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REVIEW PAPER

Ear photosynthesis in C_3 cereals and its contribution to grain yield: methodologies, controversies, and perspectives

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Abstract

In C_3 cereals such as wheat and barley, grain filling was traditionally explained as being sustained by assimilates from concurrent leaf photosynthesis and remobilization from the stem. In recent decades, a role for ear photosynthesis as a contributor to grain filling has emerged. This review analyzes several aspects of this topic: (i) methodological approaches for estimation of ear photosynthetic contribution to grain filling; (ii) the existence of genetic variability in the contribution of the ear, and evidence of genetic gains in the past; (iii) the controversy of the existence of C_4 metabolism in the ear; (iv) the response of ear photosynthesis to water deficit; and (v) morphological and physiological traits possibly related to ear temperature and thermal balance of the ear. The main conclusions are: (i) there are a number of methodologies to quantify ear photosynthetic activity (e.g. gas exchange and chlorophyll fluorescence) and the contribution of the ear to grain filling (individual ear shading, ear emergence in shaded canopies, and isotope composition); (ii) the contribution of ear photosynthesis seems to have increased in modern wheat germplasm; (iii) the contribution of the ear to grain filling increases under resource-limitation (water deficit, defoliation, or pathogen infection); (iv) there is genetic variability in the contribution of the ear in wheat, opening up the possibility to use this trait to ameliorate grain yield; (v) current evidence supports the existence of C_3 metabolism rather than C_4 metabolism; (vi) the ear is a 'dehydration avoider organ' under drought; and (vii) thermal balance in the ear is a relevant issue to explore, and more research is needed to clarify the underlying morphological and physiological traits.

Keywords: C_4 metabolism, ear photosynthesis, grain filling, spike, wheat.

Introduction

The ear is more than a container for grains

In bread and durum wheat, as well as in rice and barley, grain filling was traditionally explained as being sustained by assimilates from concurrent flag leaf photosynthesis and the retranslocation of photoassimilates (mainly fructans) stored in stems before anthesis. In recent years, the role of ear photosynthesis (or panicle photosynthesis in rice) has become increasingly recognized (e.g. Maydup *et al.*, 2010, 2012, 2014; Sanchez-Bragado *et al.*, 2014a, b; Kong *et al.*, 2016; Wang *et al.*, 2016; Vicente *et al.*, 2018). The possible advantage of the ear as

a photosynthetic (i.e. source) organ may be explained by: (i) its proximity to the grains, which are the final sinks; (ii) it being the last photosynthetic organ to senesce during grain filling (Martinez *et al.*, 2003); (iii) the positioning of the photosynthetic tissues of the ear (such as glumes, the outer bracts in the spikelet, lemmas, inner bracts, covering the grain and awns, and filiform prolongations of the lemma) at the top of the canopy, under higher irradiance than the leaves; (iv) its capacity to re-assimilate respired CO_2 (see Tambussi *et al.*, 2007); and (v) some tolerance to water stress compared with the flag leaf (Martinez *et al.*, 2003; Tambussi *et al.*, 2005; Maydup *et al.*, 2014). Additionally, there is a series of reports that claim the ear as a C_4 or intermediate C_3–C_4 organ (in wheat, Singal *et al.*, 1986; Ziegler-Jöns A. 1989; Rangan *et al.*, 2016*a*, b; in barley, Nutbeam *et al.*, 1976), although this issue is still controversial (see below). This review will consider these aspects of ear photosynthesis and its contribution to grain filling. We will not describe the refixation of respired CO_2, because this issue was widely reviewed in a previous work (Tambussi *et al.*, 2007) and no advances have subsequently been reported. After a brief introduction, we will discuss: (i) the methodological approaches to study the contribution to grain filling; different methodologies (e.g. isotope composition) have arisen to quantify contribution of ear photosynthesis to grain filling, opening up the possibility of analyzing the genotypic variability of this trait in breeding programs (methods concerning the quantification of ear photosynthesis are depicted in Box 1); (ii) the controversy about the existence of C_4 metabolism in ear parts which has resurfaced recently, with several lines of evidence presented about this important unresolved topic, and alternative explanations proposed about the information found in the scientific literature; (iii) the role of ear photosynthesis under stress conditions, in particular its 'water deficit tolerance' and thermal balance, and its possible impact on grain yield in adverse conditions (such as drought); (iv) the supposition that the contribution of the ear to grain filling has increased during breeding, at least in wheat (retrospective studies of historical series of old and modern cultivars), opening up the prospect of using this trait as a selection criterion; and, finally, (v) we will distil the main conclusions of the review and consider future perspectives for this field of research.

Methodological approaches to estimate the contribution of the ear to grain filling

Methodologies to measure photosynthetic activity are relatively known in general terms (mainly for leaves), and we detailed its adaptation to the peculiarities of the ear in Box 1 (with several methodological details and tips). Regardless of the photosynthetic rate of the ear, its actual contribution to grain filling will also depend on several other factors such as the translocation of assimilates to the sinks (kernels in this case) and the relative contribution of other sources (e.g. flag leaf photosynthesis and stem retranslocation). Various approaches have been used to estimate the photosynthetic contribution of the ear to grain filling: (i) shading individual ears (e.g. Asana *et al.*, 1950; Araus *et al.*, 1993*a*, *b*; Maydup *et al.*, 2010, 2012, 2014); (ii) ears emerging into full sunlight, with the rest of the canopy shaded with mesh (Maydup *et al.*, 2010; Serrago *et al.*, 2013); (iii) a pharmacological approach, inhibiting ear photosynthesis with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Maydup *et al.*, 2010; Molero *et al.*, 2014; Sanchez-Bragado *et al.*, 2016); and (iv) estimation by isotopic discrimination of ^{13}C (Sanchez-Bragado *et al.*, 2014*a*, *b*, 2016).

Concerning the first approach (i.e. 'ear shading'), it has been most widely used to estimate the contribution of the ear (e.g. Asana *et al.*, 1950; Araus *et al.*, 1993*a*, *b*; Maydup *et al.*, 2010, 2012, 2014). Commonly, the ear is covered during grain filling with aluminum foil, with holes inserted to avoid overheating and the accumulation of gases such as ethylene (shading with textile, black inside and white outside, has also been used; Molero *et al.*, 2014, 2020). It is essential that the treatment begins 7–10 d after anthesis (not earlier or at anthesis, as in Abdoli *et al.*, 2013), in order to avoid a decrease in potential grain weight (note that in the first days after anthesis, endosperm cells are formed). An increase in ear temperature might be one caveat of this methodology, but Maydup *et al.* (2010) found no appreciable difference when using thermocouples to measure temperature throughout the day in shaded and control ears.

In this approach, the contribution of the ear to grain filling can be calculated as:

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

where GW_{ear} is the total grain weight of the ear.

The second approach consists of comparing grain weight in plots where the whole canopy is shaded versus plots where leaves are shaded, with the ears emerging through the mesh (light extinction ~90%) into full sunlight. As far as we know, this approach was first used by Maydup *et al.* (2010), and later by Serrago *et al.* (2013).

In this case, the contribution of the ear is calculated as:

$$= \left[\frac{(GW_{ear} \text{ of emerging ear} - GW_{ear} \text{ of } \textit{all} \text{ shaded canopy}) \times 100}{GW_{ear} \text{ of intact ear}} \right]$$

As with ear shading, treatments must begin 7–10 d after anthesis. Data obtained with this and the ear shading methodology showed similar tendencies (e.g. differences between cultivars), although absolute values were not coincident.

The third method to quantify the contribution of the ear is a pharmacological approach, inhibiting PSII [and, thus, the electron transport rate (ETR)] with localized application of ~100 μM DCMU in the ear, with a surfactant such as Tween-20 or similar (Maydup *et al.*, 2010). Unwanted spillage of the inhibitor on the flag leaf must be avoided, for example by enclosing the ear in a plastic bag during application. The lack

Box 1 Methods to measure ear photosynthesis

As far as we know, photosynthetic activity of ear parts has been estimated with three methodologies: (i) direct quantification of CO_2 assimilation by IRGA (Maydup *et al.*, 2010); (ii) O_2 emission using oxygen electrodes (Clark type); and (iii) indirect measurement of thylakoid activity through modulated chlorophyll fluorescence (Maydup *et al.*, 2010, 2014). We will briefly comment on the peculiarities of these methods and as they apply to ear photosynthesis, in particular their possible limitations and drawbacks.

(i) The main constraint of IRGA is the availability of a suitable chamber to enclose the ear. In some cases, commercial IRGA equipment has accessories that can serve this purpose, such as the 'conifer chamber' for the LICOR 6400 (Tambussi *et al.*, 2005) and the chamber of the old LICOR 6200 model (Inoue *et al.*, 2004). Also, users frequently customize their own chambers (e.g. Maydup *et al.*, 2010; Molero *et al.*, 2020). In addition, a fan and a cooling system (e.g. Peltier) should be included in the design of the chamber (made of methacrylate, for instance), in order to avoid overheating of the ear (in particular, if a 'warm' lamp such as a halogen lamp with a dichroic mirror or similar light source is used, a water filter positioned above the chamber can be used to remove heat radiation; Tambussi *et al.*, 2005). In addition, because of the higher volume of these chambers (compared with leaf clips) a higher air flow rate (e.g. 400–500 ml min^{-1}) must be set. Depending on the chamber design, the light source should be placed laterally or overhead (e.g. for the LICOR 6400 conifer chamber). Recently, an innovative chamber for 3D organs (such as cereal ears and grapevine clusters) has been developed (Fortineau and Bancal, 2018). This device has a prismatic (decagonal) light source (red and blue LEDs) surrounding the methacrylate chamber. Comparing light response curves in conventional (conifer chamber, illuminated from one side) versus the prototype 3D chamber, the authors reported that although the light-saturated photosynthetic rate (A_{sat}) was identical in both chambers, saturation occurred at lower irradiances in the 3D chamber (Fortineau and Bancal, 2018). Although the authors reported that the 3D illumination is more homogenous and measurements are more accurate with this chamber, 3D illumination does not truly represent light distribution in the field, where, beyond diffuse light, illumination is mainly directional. Since the ear intercepts zenithal or lateral light (depending on the time of the day), measurements with the 3D chamber should be considered only comparative (between genotypes, treatments, etc.) and not representative of actual ear photosynthetic rates. Recently, Molero and Reynolds (2020) also reported work with bilateral illumination in the chamber provided by LEDs (90% red, 10% blue).

An important consideration is how to express the photosynthetic rate of the ear; that is, is it best on a per area, weight, chlorophyll, or organ basis (see Tambussi *et al.*, 2007)? Although photosynthesis has been calculated on a per area basis in many reports (e.g. Serrago *et al.*, 2013; Jia *et al.*, 2015; Fortineau and Bancal, 2018), an organ or weight (at anthesis) basis is more appropriate. The irregular surface of the ear makes it difficult to calculate a realistic photosynthetic area, and absolute comparisons of photosynthetic rate (on an area basis) between ears and leaves should be avoided. Considerations about ear area are more critical if zenithal light is used because there will be no correspondence between the green area making photosynthesis and the estimated total (projected) ear area. This may be relevant when deploying remote-sensing approaches based on zenithal images (Sanchez-Bragado *et al.*, 2020b). When results are expressed on an organ basis, net photosynthetic rates ranging between 5 nmol and 20 nmol $organ^{-1}$ s^{-1} have been reported, depending on the cultivar and the stage of grain filling (e.g. Maydup *et al.*, 2010, Sanchez- Bragado *et al.* 2014a, b, and Fortineau and Bancal, 2018 reported ~20 nmol ear^{-1} s^{-1}). It must be noted that photosynthetic rates expressed on an organ basis can obscure the interpretation of the data when several cultivars (for instance with different ear sizes) are compared. In this case, the dry weight of the ear around anthesis (i.e. before grain growth) might be a better option.

Another important point is that IRGA measures the net exchange of CO_2—the balance between gross CO_2 assimilation minus the CO_2 emitted by 'dark' respiration and photorespiration. In a leaf, dark respiration is commonly low compared with the photosynthetic rate (e.g. Evans and Rawson, 1970). In the ear, however, the emission of CO_2 (mainly from the grains) is high (see Tambussi *et al.*, 2005, 2007), and this can obscure the interpretation of the results. For instance, Serrago *et al.* (2013) reported that ear photosynthesis increased in defoliated compared with intact plants. However, the authors only measured net photosynthesis, and this parameter can change (increase) if grain respiration is reduced.

Box 1 Continued

In fact, Sanchez-Bragado *et al.* (2014a) showed that such a decrease in ear respiration occurs when shading eliminates leaf photosynthesis. In short, changes in the rate of net photosynthesis in the ear can be sensibly influenced by dark respiration. In some cases, the sum of net photosynthesis and dark respiration rates has been considered as an estimation of 'gross photosynthesis' (e.g. Araus *et al.*, 1993*a*, *b*; Tambussi *et al.*, 2005; Sanchez-Bragado *et al.*, 2014a; Molero and Reynolds, 2020). However, the situation is more complex for two reasons: (i) refixation (i.e. re-assimilation of respired CO_2 emitted by the grain) takes place in the ear (e.g. see Tambussi *et al.*, 2007, and references therein; Bort *et al.*, 1996); and (ii) we do not know whether the respiration rate is the same under light and dark conditions, and this could obscure the results (however, it might be possible to ignore this concern because the main respiration in the ear occurs in heterotrophic tissues and is perhaps not modified by light). In brief, the values of net photosynthesis in the ear should be interpreted with caution, in particular if phenological differences are involved and keeping in mind changes in the kernel respiration rate as grain filling progresses (e.g. Tambussi *et al.*, 2005).

(ii) Quantification of photosynthetic activity in ear parts (e.g. awns) has also been carried out with O_2 Clark-type electrodes (Li *et al.*, 2006; Xiong *et al.*, 2013; Kong *et al.*, 2016). Because the system works in a close configuration—and due to the small size of the electrode chamber—a source of CO_2 must be provided (commonly, a solution of 20 mM sodium bicarbonate; e.g. Li *et al.*, 2006; Kong *et al.*, 2016). The main limitation of this method is the destructive nature of the measurements, because small (detached) parts are placed in the illuminated chamber. It should be noted that in a similar way to chlorophyll fluorescence, the measurement is related to linear electron transport in the thylakoids and it is not a direct quantification of CO_2 assimilation. In addition, it is an estimation of maximum activity (not actual activity), especially when CO_2 and light are not limiting during the measurement. Because of the destructive nature of the measurement, and the particular conditions in the chamber (e.g. high CO_2 levels supplied by a sodium bicarbonate solution), this methodology provides only comparative data.

(iii) Modulated chlorophyll fluorescence is a widely used technique to evaluate photosynthetic performance in plants. Here we will only address modulated chlorophyll fluorescence in relation to ear photosynthesis (Maydup *et al.*, 2014; for an exhaustive description of this methodology, see Baker, 2008; Maxwell and Johnson , 2000). The 'saturating pulse method' allows the measurement of several parameters including the actual quantum yield of PSII, with the ensuing calculation of the ETR:

$$\mathrm{ETR} = \phi_{\mathrm{PSII}}.\mathrm{PPFD}.a.0.5 \ (\mathrm{units} = \mu\mathrm{mol\ electrons\ m^{-2}s^{-1}})$$

where ϕPSII is the quantum yield of PSII, PPFD is the photosynthetic photon flux density (measured with a PAR sensor), 'a' is the absorptance of the organ (i.e. the proportion of incident PPFD that it is actually absorbed), and the coefficient 0.5 assumes similar partitioning of photons between PSI and PSII. Strictly speaking, the absorptance ('a' in the equation) should be measured with an integrating sphere (Maxwell and Johnson, 2000); however, in a few plant organs, absorptance values around 0.8–0.85 are common (Björkman and Demmig, 1987). Nevertheless, if the organ (in this case, the ear) has a reflective surface (e.g. due to the presence of epicuticular waxes) or if the chlorophyll content decreases due to senescence, the absorptance value could change. In experiments where treatments can modify organ reflectance (for instance, if cultivars with different glaucousness are compared) or chlorophyll content (e.g. during senescence), absorptance measurements are needed in order to calculate the ETR. In addition to this, two important points for a realistic ETR calculation are: (1) the correct measurement of PPFD and (2) the need to achieve the steady state of photosynthetic activity (in relation to the current and previous PPFD that was present in the organ); that is, a suitable acclimation time should be considered. Concerning the first point, since the PPFD sensor is horizontally displaced relative to the sector of the photosynthetic area being measured, there may be differences between the measurements recorded by the sensor and the PPFD actually incident on the sector of leaf or ear (for instance, the optical fiber can eventually shadow the green area if it is placed incorrectly in the fluorimeter clip). Concerning the second point, one way to be sure about the steady state is to check the actual fluorescence signal in the equipment's display (this value should not change over time, and particular care should be taken if changes in irradiance occur).

Box 1 Continued

It must be remembered that (as in the case of O2 evolution), the ETR represents thylakoid activity and is not an actual measurement of net CO2 assimilation (An). Alternative electron sinks (e.g. photorespiration, the Mehler reaction, and nitrate reduction) can modify the relationship between the ETR and An (Kalaji *et al.*, 2016), and thus these results must be interpreted with caution. In addition, chlorophyll fluorescence measured by this methodology originates from chloroplasts positioned in the upper layers of the photosynthetic tissues (in contrast, assimilation of CO2 assessed by IRGA integrates the net photosynthetic activity of the whole organ). In spite of these considerations, this parameter can be a very useful indicator of quantum yield of PSII photosynthetic activity and linear electron transport, although it should not to be used as a proxy of the absolute photosynthetic rate (Baker, 2008).

of any effect on the photosynthetic activity of the leaf and the inhibition in the ear must be checked, for instance by modulated chlorophyll fluorescence (see Box 1). When we compared the reduction in total grain weight per ear in shaded versus DCMU-treated ears, similar results were observed (Maydup *et al.*, 2010). Molero *et al.* (2014) also compared both methods (shading with textile versus inhibition with DCMU); the results were similar but not completely identical (see also Molero *et al.*, 2020).

The former three approaches have been indicated as 'intrusive' (Sanchez-Bragado *et al.*, 2014a, b), and compensations could occur; for example, increasing the contribution of non-treated organs when ear photosynthesis is reduced. Even if such compensations take place, the contribution of the ear (evaluated with such intrusive approaches) should be considered as a minimum.

Finally, the contribution of the ear has been estimated by a non-intrusive approach, namely the isotopic composition of ^{13}C (Sanchez-Bragado *et al.*, 2014a, b). In this method, the ^{13}C composition ($\delta^{13}C_{grain}$) of the mature kernel, and the water-soluble fractions (WSFs) of the flag leaf and the green parts of the ear (glumes, lemmas, and awns) were measured by MS. The contribution of the ear was calculated by these authors from the following equation:

$$\delta^{13}C_{grain} = a \times \delta^{13}C_{ear} + (1 - a) \times \delta^{13}C_{flag}$$

(Sanchez-Bragado *et al.*, 2014a)

where '***a***' is the contribution of the ear to grain filling, $\delta^{13}C_{grain}$ is the ^{13}C composition of the mature kernels, $\delta^{13}C_{ear}$ is the ^{13}C composition of the green tissues of the ear (WSF), and $\delta^{13}C_{flag}$ is the ^{13}C composition of the flag leaf (WSF). As the authors acknowledge (Sanchez-Bragado *et al.*, 2014a), the main drawback of this novel methodology is that the contribution of stem retranslocation (fructan reserves) is not considered. In another study, Sanchez-Bragado *et al.* (2014b) proposed a second, more sound, approach using the isotopic composition of ^{13}C of the WSF of the peduncle (i.e. which includes both current assimilates from leaves as well as stem reserves) compared with the composition of ^{13}C in mature kernels. This approach assumes that no post-photosynthetic fractionation occurs, for example in sugar loading/unloading in the phloem. Apparently, some evidence has indicated that such fractionation is not relevant (see references in Sanchez-Bragado *et al.*, 2014a, b). As we will discuss below, the values of the contribution of the ear evaluated by isotopic composition are higher than by other methods; we ignore the causes of this, but possible compensations that occur during intrusive methods (e.g. the increase in the contribution of other sources in shaded ears) might be implicated.

Estimation of the contribution of the awn

The photosynthetic contribution of awns to grain filling has been assessed by (i) de-awning (i.e. cutting off the awns with scissors 7 d after anthesis; e.g. Maydup *et al.*, 2014), or (ii) comparing the grain yield per spike in near-isogenic lines (NILs) with awned versus awnless ears (e.g. Bort *et al.*, 1994; Weyhrich *et al.*, 1995; Rebetzke *et al.*, 2016). The first approach should be carried out with caution, because we have observed premature senescence in the body of the ear (i.e. glumes and lemmas) in some cases. The second approach seems more realistic and (prima facie) 'cleaner' from an experimental viewpoint; however, it is difficult to apply when analyzing many cultivars due to the need for NILs of each genetic background (Sanchez-Bragado *et al.*, 2020a). In addition, as pointed out in Tambussi *et al.* (2007), pleiotropic effects could obscure the interpretations of results.

Overview of the section

We have referred to several methodologies to evaluate ear photosynthesis (see Box 1) and its contribution to grain filling, from the simple (although laborious) technique of 'individual shading' to the more sophisticated (but somewhat expensive and still laborious) approach of determining isotope composition ($\delta^{13}C$). It is clear that in order to analyze many genotypes (e.g. hundreds) simultaneously in breeding programs, it is crucial to have some simple and realistic 'proxy' of ear photosynthesis and its contribution. To date, isotope techniques are possibly the most promising approach to evaluate the contribution, even though they are possibly not adequate for high-throughput applications (see Sanchez-Bragado *et al.*, 2020b).

Photosynthetic metabolism: the long and unresolved discussion of C_4 versus C_3 metabolism in the ear

The existence of C_4 metabolism in ear parts of wheat and other C_3 cereals has been discussed for years (Tambussi *et al.*, 2007, and references therein). The first reports suggesting C_4 metabolism (or intermediate C_3–C_4) in the ear parts of some C_3 cereals (Nutbean *et al.*, 1976 for barley pericarp; Singal *et al.*, 1986 and Ziegler-Jöns, 1989 for wheat; Imaizumi *et al.*, 1990 for rice) were published in the 1970s and 1980s, but some later evidence did not support these finding (Tambussi *et al.*, 2007). Recently, the issue has been revived by transcriptomic studies suggesting C_4 metabolism in the pericarp of grain (Rangan *et al.*, 2016*a*, b). Although new findings could be interesting, several pieces of evidence are needed to claim that a cycle is C_4, such as: (i) the activity of C_4 enzymes and, more importantly, levels of C_4 metabolites; (ii) the existence of compartmentalization of C_4 and C_3 reactions (at a histological or a one-cell scale); (iii) the photosynthetic activity must be insensitive to a decrease in oxygen levels, for example from 21% to 2% (i.e. non-photorespiratory conditions); and (iv) depending on the fugacity of the 'gas-tight compartment', and because the ^{13}C discrimination of phosphoenolpyruvate carboxylase (PEPC) is lower than that of Rubisco, the ^{13}C content in a C_4 organ should be higher than for typical C_3 plants. In fact, the above-mentioned items comprise the common set of criteria for separating C_3 from C_4 species. We will discuss these items in the following subsections.

Enzyme activities and C_4 metabolites in the ear

Various publications have shown the activity of some 'C_4-related enzymes' in ear parts (e.g. Jia *et al.*, 2015; Zhang *et al.*, 2019). However, some considerations should be mentioned about these finding. First of all, enzymes such as PEPC are not exclusive to C_4 metabolism. This enzyme, for instance, is found even in non-photosynthetic tissues of plants (e.g. roots) and functions anaplerotically in diverse tissues and organs (Chollet *et al.*, 1996). Another relevant aspect is the timing of enzymatic activity during grain filling. If an enzyme increases its activity at the end of grain filling (e.g. Li *et al.*, 2006), it is unlikely to be related to photosynthetic activity. One example of this can be viewed in Jia *et al.* (2015). The activity of PEPC (and other photosynthetic enzymes) begins to decline 6 d after anthesis (see fig. 4 in Jia *et al.*, 2015). Indeed, it is difficult to understand the C_4 photosynthetic function of the enzymes in these cases because their activity decreases when grain filling takes place. In an older study, Wirth *et al.* (1977) found high PEPC activity in the pericarp (and, at a lower level, in the glumes) of wheat. When the authors compared the Rubisco/PEPC activity ratio, they found a decrease in ear parts compared with the flag leaf. As pointed out by Bush *et al.* (2016), the C_4 carbon-concentrating mechanism requires a PEPC/Rubisco activity ratio of ~1:1. Because the catalytic activity of PEPC is 100 times higher than that of Rubisco, a high PEPC/Rubisco ratio is not suitable for C_4 metabolism. A PEPC/Rubisco ratio of ~0.9 (or lower, depending on the time, organ, and cultivar) was reported by Xu *et al.* (2003) in ear parts, mainly under heat stress conditions. Although these data could be interpreted as 'C4 compatible', the increase in the ratio seems to be linked to Rubisco degradation by senescence (see fig. 1 in Xu *et al.*, 2003). However, the high activity of PEPC in ear parts compared with the flag leaf is very interesting and its role should be clarified.

Concerning the analysis of metabolites indicating C_4 photosynthesis, there are contradictory results. While Singal *et al.* (1986) reported malate labeled with ^{14}C in pulse–chase experiments, Bort *et al.* (1995) found typical C_3 metabolites. As highlighted by Bort *et al.* (1995), isolated ear parts were incubated on a moist support in the experiments of Singal *et al.* (1986), which may have increased the amount of inorganic ^{14}C present in the buffer as bicarbonate (i.e. the substrate of PEPC). Thus, the presence of high C_4 acid (i.e. malic) levels could be an artifact, although this is speculative. The study of Singal *et al.* (1986) indicated that PEPC could re-assimilate respired CO_2 (refixation), although we believe this would not be C_4 photosynthesis *per se*. A recent work shows an association between 'C_4-photosynthetic enzymes' (PEPC, PPDK, NADP-ME, and NADP-MDH) and Rubisco (activity and gene expression) with the enhanced content of organic acids (malic, oxaloacetic, citric, and fumaric acid) in glumes and lemmas compared with leaves under water stress (Zhang *et al.*, 2019). The authors suggest that this metabolic pattern is involved in the spike drought tolerance through increasing the NADPH content for antioxidative system and sustaining the tricarboxylic acid cycle. Further research is necessary to test this hypothesis.

Lack of evidence of compartmentalization of C_3–C_4 cycles

There are almost no studies where the possibility of compartmentalization has been analyzed in the ear. Using immunogold labeling (electron microscopy), Araus *et al.* (1993*a*) reported that PEPC in glumes (durum wheat) is localized to 'vesicles' in the cytoplasm of mesophyll cells. On the other hand, in immature kernels, labeling localized PEPC in the aleurone layer. This and other evidence suggests an anaplerotic (rather than photosynthetic) role for PEPC, as has been reported in other cases (e.g. the bundle sheath of barley leaves; Leegood, 2008). In the ears of durum wheat, Rubisco has been localized (and uniformly distributed) in all cells of the mesophyll of glumes, lemmas, and the green pericarp of immature kernels (Tambussi *et al.*, 2005). In other words, no evidence of C_4 metabolism regarding 'compartmentalization' (at either the histological or the cellular level) was presented in this research.

Rangan *et al.* (2016*a*, *b*), based on old transmission electron micrographs of pericarp chloroplasts (Morrison, 1976), postulated some compartmentalization in the pericarp between cross and tube cells (i.e. two adjacent layers of the

green pericarp in wheat). However, clear evidence (e.g. no immunolocalization of relevant enzymes was carried out) is lacking. Immunolocalization studies are needed to determine compartmentalization of the enzymes and biochemical reactions (e.g. Rubisco and PEPC), as was pointed out by Bush *et al.* (2016). On other hand, another criticism of this hypothesis is the apparent lack of plasmodesmata between cross and tube cells (see the photographs in Morrison, 1976), which is a *sine qua non* condition for symplastic transport of metabolites in C_4 metabolism.

In summary, although the results found by Rangan *et al.*, (2016*a*, *b*) could be interesting (see the discussion in Henry *et al.*, 2017), the actual existence of C_4 photosynthesis in the pericarp is still somewhat speculative and lacks clear-cut evidence.

Sensitivity of photosynthesis to oxygen

It is well known that photorespiration in C_4 plants is actually suppressed by higher CO_2 and/or lower O_2 concentrations at the carboxylation site of Rubisco (in typical C_4 plants, Rubisco is localized to the bundle sheath chloroplasts, where there is a high CO_2/O_2 ratio). Thus, the photosynthetic sensitivity to oxygen (i.e. measurements at 21% compared with 2% O_2) is considered as evidence of C_3 metabolism (note that although oxygen sensitivity suggests C_3 metabolism, the opposite is not necessarily true, because some C_3 plants can show insensitivity to oxygen when phosphate is limiting; e.g. Sharkey *et al.*, 1986). As far as we know, the first evidence for oxygen sensitivity of ear photosynthesis was reported by Bort *et al.* (1995), who showed that the CO_2 compensation point changed linearly with O_2 concentration (clear evidence of C_3 metabolism). The overall ear photosynthesis of durum wheat [measured by an infrared gas analyzer (IRGA)] was oxygen sensitive, in the same way as the flag leaf (Tambussi *et al.*, 2005). Glumes (the outer bracts in the spikelet) and lemmas (inner bracts, covering the grain) also showed oxygen sensitivity of the photosynthetic ETR (measured by modulated chlorophyll fluorescence). Considering all earlier work (see also Tambussi *et al.*, 2007), the existence of C_4 metabolism is not supported by this evidence.

In the particular case of the pericarp (which must be discussed separately due to the possible effects of the grain as an internal source of CO_2), Tambussi *et al.* (2007) reported that the ETR of the green layer was less sensitive (compared with glumes and lemmas) to CO_2 changes (at atmospheric oxygen concentration, i.e. ~21%). Conclusive evidence of C_4 metabolism cannot be inferred from these results, because in the kernel there is an internal source of CO_2. At least in bracts, the oxygen sensitivity of photosynthesis is clear; in the pericarp, the data are more controversial. The oxygen sensitivity of photosynthesis in ear bracts suggests that photorespiration occurs in ear parts. In contrast, Balaur *et al.* (2018) reported the absence of a 'photorespiratory burst' (a peak emission of CO_2 linked to decarboxylation of glycine during a light–dark transition; Sharkey, 1988) in the ear. In addition, these authors observed very low glycolate oxidase activity (a key enzyme in the photorespiratory pathway that eliminates toxic glycolate) in bracts of the ear. However, the reason for this finding is obscure, because even C_4 species (such maize) have high glycolate oxidase activity (Zelitch *et al.*, 2009), and more research is needed to elucidate this interesting question.

^{13}C composition in ear parts

Concerning the ^{13}C content in ear parts, the evidence (e.g. Araus *et al.* 1992a, b; Gebbing *et al.*, Schnyder, 2001; Sánchez-Bragado *et al.*, 2014b; Vicente *et al.*, 2018) is completely consistent with the typical range of C_3 plants (Pate *et al.*, 2001). It must be noted that in some of these works, soluble (non-structural) carbohydrates were extracted to evaluate ^{13}C (since structural carbon mostly represents the photosynthetic ^{13}C signature of the leaf assimilates that contributed to form the ear and not the signature of ear metabolism itself). Although the ^{13}C content is higher (i.e. lower discrimination) in ear parts than in the flag leaf (e.g. Vicente *et al.*, 2018), this can be simply explained by a lower stomatal conductance (i.e. ^{13}C content in the normal range of C_3 plants; Pate, 2001). In summary, the nature of the carbon isotopic composition also supports the idea of C_3 metabolism in green parts of the ear.

Overview: C_3 or C_4 metabolism in the ear?

A number of aspects complicate the elucidation of photosynthetic metabolism in the ear of C_3 cereals (wheat, barley, and rice). First, some studies are still fragmentary, for example detailed transcriptomic analysis without quantification of actual protein levels and compartmentalization studies (e.g. Rangan *et al.*, 2016*a*, b). Secondly, genotypic variability (i.e. differences between species and cultivars) could play some role in these discrepancies. An in-depth and integrative study (using the approaches mentioned above, i.e. analysis of enzyme activity and compartmentalization, photosynthetic sensitivity to oxygen, isotope discrimination, and metabolite levels) is virtually impossible (or at least, expensive and very difficult) with many genotypes. Finally, due to the heterogeneous nature of the ear, the different photosynthetic parts should be discussed separately, in particular the green pericarp versus bracts (glumes and lemmas). In addition, the theoretical complexity of the issue is exacerbated because some results have been poorly discussed in the scientific literature, where some data have been misinterpreted in many cases. For instance, in a recent report, the presence of differentiated bundle sheaths in bracts of the ear is discussed as a C_4 trait (Balaur *et al.*, 2018). However, the presence of larger cells in the bundle sheath is actually only considered as a trait known as 'proto-Kranz anatomy' (Sage *et al.*, 2012) and not a C_4 trait *per se*. In fact, many typical C_3 plants have this anatomy (Sage *et al.*, 2012). One possibility that we cannot rule out is that C_4 metabolism operates with low activity in ear parts, hindering its detection. In the past few

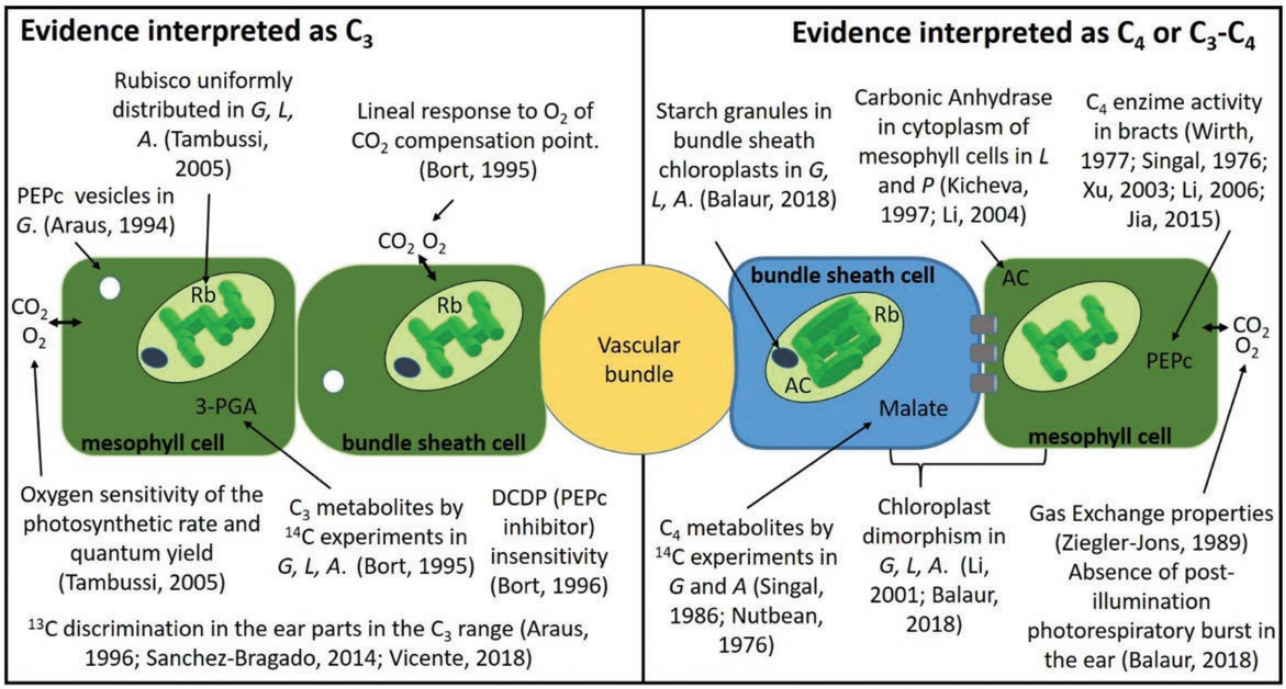

Fig. 1. Diagram summarizing the evidence that suggests C_3 versus C_4 (or intermediate C_3–C_4) metabolism in the ear parts of wheat (and other C_3 cereals). Abbreviations: CA, carbonic anhydrase; *G*, *L*, *A*, and *P* denote glume, lemma, awn, and pericarp, respectively; Rb, Rubisco; PEPC, phosphoenolpyruvate carboxylase. Footnotes to some bibliographic references in the diagram (for more details, see the main text): 'C_4 enzyme activity in bracts' (Wirth *et al.*, 1977; Singal *et al.*, 1986; Xu *et al.*, 2003; Jia *et al.*, 2015); 'C_4 enzymes' (e.g. PEPC, pyruvate orthophosphate dikinase) are actually present in C_3 plants (perhaps with anaplerotic functions as suggested by Bort *et al.*, 1994); 'Absence of glycolate oxidase (GO) activity in bracts' Balaur *et al.* (2013): the interpretation of this finding is controversial as evidence of C_4 metabolism because GO has high activity even in C_4 plants, such as maize; Balaur *et al.* (2018); 'Starch granules in bundle sheath chloroplasts in glumes': the observation is interesting (suggesting compartmentalization?) although only one chloroplast is shown in this article, without any quantification; 'C_4 metabolites by $^{14}CO_2$ experiments in *G* and *A*' (Singal *et al.* 1986). Bort *et al.* (1995) pointed out that the experimental design could lead to artifactual results: the ear was incubated on a moist support which may have increased the amount of inorganic ^{14}C present in the buffer as bicarbonate, the substrate of PEPC (artificially increasing high C_4 acid levels).

years, a novel method was proposed (Cantabrana-Alonso *et al.*, 2016) combining tunable diode laser absorption spectroscopy (named TDLAS) coupled with gas exchange systems to characterize C_4 or C_3–C_4 intermediate plants. This might offer a novel strategy to elucidate the enigma of C_4 photosynthesis in ears of C_3 species.

In summary, current evidence is controversial and some aspects should be clarified (e.g. high activity of enzymes such as PEPC), but the existence of C_4 cannot be discarded. In fact, a C_4 metabolism has been postulated in other non-leaf organs (Hibberd and Quick, 2002). Much of the evidence interpreted as C_3 versus C_4 metabolism in the ear is summarized in Fig. 1.

The ear as a stress-resilient photosynthetic organ

Dehydration avoidance

There is some evidence that ear photosynthesis is less affected by water deficit than assimilation in the flag leaves. This has been reported for bread wheat (Xu *et al.*, 1990), durum wheat (Abbad *et al.*, 2004; Tambussi *et al.*, 2005), and barley (Sánchez-Diaz et al., 2002). Direct IRGA measurements have shown that the reduction in the net assimilation rate of the ear under drought is lower than in flag leaf photosynthesis (e.g. Tambussi *et al.*, 2005; Hein *et al.*, 2016). This has also been measured by modulated chlorophyll fluorescence in ear bracts (Martinez *et al.*, 2003) and awns (Maydup *et al.*, 2014). In particular, under water stress conditions, the awns maintain a higher ETR than the flag leaf (Maydup *et al.*, 2014). From a mechanistic viewpoint, the better photosynthetic performance of ear parts under drought could be explained by their higher relative water content (RWC) (specifically glumes and awns) under water deficit (Tambussi *et al.*, 2005; Maydup *et al.*, 2014). Thus, the maintenance of a higher water status seems to suggest that the ear is a 'dehydration avoider' organ (rather than 'water stress tolerant' *per se*) (see below; Tambussi *et al.*, 2007). Under drought, a better water status might also be explained by a greater osmotic adjustment in the ear parts, which was reported by Morgan (1980) and Tambussi *et al.* (2005). The chemical nature of the accumulated osmolytes is not known, although the accumulation of proline under water stress has been reported in ear parts of barley (Bergareche *et al.*, 1993).

Barley shows higher RWC, greater osmotic adjustment, and, compared with the leaves, higher photosynthetic activity in the ear parts of water-stressed plants (Hein *et al.*, 2016). However, in this latter work (carried out under controlled conditions in small pots), the awns only had a clear advantage with respect to the leaf at incipient stages of stress. Under more severe stress, the stomata closed in the awns and photosynthesis declined. The RWC in plant parts (leaves and ear parts, i.e. glumes, lemmas, and awns) under drought has been negatively correlated with the percentage water content of each organ under control (i.e. well-irrigated) conditions (Tambussi *et al.*, 2005). Although the mechanistic relationship between both parameters is not clear, xeromorphic characteristics of the ear parts (mainly the awns) could be implicated (Tambussi *et al.*, 2007). It is largely unknown if there is genotypic variability in osmotic adjustment capacity in ear parts, a topic that could be worthwhile exploring in the future.

The higher RWC under drought is a key aspect of the 'drought avoidance' behavior of the ear, although this is neglected in some studies. For instance, Lou *et al.* (2018) reported that the ear maintains a better RWC and higher photosynthesis under drought, but the authors try to explain the higher photosynthesis in the ear based on its antioxidant capacity. From a parsimonious point of view, the higher RWC (and, ultimately, osmotic adjustment; Tambussi *et al.*, 2005) could be a more realistic explanation of the better photosynthesis of the ear under water stress conditions.

Another aspect related to water stress avoidance is delayed senescence. In many crops, senescence (in this context, the degradation of chloroplast components) is accelerated under drought conditions (e.g. Pic *et al.*, 2002). Clearly, ear senescence occurs later than flag leaf senescence (e.g. Martinez *et al.*, 2003; Lou *et al.*, 2018), and this behavior is more marked under drought (Lou *et al.*, 2018). Green parts of the ear are the last photosynthetic organs in the ontogeny of wheat, and senescence (protein and chlorophyll degradation) occurs later than in the flag leaf (Martinez *et al.*, 2003). Several key components of the photosynthetic apparatus [such as Rubisco and light-harvesting complex II (LHCII)] are retained in bracts (compared with the flag leaf) throughout grain filling, and some reports have shown that the 'stay-green behavior' of the ear is not cosmetic, because photosynthesis is also maintained (e.g. Martinez *et al.*, 2003; Maydup *et al.*, 2014). Beyond being the last organ to develop, other factors could be involved in the delayed senescence of ears. The higher RWC maintained by the ear parts (mainly bracts and awns) under water deficit could delay senescence onset and/or decrease the senescence rate. On the other hand, thermal balance in the ear could also modify the senescence pattern, and the existence of germplasm variability (genotypes with lower versus higher temperatures in the ear) should be explored (we will briefly discuss this issue in the following section).

One pertinent question is whether the higher photosynthetic rate of the ear under water stress is reflected in grain yield. As far as we know, there is only one study (analyzing a few durum wheat genotypes at different levels of available water in the substrate under controlled conditions) that shows a strong correlation between assimilation rate (measured by IRGA) of the ear versus grain yield (Abbad *et al.*, 2004). In contrast, the correlation between flag leaf photosynthesis and grain yield was very weak in that study. Although correlations do not necessarily imply causal relationships, these results suggest that ear photosynthesis could be implicated in water stress tolerance in wheat at the crop level (Abbad *et al.*, 2004).

Heat stress: thermal balance of the ear

Ear temperature (and its genotypic variability) could be a relevant trait to study because kernel weight is negatively correlated with temperature in winter cereals. In wheat, kernel weight decreases by 3–5% for each degree Celsius above 15 °C (Savin, 2010). In general terms, temperature depression (TD; i.e. the difference between air and organ temperature) seems to be lower for the ear than the flag leaf (i.e. ears are warmer than leaves). This could be explained by a lower transpiration rate (therefore, less of a cooling effect from latent heat flux; Blum, 1985), although a higher absorption of radiation cannot be dismissed (Vicente *et al.*, 2018).

There are several interesting questions about ear temperature, such as: (i) is there genotypic variability in ear temperature; (ii) what plant traits (peduncle length, stomatal density, awn length, etc.) might be implicated in ear temperature variability; (iii) what physiological—and, more importantly—what agronomic consequences could differences in ear temperature have?. The subjacent causes of differences in ear temperature are unknown, but the transpiration rate and water content might be implicated. Concerning question (ii), there are contradictory results on the role of awns because in some cases the presence of awns seems to increase ear temperature (Maydup *et al.*, 2014), whereas in other cases awns seem to lead to a decrease (Ayeneh *et al.*, 2002; Motzo *et al.*, 2002). Another trait that has been correlated with ear temperature is peduncle length (Ayeneh *et al.*, 2002). Ayeneh *et al.* (2002), analyzing 13 cultivars of durum wheat, reported a positive correlation between TD of the ear and awn length, while TD was negatively correlated with peduncle length (i.e. genotypes with shorter awns or longer peduncles had higher temperatures in the ear). In this study, grain yield was positively correlated with ear TD and CTD ('canopy temperature depression').

Breeding for yield potential and stress adaptation: incorporating ear photosynthesis

Shading individual ears during grain filling allowed the detection of differences in the contribution of the ear to grain yield between cultivars (e.g. Maydup *et al.*, 2010). The explanation

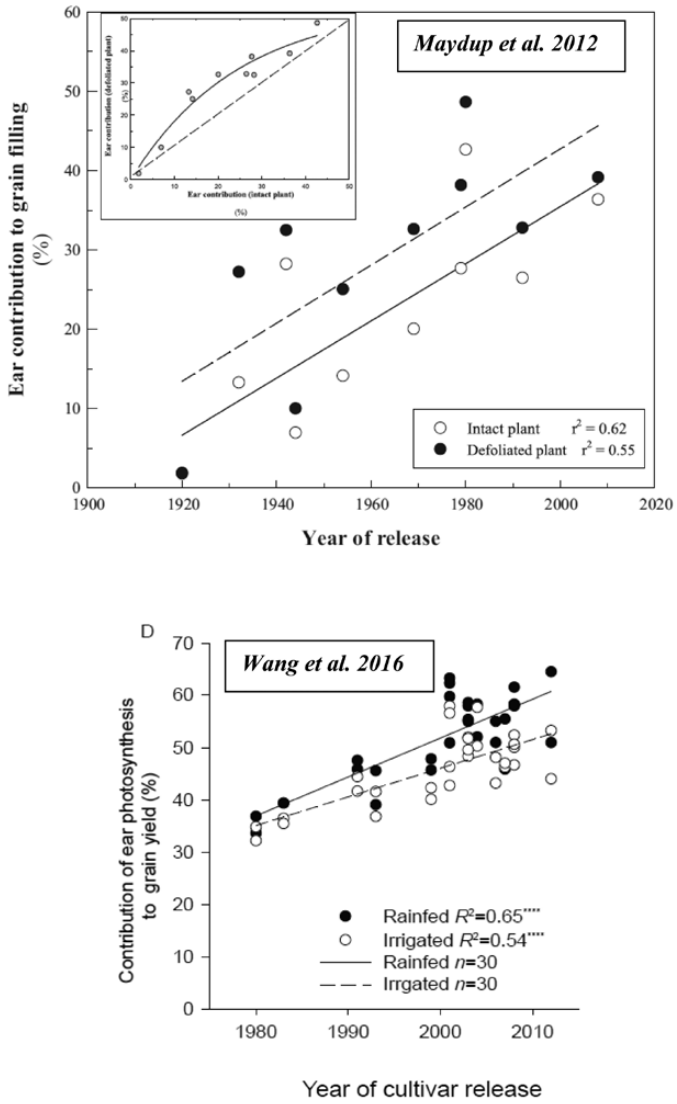

Fig. 2. Increase in the contribution of the ear to grain filling in germplasm from Argentina and China in two retrospective studies (i.e. simultaneous analysis of old and modern cultivars). Upper panel: results of Maydup *et al.* (2012) for 10 cultivars of the Argentina germplasm. Values for intact (open symbols) and defoliated (filled symbols) plants are shown. Each point represents the mean of each cultivar. Inset: the relationship between the contributions of the ear to grain filling in intact versus defoliated plants. The dotted line is the 1:1 relationship. From Maydup *et al.* (2012). 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. Field Crops Research 134, 208–215, Copyright (2012), with permission from Elsevier. Lower panel: results of Wang *et al.* (2016) in China for 15 cultivars under rainfed (filled symbols) and irrigated (open symbols) conditions in two years. Each point represents the mean of four replicates for each cultivar. From Wang *et al.* (2016). Contribution of ear photosynthesis to grain yield under rainfed and irrigation conditions for winter wheat cultivars released in the past 30 years in North China Plain. Journal of Integrative Agriculture 15,2247–2256, Copyright (2016), with permission from Elsevier. In both panels, the contribution of the ear (%) was calculated as described the text (for more details, see Maydup *et al.*, 2012).

of these differences is far from clear, although awn size could be a factor in some cases (Maydup *et al.*, 2010). The correlation between the contribution of the ear and awn size is, however, moderate in some studies (Maydup *et al.*, 2014), suggesting that other factors (e.g. refixation rate) are implicated. The contribution of ear photosynthesis seems to increase under conditions where source activity is decreased, such as water deficit (e.g. Maydup *et al.*, 2010, 2014; Wang *et al.*, 2016) and artificial defoliation (Maydup *et al.*, 2010).

One study indicated that there is genotypic variability in ear photosynthesis (and its contribution to grain filling) in wheat (Molero *et al.*, 2014), and the authors proposed that this trait could be a potential target for breeding programs. In that work, some identified QTLs (quantitative trait loci) were associated with ear photosynthesis, which might be used as a selection criterion (*ex ante*) in the future. However, we are aware that the contribution of the ear could be a complex trait, because intrinsic (i.e. ear photosynthesis *per se*) and extrinsic (i.e. relative contribution of other sources) factors could be involved. In that sense, a recent study from the same team has reported QTLs for the photosynthetic contribution of spikes to grain filling co-located with yield and yield-related traits (Molero *et al.*, 2020).

An increase in the contribution of the ear to grain filling by breeding was first reported in Argentinean germplasm, in a retrospective study of a historical series (i.e. the simultaneous analysis of representative cultivars released in different years) (Maydup *et al.*, 2012). Clearly, modern cultivars had higher contributions of the ear than the older varieties (see Fig. 2, upper panel), and at the same time the contribution of the ear showed a negative correlation with stem weight (i.e. old cultivars with longer and heavier stems showed a lower contribution of the ear than modern cultivars). Thus, it is possible that the increase in the contribution of the ear is a secondary consequence (or compensation) for the decrease in the stem contribution (retranslocation). Consistent with this, when comparing several lines, Molero *et al.* (2014) also found a negative correlation between the grain-filling contribution of assimilates stored pre-anthesis in the stems versus the contribution of the ear. In short, the increase in the contribution of the ear in modern cultivars could be a pleiotropic effect of the presence of *Rht* alleles (dwarfing alleles). Although correlative results do not imply cause–effect relationships, the analysis of the contribution of the ear in NILs (differing in the presence of *Rht* alleles) suggests that this hypothesis is plausible (Maydup *et al.*, 2012). Interestingly, Wang *et al.* (2016) reported similar results (Fig. 2, lower panel) (i.e. gains in contribution of the ear associated with breeding) through analysis of a historical series of Chinese wheat germplasm. Some of this evidence (see also Zhang *et al.*, 2013) is summarized and combined into a 'working hypothesis' in Fig. 3.

As far as we know, there is only one study where the contribution of the ear of landraces (local varieties used by farmers)

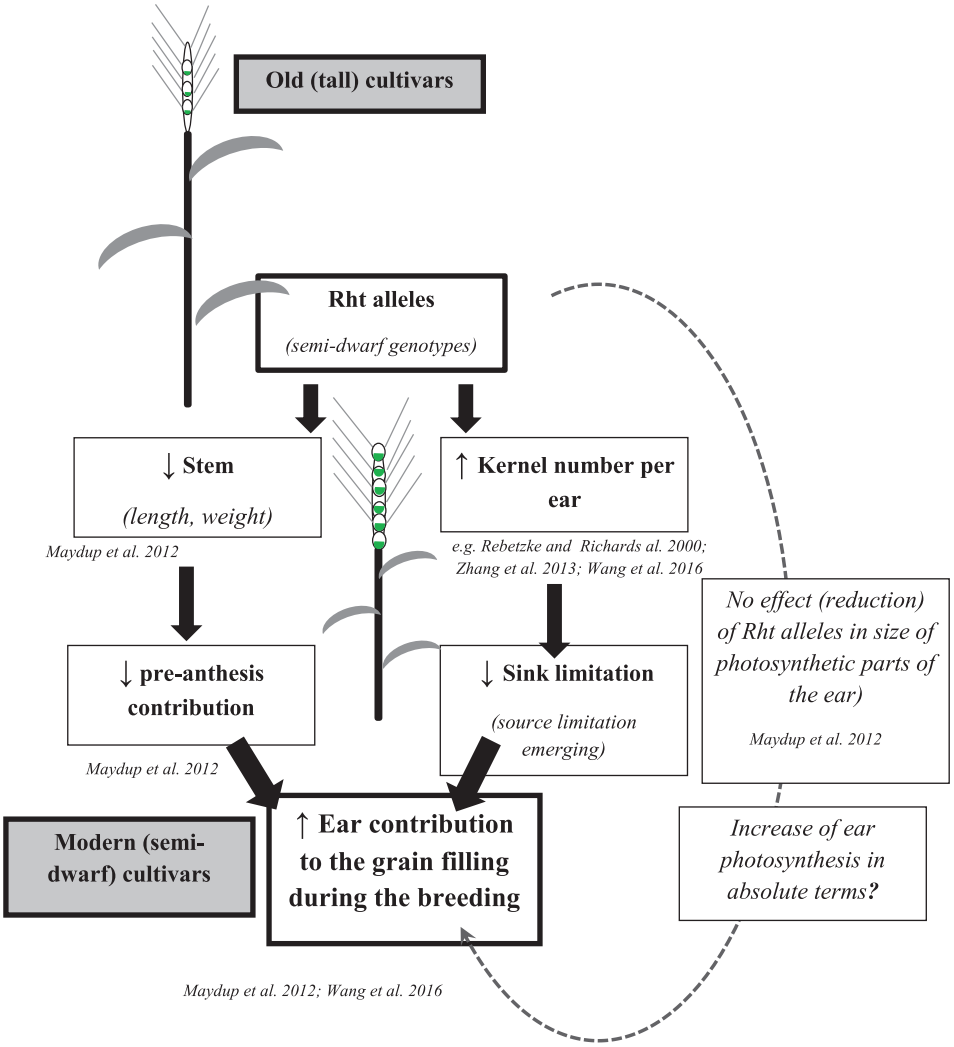

Fig. 3. Conceptual diagram (a working hypothesis with some experimental support) showing the possible relationships between the introgression of dwarfing alleles (during the Green Revolution) and the relative contribution of ear photosynthesis to grain filling in wheat. Thin arrows (↓,↑) inside the boxes denote a relative decrease or increase, respectively. Thick arrows (↓,↙, ↘) indicate possible causal relationships between boxes. The dashed arrow denotes traits possibly concomitant with the introgression of dwarfing alleles. The green area in the kernels (see ears diagram) suggests the proportional contribution of ear photosynthesis to grain filling in old (tall) and modern (semi-dwarf) cultivars of wheat reported by Maydup *et al.* (2012) and Wang *et al.* (2016).

of durum wheat has been compared with modern cultivars (Sanchez-Bragado *et al.*, 2014a). Surprisingly, landraces showed higher contributions of the ear than commercial cultivars; when the harvest index was compared with the contribution of the ear (including all commercial genotypes and landraces in the analysis, and under different environmental conditions), a negative correlation was found. It is surprising that a very high contribution of the ear (quantified with isotopic composition) was found in that study (reaching in some cases values of ~90% in landraces in particular). In general, values of the contribution of the ear quantified via isotopic composition seem to be higher than with other methodologies (see Sanchez-Bragado *et al.*, 2014a, b). As mentioned above, compensatory mechanisms (i.e. the increase in other sources when ear photosynthesis is reduced), which may occur in intrusive approaches, were absent in non-intrusive methods and could explain the higher contribution values found with the isotopic method.

The contribution of the ear to grain filling increases under a number of conditions (particularly those involving stress), such as water deficit (e.g. Maydup *et al.*, 2010; Wang *et al.*, 2016) and artificial defoliation (Maydup *et al.*, 2012), Interestingly, some fungal pathogens (e.g. yellow spot and rust) do not affect the green parts of the ear. One question to be resolved is whether the increase of the contribution of the ear is a secondary consequence of the reduction of another sources (e.g. flag leaf photosynthesis) or if there are increases in ear photosynthesis *per se*. Serrago *et al.* (2013) reported that ear photosynthesis (measured by IRGA) increased when the canopy (except the ear, as mentioned above) was shaded. Thus, the increase in the contribution of the ear (when the source is restricted) may not be simply due to a relative decrease in other contributions.

Finally Sanchez-Bragado *et al.* (2014a) also found a higher contribution of the ear (measured through isotopic composition) under high nitrogen fertilization (compared with plants without nitrogen fertilization, see above). Consistent with this, Olszewski *et al.* (2014) reported in an experiment comparing low versus high nitrogen inputs (60 kg N ha^{-1} versus 120 kg N ha^{-1}) that the increase in the photosynthetic rate under high nitrogen was larger in the ear than in the flag leaf. This might be a direct effect on photosynthesis, or an indirect effect mediated by sink demand under high nitrogen input conditions. In fact, we observed a positive correlation between kernel number per area and the contribution of the ear , suggesting that the photosynthetic activity of the ear might adjust to sink demand.

On the other hand, recently an article reported an increase in ear photosynthesis in transgenic lines overexpressing the enzyme SBPase (sedoheptulose-1,7-bisphosphatase, a Calvin cycle enzyme implicated in ribulose-1,5-bisphosphate regeneration; Simkin *et al.*, 2020). In these transgenic lines, the ear photosynthetic rate increased ~21% with respect to the wild type, an increase even higher than in the flag leaf (Driever *et al.*, 2017). This work reinforces the importance of ear photosynthesis, opening up the possibility for future amelioration of this trait in wheat.

The most extensive and detailed work analyzing the possible relationship between ear photosynthesis and grain yield was recently published by Molero *et al.* (2020). This study explores the contribution of the ear in different sets of germplasm and across several environments (differing in both yield potential and heat conditions), and the authors found greater phenotypic variability in this trait than in flag leaf photosynthesis (2- and 1.4-fold, respectively). A correlation between ear photosynthesis and grain yield was only observed in a set of germplasm (mapping populations) with very contrasting lines, opening up the possibility of exploring this trait (i.e. ear photosynthesis) in potential parents for strategic crosses (Molero *et al.*, 2020).

Awn photosynthesis and contribution: are they actually relevant to grain yield?

Photosynthesis of the awn makes up a substantial percentage of the net assimilation rate of the ear (Tambussi *et al.*, 2007, and references therein). However, its actual contribution to grain yield is more controversial and has been discussed for years (e.g. Patterson *et al.*, 1962; McKenzie 1972; Bort *et al.*, 1994; Weyhrich *et al.*, 1995; Rebetzke *et al.*, 2016; Sanchez-Bragado *et al.*, 2020a). Although de-awning experiments have clearly shown that the contribution of the awn to grain filling can be important (~10% or more, depending on the species and cultivar; e.g. Maydup *et al.*, 2014), comparisons between awned and awnless NILs have shown advantages (e.g. Patterson *et al.*, 1962), no difference (Weyhrich *et al.*, 1995; Rebetzke *et al.*, 2000), or even adverse effects on grain yield in awned isolines (Patterson *et al.*, 1962; McKenzie, 1972). Despite the positive effect of awns on grain filling and kernel weight, there are reports where a reduction in grain number per spike has been observed (e.g. Rebetzke *et al.*, 2016 in wheat; Bort *et al.*, 1994 in barley). Thus, the (putative) positive effect of awns on grain filling might be counterbalanced by a negative effect on kernel number. In a recent article, Sanchez-Bragado *et al.* (2020a) (meta-analyzing previous work and adding their own data) reported no advantage in grain yield in awned versus awnless isolines (NILs). Although an increase in average grain weight in awned (compared with awnless isolines) was found, no effect (or even negative effects) on grain yield were observed. On the other hand, awned and awnless NILs did not show any differences in response to source manipulations (defoliation), suggesting that the effect of awns on average grain weight is not related to source strength. Sanchez-Bragado *et al.* (2020a) proposed that the increase in grain weight in awned isolines is not linked to the awns as a source, but rather explained by the reduction of kernel numbers in distal (smaller) positions of the ear. Thus, the presence of awns may lead to an increase in the average grain weight via effects independent of source strength (an indirect rather than a direct effect). This hypothesis is attractive; however, it does not explain why the increase in average grain weight is still observed in cultivars where kernel number increases (rather than decreases; see cv. Westonia2 in table 2 of Sanchez-Bragado *et al.*, 2020a). In addition, the interpretation of the effects of awns on grain yield (expressed per area) is uncertain because of possible pleiotropic effects (e.g. spike number per m^2 decreases in some awned lines; Sanchez-Bragado *et al.*, 2020a).

Finally, it has been mentioned in the literature that the beneficial influence of awns could depend on environmental conditions (see Tambussi *et al.*, 2007), although no clear evidence has been presented. In the article cited above, where a number of sites were tested, Sanchez-Bragado *et al.* (2020a) reported that the influence of awns was independent of environmental conditions. In another recent study, Molero *et al.* (2020) did not find any relationship between awn length and the contribution to grain yield across several environments.

In summary, the role of awns in contributing to grain yield is still obscure, although some recent evidence seems to suggest that their influence is marginal. Because it is a relatively simple trait to select in breeding programs, sorting out the role of awns during grain yield (grain weight and grain number) is important knowledge for incorporation into wheat germplasm programs.

Concluding remarks and future perspectives

- There are a few methodologies to quantify ear photosynthetic activity (e.g. IRGA and chlorophyll fluorescence) and the contribution of the ear to grain filling (e.g. individual ear shading, ear emergence in shaded canopies, and isotope composition). Further research is needed to clarify some

discrepancies in values of the contribution of the ear assessed by different approaches.

- The existence of C_4 metabolism in the ear is still controversial; although some current evidence (oxygen sensitivity of photosynthesis, isotope composition, and metabolites) suggests the operation of C_3 metabolism, other evidence could suggest the opposite, and the discussion remains open. The identity of photosynthetic metabolism of the ear parts (C_3 versus C_4) needs to be clarified, and possible alternative pathways (e.g. refixation by PEPC of respired CO_2 and malate formation) should be analyzed.
- The contribution of ear photosynthesis seems to have increased in modern germplasm of wheat (at least in Argentina and China), which might be related to the decrease in stem weight that has emerged during breeding (e.g. introgression of dwarfing alleles).
- The observed increase in the contribution of the ear to grain filling in historical series of cultivars (retrospective studies) is still restricted to only two countries, and more investigations should be carried out in order to establish the universality of this phenomenon in wheat germplasm. One important aspect is whether the contribution of the ear has been maximized or if it is possible to further improve this trait in wheat germplasm in the future.
- The contribution of the ear to grain filling increases under source-limiting conditions (e.g. water deficit, defoliation, or presence of pathogens). Recent evidence suggests that the contribution of the ear also increases when sink limitation is reduced (increase in kernel number under high nitrogen input).
- There is genetic variability in the contribution of the ear, opening up the possibility to use this trait to enhance grain yields.
- Ear photosynthesis is resilient, showing tolerance (or, rather, 'dehydration avoidance') to water deficit (compared with the flag leaf), a fact possibly related to osmotic adjustment and the maintenance of RWC in glumes, lemmas, and awns. Further studies are needed to explore whether there is genotypic variability (e.g. in osmotic adjustment capacity) in wheat germplasm and what impact this has on grain yield.
- Thermal balance in the ear is an interesting issue to explore, and more research is needed to clarify the underlying morphological (e.g. awn length and stomatal density) and physiological traits (evaporative cooling by transpiration, etc.) causally related to ear temperature. Knowledge about these traits could be used by breeders to improve grain yield under both optimal and stressed conditions (e.g. water deficit or heat stress).

Photosynthesis of the ear (and perhaps the panicle in rice) is an issue of great relevance in agricultural science, in particular in the context of 'food security' and increases in the global demand for cereals. In fact, source limitation is emerging in wheat germplasm that is presently available (i.e. more assimilates will be required by the crop in the future), and green parts of the reproductive structures might contribute towards grain yield improvements under both optimal and stressful conditions.

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Author contributions

EAT, MLM, CC, JJG, and JLA: conceptualization; EAT writing original draft; MLM, CC, JJG, and JLA writing (review and editing); EAT and JLA funding acquisition and project administration; EAT and CC drawing the figures.

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