in non-defoliated plants. However, there was no consistent relationship between stem weight of the near-isogenic lines and their respective contributions of ear photosynthesis, suggesting that other factors (beyond the *Rht* alleles) are involved in the increase of ear contribution in the retrospective study.

1. Introduction

In wheat and other C_3 cereals, grain filling is sustained by current photosynthesis of the upper parts of the plant, i.e. the flag and penultimate leaves *plus* the ear (see Tambussi et al., 2007 and references cited therein) and by redistribution of assimilates stored in the stem (Álvaro et al., 2008a; Blum, 1998; Ehdaie et al., 2006; Foulkes et al., 2007). Several studies analyzed ear photosynthesis from a physiological viewpoint (see references in Tambussi et al., 2007), however, its contribution to grain filling has been explored less. The contribution of ear photosynthesis can be high (ranging from 20 to 40% of mature grain dry mass), depending on cultivar and growing conditions (e.g. Araus et al., 1993; Maydup et al., 2010). Several traits have been linked with the photosynthetic performance of the ear, such as a degree of drought 'tolerance' (Tambussi et al., 2005), refixation of respired CO_2 (e.g. Bort et al., 1996) and the xeromorphic anatomy of awns (e.g. Li et al., 2006). The role of awns (sclerophyllous threadlike extensions of lemmas) in ear photosynthesis of C_3 cereals has been recognized for years (e.g. Blum, 1985; Bort et al., 1994; Evans et al., 1972; Jiang et al., 2006; Li et al., 2006; Olugbemi et al., 1976), but its actual contribution to grain yield is less clear (see Tambussi et al., 2007).

Grain yield of wheat is often limited by sink strength rather than by the availability of assimilates (i.e. the 'source', Cartelle et al., 2006; Slafer and Savin, 1994). This limitation due to sink capacity

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depends on the environment (Blade and Baker, 1991). Therefore, if there is an excess of assimilates during grain filling (Borrás et al., 2004), photosynthesis of ear parts could be of minor importance. Defoliation or de-graining experiments in old germplasm of bread wheat did not show source limitation to grain filling (e.g. Kruk et al., 1997). However, recent papers indicate that some source limitation could be emerging in modern cultivars of durum and bread wheat (e.g. Álvaro et al., 2008a; Maydup et al., 2010). The increase of sink strength (i.e. a higher number of kernels in the spike; Miralles and Slafer, 1996) and a possible decrease of source size (in particular a decrease in stem dry weight) could be leading to source limitation in modern cultivars of bread wheat. One of the most important and known traits that have been selected in wheat breeding programs is plant height with the introduction of semi-dwarfism (i.e. *Rht*) alleles (Hanson et al., 1982). Wheat breeding increased potential yield (mainly due to a higher number of kernels per spike; e.g. Börner et al., 1993; Miralles and Slafer, 1996) and decreased lodging (e.g. Berry et al., 2007) through a reduction of stem height. However, the decrease of plant height and the concomitant loss of retranslocatable stem biomass could impose penalties on grain filling, mainly under stress conditions, such us drought or heat stress (e.g. Butler et al., 2005). In fact, in low yielding (i.e. stressful) environments, semi-dwarf alleles seem to have neutral (or in some cases, even negative) effects on yield (Chapman et al., 2007; Mathews et al., 2006).

In a context in which wheat breeding has decreased vegetative biomass, and presumably remobilization of stem carbohydrates, it is interesting to analyze whether the contribution of ear photosynthesis has increased in modern Argentinean cultivars of bread wheat.

In summary, the main objectives of this work were: (i) to analyze if empirical breeding has changed the contribution of ear photosynthesis to grain filling through a retrospective study of cultivars of bread wheat released in Argentina between 1920 and 2008, (ii) to analyze whether any changes in the contribution of the ear to grain filling correlate with the size of awns, (iii) to study if the introgression of dwarfism alleles has changed the contribution of the ear to grain filling using four near-isogenic lines (differing in absence/presence in *Rht* alleles) of a cultivar of bread wheat. As far as we know, this is the first study to address the impact of genetic improvement of bread wheat on the photosynthetic contribution of the ear to grain filling.

2. Materials and methods

2.1. Retrospective study

2.1.1. Plant material

Ten cultivars of bread wheat (*T. aestivum* L.) released in different years between 1920 and 2008 were used in this work. The cultivars were: Klein Favorito (1920), Klein 32 (1932), Klein Cometa (1942), Klein Orgullo (1944), Klein Rendidor (1954), Klein Toledo (1969), Klein Chamaco (1979), Buck Pucará (1980), Klein Cacique (1992) and Buck Taita (2008). This historical series includes genotypes released before and after the introduction in Argentina of Mexican germplasm with dwarfism alleles. Seeds were obtained from germplasm banks of Criadero Klein S.A. and Buck Semillas S.A.

2.1.2. Experimental setup

Seeds of the ten cultivars were planted on July 7 2009 in La Plata, (34°54′24″S; 57°55′56″W, Argentina) in the Experimental Field of Facultad de Ciencias Agrarias y Forestales (Universidad Nacional de La Plata) under rainfed conditions. The cultivars were planted in plots (1 × 3.4 m per plot; five rows, 20 cm apart). Sowing density was 260 plants/m^2. Plots were arranged in randomized blocks with three replicates. The soil was a typic argiudol (USDA classification). Fertilization was applied as 100 kg ha^{-1} diammonium phosphate immediately after sowing, and urea (250 kg ha^{-1}) 75 days after sowing, near tillering. Emergence took place on July 22.

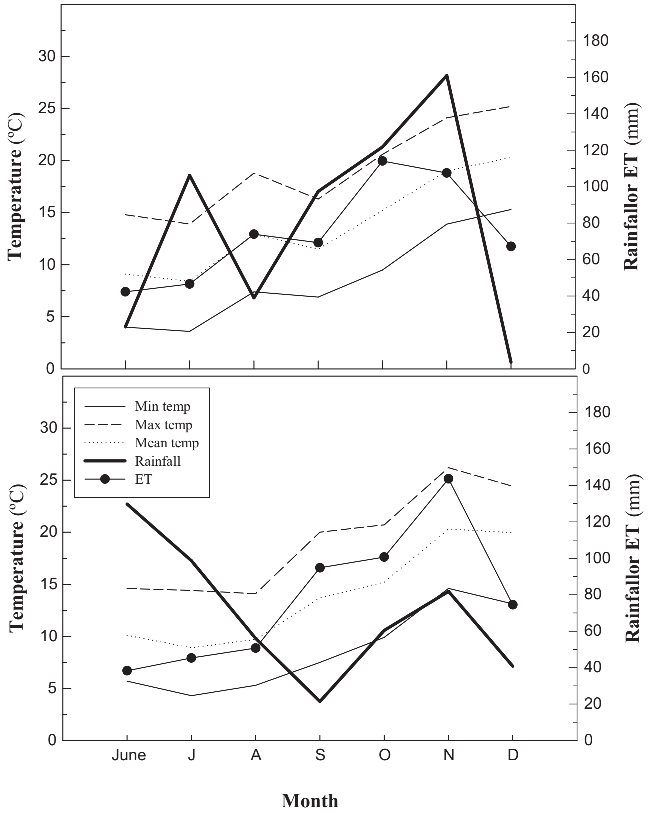

Fig. 1. Rainfall, potential evapotranspiration (ET), and minimum, maximum and average temperatures recorded during the retrospective study (upper panel), and the experiment analyzing the role of *Rht* alleles in photosynthetic contribution of ears to grain yield (lower panel).

Weather conditions are shown in Fig. 1 (upper panel). Seasonal rainfall from sowing to harvest was high (>400 mm), and temperatures were moderate (mean temperature ca. 16 °C) throughout the grain filling period (November and the first half of December).

Near anthesis (GS69, Zadoks et al., 1974), four treatments were imposed in the three central rows of each plot. These treatments were combinations of defoliation and ear shading. Defoliation consisted of the removal of all leaves of the culms in 1-m sections of the rows. Ears were shaded by wrapping them in aluminum foil as in Maydup et al. (2010). In order to prevent the accumulation of ethylene and to allow for convective heat flux, several holes (diameter ca. 5 mm) were made in the aluminum foil covers. Ear shading treatments were imposed at random on ten ears from different rows within each plot. In a previous article (Maydup et al., 2010), we demonstrated that this methodology does not produce artifactual effects on ear temperature.

2.2. Quantification of stem biomass and stem/ear contribution to grain filling

In the retrospective study, five days after anthesis six main stems per plot and cultivar were harvested and dry weight (60 °C until constant weight) was determined. At maturity, shoot dry weight, number of kernels *per* ear, and total grain weight were measured in six culms and ears. The average weight of grains from specific positions within the central spikelet (G1–G3, from the proximal to the distal kernel; Kruk et al., 1997) was also determined.

We calculated the ear contribution to grain filling (Maydup et al., 2010) as:

$$= \left[\frac{(GW_{ear}\text{ of non-shaded ear} - GW_{ear}\text{ of shaded ear})}{GW_{ear}\text{ of non-shaded ear}} \times 100\right]$$

where GW_{ear} is total grain weight per ear.

Dry matter translocation (DMT) was calculated as the decrease of stem (*plus* leaf sheaths) dry weight between five days after anthesis and the end of grain filling (maturity), as an estimate of C remobilization to the grains. In all cases six culms per plot were sampled and stem dry matter (DM) was determined. Dry matter translocation efficiency (DMT_e, %) and the contribution of preanthesis stem assimilates to grain yield (CPA) were calculated as in Álvaro et al. (2008a):

$$DMT_e = \frac{DMT}{DM_{anthesis}} \times 100$$

$$CPA = \frac{DMT}{GW_{ear}} \times 100$$

where $DM_{anthesis}$ was the dry weight of the stem at anthesis and GW_{ear} was the total grain weight per ear.

In some cultivars of wheat, assimilate accumulation in vegetative parts (and, hence the increase of stem weight) may continue in the first days of grain filling (e.g. Ehdaie et al., 2006), therefore we also measured the weight of stems 10 days after the first measurement (i.e. fifteen days after anthesis). To calculate DMT_e and CPA (see above) the maximum value reached by the stem weight (5 or 15 days after anthesis) was taken as reference. In our case, stem dry weight increased after anthesis only in two cultivars. In this article, we use the term 'preantesis assimilates', although we are aware that part of this may involve assimilates stored early in post-anthesis.

2.3. Ear contribution in near-isogenic lines differing in dwarfism alleles

2.3.1. Plant material

Four near-isogenic lines of the Brazilian cultivar Maringa were used. These lines, which are homozygous at over 99% of the loci unlinked to *Rht* (Miralles and Slafer, 1996), included the standard height (*rht1 rht1 rht2 rht2*; 'tall'), two semidwarf (*Rht1 Rht1 rht2 rht2* and *rht1 rht1 Rht2 Rht2*; referred to as '*Rht1*' and '*Rht2*', respectively) and one dwarf line (*Rht1 Rht1 Rht2 Rht2*; referred to as '*Rht1* + *Rht2*'). Although the modern names of *Rht1* and *Rht2* are *Rht-B1b* and *Rht-D1b* respectively, for simplicity we use the former names in the text. These lines were produced by Dr. M.D. Gale (Plant Breeding Institute, Cambridge) through seven backcrosses from CIMMYT lines carrying the semi-dwarf genes, followed by selfing to produce *Rht* and *rht* homozygotes of Maringa, and were generously supplied by Dr. Gabriela Tranquilli (INTA - Instituto de Recursos Biológicos, Argentina).

2.3.2. Experimental setup

Seeds of the four near-isogenic lines were planted on July 01, 2011 in La Plata under rainfed conditions. Seeds were planted in plots (1 m × 3.4 m per plot; five rows, 20 cm apart). Sowing density was 260 plants/m^2. Plots were arranged in randomized blocks with three replicates. The soil was a typic argiudol (USDA classification). Fertilization was applied as 100 kg ha^{-1} diammonium phosphate immediately after sowing and urea (250 kg ha^{-1}) 75 days after sowing, near tillering. Emergence took place on July 15. Weather conditions are shown in Fig. 1 (lower panel). Mean temperatures were high (ca. 20 °C) throughout the grain filling period (November and the first half of December), and rainfall was adequate (ca. 350 mm from sowing to harvest, which is close to the average for La Plata, i.e. ca. 380 mm from July to November for the period 1969–2009).

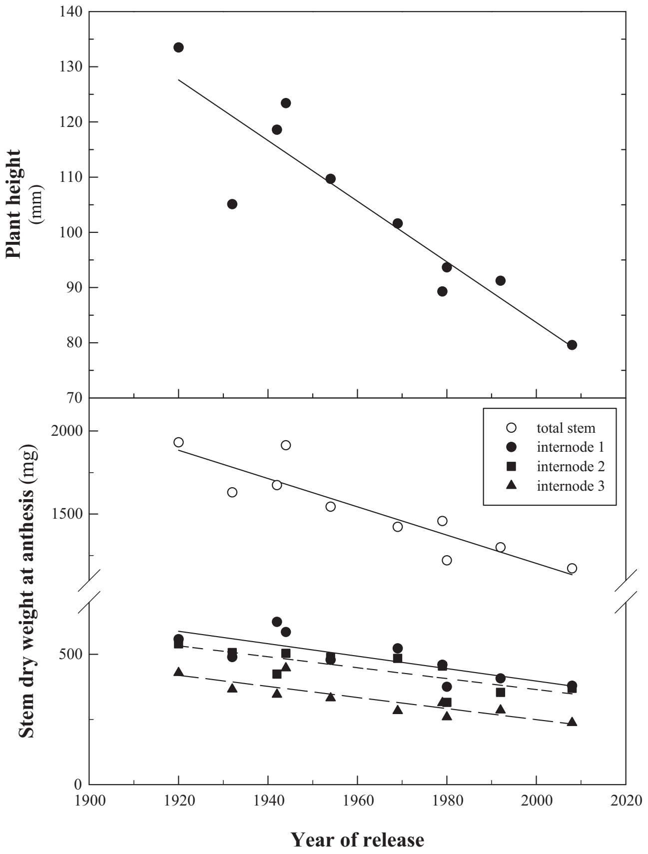

Fig. 2. Plant height (upper panel), total stem biomass near anthesis (lower panel) and internode biomass (1–3 numbered from the uppermost internode, internode 1 includes the ear peduncle; lower panel) vs. year of release in the ten cultivars of the retrospective study. Each value represents the mean of three replicates (=plots) per cultivar. In each plot, six culms were analyzed and averaged. For more details see Section 2.

Five days after anthesis, we applied treatments of defoliation and ear shading identically as in the retrospective study. Contribution of the ear to grain filling was calculated as mentioned above.

2.4. Statistical analysis

Statistical analyses were carried out using the Statistica 5.1 software (StatSoft, Inc.). Differences were analyzed by ANOVA. Means were compared by the LSD test ($p < 0.05$).

3. Results

3.1. Retrospective study

Plant height (from the ground to the terminal spikelet) showed a lineal decline ($r^2 = 0.83$) from older to more modern cultivars, with a decrease of 0.55 cm *per* year of release (Fig. 2, upper panel). This trend led to a decrease of ca. 36% in plant height of modern cultivars. In parallel, stem weight showed a similar decrease with year of release ($r^2 = 0.81$), of about 8.5 mg of stem DW *per* year (Fig. 2, lower panel), with a drop in stem weight of ca. 39% (comparing the oldest vs. modern cultivars). All internodes were similarly affected (Fig. 2, lower panel).

Defoliation reduced grain weight per ear (GW_{ear}) to different extents depending on the cultivar, with the decrease ranging from 0 to ca. 30% in some cases (compare the black bars in each panel in

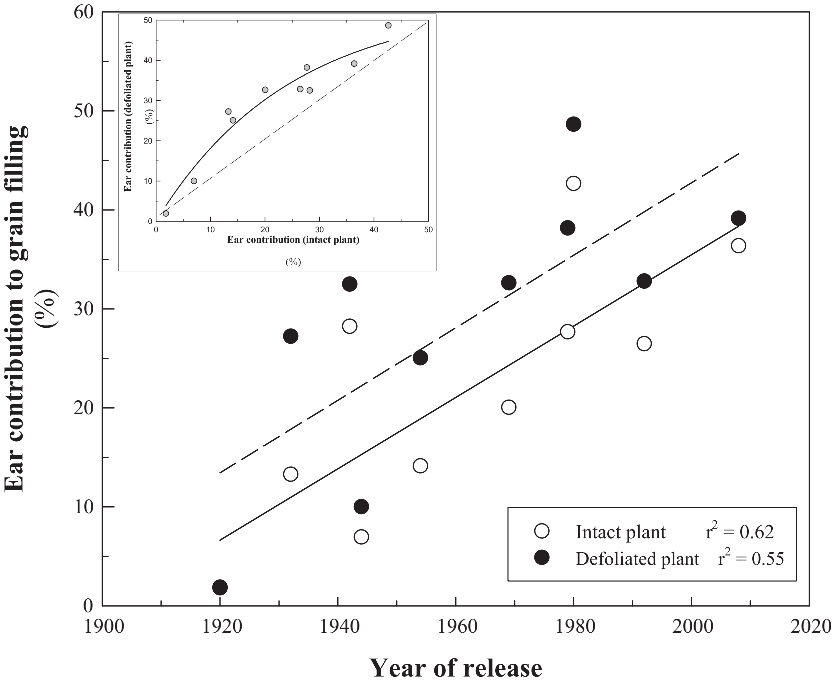

Fig. 3. Contribution of ear photosynthesis to grain filling in the ten cultivars of the retrospective study. Each point represents the mean of each cultivar. Values for intact (open symbols) and defoliated (closed symbols) plants are shown. Ear contribution (%) was calculated as described in Section 2. *Inset*: the relationship between the contributions of the ear to grain filling in intact vs. defoliated plants. Dotted line is the 1:1 relationship.

Supplementary material Fig. 1). Supplementary Fig. 1 also shows the effects of de-awning and ear shading on GW_{ear}. In general, ear shading caused an increasing effect from older to modern cultivars (for instance, compare the effect of ear shading in the modern cv. Taita vs. the old cv. Favorito; Fig. 1 in Supplementary material, first and last panel, respectively). In the modern cv. Taita, the decrease of grain weight in shaded ears was around 40%, both in intact as in defoliated plants. In this cultivar we analyzed the effects of treatments on grain weight in several positions in the central spikelets, as in Kruk et al. (1997). The grains in distal positions in the spikelet (i.e. G3) were most strongly affected by ear treatments, both in intact and defoliated plants (data not shown).

In order to quantify the trend for a larger photosynthetic contribution of the ear in modern cultivars, we calculated ear contribution to grain filling for each cultivar. Ear contribution showed a clear increase with year of release ($r^2 = 0.55$ and 0.6 in intact and defoliated plants respectively; Fig. 3), and this contribution was higher in defoliated than intact plants (Fig. 3, inset). When photosynthetic ear contribution was related to plant height or stem weight of the cultivars, we found a strong negative correlation between these parameters (Fig. 4). In particular, there was a strong negative correlation ($r^2 = 0.75$ and $r^2 = 0.8$ in intact and defoliated plants, respectively) with stem weight near anthesis (Fig. 4 lower panel). In short, plant breeding resulted in an increased contribution of the ear to grain filling concomitant with the decrease of stem biomass (Fig. 4, lower panel). Fig. 5 shows dry matter translocation efficiency (DMT_e) and the contribution of preanthesis assimilates to grain filling (CPA; i.e. the ratio of stem mobilized reserves to GW_{ear}). In our study, DMT_e did not show a consistent change for cultivars released in different eras (upper panel) but CPA clearly decreased with the year of cultivar release (Fig. 5, lower panel).

In this historical set, old cultivars are either awnless or have a low FW of awns per spikelet (data not shown). The correlation between awns size and ear contribution was high if all cultivars are included in the analysis ($r^2 = 0.85$ and 0.67 in intact and defoliated plants, respectively; see inset in Fig. 6). Nevertheless, if awnless cultivars are excluded from the analysis, the correlation is weaker ($r^2 = 0.46$ and 0.42; Fig. 6; main panel).

3.2. Ear contribution in cultivars differing in Rht alleles

Lines carrying an *Rht* allele (either *Rht1* or *Rht2*) are 'semi-dwarf' showing a decrease in plant height and stem dry weight. Line *Rht 1 + Rht 2* is dwarf (plant height ca. 50 cm), with a 45% decrease in stem weight with respect to the tall line cv. Maringa (Fig. 7, upper panel).

The photosynthetic contribution of the ear is higher when dwarfism alleles are present, mainly in intact (non-defoliated) plants (Fig. 7; lower panel). In non-defoliated plants, ear contribution was ca. 4, 22, 16 and 16.5% in tall, *Rht1*, *Rht2* and *Rht 1 + 2* respectively. The presence of two dwarfism alleles in *Rht1* and *Rht2* (i.e. the dwarf line *Rht 1 + 2*) did not cause any change in ear contribution compared to semi-dwarf lines.

4. Discussion

4.1. Is there a source-limitation in modern cultivars of wheat?

Typically, it has been considered that grain yield of wheat is limited by sink strength rather than by the availability of assimilates during the grain filling period (i.e. the source; Borrás et al., 2004; Savin and Slafer, 1991). Defoliation experiments in wheat (e.g. Kruk et al., 1997) and barley (e.g. Dreccer et al., 1997) showed no evidences of significant grain weight reduction, in particular in old cultivars. However, modern varieties could be less sink limited than old ones (Monneveux et al., 2005), i.e. the degree of sink-limitation for yield might have been reduced by breeding. Indeed, the existence or not of source limitation in wheat is controversial, and evidences in one direction or the other have been presented (e.g. Ahmadi et al., 2009; Álvaro et al., 2008a,b; Cartelle et al., 2006). In our work defoliation caused a reduction of up to 30% in the weight of grains per spike in some varieties. Recent works in durum wheat using sink manipulations such as de-graining

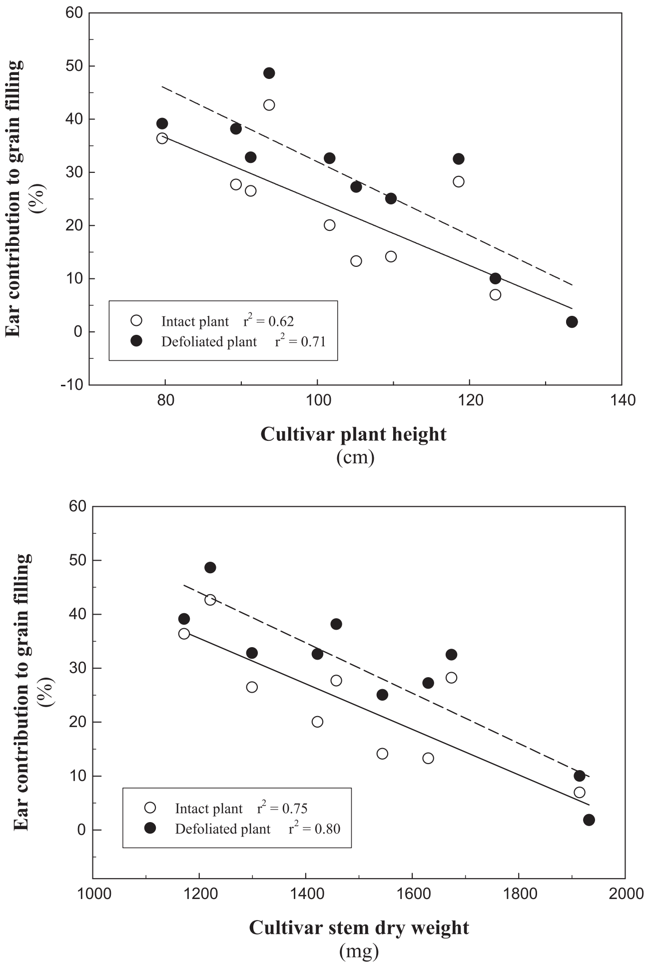

Fig. 4. Relationship between plant height (upper panel) or stem dry weight (lower panel) vs. ear contribution to grain filling in intact (open symbol) and defoliated plants (closed symbols) of the ten cultivars (retrospective study). Each point represents the mean of three replicates (=plots). In each plot, six culms or ears were analyzed and averaged. The determination coefficients (r^2) for each relationship are shown.

indicate that some source-limitation could be emerging in modern cultivars (e.g. Álvaro et al., 2008a,b). Discrepancies between different reports could be explained by germplasm, environmental conditions or experimental approaches. On the other hand, the apparent lack of ‘source-limitation’ of grain yield in previous works may be partly due to the fact that the photosynthetic role of the ear was not taken into account (e.g. Kruk et al., 1997). In a comprehensive meta-analysis of literature data, Borrás et al. (2004) show that the increase in grain weight with increasing source-sink ratio is lower than the expected response for a 1:1 relationship. However, the interpretation of source manipulation treatments, particularly defoliation, must take into account compensatory mechanisms that may occur in the remaining photosynthetic tissues (e.g. Chanishvili et al., 2005). Thus, photosynthetic rate may increase, compensating for the decrease of the photosynthetic area. In other words, the actual decrease of assimilate availability for grain filling cannot be simply predicted from the reduction of green leaf area, as assumed in some earlier works (see references above). If such compensations occur in our case (e.g. if leaf photosynthesis increases to compensate for ear shading), this means that calculated contributions of ear photosynthesis are conservative, i.e. they may represent an underestimation.

Several abiotic (e.g. water deficit; Araus et al., 2002) and biotic (foliar diseases; Serrago et al., 2011) stresses could impose a shortage of assimilates and therefore, source-limitation for grain filling. Taken together the increase of sink size in current wheat varieties and the impact of stresses that decrease source activity, photosynthesis of the ear may have an increasingly important role during grain filling of wheat.

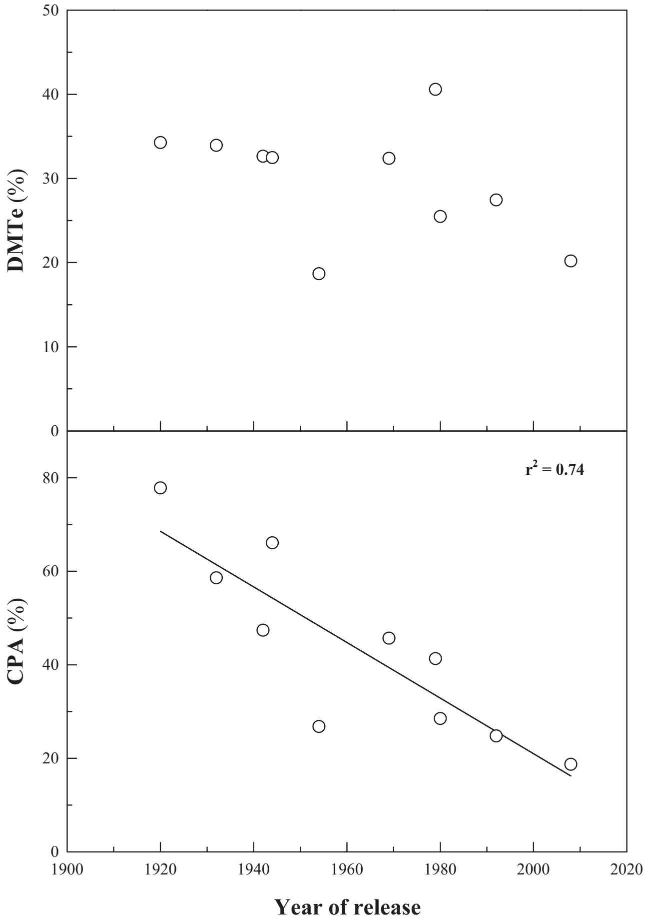

Fig. 5. Dry matter translocation efficiency (DMT$_e$, upper panel) and contribution of pre-anthesis assimilates to grain filling (CPA, lower panel) vs. year of release in the ten cultivars of the retrospective study. DMT$_e$ and CPA were calculated as indicated in Section 2.

4.2. Dwarfism alleles and ear contribution

Decreased height has played a significant role in genetic improvement of yield of wheat. In several regions of the world (e.g. Ganeva et al., 2005; Hanson et al., 1982; Knopf et al., 2008; Zhang et al., 2006), this reduction is largely explained by the introduction of *Rht* alleles from the Norin 10 and other japanese cultivars. In Argentina, Mexican germplasm carrying dwarfism alleles was introduced in the mid-1960s (e.g. Hanson et al., 1982). Although we do not know the allelic composition of the *Rht* loci of the cultivars used in this retrospective study, there is clear trend for decreased plant height and stem dry weight at anthesis (SDWA) with year of release of the cultivars (see Fig. 2). Unexpectedly, there was a gradual decrease in both parameters (i.e. plant height and SDWA) rather than an abrupt change in the decade when dwarfism alleles were introduced in Argentina. In addition to decreased plant height, the presence of dwarfism alleles may lead to reduced leaf area, particularly in pre-anthesis (Calderini et al., 1996). In pre-anthesis period, radiation use efficiency is lower in dwarf lines, possibly due to a poor canopy architecture linked to decreased length of internodes (Miralles and Slafer, 1997). Taken together, these effects may lead to

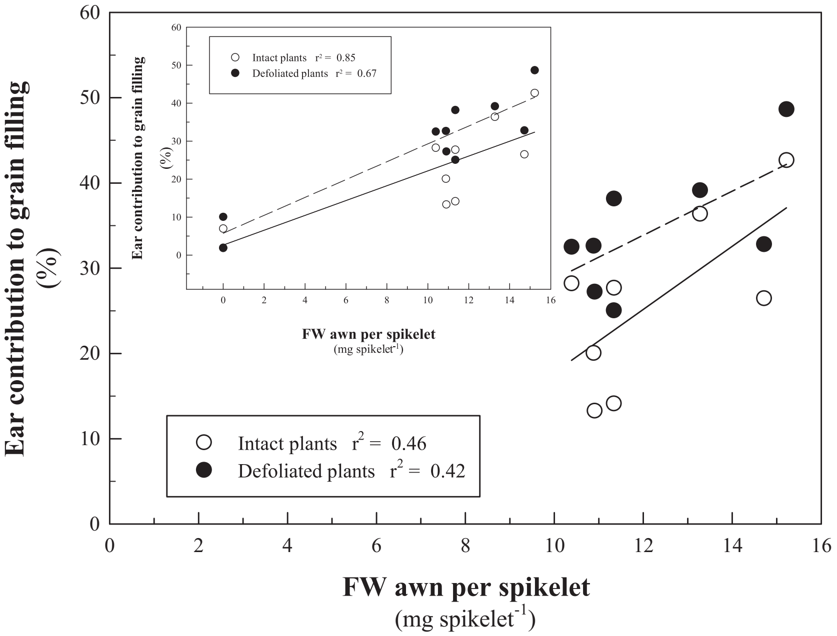

Fig. 6. Relationship between awn size (mg awn FW spikelet^{-1}) and ear contribution to grain filling in the ten cultivars of the retrospective study. In the inset of the figure, all cultivars were included in the analysis. In the main part of the figure, two awnless cultivars (i.e. cv. Favorito and cv. Orgullo) are excluded. Determination coefficients for each case are shown.

a decrease in source capacity. As mentioned above, a main objective of this study was to assess whether the decrease of stem biomass associated with modern breeding of wheat is offset by an increase in the contribution of photosynthesis of the ear.

Retranslocation of water soluble carbohydrates stored in the stem is an important source of assimilates for grain filling in wheat (e.g. Álvaro et al., 2008a,b; Blum, 1998; Cruz-Aguado et al., 2000; Plaut et al., 2004). *Rht* alleles may reduce the amount of carbon stored in the stem (e.g. Borrell et al., 1993). Although the magnitude of this decline varies depending on the genetic background, Borrell et al. (1993) reported that a 21% drop in height decreased the absolute amount of carbon stored by 35%. In our study, the decrease in plant height was close to 35% (see Fig. 2, upper panel), thus, the impact on stem reserves can be important.

Other studies seem to indicate the opposite (e.g. Shearman et al., 2005). In durum wheat, Álvaro et al. (2008a) reported that the contribution of preanthesis assimilates increased in modern germplasm of durum wheat; these authors speculate that the higher sink demand for assimilates of these cultivars could enhance reserve mobilization from the stem. In short, despite the decrease of stem biomass in modern germplasm of durum wheat, the efficiency of translocation (DMT_e, i.e. the ratio between translocated stem dry mass to stem dry mass at anthesis) increased in recent cultivars of durum wheat (Álvaro et al., 2008b). For this reason, we analyzed DMT_e and CPA (i.e. the ratio of stem mobilized reserves to GW_{ear}). In the present set of bread wheat cultivars, we did not find a clear relationship of DMT_e vs. year of release, but CPA clearly decreased in modern cultivars. We do not know the underlying causes of the discrepancies between different studies about changes in CPA (see references above). Species (durum vs. bread wheat) or germplasm (breeding programs) differences could be involved. We speculate that in the case of durum wheat (with long awns and high ear contribution to grain filling per se, Araus et al., 1993) an increase in DMT_e (and concomitantly, a higher CPA) rather ear contribution may have been selected during the breeding. In our study, the decline in the contribution of stem assimilates seems to be compensated for by ear photosynthesis in modern cultivars.

Since the presence of dwarfism alleles could have several pleiotropic effects (e.g. higher kernel number per ear; Börner et al., 1993) we cannot rule out that the correlation between stem weight (and plant height) vs. ear contribution is merely coincidental. For example, the existence of a greater sink (a higher kernel number) can increase ear photosynthetic rates and result in a greater ear contribution to the final weight of the grains. Thus, pleiotropic effects cannot be ruled out. In addition, there is no consistency between stem weight of the near-isogenic lines and their respective photosynthetic contribution of the ear (see Fig. 7), suggesting that other factors (beyond the *Rht* alleles) are implicated. Unexpectedly, ear contribution in the near-isogenic lines (up to 22%, depending on the line; Fig. 7) was lower than in some cultivars of the retrospective study (ca. 40% in modern cultivars, see Fig. 3). However, it must be noted that the genetic backgrounds are different in both studies, e.g. cv. Maringa may be more sink-limited than modern Argentinean cultivars of wheat, or its capacity to compensate for a decrease of source capacity could be lower.

4.3. *Role of awns size in grain filling*

Awns contribute about 40–50% of net assimilation of external (atmospheric) CO_2 in the ear of C_3 cereals, depending on genera (*Triticum* vs. *Hordeum*), species (*T. aestivum* vs. *T. turgidum*) and cultivar (e.g. long vs. short awns) (Blum, 1985). The presence of awns increases the photosynthetic area of the ear (30–50%, depending on the genotype; see Ali et al., 2010 and references cited therein) and the photosynthetic rate per organ. Another advantage of awns, compared to the flag leaf, is that their chloroplasts remain intact and active during the grain filling. By contrast, chloroplasts in flag leaves are almost completely degraded at the dough-development and ripening stage (Li et al., 2006). A delayed senescence has been reported also for other organs of the ear (Martinez et al., 2003).

Although the photosynthetic role of awns of cereals has been studied for a long time (e.g. in barley see Jiang et al., 2006; Li et al., 2006; Olugbemi et al., 1976), its contribution to grain filling is not known, and even controversial (e.g. Mc Kenzie, 1972; see Tambussi et al., 2007 and references therein). The presence of long awns on

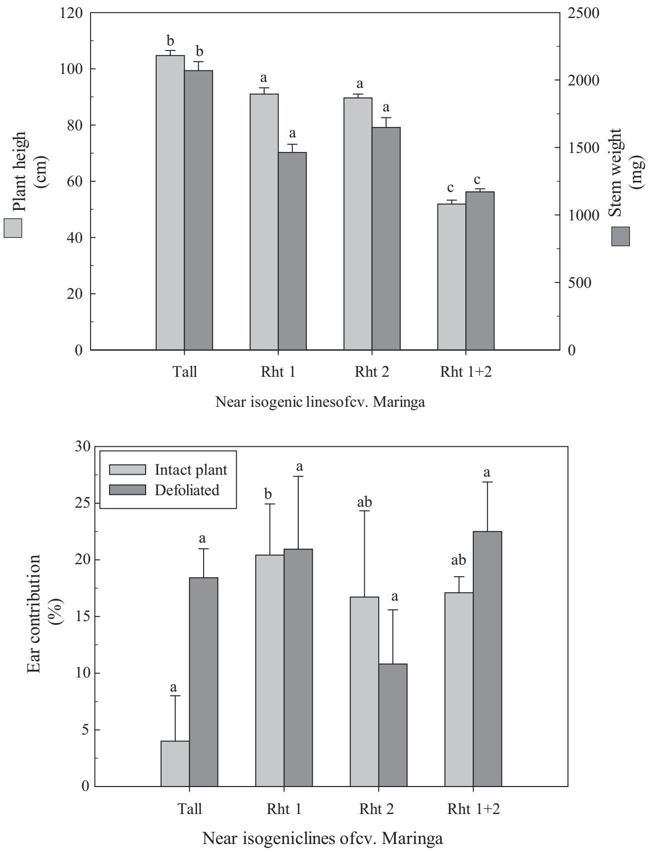

Fig. 7. *Upper panel:* Plant height (light gray bars) and stem dry weight (near anthesis; dark gray bars) of the four near-isogenic lines of cv. Maringa differing in *Rht* alleles: the standard height (*rht1 rht1 rht2 rht2*; referred to as 'tall'), two semidwarf (*Rht1 Rht1 rht2 rht2* and *rht1 rht1 Rht2 Rht2*; referred to as '*Rht1*' and '*Rht2*' respectively) and a dwarf line (*Rht1 Rht1 Rht2 Rht2*; referred to as '*Rht* 1 + 2'). Each value represents the mean ± s.e. of three replicates (=plots) per treatment. In each plot, six ears were analyzed and averaged. For each line, different letters indicate significant differences between groups ($p \leq 0.05$) according to the LSD test. For more details see Section 2. *Lower panel*: Ear contribution to grain filling in four near-isogenic lines of cv. Maringa (see legend in the upper panel) in intact (light gray bars) and defoliated (dark gray bars) plants. Ear contribution (as %) was calculated as in Fig. 5. Each value represents the mean ± s.e. of three replicates (=plots) per treatment. In each plot, six ears were analyzed and averaged. For each bar, different letters indicate significant differences between groups ($p \leq 0.05$) in each treatment (intact and defoliated plant) according to the LSD test. Between intact vs. defoliated plants, only in the 'tall' line the differences between means were significant ($p \leq 0.05$). For more details see Section 2.

the spike is part of the current wheat ideotype, in particular for drought conditions (Reynolds and Tuberosa, 2008), but there is no clear evidence of a correlation between awn size and ear contribution to grain filling. For instance, although Ali et al. (2010) found a decrease of total grain weight in de-awned ears, there is no association between cultivar differences in awn size and percentage ear contribution to grain filling. In fact, there are reports where the presence of awn has a positive effect on grain yield (Motzo and Giunta, 2002) and others where it is detrimental (Mc Kenzie, 1972). In the retrospective study presented here, the correlation between awn size of the cultivars and ear contribution to grain filling was not strong, although this could be explained by the limited range of variability of awn size in the historical set of cultivars employed here.

Thus, although awns size could be relevant for total ear photosynthesis (see Tambussi et al., 2007), the results of the retrospective analysis suggest that the increase in the contribution of the ear to grain filling during this breeding period cannot be explained only by this trait (i.e. awn length and mass). Assimilation in the body of the ear (glumes, lemmas, green pericarp; including both fixation of atmospheric CO_2 and re-fixation of respired CO_2) could be involved (see Tambussi et al., 2007). Further research is needed to solve this question.

4.4. *Concluding remarks*

In this paper we show that: (1) the photosynthetic contribution of the ear to grain filling of bread wheat increased during the breeding period 1920–2008 in Argentina; (2) there is a strong negative correlation between ear contribution and stem dry weight of the cultivars released in this period; (3) the introduction of *Rht* alleles could be involved in this increase of ear contribution to grain filling, although our data are not conclusive in this regards; (4) the increase of ear contribution to grain filling in our retrospective analysis does not seem to be explained only by an increase in awn size during this period. Other traits (e.g. photosynthetic re-fixation in the body of the ear) could be involved and further research is needed to elucidate this question.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2012.06.008.

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