New avenues for increasing yield and stability in C3 cereals: exploring ear photosynthesis

Rut Sanchez-Bragado[a,b], Rubén Vicente[c], Gemma Molero[d],
Maria Dolors Serret[b], María Luján Maydup[e] and
José Luis Araus[b]

Small grain cereals such as wheat, rice and barley are among the most important crops worldwide. Any attempt to increase crop productivity and stability through breeding implies developing new strategies for plant phenotyping, including defining ideotype attributes for selection. Recently, the role of non-foliar photosynthetic organs, particularly the inflorescences, has received increasing attention. For example, ear photosynthesis has been reported to be a major contributor to grain filling in wheat and barley under stress and good agronomic conditions. This review provides an overview of the particular characteristics of the ear that makes this photosynthetic organ better adapted to grain filling than the flag leaf and revises potential metabolic and molecular traits that merit further research as targets for cereal improvement. Currently, the absence of high-throughput phenotyping methods limits the inclusion of ear photosynthesis in the breeding agenda. In this regard, a number of different approaches are presented.

Addresses
[a] Department of Crop and Forest Sciences, University of Lleida - AGROTECNIO Center, Av. R. Roure 191, 25198 Lleida, Spain
[b] Secció de Fisiologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Barcelona, and AGROTECNIO Center, Lleida, Spain
[c] Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam, Germany
[d] Global Wheat Program, International Maize and Wheat Improvement Centre (CIMMYT), Texcoco, Mexico
[e] National Council of Scientific and Technological Research, CONICET, La Plata University- Plant Physiology Institute INFIVE, Argentina

Corresponding author: Araus, José Luis (jaraus@ub.edu)

Introduction

The main staple crops at a global level are wheat followed by rice, while other small grain cereals such as barley are also among the most cultivated. Annual genetic gains for wheat have been reported as ~1 % on average [1,2] not sufficient to meet the projected global demand, which increases by 1.7% annually [3]. Indeed, progress from the main traits that contributed to the *Green Revolution*, such as harvest index, have become exhausted. Therefore, yield improvements will need to come from technological innovations together with new traits not considered before.

Climate change predictions for the coming decades include increases in temperature as well as longer and more numerous droughts in many regions, which together with other global change challenges (demography, dietary habits, etc.), demand increases to the productivity and stability of wheat. Increasing the photosynthetic efficiency of leaves, which frequently includes genetically modified organisms (GMO) approaches, has been argued as one of the logical avenues to tackle the problem and achieve the so-called second 'Green Revolution'. However, in spite of the abundant literature produced [4,5], gene transformation approaches have not yet delivered their promise [6]. In fact, the most solid evidence related to an increase in yield being associated with increases in photosynthesis originates from retrospective studies where genetic advances in photosynthesis have been paralleled by increases in stomatal conductance [7], while in other cases stay-green is the main factor [8]. However, in some cases, like durum wheat under Mediterranean conditions, stay green has not necessarily been a positive trait [9].

In any case, rather than individual processes such as the photosynthetic capacity of single leaves, it is likely that the integrated canopy photosynthesis may be more relevant for determining final yield [10]. Canopy photosynthesis is not only dependent on the photosynthesis of individual leaves but also on the non-laminar parts, including reproductive organs such as the ears or the panicles when cereals are considered.

Ear photosynthesis and grain filling

Surprisingly, the photosynthetic contribution of non-laminar parts and particularly of the inflorescence (the ear in the case of wheat or barley, and the panicle for rice or oats) has been barely considered. In fact, grain set and further filling occurs by acquisition of

assimilates from (i) flag leaf photosynthesis (blades and sheaths), (ii) ear photosynthesis and (iii) remobilisation of assimilates stored in the stem (mostly) before anthesis [11,12••]. The contribution of each component depends on the plant species, cultivar and environment. However, different lines of evidence, including diverse methodological approaches, have concluded that the ear may be the main contributor to grain filling. Methodological approaches include not only disrupting treatments, consisting of specific elimination of the photosynthetic activity of the ear, the leaves or other parts of the plant [13••,14•] but also using non-disruptive approaches [15,16]. Moreover, by using measurements of N content and the natural abundance of $\delta^{15}N$, a potential contribution of ears in providing N has been determined in wheat, and it is at least similar to the contribution of leaves and even more relevant under water-limiting and nitrogen-limiting conditions [17]. Accordingly, Lopes *et al.* [18] proved that glumes participate in the storage and remobilisation of N to the grain.

The net photosynthesis rate on a per unit area basis in the ear is considerably lower than that of leaves (flag and penultimate leaf). However, its real photosynthetic importance may in fact be underestimated by the high rates of respiration of the ear [19]. The high rates of respiration in the ear are due to the presence of heterotrophic tissues and the respiration of the developing grains [12••,20•]. Overall the photosynthetic capacity of the ear as a whole organ may reach values close [21•] or even higher to that of the flag leaf [19,24]. Moreover, while wheat leaf photosynthesis declines after anthesis, other organs such as the ear, stem and leaf sheath may remain comparatively photosynthetically active during the second half of grain filling, contributing photoassimilates to growing grains [22•]. Because of its proximity to developing grains and its continuing photosynthetic capacity during grain filling, the ear seems to be one of the main photosynthetic contributors during grain filling [13••,20•,22•,23]. In fact, theoretical capacity of the ear to produce assimilates along the grain filling equates the total weight of the grains in the ear [14•]. However, although a positive relationship has been reported between ear photosynthesis and grain yield in wheat [11,24], no clear links have been found in retrospective studies between the genetic advance in grain yield and ear photosynthesis [13••,25].

It has been proposed in wheat using C isotope composition, among other methodologies, that the C assimilated by flag leaves is mainly used for shoot growth [26], while a significant part of the C (and N) accumulated in grains of wheat, barley and rice comes from the ears and panicles when good agronomic conditions exist [16,26,27]. This is even more relevant under water-limiting and N-limiting conditions [11,15–17,20•,28].

Altogether, the photosynthetic contribution of inflorescences in C_3 cereals is a key factor for grain yield during grain filling, and this is especially so under heat-stress and water-stress conditions that will be more frequent and more severe with climate change in the Mediterranean basin. For example, in the case of wheat, different transcriptomic, metabolomic and physiological approaches show that the ears are more stress resistant than the leaves [29••]. However, recent evidence indicates that the contribution of ear photosynthesis to grain filling may also be important under good agronomic conditions [13••,14•,15,16]. These aspects make ear photosynthesis an important target for increasing yield potential and stability. The particular characteristics of the ear as a photosynthetic organ may confer some advantages compared to the flag leaf and the rest of foliar canopy [12••].
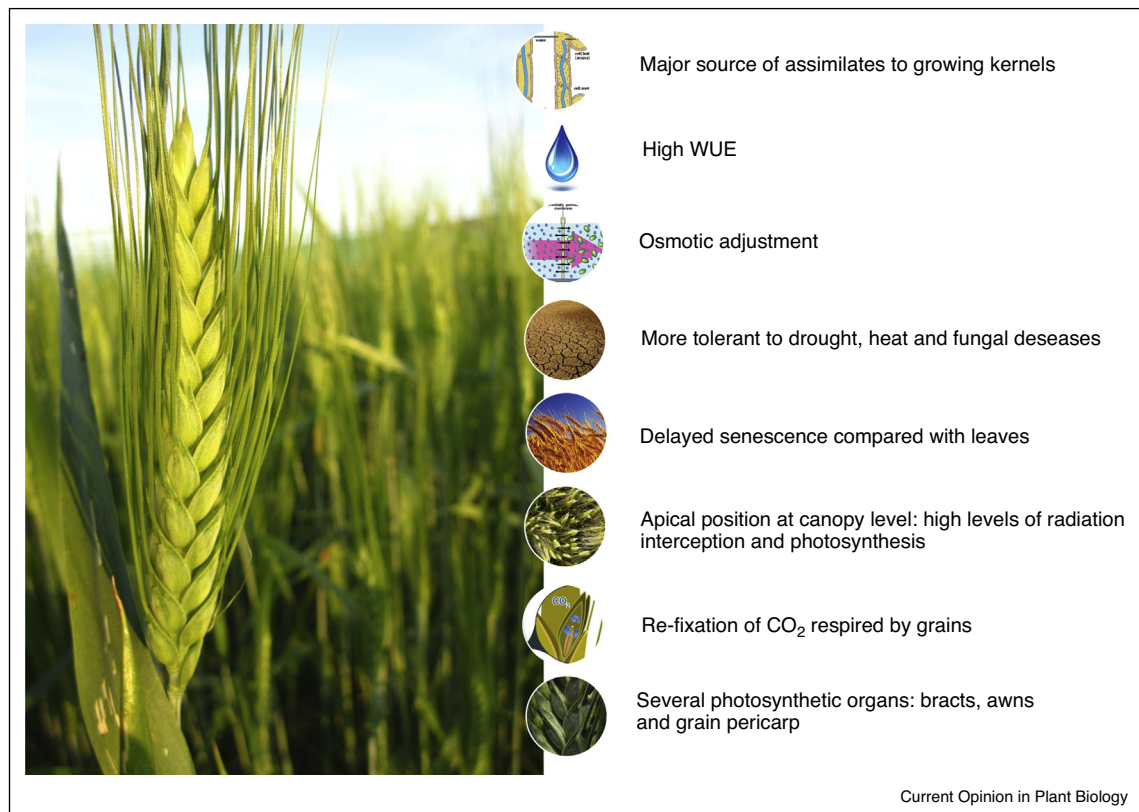
A recent study has identified several QTLs associated with ear photosynthesis including a common QTL for yield potential and heat stress on chromosome 5B in a mapping population developed from a cross between durum and emmer wheat [30]. The QTLs identified for ear photosynthesis in the cited study co-located with previous QTLs identified for yield, grain weight, grain number, root traits and photochemical reflectance index, and confirmed the contribution of ear photosynthesis to these traits at a genetic level.

The ear is a 'constitutively' stress-adapted organ

Ears have evolved under full exposure to solar radiation, unlike the rest of the canopy. In the case of wheat, ears intercept up to 30% of the incident radiation [16], and in the case of two row barley with its prostrated ears probably far more (Figure 1). Moreover, because of its apical position, the ear is far less susceptible to possible shading events in front of frequent fluctuations of light caused by wind-induced movements [31] compared with wheat leaves [32]. Excessive light absorption by the ears may be mitigated by the presence of epicuticular waxes, which in this organ have a special structure favouring the reflectance of radiation [33], and in the case of awned ears, the awns diffuse the incoming light. Nevertheless, regardless of the water regime, under sunny conditions ears may exhibit temperatures that are several degrees higher than the flag leaves or the rest of the canopy [29••].

Ear photosynthesis, including Rubisco, chlorophylls, chlorophyll fluorescence parameters and stomatal conductance, is less adversely affected by drought than leaves [34,35•,36,37], even considering that under such conditions the ear temperature may be several degrees higher than the leaves (Figure 2). Interestingly, awns have more heat-stable photosynthesis and a higher optimum temperature than flag leaves [38]. Additionally, ear organs exhibit delayed senescence [12••,22•,29••,35•]. Therefore, ear photosynthesis may

Figure 1

- Major source of assimilates to growing kernels
- High WUE
- Osmotic adjustment
- More tolerant to drought, heat and fungal deseases
- Delayed senescence compared with leaves
- Apical position at canopy level: high levels of radiation interception and photosynthesis
- Re-fixation of CO_2 respired by grains
- Several photosynthetic organs: bracts, awns and grain pericarp

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The particular physiological and morphological characteristics of the ear makes this organ especially advantageous, not only under drought and heat conditions but also under good agronomic conditions: (i) the proximity to the sink organs facilitates the transport of assimilates compared to other source organs; the xeromorphic anatomy of the ears, together with (vii) the re-fixation of CO_2 respired by grains may grant the ear (ii) a higher water use efficiency (WUE) [20[•],24], which combined with (iii) a higher osmotic adjustment [37] provides greater capacity to maintain higher relative water content with respect to the rest of the plant [39,75]. As a consequence, this may avoid dehydration of the bracts and awns [76] therefore preventing (iv–v) drought or heat-induced senescence compared to the flag leaves [37] or the incidence of certain fungal diseases that can affect the leaves much more than the ears [76,77]. The ear (vi) is the organ with the greatest exposure to radiation due to its apical position in the plant, and consequently it experiences the most heat, increasing respiration losses. Nevertheless, the excessive light absorption may be mitigated by the presence of epicuticular waxes [33]. The refixation of CO_2 (vii) respired by the growing grains is another characteristic that makes the ear photosynthesis different from that of the leaves [20[•]]. In fact (viii) the awns and external surfaces of the glumes are considered the main photosynthetic organs in ears fixing external CO_2, whereas internal surfaces of ear bracts (i.e. lemmas and paleae) and the green pericarp probably have a role more closely related to recycling CO_2 respired by the grains [12[••],29[••],41,78].

have special morphological, physiological and metabolic features that confer tolerance to heat and water stress more than other parts of the plant.

Ears exhibit better performance under water stress than leaves due to better nitrogen and water status, photochemical efficiency, stability of the photosynthetic apparatus, lower diffusive conductance, and high expression of key genes for primary metabolism as well as for drought-stress responses [29[••],36]. Better water status in ear bracts and awns under water stress has been correlated with higher osmotic adjustment and a higher Relative Water Content (RWC) compared to the leaves [20[•],37–39]. Osmotic adjustment (accumulation of osmolytes) can ensure maintenance of turgor and gas exchange under

water stress [37]. These drought tolerance traits are related to the xeromorphic anatomy of ear, which exhibits thick epidermis and cuticle on the dorsal side of bracts [20[•],36,40]. Moreover, because of the excess light intercepted (more than needed for photosynthesis) and the presence of high temperatures, ears exhibit higher non-photochemical quenching (NPQ) and xanthophyll cycle-dependent photoprotection than leaves to dissipate the excess energy and heat and thus protect the photosynthetic apparatus [35[•]]. In fact, the chloroplasts in ears remain more structurally preserved than leaves under water stress [35[•]].

Because of their anatomy and proximity to respired CO_2 released during grain endosperm respiration, ear bracts

Figure 2

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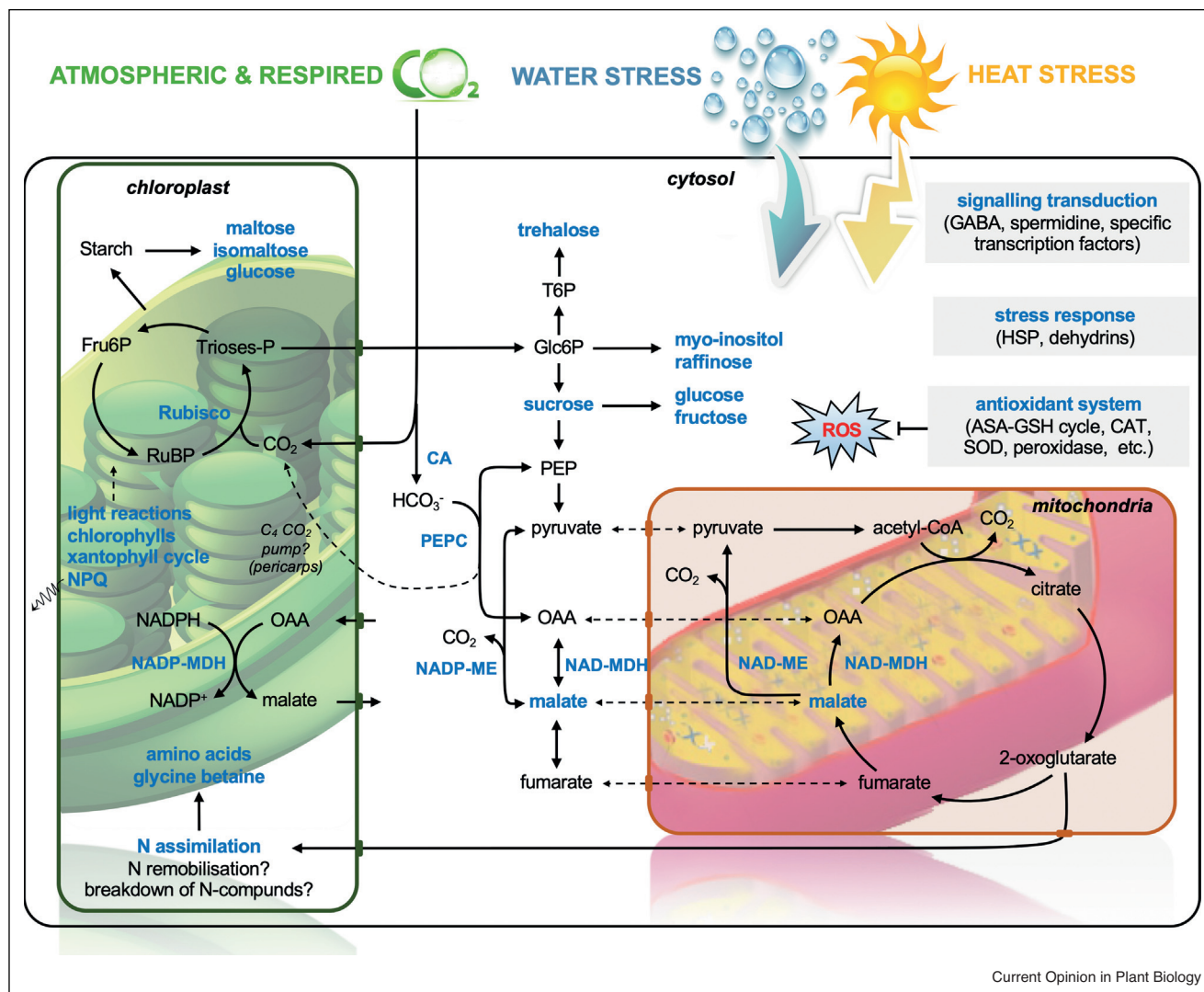
Overview of the most relevant changes observed in ear organs under water and/or heat stress. The components and processes that showed upregulation (in terms of either higher content, activity or gene expression) and/or better performance (i) in ears compared to leaves or (ii) in ears under water stress compared to optimal conditions are highlighted in blue. However, under water stress a lower accumulation of ROS was observed in ears compared to leaves (highlighted in red) [22•,29••,35•,36,44,46,47••,52•,53••,54••,55•,56••,57••]. These components are potential targets that can be used in breeding programmes to select genotypes not only possessing ears with better stress adaptation but eventually to improve the resilience of the whole plant to stress conditions in C3 cereals. The ear organs can fix CO_2 more efficiently than leaves under stress conditions due to several factors, for example, their xeromorphic anatomy, better water status, greater uptake of respiratory CO_2 from grains and delayed senescence. The proportion of atmospheric and respired CO_2 used varies between organs and depends on their proximity to the developing grains and their different tolerance to stress conditions. Awns exhibit the highest photosynthetic capacity in ears and this is based on the uptake of atmospheric CO_2. Although ear bracts can also fix atmospheric CO_2 (mainly the glumes), a high capacity for the refixation of CO_2 is expected. PEPC facilitates anaplerotic reactions for the synthesis of amino and fatty acids needed for the growing grain. Nevertheless, the CO_2 fixed by PEPC may be used via a "C4 pathway" in wheat pericarps to concentrate CO_2 around Rubisco, although current evidences are very weak in this sense. Acetyl-CoA, acetyl coenzyme A; ASA-GSH, ascorbate-glutathione; CA, carbonic anhydrase; CAT, catalase; Fru6P, fructose 6-phosphate; GABA, gamma-aminobutyric acid; Glc6P, glucose 6-phosphate; HSP, heat shock protein; MDH, malate dehydrogenase; ME, malic enzyme; NAD, nicotinamide adenine dinucleotide; $NADP^+$/NADPH, oxidised/reduced nicotinamide adenine dinucleotide; NPQ, non-photochemical-quenching; OAA, oxaloacetate; PEP, phosphoenolpyruvate; ROS, reactive oxygen species; Rubisco, RuBP carboxylase oxygenase; RuBP, ribulose-1,5-bisphophate; SOD, superoxide dismutase; T6P, trehalose 6-phosphate.

Figure 3

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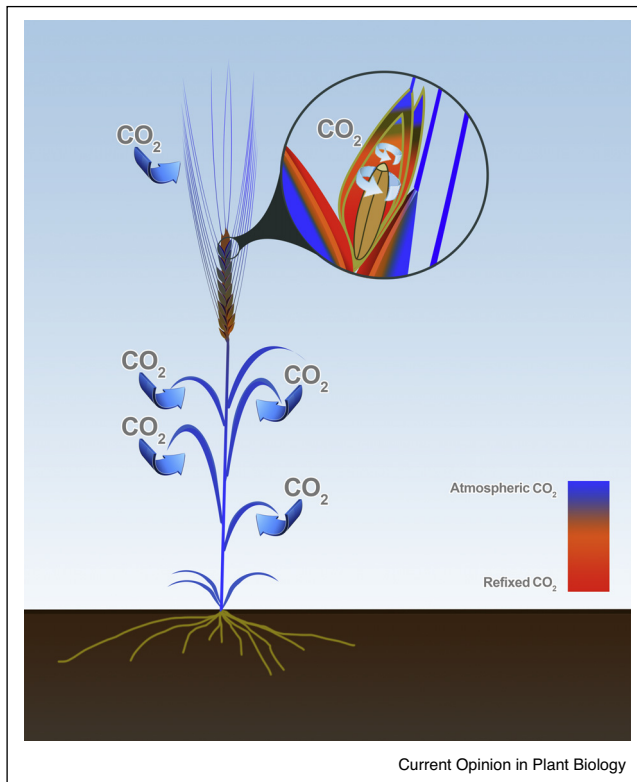
Provenance of the CO_2 taken up by different organs in a wheat plant. Red represents organs and sites that mainly refix CO_2 respired by developing grains, whereas blue represents organs and sites of net fixation of atmospheric CO_2. The awns and the glumes (represented as blue in the figure) are putative sites for net fixation of atmospheric CO_2 (stomata in these sites are abundant) [37]. By contrast, ear bracts (i.e. lemmas and paleae) and the green pericarps of the grains are mainly involved in recycling respiratory CO_2 [12**,29**,41]. Because of their anatomy and proximity to respired CO_2 released during grain endosperm respiration, ear bracts and green pericarps exhibit a higher capacity for CO_2 refixation (organs represented in red) than leaves and awns, which mainly fix atmospheric CO_2 (organs represented in blue) [12**,40,41]. This can reduce respiratory loss and increase the water use efficiency of the ear as a process independent of gas exchange.

and green pericarps show a higher capacity than leaves and awns for refixation of CO_2 [12**,40,41] (Figure 3). This can reduce respiratory losses and increase the water use efficiency of the ear as a process independent of gas exchange with the external atmosphere. Wheat and barley ears can refix 55–75% of the respired CO_2 and this process can significantly contribute to grain yield [41–43]. When grains are bigger, they can block the stomata (ventral-side) of ear bracts and the uptake of CO_2 from the atmosphere; however, this does not result in lower photosynthesis because there is compensation from the higher uptake of respiratory CO_2 from grains and better exposure to light of the apical parts of the bracts after the spikelet structures are pushed apart by the growing grains [12**,40]. High carbonic anhydrase (CA) activity was

found in glumes, lemmas, paleae and pericarps during grain filling, which can catalyse the hydration of CO_2 to HCO_3^- and provide the substrate for phosphoenolpyruvate carboxylase (PEPC) [44]. There exists a controversy about whether photosynthetic metabolism in ears is typically C_3 or whether there is some degree of C_4, C_3–C_4 intermediate or CAM, either constitutive or more apparent under drought conditions. C_4 photosynthesis would provide clear advantages as an efficient form of carbon assimilation minimising carbon losses. Ziegler-Jons [45] proposed that wheat ear bracts exhibit an anatomy-like intermediate C_3–C_4, with the presence of a second type of cell surrounding the vascular bundles with evenly distributed chloroplasts, designated as bundle-sheath cells. Jia *et al.* [22*] suggested that water deficit induced the activity of C_4 pathway enzymes (PEPC; NADP-malate dehydrogenase, NADP-MDH; NAD(P)-malic enzyme, NAD(P)-ME; and pyruvate orthophosphate dikinase, PPDK) in ear bracts, especially in glumes and lemmas and during early grain filling, while at the same time Rubisco activity declined significantly (high PEPC/Rubisco ratios). Supporting this, Singal *et al.* [46] showed that most of the CO_2 fixed by ears in wheat was mainly accumulated in malate and C_4 enzymes (i.e. PEPC, NAD(P)-MDH, NAD(P)-ME, aspartate aminotransferase and alanine aminotransferase), and the quantities of these were generally greater in ear organs than in flag leaves. Rangan *et al.* [47**] revealed that at the transcript level there is a complete set of specific C_4 pathway enzymes in the pericarp of the developing wheat seed, and these are expressed at optimal levels during early to mid-grain filling. However, it is unlikely that CAM or C_4 metabolism occur in ears (at least in the bracts) under optimal or water-limited conditions as has been demonstrated by (i) the O_2 sensitivity of the assimilation rate and electron transport, (ii) the lack of Rubisco compartmentalisation in the mesophyll tissues and Kranz anatomy (although this is not strictly necessary), (iii) the gas-exchange pattern at night [37], (iv) the absence of any effect of a PEPC inhibitor on ear photosynthesis [48], (v) the low amounts of C_4 acids (malate and aspartate) produced by photosynthesis [49], and (vi) the similarity of $\delta^{13}C$ values in ear organs to other C_3 plants [20*,48]. Moreover, immunolabelling studies suggest that PEPC is involved in anaplerotic reactions that supply four-carbon skeletons for the large demand for amino and fatty acids rather than the synthesis of carbohydrates in wheat grains [48,50], which is a conclusion that could be extended to the other C_4 enzymes reported above. Nevertheless, whether C_4 photosynthesis happens in wheat seeds is still a matter of debate [51] and further experimental evidence is required.

Osmolytes play an important role by maintaining cell turgor and preventing protein degradation and cells from oxidative damage by scavenging reactive oxygen species (ROS). Comparing ear organs in barley, Abebe *et al.* [52*]

showed that the effects of drought stress on gene expression were more evident in the awns, followed by lemmas and paleae, while little effect was observed in developing grains. Genes involved in the synthesis of osmolytes (proline, glycine betaine, spermidine, GABA, raffinose and trehalose) were upregulated under water stress. Moreover, alternative oxidase was reported as being upregulated in the lemmas, paleae, and awns, and this can reduce accumulation of ROS [52[•]]. Genes for the drought stress response were upregulated in both wheat and barley ears [29[••],52[•]], including heat shock proteins, LEA (dehydrins), antioxidant system enzymes, and so on, which can play a role in stabilising proteins, preventing water loss and reducing ROS, among other functions.

Under water stress, Vicente *et al.* [29[••]] observed an upregulation of genes involved in respiration, CO_2 refixation via PEPC and nitrogen assimilation in glumes during the grain filling period, while genes related to light reactions and the Calvin-Benson cycle were less repressed than in leaves. Changes at the transcript level have been corroborated for metabolite and enzyme activities. Metabolome analysis of durum wheat leaves and ear bracts showed that respiratory intermediates and most amino acids increased in ear bracts but not in leaves under water stress [53[••], Vergara-Diaz *et al.* unpublished results]. These changes highlight the active role of ear bracts in producing organic and amino acids for the growing grain and are in line with the high refixation rates of respiratory CO_2 in these organs and the upregulation of C–N metabolism genes. Several sugars increased in ears, which can be associated with better C assimilation via photosynthesis or greater breakdown of complex sugars to increase osmolytes (raffinose, trehalose, malate, maltose and isomaltose) and improve drought tolerance (Figure 2). The accumulation of most of the amino acids in ears can also indicate a better tolerance to stress. Although some components can be involved in other functions, increases in glycine, serine, glycerate and glycolate suggest that photorespiration is induced to protect ears from photoinhibition under water or heat stress. Ascorbate metabolism, which is involved in the antioxidant system, was also induced in ears. In agreement with these observations, Impa *et al.* [54[••]] showed an increase in proteinogenic amino acids (glycine methionine, and alanine) and carbohydrates (sucrose, glucose, fructose, myo-inositol, raffinose, and maltose) in wheat ears subjected to high night temperatures after heading, changes that were mostly not observed in leaves. Again, these changes can be related to stress-induced breakdowns or stress-adaptive processes (accumulation of osmolytes to maintain cell turgor, stabilise cell membranes, and prevent protein degradation). In barley, Hein *et al.* [55[•]] indicated that several amino acids (glycine, valine, isoleucine, threonine, and phenylalanine) and sugars (glucose, fructose, sucrose and malate) accumulated in ear organs in response to water stress, although their changes

were organ-specific. Furthermore, the combination of water and heat stress led to changes in the rice metabolome that are dependent on the cultivar, the organ and the severity of the water stress [56[••]]. Interestingly, mild water stress did not modify the metabolome of flowering spikelets when all the cultivars were grouped together, whereas several sugars and amino acids related to stress were modified in leaves. Under severe drought, more sugars and amino acids related to stress increased in leaves compared to mild stress, while respiratory intermediates decreased. However, in spikelets severe water stress only induced a few amino acids (isoleucine, leucine and threonine) and polyols. In developing seeds water stress increased some amino acids and decreased succinic acid, glucose-6-phosphate, erythronic acid and erythritol. Proline, which is involved in stress responses as an osmolyte and antioxidative defence and signalling molecule, was generally accumulated under water stress in both leaves and ears [53[••],55[•]].

Regarding enzyme activities, we described above stimulation of 'C4 enzymes' that could reflect induction of organic and amino acid synthesis in ears for transport to developing grains [22[•],44,46]. Additionally, Kong *et al.* [35[•]] and Lou *et al.* [57[••]] reported that glumes and lemmas have higher antioxidant enzyme activities (their gene expression was also induced) and lower accumulation of ROS than flag leaves in wheat under water stress. Overall, the data support the idea of an active metabolism in ear organs (one that is efficiently protected against water deficit) that significantly contributes to grain filling, especially during the late stages when leaves are more senescent. In fact, the ears are the youngest photosynthetic organs and the last to develop, and as water stress progresses they show a slower decline in their photosynthetic apparatus than the leaves, which is mainly due to their better water status.

Metabolic/molecular clues for improvement

A complete understanding of the mechanisms of carbon assimilation and drought tolerance in the different ear parts is necessary for the identification of new traits as selection criteria for breeding programs. The variability in CO_2 refixation between cultivars and its role in grain yield is still not well studied. This trait can positively impact grain yield by ensuring the provision of CO_2 for ear photosynthesis and minimising the loss of CO_2 released by grains, while ensuring a high WUE because transpiration through stomata is prevented.

Furthermore, it seems that there are differences between the genes expressed in ears and leaves [47[••],58], which suggests that some enzyme isoforms are organ-dependent. For example, C4-specific forms of several enzymes were observed in wheat pericarps and differed from the typical C3 forms found in leaves [47[••]]. The conversion of C3 crops towards C4 photosynthesis is a long-standing but

challenging goal due to the specialised anatomy of the C_4 pathway, which requires further research as well as improvements to the current engineering tools to enable introduction into the C_3 cereals [59]. In the shorter term, the identification of specific isozymes in ears, which can have an advantage under drought and heat stresses, are potential targets for introduction into leaf tissues. The recent advances in genome editing through CRISPR technology may greatly accelerate the engineering of cereals, even in the case of wheat with its large genome and recalcitrant nature [60]. The 'omic' studies have significantly contributed to the identification of new candidate genes, metabolites and proteins in ears that can be used in breeding programmes to improve drought and heat stress tolerance. For example, metabolites with osmolyte functions, TCA cycle intermediates, antioxidant enzymes, or stress response genes upregulated in ears can be used as target proxies to screen diverse germplasm or mapping populations to identify important genomic regions and develop gene-based markers to target-specific enzymes [54••]. Much effort has been invested in studying ear carbon metabolism, yet the potential contribution of ears towards supplying nitrogen might also be a positive trait for grain yield [17]. Knowledge of the role that ears play in nitrogen assimilation, translocation and remobilisation during grain filling is still scarce, but it seems to be relevant [18,29••].

Phenotyping ears

Many questions remain open about the mechanisms of ear photosynthesis, such as the contribution of CO_2 refixed from growing grains versus that from the surrounding atmosphere, or even the photosynthetic metabolism of the different ear parts [49,50] compared to laminar parts of the plant, which are crucial to understanding the adaption of the ear to stress conditions. The awns are the main photosynthetic organ in ears that fix external CO_2, followed by the glumes, whereas photosynthesis in the ear bracts (i.e. lemmas and paleae) and green pericarps is mainly related to recycling respiratory CO_2 [12••,29••,41]. However, the relative importance of the external and internal sources of CO_2 to ear photosynthesis has not been resolved and genotypic variability may be expected. This is not trivial since, for example, an internal source of CO_2 may be associated with lower photorespiration losses, while fixation of the external CO_2 is related to transpiration and ear cooling, which is positive, but also a negative factor with respect to the associated water loss. Besides that, the relative contribution of both sources may change through time (basically increasing the importance of refixed CO_2) as the grains grow and sit tightly against the internal bracts. The search for phenotypic approaches to discern the relative contribution of both sources of CO_2 in the ear photosynthesis continues.

On a different level, there is not yet a high-throughput phenotyping approach to assess the photosynthetic contribution of the ear to the growth and development of wheat grains (Figure 4). The protocols available are either intrusive and/or low throughput, which has to date made ear phenotyping impractical on a large scale and in general terms only feasible for selecting parental lines for further crossings (Figure 4). In other words, the nature of these phenotypical approaches makes them difficult to upscale and to apply in genomic selection (that models markers of largely unknown effect to predict yield, and QTL) or in genome wide association studies (GWAS, that help to dissect complex physiological traits). Evidence for this is the existence of only one study that has reported the use of these techniques for GWAS [30]. Besides that, molecular markers associated with ear morphological traits such as ear length, awn length and number of spikelets per ear have been identified in wheat [61–63], barley [64] or wheat wild relatives [65]. Nevertheless, the identification of molecular markers associated with ear photosynthesis shows promise as a breeding tool for use in marker-assisted selection and would represent a step forward in the status of the ear as a photosynthetic organ.

Different remote sensing and analytical approaches are emerging that allow phenotyping of the photosynthetic performance of ears in a more high-throughput manner. For example, the use of the hydrogen stable isotope signature ($\delta^2 H$) in its natural abundance has been proposed as an indicator of the degree of photoautotrophy in different organs of wheat (including the ear) [66••], in addition to its role as an indicator of transpiration and thus water status.

Moreover, as stated above, different lines of evidence suggest that higher levels of stomatal conductance and transpiration are associated with genetic advance in yield [7,9,67]. Therefore, it is expected that the best performing wheat genotypes will maintain ears at lower temperatures as an indicator of better water status and higher levels of ear photosynthesis. In addition, it has been proposed that ear temperature may also indicate the phenological stage and genotypic heat stress tolerance [68]. Therefore, it is necessary to develop phenotyping protocols that are able to measure ear temperature in the field while discerning it from the rest of the canopy. This may be achieved through direct measurement of ear temperature in the field using algorithms based on thermal images alone [69] or combined with high-resolution RGB images [70•], and thus could select the ears for data extraction (e.g. temperature). In this sense, the automatic extraction of specific information of a given plant part from any RGB, spectral or thermal image acquired on the ground or even from an unnamed aerial platform (e.g. drone) is already a feasible technological target. High-resolution RGB images are ideal candidates for practical large-scale implementation of remote sensing-based phenotyping of ears. The main challenges to be accomplished are the development of specific algorithms, so that RGB images can be used alone or in combination with thermal and spectral information, and then applied to analyse the

Figure 4

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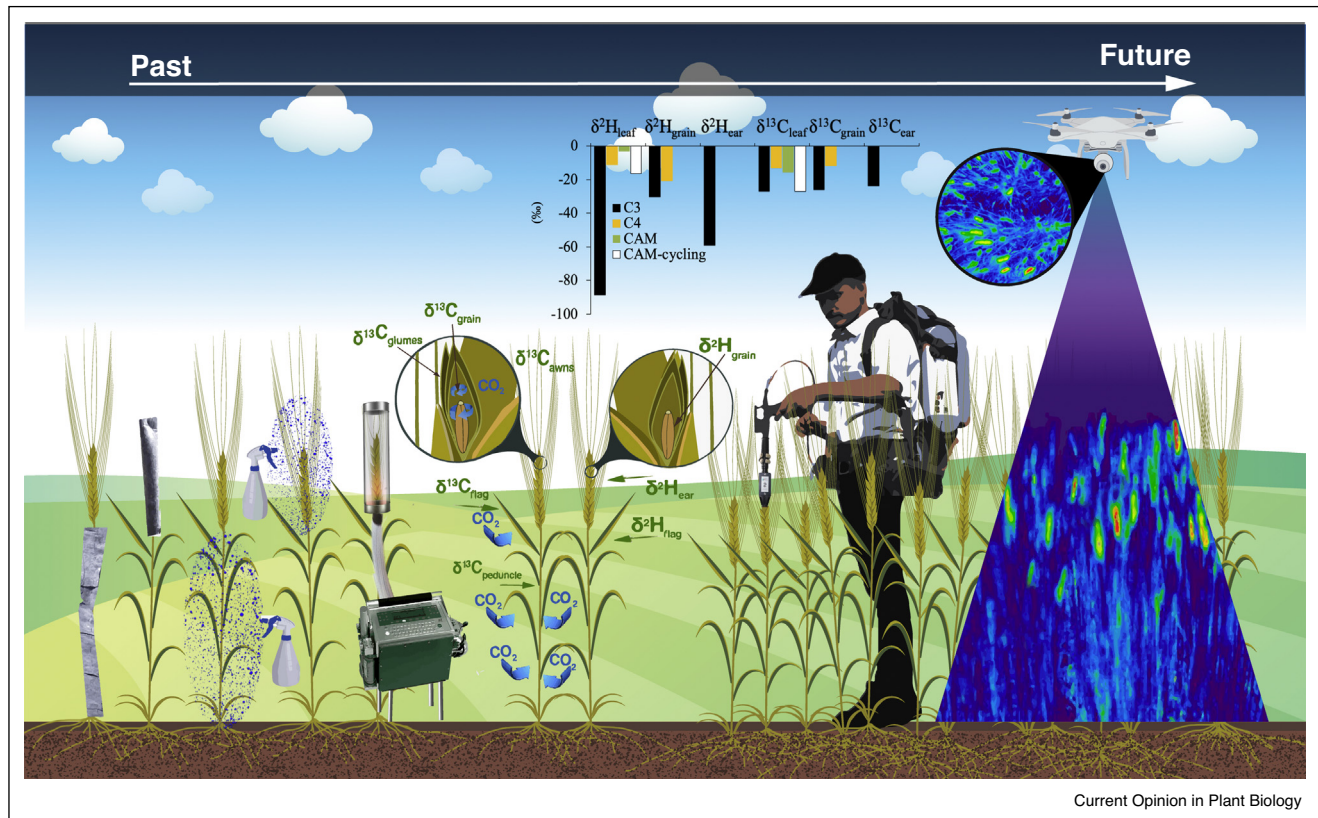
Different strategies for ear phenotyping have been developed over time and will continue being developed into the future. Approaches developed in the past to estimate relative contributions of the ear and other parts of the culm to grain filling include organ-specific inhibition of photosynthesis through different approaches such as: i) application of herbicides [14•,79], desiccants such as potassium iodide [80]; ii) shading or source/sink manipulation treatments, including organ removal [23,81–83]; and iii) an approach using the $\delta^{13}C$ of assimilates that allows inference of the relative contribution of the awns, glumes and peduncle to grain filling [14•,15,16]. The above approaches may be combined with gas exchange measurements (net and gross photosynthesis and respiration) of the ear, using specially designed photosynthetic chambers and portable gas analysers. Recently, other approaches have been proposed if not to assess the relative contribution of each plant part to grain filling, at least to characterise relevant photosynthetic attributes of the ears. This is the case of the analysis of the stable hydrogen isotope composition (δ^2H) in its natural abundance when analysed from different plant parts such as the ear, flag leaf and mature grains [66••]. Thus δ^2H may provide simultaneous time-integrated records of the photosynthetic and evaporative performance of the plant during crop development based on, among other aspects, its tighter association with $\delta^{13}C$ than with $\delta^{18}O$ [66••]. δ^2H may be further strongly affected by the trophic (photoautotrophic versus heterotrophic) nature of the plant part considered [84–90], as well as being affected by potential differences in their photosynthetic metabolism. A step forward in terms of high throughput methodologies for ear phenotyping implies to explode the potential of spectroscopy for *in situ* (i.e. field) prediction in a non-destructive manner of the profiles of key metabolites in specific organs such as the ear bracts and the flag leaf using a portable spectroradiometer [29••,53••]. While this technique has proved feasible, it is necessary to upscale it even further in terms of high throughput. In that context it is necessary to develop-specific algorithms for image data extraction of specific plant parts such as the ear from the remaining canopy (i.e. leaves) and subsequently analysing the ear. The relevant information amenable to specific-organ extraction is colour (RGB images), temperature (thermal images) and spectral signatures (multispectral cameras). In that sense, the use of RGB images alone (to assess the ear colour) or combined (for organ detection and area profiling) with thermal and multispectral/hyperspectral images, to extract-specific information from the ears is a goal that is already technically achievable. That may allow in the near future the phenotyping of ears from canopy images acquired at ground level or from an unmanned aerial vehicle. To that end, developing algorithms for automatic ear detection [69] and further contour delimitation, preferably based on the use of high-resolution RGB images, is the step to follow. The insight histogram near the δ^2H refer to the range of values reported for different plant parts of wheat [66••] or among species with different photosynthetic metabolism (Sanchez-Bragado unpublished results).

colour characteristics of the ears or the definition of the contours or these organs for further extraction of thermal or spectral information. An example of a rather simple trait for phenotyping, through RGB images taken from an aerial platform, is the stay-green' attribute in the ear.

The development of high-resolution spectral systems has been promoted for quantitative estimation of plant bio-chemical and physiological processes. Recent studies have addressed the use of hyperspectral reflectance for the evaluation of diverse physiological traits such as

photosystem functioning, maximum Rubisco activity and dark respiration [71–73]. In contrast, close relationships between the plant metabolome, yield performance and stress resilience have been reported [53**,74]. In this sense, the possibility of predicting certain leaf and ear metabolites from remotely sensed data, particularly under field conditions, might enable *in vivo* metabolomics (metabolite characterisation) and open the door to a new generation of plant phenotyping approaches. Developing prediction models in metabolites of ear tissues using hyperspectral information has already been reported [53**]. However, its implementation as a high throughput technique in the field, either using sensors or imagers, is necessary if hyperspectral measurements are to be adopted as a phenotyping technique. It is in this context that the integration of organ recognition algorithms for data extraction from the canopy makes becomes relevant.

Concluding remarks

The cereal inflorescence has particular characteristics that overpass the traditional perception of a sink organ where grains are formed. In that sense, inflorescence photosynthesis (and in the case of wheat or barley, ear photosynthesis) plays a key role in determining yield during the last part of the crop cycle. The identification in the field of lines with higher ear photosynthesis rates and/or contributions to filling the grains, the reasonably heritability values of these traits, their correlation with yield or yield component and new phenotypic techniques to determine the contribution of ear photosynthesis towards grain yield will justify the inclusion of this photosynthetic organ in breeding programs.

Declaration of interests

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.

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