



Are awns truly relevant for wheat yields? A study of performance of awned/awnless isogenic lines and their response to source–sink manipulations

R. Sanchez-Bragado^{a,b}, J.W. Kim^a, C. Rivera-Amado^b, G. Molero^b, J.L. Araus^c, R. Savin^a, Gustavo A. Slafer^{a,d,*}

^a Department of Crop and Forest Sciences, University of Lleida – AGROTECNIO Center, Av. R. Roure 191, 25198 Lleida, Spain

^b Global Wheat Program, International Maize and Wheat Improvement Centre (CIMMYT), Texcoco, Mexico

^c Secció de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Barcelona and AGROTECNIO Center, Lleida, Spain

^d ICREA, Catalanian Institution for Research and Advanced Studies, Barcelona, Spain



ARTICLE INFO

Keywords:

Awns
NILs
Defoliation
Degraining
Source-sink

ABSTRACT

The introgression of awns as a source of photoassimilates during grain filling has received long been of particular attention in wheat. Although the benefits for average grain weight (AGW) seem consistent, those for yield still remain unclear, and the causes for the improved AGW have not been examined. In this study we carried out three field experiments. In two of them, one in CIMMYT (Mexico) and the other in Lleida (Spain), we compared awned-awnless near isogenic lines (NILs) subjected to defoliation and degraining during the effective period of grain filling, whilst in another experiment we analysed the responses of modern cultivars to awn detachment 10 days after anthesis under two nitrogen levels. There were not consistent differences in yield between the awned-awnless NILs neither across the locations nor across genetic backgrounds. This lack of effect on yield was associated with a compensatory effect of the presence of awns on decreasing grain number while increasing AGW. The manipulation treatments did not consistently affect AGW neither in the NILs nor in the modern cultivars. Focusing on the responses of the NILs, firstly there were some statistically significant responses in AGW but they were both mostly very small and inconsistent between defoliation and degraining treatments as well as between genetic backgrounds. Secondly these responses failed to be consistently more noticeable in the awnless than in the awned lines (as expected if the presence of awns increases AGW through increasing photosynthetic capacity during grain filling). The increase on AGW due to the presence of awns seemed ascribed to both an indirect and a direct effect. The former would simply reflect the consequence of awns reducing grain number: this would naturally be the consequence of an increased failure of distal florets to become fertile which would have reduced the proportion of grains constitutively smaller as well. A direct true effect on the capacity of the grains to grow also emerged with the analysis of individual size of each particular grain for the awned-awnless NILs revealing a constitutively higher potential size in the awned NILs, even for the largest grains.

1. Introduction

Plant adaptation has developed the presence of awns in many wild grasses to prevent herbivory and to induce the motility required for seed dispersal (Elbaum et al., 2007; Li et al., 2010). Wheat domestication first and human selection later developed wheat plants with shorter, or even without, awns (Mach, 2015). Despite that domestication in some environments went against awns (not having sense to avoid herbivory or benefit seed dispersal in an agricultural condition), it seems possible that they may bring about other advantages for the crop. Indeed, modern wheat breeding favoured the presence of awns in

many regions, particularly under stress conditions (Guo and Schnurbusch, 2016), suggesting they may contribute to yield through adding photosynthetic and carbohydrate storage capacities (Evans and Rawson, 1970; Weyhrich et al., 1995; Li et al., 2006; Tambussi et al., 2007). There have been several studies reporting on the benefits of awns based on unrelated genotypes (Evans et al., 1972; Knott, 1986; Li et al., 2006; Elbaum et al., 2007; Maydup et al., 2014), but such studies may always carry confounding effects. Direct assessments of the role of awns in grain weight and yield determination using near isogenic lines (NILs) are scarce. Indeed, not only are the positive or negative effects of awns on wheat yield scarcely reported but also the possible causes for

* Corresponding author at: Department of Crop and Forest Sciences, University of Lleida - AGROTECNIO Center, Av. R. Roure 191, 25198 Lleida, Spain.

E-mail addresses: ruth.sanchez@udl.cat (R. Sanchez-Bragado), jin.kim@udl.cat (J.W. Kim), A.RIVERA@cgiar.org (C. Rivera-Amado), G.Molero@cgiar.org (G. Molero), jaraus@ub.edu (J.L. Araus), roxana.savin@udl.cat (R. Savin), gustavo.slafer@udl.cat (G.A. Slafer).

<https://doi.org/10.1016/j.fcr.2020.107827>

Received 9 December 2019; Received in revised form 25 April 2020; Accepted 26 April 2020

0378-4290/ © 2020 Elsevier B.V. All rights reserved.

such claims are not well known, and arguments about their cost-benefit are still ongoing (Guo and Schnurbusch, 2016). Some studies reported that presence of awns could be either positive (Martin et al., 2003; Weyhrich et al., 1994) or negative (McKenzie, 1972) for wheat yield, depending on growing conditions (Knott, 1986; Motzo and Giunta, 2002; Maydup et al., 2010) and genotypes (Martin et al., 2003). In a study developed by Rebetzke et al. (2016) with NILs, the presence of awns was associated to larger average grain size (confirming previous studies: Olugbemi and Bush, 1987; Bort et al., 1994; Weyhrich et al., 1995) though not necessarily increasing yield as grain number was reduced. Reduction in grain number was interpreted to be related to the cost of assimilates destined to awn development (Rebetzke et al., 2016). Therefore, spike fertility would be decreased (Bort et al., 1994) due to a reduction in assimilate allocation to the development of floret primordia (Guo and Schnurbusch, 2016). This would be in turn compatible with the fact that development of labile florets is strongly sensitive to availability of resources (Ferrante et al., 2013a; Guo et al., 2016; Guo and Schnurbusch, 2015). The negative effects of awns on spike fertility seemed in line with a negative correlation between dry matter allocated in the awns and fruiting efficiency found at CIMMYT, while no effects were observed with biomass allocated in other parts of the spike (Sierra-Gonzalez, Molero, Rivera-Amado, Reynolds and Foulkes; unpublished data). On the other hand, the presence of awns has been observed to increase the spike photosynthetic area up to 50% (Blum, 1985; Motzo and Giunta, 2002) potentially increasing crop photosynthesis and light interception, although this may depend on awn length (Maydup et al., 2014), and functionality (Motzo and Giunta, 2002). Indeed, awns, when present, seem to be a major contributor to spike photosynthesis (Evans and Rawson, 1970; Li et al., 2006, 2010; Maydup et al., 2014; Tambussi et al., 2007).

It has been postulated that grain growth of wheat is often limited by sink strength (Slafer and Savin, 1994a; Borrás et al., 2004), as the availability of assimilates seems to be in excess compared with the sink capacity to store them (Borrill et al., 2015; Reynolds et al., 2005; Serrago et al., 2013) and potential grain weight is determined prior to grain filling (Calderini et al., 2001; Fahy et al., 2018; Xie et al., 2015). That is why breeding has improved yield through consistently increasing sink strength (Calderini et al., 1997; Foulkes et al., 2007; Reynolds et al., 2009; Shearman et al., 2005). However, precisely for that consistent improvement in sink strength, more modern cultivars and elite lines might have become to a certain extent source-limited. Indeed, it has been recurrently suggested that some degree of source limitation could be emerging in elite wheat germplasm (Acreche and Slafer, 2011; Álvaro et al., 2008; Kruk et al., 1997; Pedro et al., 2011; Serrago et al., 2013). Thereby, should there be a shortage of photo-assimilates during post-anthesis to maximize grain filling, the presence of awns increasing the source-strength of the spikes would be relevant. This would be the most common explanation for the heavier grains in the awned than in the awnless lines (Maydup et al., 2014; Motzo and Giunta, 2002). In addition to the overall extra photosynthetic capacity provided by awns, they would feed grains from their actual photosynthesis during grain filling more efficiently than leaves due to closeness, implying a minimal pathway for carbon movement (Evans et al., 1972).

However, the presence of awns may affect grain size independently of their contribution as a source of photoassimilates during grain filling. As awn and floret development overlap in the juvenile spikes before anthesis, awn growth might affect not only floret fertility but also potential grain size (Mitchell et al., 2013, 2012; Schaller and Qualset, 1975), which is partly determined by the size of the carpels (Calderini et al., 1999; Hasan et al., 2011; Xie et al., 2015). In this context, evaluation of the causes for any possible effects of awns on grain weight would require not only the determination of grain size in awned/awnless NILs but also a quantification of the sensitivity of grain size in these contrasting NILs to manipulation of source- and sink-strengths during grain filling. In wheat, studies of the response of grain weight to

manipulations of source-sink balances have been deployed in the past to determine whether or not there is source-limitation for grain filling. Fischer and HilleRisLambers (1978) first showed that these techniques were complementary, and that there were consistent cultivar and year effects, which could be explained by physiological inferences. Thereby, manipulation treatments such as defoliation (Ahmadi et al., 2009; Kruk et al., 1997), and degreasing (Calderini et al., 2006; Serrago et al., 2013; Slafer and Savin, 1994b) have been shown to be suitable to quantitatively estimate the responses of grain weight to different source-sink balances. But to the best of our knowledge the response of grain size to such manipulations in awned/awnless NILs has never been done. The true relevance of awns as a critical source of assimilates to fill the grains could be complemented by analyzing the response of grain weight to awns removal at the onset of grain growth, when any effects of awns on grain number or potential grain size would interfere on final average grain weight. Again, we are not aware of such manipulation studies within the mainstream literature in modern elite wheat germplasm.

The main objective of this study was to quantify the influence of awns on grain weight of elite germplasm of wheat; for which we determined under field conditions the agronomic components of yield in (i) two NILs for the presence and absence of awns in two contrasting conditions in terms of growing temperature, (ii) a set of six NILs with different genetic background, and (iii) four modern high-yielding cultivars to which awns were left unmanipulated or removed during the effective period of grain filling. In addition, we tested if the cause of any effect of awns on grain weight was due to improved source of photo-assimilates for grain filling. This was possible through the quantification of the grain weight response in awned and awnless NILs to defoliation and degreasing treatments imposed at the onset of grain filling, complemented with the analysis of differences in individual grain sizes between awned and awnless NILs.

2. Materials and methods

2.1. General conditions

We carried out three field experiments, one of them in the 2017/2018 growing season at CIMMYT's Experimental Station, Norman E. Borlaug (CENEB), near Ciudad Obregon, Mexico (27°24' N, 109°56' W, 38 m asl), while the other two in farmers' fields close to Bell-lloc d'Urgell (41°38' N, 0°47' E, 196 m asl), in Lleida, Catalonia, North-East Spain, in the 2017/2018 and 2018/2019 growing seasons (Table 1). The soil in Obregon was a typical calciorthid (Sayre et al., 1997), with a plant-available water-holding capacity of about 200 mm (Lopes and Reynolds, 2010). The soil in Lleida was a typical Calcixerept fine-loamy over sandy skeletal, mixed and thermic (FAO, 1990).

The experiments were sown on 23 November 2017 in Obregon (with a seed density of 110 kg ha⁻¹), and on 17 November 2017 (seed density was deliberately high, 280 kg ha⁻¹, but then seedlings were thinned to leave a uniform plant density of 250 plants m⁻²) and 20 December 2018 in Lleida (seed density of 124 kg ha⁻¹). All experiments were irrigated and fertilised (Table 1). Fertilisation included N and P in Obregon but only N in Lleida where fields had high levels of soil P. Experiment plots were kept free of weeds, insect pests, and disease by recommended chemical measures.

Weather conditions were rather contrasting between Obregon and Lleida (Fig. 1). Disregarding differences in rainfall (as experiments were irrigated to avoid water stress), as expected by their latitudes Obregon during the growing season was warmer than Lleida. Consequently, the photothermal quotient (PTQ, here simply calculated as the ratio between solar radiation and mean temperature) was always higher in Lleida than in Obregon.

Table 1

Description of the experiments of the study, including conditions, treatments and genotypes.

Year	Location	Irrigation	Fertilisation	Treatments		
				Genotypes	N application	Source-sink
2017/2018	Obregon	Fully irrigated (500 mm in total), last 11 days after anthesis	200 kg N ha ⁻¹ 50 kg P ha ⁻¹	NILs Janz + ^a Janz –	No N treatment	Unmanipulated Defoliation Degraining
2017/2018	Lleida	Supplementary irrigated once at anthesis (80 mm)	Different N treatments	MarcoPolo + Elite lines Avelino + Pistolo + Ingenio +	N0 = Unfertilised N1 = 200 kg N ha ⁻¹	Unmanipulated Awns detached
2018/2019	Lleida	Supplementary irrigated twice at tillering (80 mm) and at anthesis (80 mm)	200 kg N ha ⁻¹	NILs Frame + Frame – Janz + Janz – Westonia 2 + Westonia 2 – Westonia 4 + Westonia 4 – Westonia 8 + Westonia 8 – LSP + ^b LSP –	No N treatment	Unmanipulated Defoliation Degraining

^a + = awned; – = awnless.^b LSP = Large spike line developed at CIMMYT.

2.2. Treatments and experimental design

All experiments included a factorial combination of genotypes and source-sink manipulations during grain filling, and in one of them there was an additional treatment of contrasting N availability (Table 1). The experiment in Obregon comprised the factorial combination of two genotypes (NILs varying for the presence and absence of awns in the background of Janz, a commercial wheat cultivar from Australia) and three source-sink manipulations (an unmanipulated control, a de-grained and a defoliated treatment, see below). The experimental design was a split plot with genotypes assigned to main plots and manipulations to subplots; main plots were arranged in a randomised α -lattice with four replicates on raised beds with 0.8 m wide (comprising two adjacent beds per plot), two rows per bed (0.24 m between rows) and 4 m long accounting for a total plot area of 6.4 m². The experiment in Lleida 2017/2018 comprised the factorial combination of four different awned elite lines (all commercial cultivars), two contrasting levels of soil N (unfertilised, N0 and fertilised with 200 kg N ha⁻¹, N1) and two source-sink manipulations (an unmanipulated control and a treatment in which awns were removed at the onset of grain filling, see below) (Table 1). The experimental design was a split plot with genotypes and N fertilisation assigned to main plots and manipulations to subplots; main plots were arranged in a randomised complete block

design with three replicates, with plots of 4.8 m²: 1.2 m width (six rows, 0.2 m apart) and 4 m long. The experiment in Lleida in 2018/2019 replicated that in Obregon the year before (although in Lleida plots were in flats instead of beds) (i.e. NILs $\times$ source-sink manipulations), though expanding the genotypes to 12: six pairs of NILs, one pair was the same used in the previous experiment and the other five had other genetic backgrounds, all elite germplasm (four from Australian commercial cultivars: Frame and Westonia; and the fifth was an advanced line developed at CIMMYT in the 1990s characterized for having a large spike phenotype; LSP) (Table 1). The experimental design was a split plot with genotypes assigned to main plots (arranged in a randomised complete block design with three replications) and the three source-sink manipulations to sub-plots (Table 1). Main plots (4.8 m²) consisted of six rows, 0.2 m apart, and 4 m long.

The development of the NILs is explained elsewhere (Rebetzke et al., 2016). Briefly, Rebetzke et al. (2016) initially crossed the awned commercial cultivars with an awnless line, derived from a cross between Halberd and Mara. Then backcrossed to the corresponding commercial parents and thereafter progressed with a single-seed descent for a number of generations. The number following Westonia stands for the filial generation and in the case of Janz it was BC1F4 and in Frame it was BC1F3. The LPS NILs were developed as indicated in Gaju et al. (2009) and are characterized by having a large spike using a

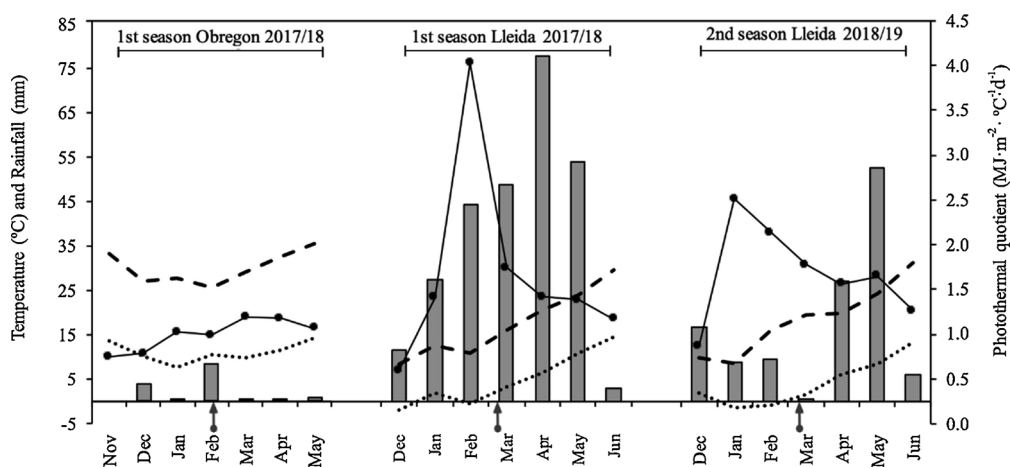


Fig. 1. Monthly accumulated rainfall (bars), mean maximum and mean minimum temperatures (dashed and dotted lines), the mean daily photo-thermal quotient (estimated as the ratio between daily radiation and average day temperature; closed circles, plain lines) and the average anthesis date (grey arrows) at Obregon 2017/2018, and Lleida 2017/2018 and 2018/2019.

combination of backcrossing and recurrent selection from different sources and tiller inhibition gene on chromosome 1AS (Richards, 1988).

Source-sink manipulation treatments were imposed on at least 3 main tillers per subplot (i.e. 9 plants per treatment) in Lleida 2017/2018 and 7 main tillers per subplot (i.e. 21 plants per treatment) in Obregon 2017/2018 and Lleida 2018/2019. These main-tillers spikes represented well those of all tillers of the crop (as it is observed by the high relationship between average grain weight of main shoots versus average grain weight of the whole canopy; $R^2 = 0.84$, $P < 0.001$, Supplementary Fig. S1). In all experiments, source-sink manipulations were imposed in main shoot spikes that were all very similar at anthesis (GS65) when they were selected. For that purpose, at anthesis, 9–63 main shoots (having same height, same degree of anthesis, and same number of spikelets per spike) from central rows of each subplot were labelled, and then randomly assigned to the particular source-sink treatment (i.e. each particular source-sink treatment had a minimum of 3 spikes within each plot; a minimum of 9 spikes per genotype). In all experiments the imposition of these treatments was 10 days after anthesis. The removal of awns 10 days after anthesis was carried out in order to ensure the grain number per spike (GNS) and potential grain size were already fixed. In Lleida 2017/2018 these treatments were (i) awns removed, where the awns were clipped at the top of each lemma on every spikelet, or (ii) left unmanipulated as controls (Supplementary Fig. S2). In Obregon 2017/2018 and Lleida 2018/2019, treatments were either (i) defoliated (flag-leaf and leaf-two laminae removed), (ii) degrading, (removing all spikelets vertically along one side of the spike), or (iii) left unmanipulated as controls (Supplementary Fig. S2).

2.3. Sampling and measurements

Phenological stages were determined according the decimal code developed by Zadoks et al. (1974). All labelled spikes were harvested at physiological maturity, threshed and the grains counted and weighed. Average grain weight (AGW) was determined after oven drying them for 48 h at 65 °C in Lleida and 70 °C in Obregon. In Lleida 2017/2018, in addition we determined the weight of the proximal grains in particular. For this purpose, before threshing the spikes, we separated the two grains most proximal to the rachis (G1 and G2) in each spikelet and weighed them separately. With this procedure we could determine whether the response of AGW mimicked that of proximal grains or otherwise, being these proximal grains potentially larger than, and dominant over, the other more distal grains in each spikelet.

At maturity, in Lleida 2017/2018 and 2018/2019 a sample of 1.0 m long was taken from a central row of each experimental unit (20th and 25th of June in 2018 and 2019, respectively). As we were going to have a relatively small sample we used a protocol to minimize the noise that could be expected in small samples: soon after seedling emergence plots were inspected and 1 m long areas within inner rows (discarding border rows) were labelled for guaranteeing that the plant density and uniformity were those ideally expected both in the sample area and in its borders. Plants were cut at the soil level and taken to the lab for processing. The spike was separated from the stem and leaves and threshed, after oven-dried all tissues for 48 h at 65 °C and yield components were determined. These were the number of grains per unit land area (GN), in turn composed by the number of spikes per m² (SN) and the number of grains per spike (GNS), and the AGW. In Lleida 2018/2019 after determining AGW we scanned all the grains with the Marvin 5.0 optical seed analyser (Marvitech, GTA Sensorik GmbH, Germany) and therefore obtained morphometric measurements (width, length, and area) for each individual grain of the sample. With this it could be seen whether the effect of awns on AGW operates through affecting the size of the whole, or only a part, of the population of grains of the crop. In Obregon 2017/2018 for each plot at maturity, yield components were determined in the plot area using standard protocols (Pask et al., 2012).

2.4. Statistical analysis

Yield and agronomic components were subjected to one-way analysis of variance (ANOVA) using the general lineal model to calculate the effects of treatments, genotype, and their interaction on the studied variables. Means were compared by Tukey's HSD test and were performed on a combination of source-sink treatments and genotype. A bivariate correlation procedure was constructed to analyse the relationships between the measured traits. Frequency distribution of the grain area was calculated considering the individual area of each particular grain. Normal distribution was adjusted for the area of each individual grain within each genetic background of awned (+) and awnless (−) counterparts. Descriptive statistics were estimated, variance analysed, and bottom and top deciles calculated using the SPSS 18.0 statistical package (SPSS, Chicago, IL, USA).

3. Results

3.1. Effects of awns in Janz background in Obregon and Lleida

Yield was virtually the same in the awned and awnless NILs in Obregon, while it tended to decrease with the absence of awns in Lleida (Fig. 2), the difference was not statistically significant. Lack of a clear effect on yield was due to the fact that yield components were conflictingly affected by the presence of awns. While AGW significantly increased with the presence of awns both in Obregon (c. 27 mg grain^{−1} awnless vs. c. 29 mg grain^{−1} awned) and, particularly, in Lleida (c. 32 mg grain^{−1} awnless vs. c. 41 mg grain^{−1} awned), GN tended to decrease in both locations (from c. 14,000 to c. 13,500 grains m^{−2} in Obregon, and from c. 18,800 to c. 17,700 grains m^{−2} in Lleida) (Fig. 2). The trend to a reduction in GN produced by the presence of awns was due to a parallel trend to reducing GNS (Fig. 2).

To test whether the presence of awns would have increased AGW through its contribution of resources for grain filling, we studied the responses to source-sink manipulations (defoliation and degrading) during grain filling. In Obregon, grain weight was slightly more reduced in response to defoliation in the awnless in comparison with the awned NIL (Fig. 3, lower panel). On the other hand, in the response to degrading grain weight of the remaining grains increased more in the awned than in the awnless NILs (Fig. 3, upper panel). In Lleida, AGW slightly decreased in response to defoliation but this decrease was significant only in the awned NILs (Fig. 3, lower panel). Degrading did slightly increase the weight of the remaining grains in the awnless while not in the awned NIL (Fig. 3, lower panel).

3.2. Effects of awns across different genetic backgrounds

Yield was not significantly affected by the presence/absence of awns in any of the different backgrounds (Table 2). And non-significant trends in yield were erratic, depending on the particular background: while awned NILs tended to increase yield in the background of Janz, awns did not produce any noticeable trend in Frame, Westonia2 and Westonia8, and even tended to decrease yield in Westonia4 and LSP. Again, the lack of a clear effect on yield was due to a trade-off on yield components. Indeed, presence of awns showed a significant effect in AGW and GN (Table 2), whereas the interaction between the genetic background and the presence of awns was not significant or negligible (GN and its components) compared with the magnitude of the direct effect of the presence of awns (AGW). Therefore, the presence of awns tended to increase AGW quite consistently across all backgrounds, being the difference statistically significantly between NILs of five out of six backgrounds analysed (Table 2). But this increase in AGW seemed to be compensated by a clear trend (though not statistically significant but across all backgrounds) of awned NILs to decrease GN (though the difference for particular contrasts was only statistically significant in LSP; Table 2), mainly through parallel effects on GNS (again in all NILs,

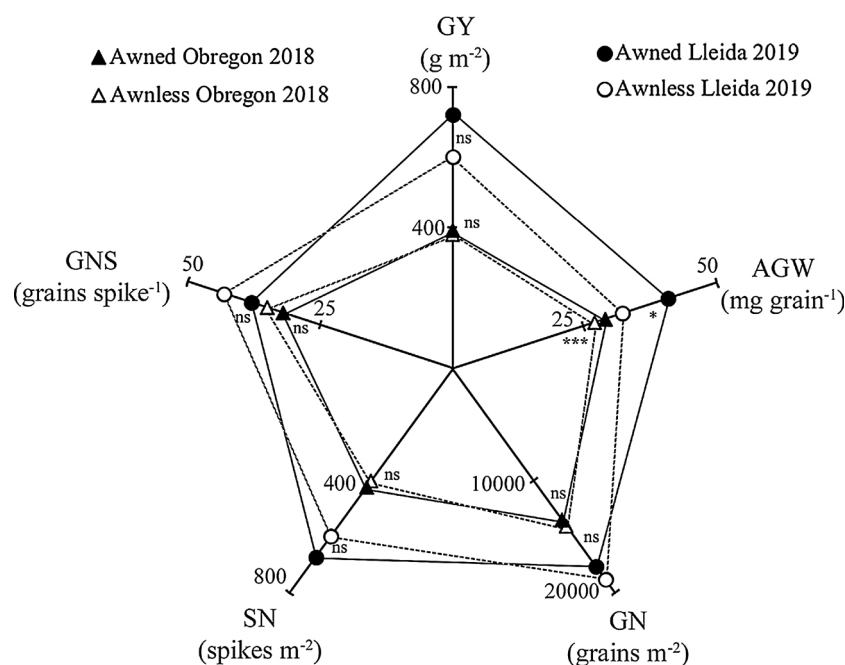


Fig. 2. Radar charts of mean values of the difference between NILs of grain yield (GY), average grain weight (AGW), grain number per unit land area (GN), spike number per unit land area (SN), number of grains per spike (GNS), of the near isogenic lines with or without the presence of awns (awned and awnless, respectively) in the background of Janz grown under good agronomic conditions in Obregon (2017/2018, triangle symbol) and in Lleida (2018/2019, circle symbol). Open symbols with dashed lines and closed symbols with continuous lines represent awnless and awned NILs, respectively. Level of significance expresses the statistical significance between awned and awnless NILs in each location, *** $P < 0.001$; ** $P < 0.05$; ns, $P > 0.05$.

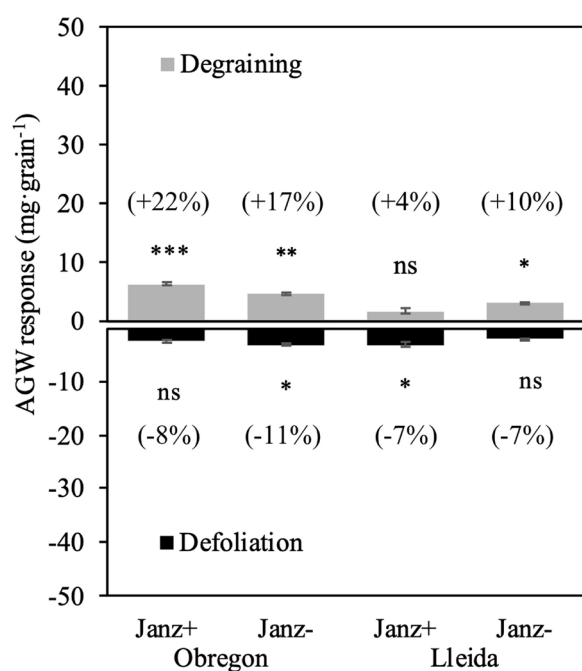


Fig. 3. Response to degrading (upper panel) and defoliation (lower panel) of average grain weight (AGW) in the near isogenic isolines with or without awns in the background of Janz (JA4+ and JA4-, respectively) grown under good agronomic conditions in Obregon (2017/2018) and Lleida (2018/2019). Response was the difference in grain weight in the manipulated spikes (either degraded or defoliated) and in the unmanipulated control. Segments on each bar stand for the standard error of the means. Values between brackets are the AGW increase (+) or decrease (-) of the treatment relative to control expressed as percentage. Level of significance: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, $P > 0.05$.

with the exception of those with the background of Frame and Westonia 4 in which the awned lines had less spikes m^{-2}).

The response on AGW to the presence of awns was strongly associated with GNS of the awnless genotype (Fig. 4, left panel), whereas no correlation was observed with the size of the grains (Fig. 4, right panel).

The consistent effect of awns on increasing AGW across genetic

backgrounds could again be interpreted as grain growth in awnless NILs being source-limited (or more source-limited than in awned NILs.). Considering the overall responses to source-sink manipulations during grain filling, there were some interactions between NILs and manipulation treatments but the magnitude of the interactions was negligible compared to the direct effect of the NILs (mean squares for NILs $\times$ manipulations and Background $\times$ NILs $\times$ manipulations were $< 2\%$ of the mean squares for the direct effects of the NILs; F-ratios of 4.2, 1.8 and 231.5, respectively; Supplementary Table S1). Although the mean square for the source-sink manipulations was significant, a large proportion of that variation came from the comparison of degraded vs. defoliated treatments. Analysing the responses respect to the unmanipulated control within each pair of NILs, the differences were therefore mostly not significant and in general rather small, relative to the magnitude of the treatments imposed. Averaging across all genotypes AGW was decreased by only $3.4 \pm 0.7 \text{ mg grain}^{-1}$, equivalent to an $8.1 \pm 1.3\%$ respect to the control, in response to defoliation, and increased by only $2.3 \pm 0.7 \text{ mg grain}^{-1}$, equivalent to an $5.1 \pm 1.5\%$ respect to the control, in response to a potential doubling of assimilate availability per grain (after halving the spikes). Thus, there were no clear indications that grain growth in the controls had been severely restricted by the availability of resources for grain filling; and even less that the presence of awns would have consistently increased grain growth respect to the awnless NILs due to reducing that limitation (Fig. 5). For instance, lines with the background of LSP did not show significant responses of AGW to defoliation or degrading, and the non-significant trends had similar magnitude in the awned and awnless NILs (Fig. 5). The presence of awns in the background of Westonia2 did not alleviate any source limitation that could have been responsible for the lower AGW in the awnless NIL, as the only significant (though minor) responses occurred in the awned lines (Fig. 5). NILs with the background of Frame, and more clearly those with the background of Westonia4 and Westonia8, had AGW that responded more in the awnless than in the awned NILs (Fig. 5), which is compatible with the assumption that the presence of awns would alleviate a certain degree of source-limitation to fill the grains, but only in three out of the six background tested (whilst the increased AGW due to the presence of awns was rather transversal across genetic backgrounds, as shown above; Table 2).

There would be, at least, two alternative explanations for the

Table 2

Grain yield (GY) and yield components, average grain weight (AGW) grain number per unit land area (GN), spike number per unit land area (SN), and number of grains per spike (GNS), of the near isogenic lines with or without the presence of awns in the different backgrounds of Frame, Westonia 2, Westonia 4, Westonia 8, LSP and Janz grown under good agronomic conditions in Lleida (2018/2019). The outputs of the ANOVAs for each trait were included at the bottom of each variable. Bold font was used when the difference between NILs was statistically significant (includes a case in which this was so at $P = 0.054$). Level of significance, *** $P < 0.001$, ** $P < 0.01$, ns $P > 0.05$.

Background	NIL	GY (g m ⁻²)	AGW (mg grain ⁻¹)	GN (grains m ⁻²)	SN (spikes m ⁻²)	GSP (grains spike ⁻¹)
Frame	Awned	600.4	43.8	13,690	463.3	30.0
	Awnless	624.2	43.3	14,402	587.5	24.4
Westonia2	Awned	527.8	47.5	11,069	413.3	25.1
	Awnless	575.2	43.1	13,355	415.0	32.5
Westonia4	Awned	602.8	43.1	14,077	441.3	45.3
	Awnless	709.8	39.7	17,935	475.3	40.4
Westonia8	Awned	640.2	48.3	13,159	422.0	31.0
	Awnless	649.2	44.0	14,775	402.3	33.9
LSP	Awned	660.8	53.1	12,652	291.7	43.3
	Awnless	751.7	40.0	18,797	360.5	54.3
Janz	Awned	721.7	40.9	17,658	673.0	38.1
	Awnless	602.3	32.2	18,810	599.0	43.2
Average	Awned	625.6	46.1	13.7	450.8	35.5
	Awnless	647.9	40.2	16.3	473.2	38.0

Source of variation			Mean squares		
Background (B)	15,234 ^{ns}	83***	25,740,153 ^{ns}	63,622***	387**
Presence of awns (A)	5814 ^{ns}	273***	57,386,365*	4,206 ^{ns}	59 ^{ns}
B × A interaction	9,541 ^{ns}	25***	5,293,607 ^{ns}	6,446 ^{ns}	59 ^{ns}
Error	18,381	5	12,880,442	6,356	82

presence of awns increasing AGW, which need not to be mutually exclusive. Firstly, the reduction in GNS (that also occurred as a consequence of introgressing awns in an awnless background with reasonable consistency; Table 2) would have naturally reduced the proportion of grains that are of a smaller potential size (as reduction of grain number is normally at the expense of reducing fertility of distal florets, and these florets produce distal grains that are constitutively smaller than the proximal ones). Secondly, there could be a pleiotropic effect of awns on the actual potential size of the grains. The analysis of the size (area) of each individual grain would shed light on this issue. Naturally the average area of the grains was highly correlated with the AGW ($R^2 = 0.86$, $P < 0.001$; Supplementary Fig. S3).

The distribution of individual grain areas showed, smaller mean grain areas for the awnless than for the awned NILs, consistently across

genetic backgrounds Fig. 6 and Table 3). In all backgrounds, there was an increase in the average grain area of the top decile (i.e. the largest 10% of the grains was consistently larger in the awned than in the awnless NILs) (Fig. 6 and Table3). In general, it seems there was a direct, pleiotropic, effect of the presence of awns on the potential size of the grains: largest grains are normally those corresponding to the proximal positions within the central spikelets and the fertility of florets in that position would never be jeopardized when the number of grains is affected. If these grains differ, it would mean that the presence of awns would have improved the potential size of the grains. The exception in the present study would be Frame, in whose background the size of the largest grains was not affected by the presence of awns. Nevertheless, the increase in average size of the grains in awned NILs would be likely due to the simple effect that when reducing the fertility

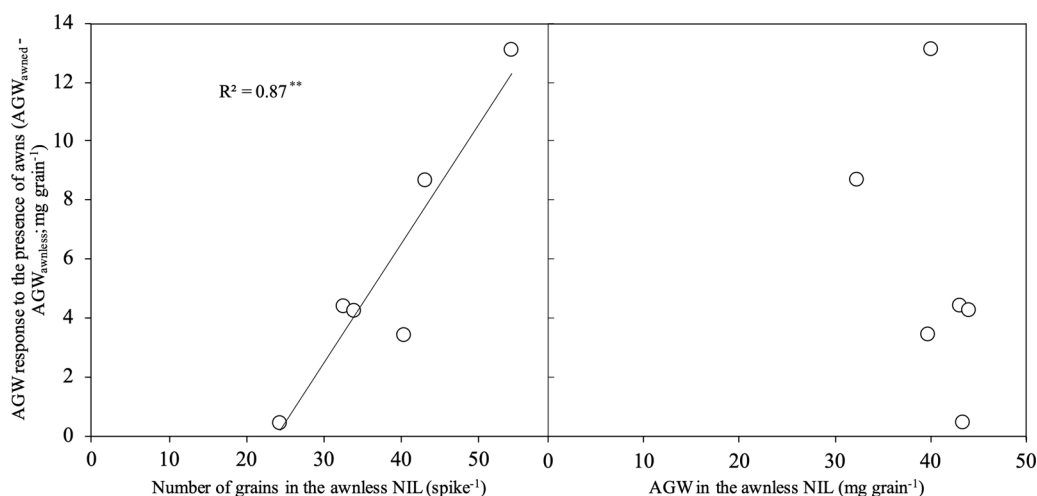


Fig. 4. Relationship between the response of the average grain weight (AGW) to the presence of awns and either the number of grains per spike (left panel) or the AGW (right panel) in the awnless sister line across the NILs with and without awns on six different backgrounds (Janz, Frame, Westonia2, Westonia4, Westonia8 and LSP) grown under good agronomic conditions in Lleida (2018/2019).

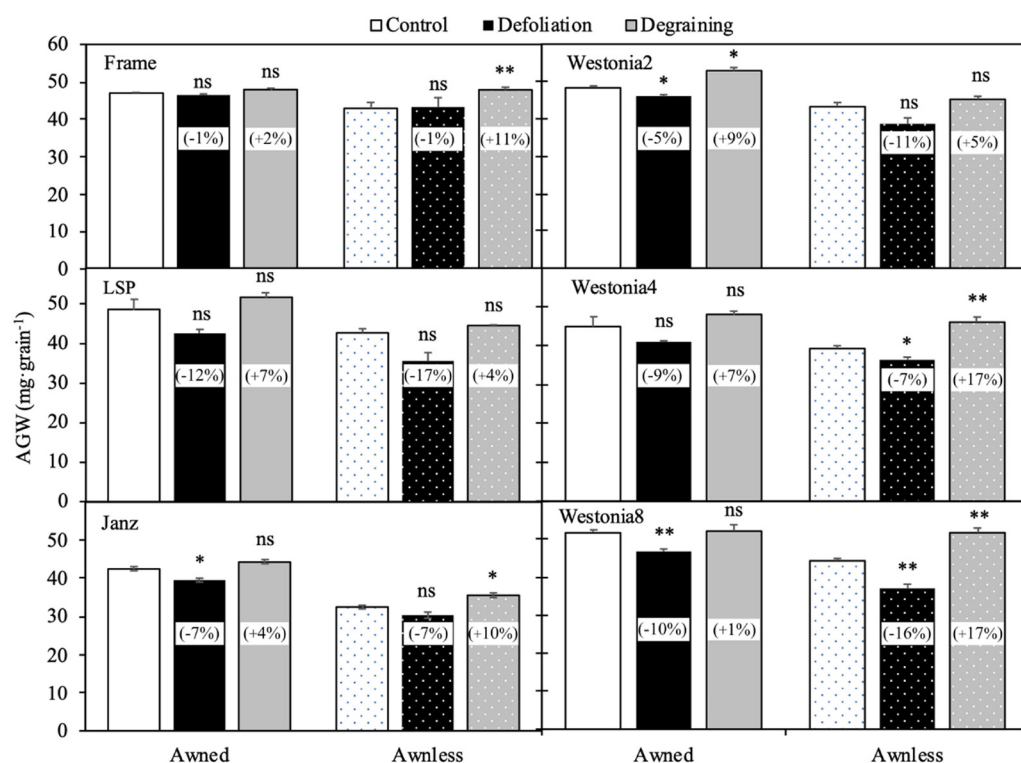


Fig. 5. Mean values of average grain weight (AGW) in control un-manipulated treatments and in plants treated with defoliation (laminae from the flag and penultimate leaves removed) or with degreasing (all spikelets removed along one side of the spike) of the near isogenic isolines with or without the presence of awns (awned and awnless, respectively) in the backgrounds of Frame, Westonia2, Westonia4, Westonia8 LSP and Janz grown under good agronomic conditions in Lleida (2018/2019). Segments on each bar stand for the standard error of the means. Values between brackets the AGW increase (+) or decrease (-) of the treatment relative to control expressed as percentage. Levels of significance, ** $P < 0.01$; * $P < 0.05$; ns, $P > 0.05$.

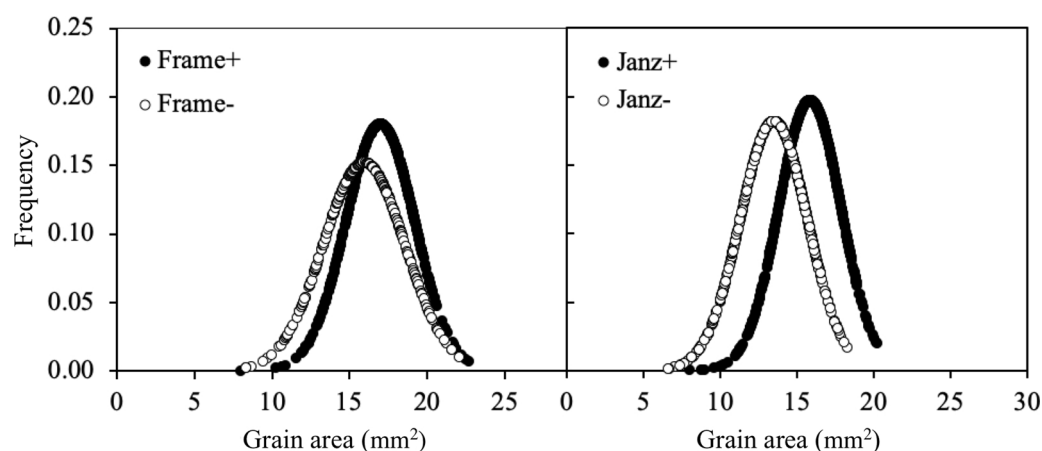


Fig. 6. Frequency distribution of grain area in the genetic backgrounds of Frame and Janz of awned (+) and awnless (-) counterparts grown under good agronomic conditions in Lleida (2018/2019).

Table 3

Average grain area ($\pm$ standard error) of all individual grains (Mean) and of the bottom (10% smallest) and top (10% largest) deciles for grain area of the near isogenic lines with or without awns in the different backgrounds of Janz, Frame, Westonia 2, Westonia 4, Westonia 8 and LSP grown under good agronomic conditions in Lleida (2018/2019).

	Janz		Frame		LSP	
	Awned	Awnless	Awned	Awnless	Awned	Awnless
Mean (mm ²)	15.89 $\pm$ 0.08	13.54 $\pm$ 0.09	17.01 $\pm$ 0.10	15.96 $\pm$ 0.14	18.46 $\pm$ 0.10	16.84 $\pm$ 0.12
Bottom decile (mm ²)	11.78 $\pm$ 0.14	9.24 $\pm$ 0.11	12.91 $\pm$ 0.16	11.18 $\pm$ 0.20	12.14 $\pm$ 0.15	9.71 $\pm$ 0.15
Top decile (mm ²)	18.89 $\pm$ 0.07	16.41 $\pm$ 0.07	20.39 $\pm$ 0.11	20.14 $\pm$ 0.13	23.07 $\pm$ 0.09	21.07 $\pm$ 0.08
	Westonia 2		Westonia 4		Westonia 8	
	Awned	Awnless	Awned	Awnless	Awned	Awnless
Mean (mm ²)	17.52 $\pm$ 0.08	16.59 $\pm$ 0.08	16.14 $\pm$ 0.10	15.20 $\pm$ 0.07	18.08 $\pm$ 0.09	16.73 $\pm$ 0.11
Bottom decile (mm ²)	14.01 $\pm$ 0.20	12.70 $\pm$ 0.18	10.79 $\pm$ 0.15	11.15 $\pm$ 0.13	13.66 $\pm$ 0.18	10.76 $\pm$ 0.15
Top decile (mm ²)	20.67 $\pm$ 0.09	19.54 $\pm$ 0.06	19.60 $\pm$ 0.08	18.17 $\pm$ 0.08	21.38 $\pm$ 0.10	20.51 $\pm$ 0.07

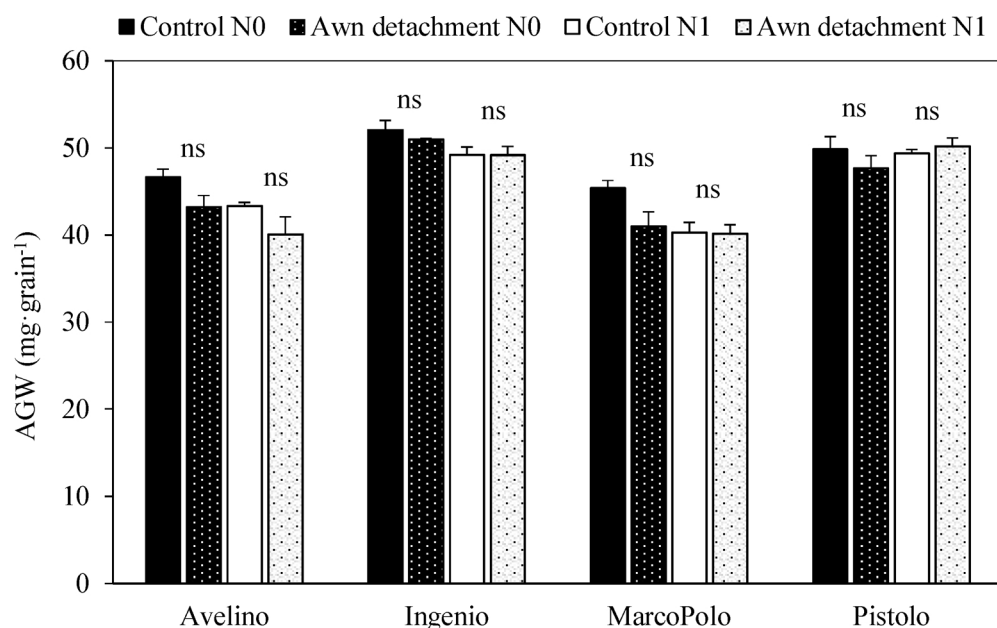


Fig. 7. Average grain weight (AGW) in control unmanipulated spikes and in spikes with awns detached 10 days after anthesis of the four elite lines: Avelino, Ingenio, MarcoPolo and Pistolo grown under two levels of nitrogen, nonfertilised (N0, closed bars) and fertilised (N1, open bars) grown under field conditions in Lleida (2017/2018). Segments on each bar stand for the standard error of the means. Level of significance, ns: $P > 0.05$.

of the spike, the distal florets do not produce grains. Therefore, reduced fertility would indirectly increase the average of all grains by reducing the proportion of potentially small grains from the population (due to the penalty imposed by awns on the number of grains).

3.3. AGW response to awn removal in current commercial cultivars

To further study any specific potential contribution of awns to AGW in absence of any pleiotropic effect on potential grain size or simultaneous changes in GNS we carried out in parallel with the experiment of Obregon another study in Lleida (2017/2018). Although there was a trend to reduce AGW by the detachment of all awns, the reduction was in all cases (i.e. across four cultivars, with clear differences in AGW, grown in two contrasting N regimes) very small and not significant (Fig. 7 and Supplementary Table S2).

Focusing on the response of the proximal grains, data points of the relationship between the responses of proximal or all grains were close to the 1:1 ratio and randomly distributed above-below it (Fig. 8).

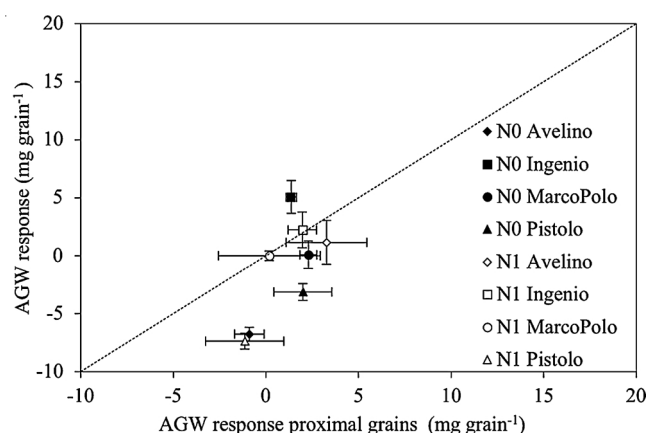


Fig. 8. Relationship between the responses of grain weight to awn detachment 10 d after anthesis averaged across the grains most proximal to the rachis and across all grains (AGW). Responses were in all cases the difference in weight between grains in the spikes with awns removed and those left unmanipulated. Four elite lines Avelino (rhombus), Ingenio (squares), MarcoPolo (circles) and Pistolo (triangles) grown under two levels of nitrogen, nonfertilised and fertilised in the field at Lleida (2017/2018).

Therefore, proximal grains did not respond consistently less than the average of all grains (as it would have been expected if awn removal would have increased the competition for limited resources among growing grains). Indeed, the overall average (across all cultivars and N conditions) response to awn removal was not significantly different from zero for either the proximal or all grains (1.1 ± 0.6 and -1.1 ± 1.5 mg grain⁻¹, respectively).

4. Discussion

The average yield of Janz NILs in Obregon was relatively low (compared with what can be achieved in that location; e.g. Molero et al., 2019) and lower than their yield in Lleida. The relatively low yields observed in Obregon reflects that Janz would not be well adapted to that growing condition (indeed, perhaps the degree of source limitation evidenced in Obregon might also be related to that lack of good agronomic adaptation). The fact that the yield in Lleida was higher is simply the reflection of large differences between these locations in photothermal quotient (balance between radiation and temperature), much lower in Obregon than in Lleida during pre-anthesis and grain filling.

4.1. Comparison with studies elsewhere

Considering all results together, there was no consistent evidences that the presence of awns confers a clear advantage for wheat yield. However, when analyzing the differences between isogenic lines there was a rather consistent effect of awns on increasing AGW across genetic backgrounds and locations. The overall increases in AGW in awned in comparison with awnless isogenic lines (that did not bring about consistent improvements in yield) are in line with previous studies using such materials (Fig. 9). Unlike the present study, those referenced together in Fig. 9 were carried out under a wide range of growing conditions, reflected by the range of yields considered (from less than 1 to more than 6 Mg ha⁻¹), produced by locations, growing seasons and management practices (in particular irrigated and/or water-stressed conditions). Fig. 9 demonstrates that (i) our results in terms of the relevance of awns for GY and AGW are consistent with those reported by a number of past publications: therefore the specific study of responses to source sink manipulations and on distributions of individual grain sizes of our work may trustworthily reflect the causes for the influence of awns on AGW more generally, (ii) the lack of clear effect of awns on

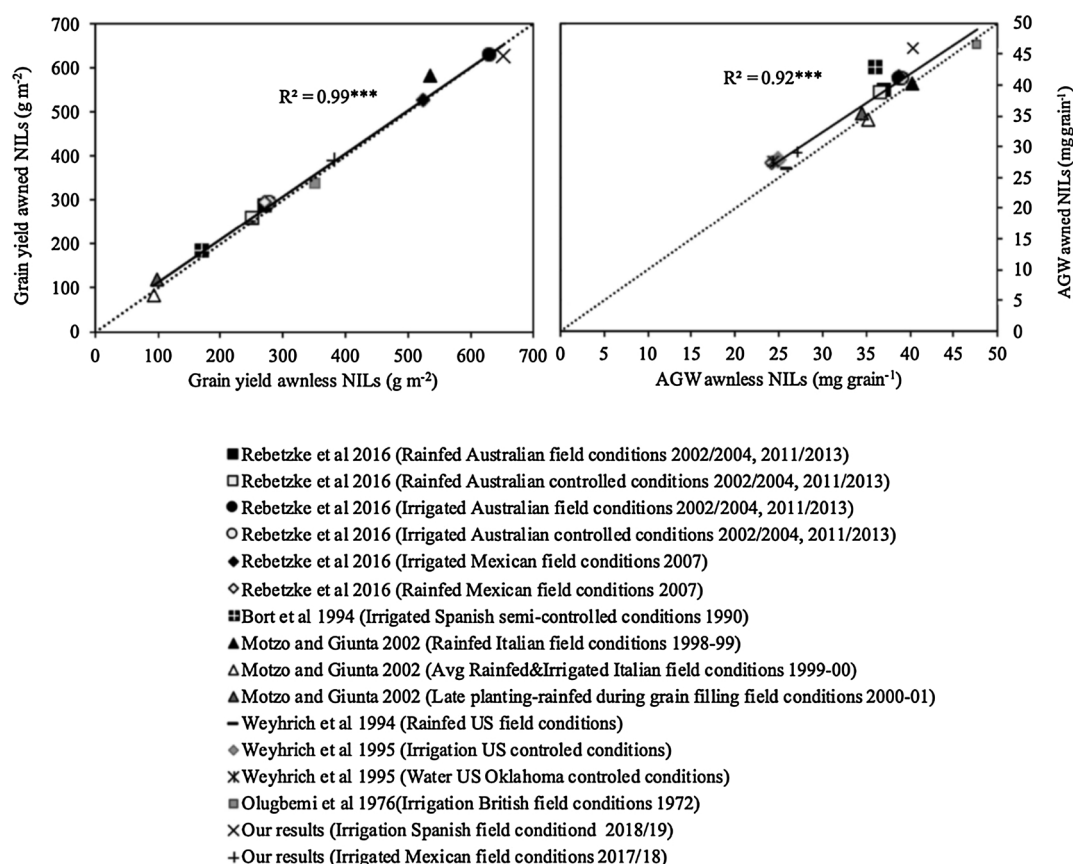


Fig. 9. Relationship in grain yield (left panel) and the average grain weight (AGW, right panel) between awned NILs vs. awnless NILs from the present and previous studies. Each symbol represents a particular environmental background (management $\times$ year $\times$ location; unless the authors published averages across more than one environment) in which the study was carried out. If more than one pair of NILs were used in a particular case the average of all backgrounds was computed.

yield found in the three field experiments cannot be ascribed just to the fact that our experiments were under irrigation: the same lack of effect was evident for a very wide range of growing conditions. Indeed, the effect of awns was unrelated to the yielding condition of the study in which it was evaluated (Supplementary Fig. S4), and (iii) the consistent effect of awns we found on AGW was similar to that more or less generally found regardless of the yielding condition of the study (Supplementary Fig. S4).

This means that the suggestion that awns would be more or less relevant depending on the growing condition, which is commonplace in the literature, seems unsupported: there was not a clear differential trend for both the effect on AGW, and the lack of effect on yield, depending on yielding conditions.

4.2. Higher AGW of awned lines do not seem related to increased source strength

The effect of awns in increasing the AGW seemed to have been rather independent of the potentially increased photosynthetic capacity during grain filling provided by awns. Beyond the fact that circumstantially some responses to source-sink manipulations during grain filling were identified as statistically significant (though even in these cases, almost without exception, they were very small compared to the magnitude of the treatment), responses of grain weight to both defoliation/degraining in the isogenic lines and to awn detachment in modern cultivars of wheat would challenge the hypothesis that improved grain weight in awned lines would be the improved source-strength during grain filling. Removing the two most critical leaves during grain filling did not produce any clear and consistent decrease in grain weight (as it would have been expected, at least in the awnless

lines, if the grains were subjected to a source limitation by the absence of awns). This lack of a clear effect of defoliation during grain filling on AGW has been observed in previous studies (e.g. Kruk et al., 1997; Maydup et al., 2010). In this sense, particularly interesting is the study by Ahmadi et al. (2009) where this lack of response of AGW to defoliation (and in this case defoliating the whole plot) was shown not only under irrigation but also under drought stress. This lack of clear response to defoliation is in agreement with evidences in the literature showing no clear responses of AGW to shading during grain filling (Maydup et al., 2010), with the exception of cases in which the intensity of shading has been too extreme (e.g. 80–90% shading intensity during grain filling: Beed et al., 2007; Sardaña et al., 2009); which would be wrongly interpreted as an evidence of grain growth being source-limited in the unshaded control (Serrago et al., 2013). Likewise removing half of the potentially competing grains did not produce a consistent increase in the weight of the remaining grains. This is also in agreement with a large body of literature showing negligible or very small increases in AGW in response to removal of competing grains during grain filling even in stressful environments (e.g. Cartelle et al., 2006; Pedro et al., 2011).

The conclusion that awns are not generally relevant to complement other sources of photo-assimilates to fill the grains seems to be not in agreement with the fact that awns contribute relevantly to both (i) the overall spike photosynthesis (as shown long time ago; e.g. Evans et al., 1972; Teare et al., 1972; Bort et al., 1994), and (ii) filling the grains (e.g. Li et al., 2006; Sanchez-Bragado et al., 2014a, 2014b, 2016). The fact that when they are present may produce those relevant contributions does not mean that if absent that contribution could not be compensated by that from other organs. This seemed to be the case as illustrated in the present study with the responses to source-sink

manipulations during grain filling, where the increase on AGW in awned vs. awnless NILs seemed to not have alleviated a conjectural source limitation in awnless genotypes (should that be the reason for their lower AGW): awnless lines should have responded more than its awned counterparts, a trend that was not consistently observed in our results.

4.3. Possible causes for the higher AGW in awned lines

If the increased AGW in the awned lines, compared to their awnless counterparts, was not a direct consequence of the photosynthetic contribution of the awns during grain filling, it means that awns may have affected grain weight distribution in the ear (Rebetzke et al., 2016). The presence of awns decreased the number of grains and whenever there is a decrease in grain number the consequence is the loss of grains in distal positions which are constitutively smaller than those in proximal positions (Acreche and Slafer, 2011; Miralles and Slafer, 1995). Therefore, the awned lines may lack of grains that are constitutively small and therefore there could be an increased AGW without any true effect on the capacity of the grains to grow, simply reflecting the failure of distal florets to become fertile. This indeed is compatible with the fact that awns use available assimilates during their development representing a significant sink competing with growing florets during ear formation, as suggested already long time ago (Schaller and Qualset, 1975), and floret development is definitively limited by availability of resources (Ferrante et al., 2013b). This indirect effect of awns on increased AGW by reducing the likelihood of distal florets to become fertile reducing GNS would be in line with what was hypothesised by Rebetzke et al. (2016). Thus, awns formation may compete with ovary growth for assimilates (Guo and Schnurbusch, 2016) as ovary size has been shown to be associated with both the likelihood of a floret primordia to become a fertile floret (Guo et al., 2015) and grain size (Calderini et al., 2006).

But our results evidenced that beyond the contribution of reducing the proportion of grains constitutively small to increase AGW, the presence of awns most frequently seemed to have a direct effect on the size of grains in given floret positions, specifically those proximal to the rachis which normally generate the largest grains. The analysis of the individual sizes of each particular grain constituting the AGW between awned and awnless isogenic lines revealed a constitutively higher potential size of the grains of awned NILs (assuming the top decile of grain sizes would reflect such potential).

4.4. Conclusions

We conclude that when analysed in NILs the presence of awns do not seem to produce any advantages in yield though consistently improving AGW at the expense of reducing the number of grains; but the effect goes beyond limiting the proportion of grains constitutively small (by increasing the failure of distal florets to set grains), increasing also the size of the largest grains, as the size of the proximal grains (represented by the top decile in grain size) in all lines was consistently larger in awned than in awnless lines.

CRediT authorship contribution statement

R. Sanchez-Bragado: Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **J.W. Kim:** Investigation, Writing - review & editing. **C. Rivera-Amado:** Methodology, Writing - review & editing. **G. Molero:** Conceptualization, Methodology, Funding acquisition, Writing - review & editing. **J.L. Araus:** Methodology, Writing - review & editing. **R. Savin:** Methodology, Writing - review & editing. **Gustavo A. Slafer:** Conceptualization, Supervision, Methodology, Visualization, Funding acquisition, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank David Bonnett and Greg Rebetzke for providing the Australian NILs and information related with them. Funding for the experimental work at Lleida was provided by project AGL2015-69595-R from the Spanish Research Agency (AEI). Part of the funding for the experiment was supported by BASF Agricultural Solutions. RSB was supported by Juan de la Cierva program JDC-Formación (FJCI-2016-28164). JWK held a pre-doctoral research contract from the Government of Catalonia. CRA and GM were supported by the Sustainable Modernization of Traditional Agriculture (MasAgro) an initiative from the Secretariat of Agriculture and Rural Development (SADER) and CIMMYT and the International Wheat Yield Partnership (IWYP). JLA acknowledges the support of ICREA Academia, Generalitat de Catalunya, Spain.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.fcr.2020.107827>.

References

- Acreche, M.M., Slafer, G.A., 2011. Lodging yield penalties as affected by breeding in Mediterranean wheats. *Field Crops Res.* 122, 40–48. <https://doi.org/10.1016/j.fcr.2011.02.004>.
- Ahmadi, A., Joudi, M., Janmohammadi, M., 2009. Late defoliation and wheat yield: little evidence of post-anthesis source limitation. *Field Crops Res.* 113, 90–93. <https://doi.org/10.1016/j.fcr.2009.04.010>.
- Álvarez, F., Royo, C., García del Moral, L.F., Villegas, D., 2008. Grain filling and dry matter translocation responses to source-sink modifications in a historical series of durum wheat. *Crop Sci.* 48, 1523. <https://doi.org/10.2135/cropsci2007.10.0545>.
- Beed, F.D., Paveley, N.D., Sylvester-Bradley, R., 2007. Predictability of wheat growth and yield in light-limited conditions. *J. Agric. Sci.* 145, 63–79. <https://doi.org/10.1017/S0021859606006678>.
- Blum, A., 1985. Photosynthesis and transpiration in leaves and ears of wheat and barley varieties. *J. Exp. Bot.* 36, 432–440. <https://doi.org/10.1093/jxb/36.3.432>.
- Borrás, L., Slafer, G.A., Otegui, M.E., 2004. Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *F. Crop. Res.* 86, 131–146. <https://doi.org/10.1016/j.fcr.2003.08.002>.
- Borrill, P., Fahy, B., Smith, A.M., Uauy, C., 2015. Wheat grain filling is limited by grain filling capacity rather than the duration of flag leaf photosynthesis: a case study using NAM RNAi plants. *PLoS One* 10, e0134947. <https://doi.org/10.1371/journal.pone.0134947>.
- Bort, J., Febrero, A., Amaro, T., Araus, J., 1994. Role of awns in ear water-use efficiency and grain weight in barley. *Agronomie* 14, 133–139.
- Calderini, D.F., Dreccer, M.F., Slafer, G.A., 1997. Consequences of breeding on biomass, radiation interception and radiation-use efficiency in wheat. *Field Crops Res.* 52, 271–381. [https://doi.org/10.1016/S0378-4290\(96\)03465-X](https://doi.org/10.1016/S0378-4290(96)03465-X).
- Calderini, D.F., Abeledo, L.G., Savin, R., Slafer, G.A., 1999. Effect of temperature and carpel size during pre-anthesis on potential grain weight in wheat. *J. Agric. Sci.* <https://doi.org/10.1017/S0021859699006504>.
- Calderini, D.F., Savin, R., Abeledo, L.G., Reynolds, M.P., Slafer, G.A., 2001. The importance of the period immediately preceding anthesis for grain weight determination in wheat. *Euphytica* 119, 199–204. <https://doi.org/10.1023/A:1017597923568>.
- Calderini, D.F., Reynolds, M.P., Slafer, G.A., 2006. Source-sink effects on grain weight of bread wheat, durum wheat, and triticale at different locations. *Aust. J. Agric.* 57, 227–233. <https://doi.org/10.1071/AR05107>.
- Cartelle, J., Pedró, A., Savin, R., Slafer, G.A., 2006. Grain weight responses to post-anthesis spikelet-trimming in an old and a modern wheat under Mediterranean conditions. *Eur. J. Agron.* 25, 365–371. <https://doi.org/10.1016/j.eja.2006.07.004>.
- Elbaum, R., Zaltzman, L., Burgert, I., Fratzl, P., 2007. The role of wheat awns in the seed dispersal unit. *Science* 316, 884–886. <https://doi.org/10.1126/science.1140097>.
- Evans, L., Rawson, H., 1970. Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. *Aust. J. Biol. Sci.* 23, 245–254. <https://doi.org/10.1071/BI9700245>.
- Evans, L.T., Sutherla, J., Jackson, P., Bingham, J., 1972. Effect of awns and drought on supply of photosynthate and its distribution within wheat ears. *Ann. Appl. Biol.* 70, 67–76. <https://doi.org/10.1111/j.1744-7348.1972.tb04689.x>.
- Fahy, B., Siddiqui, H., David, L.C., Powers, S.J., Borrill, P., Uauy, C., Smith, A.M., 2018.

- Final grain weight is not limited by the activity of key starch-synthesising enzymes during grain filling in wheat. *J. Exp. Bot.* 69, 5461–5475. <https://doi.org/10.1093/jxb/ery314>.
- Ferrante, A., Savin, R., Slafer, G.A., 2013a. Floret development and grain setting differences between modern durum wheats under contrasting nitrogen availability. *J. Exp. Bot.* 64, 169–184. <https://doi.org/10.1093/jxb/ers320>.
- Ferrante, A., Savin, R., Slafer, G.A., 2013b. Is floret primordia death triggered by floret development in durum wheat? *J. Exp. Bot.* 64, 2859–2869. <https://doi.org/10.1093/jxb/ert129>.
- Fischer, R.A., HilleRisLambers, D., 1978. Effect of environment and cultivar on source limitation to grain weight in wheat. *Aust. J. Agric. Res.* 29, 443–458. <https://doi.org/10.1071/AR9780443>.
- Foulkes, M.J., Snape, J.W., Shearman, V.J., Reynolds, M.P., Gaju, O., Sylvester-Bradley, R., 2007. Genetic progress in yield potential in wheat: recent advances and future prospects. *J. Agric. Sci.* 145, 17–29. <https://doi.org/10.1017/S0021859607006740>.
- Gaju, O., Reynolds, M.P., Sparkes, D.L., Foulkes, M.J., 2009. Relationships between large-spike phenotype, grain number, and yield potential in spring wheat. *Crop Sci.* 49, 961–973. <https://doi.org/10.2135/cropsci2008.05.0285>.
- Guo, Z., Schnurbusch, T., 2015. Variation of floret fertility in hexaploid wheat revealed by tiller removal. *J. Exp. Bot.* 66, 5945–5958. <https://doi.org/10.1093/jxb/erv303>.
- Guo, Z., Schnurbusch, T., 2016. Costs and benefits of awns. *J. Exp. Bot.* 67, 2533–2535. <https://doi.org/10.1093/jxb/erw140>.
- Guo, Z., Chen, D., Schnurbusch, T., 2015. Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. *J. Exp. Bot.* 66, 3099–3111. <https://doi.org/10.1093/jxb/erv117>.
- Guo, Z., Slafer, G.A., Schnurbusch, T., 2016. Genotypic variation in spike fertility traits and ovary size as determinants of floret and grain survival rate in wheat. *J. Exp. Bot.* 67, 4221–4230. <https://doi.org/10.1093/jxb/erw200>.
- Hasan, A.K., Herrera, J., Lizana, C., Calderini, D.F., 2011. Carpel weight, grain length and stabilized grain water content are physiological drivers of grain weight determination of wheat. *Field Crops Res.* 123, 241–247. <https://doi.org/10.1016/j.fcr.2011.05.019>.
- Knott, D.R., 1986. Effect of genes for photoperiodism, semidwarfism, and awns on agronomic characters in a wheat cross. *Crop Sci.* 26, 1158–1162. <https://doi.org/10.2135/cropsci1986.0011183x002600060016x>.
- Kruk, B.C., Calderini, D.F., Slafer, G.A., 1997. Grain weight in wheat cultivars released from 1920 to 1990 as affected by post-anthesis defoliation. *J. Agric. Sci.* 128, 273–281. <https://doi.org/10.1017/S002185960004133>.
- Li, X., Wang, H., Li, H., Zhang, L., Teng, N., Lin, Q., Wang, J., Kuang, T., Li, Z., Li, B., Zhang, A., Lin, J., 2006. Awns play a dominant role in carbohydrate production during the grain-filling stages in wheat (*Triticum aestivum* L.). *Physiol. Plant.* 127, 701–709. <https://doi.org/10.1111/j.1399-3054.2006.00679.x>.
- Li, X.F., Bin, D., Hong-Gang, W., 2010. Awn anatomy of common wheat (*Triticum aestivum* L.) and its relatives. *Caryologia* 63, 391–397. <https://doi.org/10.1080/00087114.2010.10589751>.
- Lopes, M.S., Reynolds, M.P., 2010. Dissecting drought adaptation into its phenotypic and genetic components in wheat. *Asp. Appl. Biol.* 7–11.
- Mach, J., 2015. Domesticated versus wild rice? Bring it awn! *Plant Cell* 27, 1818. <https://doi.org/10.1105/tpc.15.00504>.
- Martin, J.N., Carver, B.F., Hunger, R.M., Cox, T.S., 2003. Contributions of leaf rust resistance and awns to agronomic and grain quality performance in winter wheat. *Crop Sci.* 43, 1712–1717.
- Maydup, M.L., Antonietta, M., Guiamet, J.J., Graciano, C., López, J.R., Tambussi, E.A., 2010. The contribution of ear photosynthesis to grain filling in bread wheat (*Triticum aestivum* L.). *Field Crops Res.* 119, 48–58. <https://doi.org/10.1016/j.fcr.2010.06.014>.
- Maydup, M.L., Antonietta, M., Graciano, C., Guiamet, J.J., Tambussi, E.A., 2014. The contribution of the awns of bread wheat (*Triticum aestivum* L.) to grain filling: responses to water deficit and the effects of awns on ear temperature and hydraulic conductance. *Field Crops Res.* 167, 102–111. <https://doi.org/10.1016/j.fcr.2014.07.012>.
- McKenzie, H., 1972. Adverse influence of awns on yield of wheat. *Can. J. Plant Sci.* 52, 81–87. <https://doi.org/10.4141/cjps72-010>.
- Miralles, D.J., Slafer, G.A., 1995. Individual grain weight responses to genetic reduction in culm length in wheat as affected by source-sink manipulations. *Field Crops Res.* 43, 55–66. [https://doi.org/10.1016/0378-4290\(95\)00041-N](https://doi.org/10.1016/0378-4290(95)00041-N).
- Mitchell, J.H., Chapman, S.C., Rebetzke, G.J., Bonnett, D.G., Fukai, S., 2012. Evaluation of a reduced-tillering (tin) gene in wheat lines grown across different production environments. *Crop Pasture Sci.* 63, 128–141. <https://doi.org/10.1071/CP11260>.
- Mitchell, J.H., Rebetzke, G.J., Chapman, S.C., Fukai, S., 2013. Evaluation of reduced-tillering (tin) wheat lines in managed, terminal water deficit environments. *J. Exp. Bot.* 64, 3439–3451. <https://doi.org/10.1093/jxb/ert181>.
- Molero, G., Joynson, R., Pinera-Chavez, F.J., Gardiner, L.J., Rivera-Amado, C., Hall, A., Reynolds, M.P., 2019. Elucidating the genetic basis of biomass accumulation and radiation use efficiency in spring wheat and its role in yield potential. *Plant Biotechnol. J.* 17, 1276–1288. <https://doi.org/10.1111/pbi.13052>.
- Motzo, R., Giunta, F., 2002. Awnedness affects grain yield and kernel weight in near-isogenic lines of durum wheat. *Aust. J. Agric. Res.* 53, 1285–1293. <https://doi.org/10.1071/AR02008>.
- Olugbemi, L., Bush, M., 1987. The influence of temperature on the contribution of awns to yield in wheat. *Funct. Plant Biol.* 14, 299–310. <https://doi.org/10.1071/pp9870299>.
- Pask, A.J.D., Pietragalla, J., Mullan, D., Reynolds, M.P., 2012. Physiological Breeding II: A Field Guide to Wheat Phenotyping. CIMMYT, Mexico, DF.
- Pedro, A., Savin, R., Habash, D.Z., Slafer, G.A., 2011. Physiological attributes associated with yield and stability in selected lines of a durum wheat population. *Euphytica* 180, 195–208. <https://doi.org/10.1007/s10681-011-0352-y>.
- Rebetzke, G.J., Bonnett, D.G., Reynolds, M.P., 2016. Awns reduce grain number to increase grain size and harvestable yield in irrigated and rainfed spring wheat. *J. Exp. Bot.* 67, 2573–2586. <https://doi.org/10.1093/jxb/erv081>.
- Reynolds, M.P., Pellegrineschi, A., Skovmand, B., 2005. Sink-limitation to yield and biomass: a summary of some investigations in spring wheat. *Ann. Appl. Biol.* 146, 39–49. <https://doi.org/10.1111/j.1744-7348.2005.03100.x>.
- Reynolds, M.P., Foulkes, M.J., Slafer, G.A., Berry, P., Parry, M.A.J., Snape, J.W., Angus, W.J., 2009. Raising yield potential in wheat. *J. Exp. Bot.* 60, 1899–1918. <https://doi.org/10.1093/jxb/erp016>.
- Richards, R.A., 1988. A tiller inhibitor gene in wheat and its effect on plant growth. *Aust. J. Agric. Res.* 39, 749–757. <https://doi.org/10.1071/AR9880749>.
- Sanchez-Bragado, R., Elazab, A., Zhou, B., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., Araus, J.L., 2014a. Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: genotypic and growing conditions effects. *J. Integr. Plant Biol.* 56, 444–454. <https://doi.org/10.1111/jipb.12106>.
- Sanchez-Bragado, R., Molero, G., Reynolds, M.P., Araus, J.L., 2014b. Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ $\delta^{13}\text{C}$. *J. Exp. Bot.* 65, 5401–5413. <https://doi.org/10.1093/jxb/eru298>.
- Sanchez-Bragado, R., Molero, G., Reynolds, M.P., Araus, J.L., 2016. Photosynthetic contribution of the ear to grain filling in wheat: a comparison of different methodologies for evaluation. *J. Exp. Bot.* 67, 2787–2798. <https://doi.org/10.1093/jxb/erv116>.
- Sayre, K.D., Rajaram, S., Fischer, R.A., 1997. Yield potential progress in short bread wheats in northwest Mexico. *Crop Sci.* 114 (37), 36–42. <https://doi.org/10.2135/cropsci1997.0011183X003700010006x>.
- Schaller, C.W., Qualset, C.O., 1975. Isogenic analysis of productivity in barley: interaction of genes affecting awn length and leaf-spotting. *Crop Sci.* 15, 379–381. <https://doi.org/10.2135/cropsci1975.0011183X001500030029x>.
- Serrago, R.A., Alzueta, I., Savin, R., Slafer, G.A., 2013. Understanding grain yield responses to source-sink ratios during grain filling in wheat and barley under contrasting environments. *Field Crops Res.* 150, 42–51. <https://doi.org/10.1016/j.fcr.2013.05.016>.
- Shearman, V.J., Scott, R.K., Foulkes, M.J., 2005. Physiological processes associated with wheat yield progress in the UK. *Crop Sci.* 45, 175–185. <https://doi.org/10.2135/cropsci2005.0175>.
- Slafer, G.A., Savin, R., 1994a. Source-sink relationships and grain mass at different positions within the spike in wheat. *Field Crops Res.* 37, 39–49. [https://doi.org/10.1016/0378-4290\(94\)90080-9](https://doi.org/10.1016/0378-4290(94)90080-9).
- Slafer, G.A., Savin, R., 1994b. Postanthesis green area duration in a semidwarf and a standard-height wheat cultivar as affected by sink strength. *Aust. J. Agric. Res.* 45, 1337–1346. <https://doi.org/10.1071/AR9941337>.
- Tambussi, E.A., Bort, J., Guiamet, J.J., Nogués, S., Araus, J.L., 2007. The Photosynthetic role of ears in C_3 cereals: metabolism, water use efficiency and contribution to grain yield. *Crit. Rev. Plant Sci.* 26, 1–16. <https://doi.org/10.1080/07352680601147901>.
- Teare, I.D., Law, A.G., Simmons, G.F., 1972. Stomatal frequency and distribution on the inflorescence of *Triticum aestivum*. *Can. J. Plant Sci.* 52, 89–94. <https://doi.org/10.4141/cjps72-011>.
- Weyhrich, R.A., Carver, B.F., Smith, E.L., 1994. Effects of awn suppression on grain yield and agronomic traits in hard red winter wheat. *Crop Sci.* 34. <https://doi.org/10.2135/cropsci1994.0011183X003400040025x>.
- Weyhrich, R.A., Carver, B.F., Martin, B.C., 1995. Photosynthesis and water-use efficiency of awned and awnleted near-isogenic lines of hard red winter wheat. *Crop Sci.* 35, 172–176. <https://doi.org/10.2135/cropsci1995.0011183X003500010032x>.
- Xie, Q., Mayes, S., Sparkes, D.L., 2015. Carpel size, grain filling, and morphology determine individual grain weight in wheat. *J. Exp. Bot.* 66, 6715–6730. <https://doi.org/10.1093/jxb/erv378>.