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Photosynthesis in non-foliar tissues: implications for yield.

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Abstract

Photosynthesis is currently a focus for crop improvement, however the majority of this work has taken place and been assessed in leaves, whilst limited consideration has been given to the contribution that other green tissues make to whole plant carbon assimilation. The major focus of this review is to evaluate the impact of non-foliar photosynthesis on carbon use efficiency and total assimilation. Here we appraise and summarise past and current literature on the substantial contribution of different photosynthetically active organs and tissues to productivity in a variety of different plant types, with an emphasis on fruit and cereal crops. Previous studies provide evidence that non-leaf photosynthesis could be an unexploited potential target for crop improvement. We also briefly examine the role of stomata in non-foliar tissues and their role in gas exchange, maintenance of optimal temperatures and thus photosynthesis. In the final section, we discuss possible opportunities to manipulate these processes and provide evidence that wheat plants genetically manipulated to increase leaf photosynthesis, also displayed higher rates of ear assimilation, which translated to increased grain yield. By understanding these processes, we can start to provide insights into manipulating non-foliar photosynthesis and stomatal behaviour to identify novel targets for exploitation for on-going breeding programmes.

Introduction

Photosynthesis in leaves is a well-established and extremely well researched process whereby plants harvest the energy from sunlight and use this to convert CO_2 into soluble carbohydrates, which are subsequently used for plant growth (Calvin and Benson, 1948, Bassham and Calvin, 1960, Raines, 2003, Biel and Fomina, 2015). Therefore photosynthesis is responsible, either directly (through plant growth) or indirectly (through the food chain), for all food consumed worldwide. The majority of studies on photosynthesis often only consider photosynthesis in leaves, with little appreciation of potential carbon assimilation in other green non-foliar tissue and its contribution to overall yield. With the predicted requirement to double food production by the year 2020 (WorldBank, 2008, RSOL, 2009, Tilman and Clark, 2015, FAO, 2017) and the fact that annual genetic gains in yield, using current breeding approaches are reducing or slowing for many crops (Ray *et al.*, 2012, Ray *et al.*, 2013), research into photosynthesis and the processes associated with it are being increasingly recognized as potential novel targets for improving crop yield. Crop yield is determined by the cumulative rate of photosynthesis over the growing season. The maximum yield obtained (yield potential), defined as the yield obtainable when a crop is grown in optimal conditions with no biotic or abiotic stress (Evans and Fischer, 1999) is the result of three key determinants i) light capture; ii) radiation use efficiency (RUE) or energy conversion efficiency (the product of which is biomass) and iii) harvest index (HI, the partition of harvestable produce relative to plant biomass) (Reynolds *et al.*, 2009). Significant gains in both HI and light interception have been made over the last several decade, with considerable increases in HI following the green revolution and the introduction of dwarfing (*Rht*) genes (Gale and Youssefian, 1985, Calderini *et al.*, 1995), therefore the current focus is on RUE (Reynolds *et al.*, 2009, Parry *et al.*, 2011), which is primarily photosynthesis and conversion of light energy into fixed carbon. Several recent studies have demonstrated that improving diverse aspects of photosynthesis in leaf tissue, including altering key enzymes within the Calvin-Benson cycle (CBC) (Lefebvre *et al.*, 2005, Simkin *et al.*, 2015, Driever *et al.*, 2017, Simkin *et al.*, 2017a), electron transport (Chida *et al.*, 2007, Simkin *et al.*, 2017b, Yadav *et al.*, 2018, Ermakova *et al.*, 2019), photorespiration (Timm *et al.*, 2012, López-Calcagno *et al.*, 2018) and the kinetics of non-photochemical quenching (NPQ) (Kromdijk *et al.*, 2016, Glowacka *et al.*, 2018) can improve yield potential in both glasshouse and field grown plants (Simkin *et al.*, 2019). However, leaves are not the only location within the plant where photosynthesis occurs, with evidence that petioles and stems (Hibberd and Quick, 2002), seeds (Schwender *et al.*, 2004), fruit (Hetherington *et al.*, 1998, Carrara *et al.*, 2001, Hiratsuka *et al.*, 2015, Sui *et al.*, 2017), wheat ears (Maydup *et al.*, 2010) and the husks of corn (Pengelly *et al.*, 2011) all photosynthesize and may provide significant and alternative sources of photoassimlates essential for optimal yield.

Fig. 1 illustrates chlorophyll fluorescence imaging of the operating efficiency of PSII photochemistry (F_q'/F_m') in non-leaf tissues, which is indicative of functional electron transport in these green non-leaf organs. To date little data exist on how potential manipulation of photosynthetic processes may impact on these chlorophyll containing tissues.

The majority of studies that have examined photosynthesis in non-foliar tissue have assumed and described a photosynthetic pathway similar to that of the mesophyll. However, one key difference in non-foliar tissue photosynthesis is the fact that there are two potential major sources of CO_2. Firstly, Ribulose–1,5-bisphosphate carboxylase (Rubisco) assimilates atmospheric CO_2 that diffuses into the cells through the stomatal pores, leading to the production of sugars via the CBC, similar to the CO_2 pathway in leaf (C3) tissue. Secondly, CO_2 released by mitochondrial respiration can be the main supply of CO_2 and is re-fixed (recycling photosynthesis) (Aschan and Pfanz, 2003, Millar *et al.*, 2011) and there is limited diffusion and supply of external CO_2. Although stomata are present in various numbers on some non-foliar tissues their function has not been fully evaluated, and the amount of photosynthesis that relies on atmospheric supply of CO_2 through these pores is not currently known. In this review, we focus on photosynthesis in non-foliar tissues and the potential contribution to yield, as well as the role of stomata in this processes. Before discussing the possibility to manipulate non-foliar photosynthesis for improved productivity or nutritional quality, we first provide an overview of what is known about photosynthesis in various organs, focusing on stems and fruits as well as various organs in cereals.

Photosynthesis in stems

Stems act as both a temporary storage sites for photoassimilates from leaves and carry out photosynthesis in their own right (Aschan and Pfanz, 2003). In tomato, chlorophyll levels were found to be higher in upper parts of the stem than the lower parts (Xu *et al.*, 1997) and a comparison of the photosynthetic activity of various plant parts found the entire stem accounted for up to 4% of photosynthetic activity (Hetherington *et al.*, 1998). The contribution of stem photosynthesis to yield has been demonstrated in cotton by Hu et al. (2012) who reported that keeping the main stem in darkness reduced seed weight by 16% (Hu *et al.*, 2012). These findings were supported by Simbo et al. (2013) who showed that when light was excluded from the stem of defoliated *Adansonia digitata* L (African baobab) and *Ricinus communis* (Castor Bean), a reduction in bud dry weight was observed providing further evidence for the importance of the stem for providing photoassimilates for plant development and growth. In some plants, such as *Justicia californica,* flowers and fruits develop in the absence of leaves, where the stem is the only photosynthetically active tissue (Tinoco-Ojanguren, 2008, Ávila *et al.*, 2014),

highlighting the role of stem photosynthesis also for reproductive success. This is emphasised further by a reported stem photosynthesis equivalent to 130% of leaf levels in this species (Tinoco-Ojanguren, 2008), whilst in other species, rates of between 16% and 60% relative to leaf levels have been reported (Ehleringer *et al.*, 1987, Ávila *et al.*, 2014). In the woody plant Eucalyptus, photosynthesis in chlorophyll containing tissue, chlorenchyma, located beneath the periderm layer (Pfanz *et al.*, 2002, Manetas, 2004) known as corticular photosynthesis (CP) contributed 11% of total photosynthate to plant growth demonstrating the contribution of CP to eucalyptus growth (Cernusak and Hutley, 2011).

Stem photosynthesis is particularly important in deciduous species. In the summer-deciduous, green-stemmed Mediterranean shrub *Calicotome villosa*, total branch photosynthesis is higher in the summer due to an absence of leaves, and green stem photosynthesis outcompetes leaf photosynthesis on an annual basis (Yiotis *et al.*, 2008). In the desert ephemeral *Erigonum inflatum* substantial photosynthesis was demonstrated in the inflated stems, despite the fact that these contained only half the chlorophyll and nitrogen content of the leaves (Osmond *et al.*, 1987). Internal CO_2 concentrations in these stems was reported to be extremely high, however interestingly, fixation of this internal CO_2 was 6-10 times less than fixation of atmospheric CO_2. However, although small, this additional internal CO_2 pool facilitated high water use efficiency (WUE; measured as water lost relative to carbon gained) due to no water loss through stomata for this carbon gain. Greater WUE was further enhanced in this species by smaller stem stomata that are more responsive to temperature and high vapour pressure deficit (VPD) compared with their leaf counterparts (Osmond *et al*., 1987). The importance of stem photosynthesis in desert species, is supported by a more recent study by Avila-Lovera *et al*., (2017) who examined 11 green stemmed desert plants and showed co-ordination between stem photosynthesis and hydraulics similar to that in leaves, with an even tighter relationship during the dry season facilitating additional carbon gain and a potential mechanisms for enhanced drought tolerance. Furthermore, stem photosynthetic rates were higher during the dry season when leaves were lost and light interception by the stems was increase in the absence of foliage (Avila-Lovera *et al.*, 2017). Together these studies illustrate the importance and annual contribution of stem photosynthesis to overall carbon gain, which not only contributes to the survival of plants growing in dry and hot environments, but also supports the notion that stem photosynthesis may contribute significantly to yield, and that this contribution may be more important under conditions such as reduced water availability, high temperatures and high VPD. However, to date there have been limited studies that have evaluated the importance of stem photosynthesis to yield in key crop species. Therefore although stem photosynthesis may represent a potential novel target to support enhanced photosynthetic carbon gain, particular

under conditions of water stress (such as those predicted under climate change for certain agriculture areas), more quantitative information on stem performance in crops is needed to evaluate and fully exploit this process.

Fruit photosynthesis

Fruit photosynthesis is particularly interesting, as many species (e.g. tomato) undergo a shift from green photosynthetic (or partial photosynthetic) to fully heterotrophic metabolism on ripening (Lytovchenko *et al*., 2011). As early as 1974, Tanaka et al. (1974) conducted shading experiments on tomato fruits and showed that fruit photosynthesis contributes to net sugar accumulation and growth and from this work concluded that this photosynthesis contributed between 10% and 15% of the total fixed carbon, which was later confirmed by Hetheringon et al. (1998) and Obiadalla-Ali et al. (2004). In addition to showing similar photosynthetic function to leaves, developing tomato fruit have also been reported to have approximately 41% of the photosynthetic electron transport capacity of leaf tissue (Piechulla *et al.*, 1987). Recent proteomic analysis has demonstrated that all of the components of the CBC and photorespiratory cycle accumulate at the protein level in tomato fruit (Barsan *et al.*, 2010, Barsan *et al.*, 2012). The major light-harvesting proteins (including the thylakoid membrane light-harvesting complexes proteins of PSI (*psaA*) and PSII (*psbA*) and the chlorophyll a/b-binding proteins) have also been observed (Piechulla *et al.*, 1986, Lemaire-Chamley *et al.*, 2005), in conjunction with plastocyanin, cytochrome *f*, cytochrome *b*, ferredoxins, Rieske iron sulphur protein (Piechulla *et al.*, 1987, Livne and Gepstein, 1988, Cheung *et al.*, 1993, Aoki *et al.*, 1998) and the CBC proteins, Ribulose–1,5-bisphosphate carboxylase (Rubisco) and fructose 1,6-bisphophate aldolase (FBPaldolase) (Barsan *et al.*, 2010, Steinhauser *et al.*, 2010). Rubisco assays have also demonstrated that the enzyme is active in tomato fruit (Willmer and Johnston, 1976, Bravdo *et al.*, 1977, Laval-Martin *et al.*, 1977, Piechulla *et al.*, 1987, Sugita and Gruissem, 1987).

Despite the fact that transcriptomic and metabolomic analysis have revealed high expression levels of many of these photosynthetic genes in tomato fruit, and have shown that photosynthetic carbon assimilation in these organs makes an important contribution to early fruit development (Wang *et al.*, 2009), many studies do not agree that these fruit are net assimilators of CO_2 (see Blanke and Lenz, 1989; Carrara *et al*., 2001). Lytovchenko et al. (2011) used antisense technology to reduce expression of the chlorophyll biosynthesis gene glutamate 1-semialdehyde aminotransferase, which resulted in a reduced photosynthetic rate, however fruit size and metabolite levels remained unchanged. These authors suggested that transport of photosynthate from leaves compensated for any reduction in fruit localised photosynthetic rates and proposed that fruit photosynthesis is dispensable. However a delay in seed development

was observed, suggesting that localised CO_2 fixation/re-assimilation may be important for seed formation (Lytovchenko *et al.*, 2011). In contrast, another study demonstrated that decreased expression of fruit chloroplastic fructose-1,6-bisphosphatase (FBPase) resulted in a 15-20% negative impact on fruit development (Obiadalla-Ali *et al.*, 2004). Lytovchenko et al. (2011) suggested that these contradictory results could be explained by different promotor specificity and/or the impact of reduced FBPase activity later in the development of the fruit.

Whilst it is evident that photosynthesis occurs in fruits, the extent and importance is not clear. The fact that tomato fruit lack stomata (Vogg *et al.*, 2004) (**Fig. 2**) implies that photosynthesis in these organs relies exclusively on CO_2 liberated from mitochondria, that no 'new' carbon is fixed and that photosynthesis functions to re-assimilate CO_2 (recycling photosynthesis) that would otherwise be lost. This is supported by the reported accumulation of transcripts in tomato loculare tissue associated with photosynthesis, clearly demonstrating photosynthetic capacity, but alongside high measured respiration rates (Lemaire-Chamley *et al.*, 2005). CO_2 generated by the oxidative pentose pathway is re-assimilated by the CBC in a manner previously reported in green seeds of oil seed rape (Schwender *et al.*, 2004). It has been reported that these photosynthesis-specific transcripts are regulated by transcription factors in a similar way to those in leaf tissue (Hetherington *et al.*, 1998, Carrara *et al.*, 2001). However, a number of authors have reported the existence of some fruit-specific regulation of photosynthetic genes (Piechulla *et al.*, 1987, Piechulla and Gruissem, 1987, Sugita and Gruissem, 1987, Manzara *et al.*, 1993) and Cocaliadis et al. (2014) suggested that this is likely to optimise photosynthetic function for fruit development. This specificity therefore provides a potential route for manipulating key photosynthetic genes specifically in fruit to enhance development, yield or nutritional quality.

In summary it appears that photosynthetic carbon assimilation does take place in green immature tomato fruit and that this relies almost exclusively on respired CO_2 and that any reductions in the rate of photosynthesis in these organs can be compensated for by upregulation of leaf photosynthesis (Araujo et al., 2011; Nunes-Nesi et al., 2011) and increased imported photoassimilates from leaves. However, such import cannot compensate for the losses of fruit photosynthesis for seed set, establishment and development (Lytovchenko *et al.*, 2011). Therefore altering fruit photosynthesis could provide advantages of early seed set, as well as maintaining yield, particularly under conditions of stress when leaf photosynthesis may be compromised, however, fruit photosynthesis can continue to rely entirely on respiratory CO_2.

Tomato photosynthesis is restricted to the green phases of development up until chloroplast-to-chloroplast differentiation, which is marked by the loss of chlorophyll, the degradation of the thylakoid membranes and a strong decrease in the levels of photosynthesis

associated transcripts and proteins (Harris and Spurr, 1969a, Harris and Spurr, 1969b, Cheung *et al.*, 1993, Barsan *et al.*, 2012), after which the fruit continues to develop and ripen. This is similar for other fruits such as Pepper (*Capsicum annum*) (Steer and Pearson, 1976), Satsuma mandarin (*Citrus unshiu*) (Hiratsuka *et al.*, 2015) blueberry (Birkhold *et al.*, 1992); coffee (*Coffea arabica*) (Cannell, 1985, Lopez *et al.*, 2000); plum (Aoyagi and Bassham, 1984); the ornamental plant *Arum italicum*, (Ferroni *et al.*, 2013) and *Jatropha curcas* (Ranjan *et al.*, 2012). It has been demonstrated that in these fruit photosynthesis also occurs, is greater at low irradiances and increases with increasing [CO_2] supplied through fully developed stomata in the rind in Satsuma (Hiratsuka *et al.*, 2015). The fact that stomata can be found in density of about 72 mm^{-2} in immature Jatropha fruit suggest that new carbon can be assimilated through these tissues (Ranjan *et al.*, 2012). In this case, given the importance of fruit photosynthesis in the absence of leaves, increasing stomatal density could increase CO_2 uptake and boost photosynthetic rates in fruit with a positive impact on yield.

Cucumber (*Cucumis sativus*) is fundamentally different to tomato and other coloured fruit, remaining green through to full maturity with a surface area equivalent to a fully expanded leaf (Sui *et al.*, 2017). An analysis of gene expression found a number of CBC enzymes (SBPase, FBPase, rbcL, rbcS) and light-harvesting complex proteins of PSI (*Lhca*) and PSII (*Lhcb*) expressed in the exocarp (Sui *et al.*, 2017). Interestingly, unlike tomato, stomata are found on the epidermis of cucumbers (**Fig. 2**), although Sui et al. (2017) reported a layer of epicuticular waxes around the guard cells that may reduce function. However, the presence of these pores on the fruit surface suggests, in the case of cucumber at least, that these fruit are capable of assimilating some CO_2 directly from the atmosphere. Their physiology also suggests that photosynthesis can occur from the re-assimilation of respiratory CO_2. Cucumber fruits have been shown to have both high photosynthetic and respiratory rates (Todd *et al.*, 1961) and a recent study demonstrated that fruit photosynthesis contributed 9.4% of its own carbon requirements whilst 88% of respiratory CO_2 in fruit was re-fixed (Sui *et al.*, 2017). Improving photosynthetic efficiency in fruit therefore has the potential to increase fruit carbon contribution for growth through both recycling respiratory CO_2 and atmospheric assimilation that could in turn directly impact on WUE. Maintaining, or increasing fruit yield (or fruit size) whilst using less water cannot be underestimated given current environmental changes.

Are stomata important in fruit photosynthesis?

It is important to note that although stomata are routinely found on the surface of some fruit and are of a similar size to those found on respective leaves, the numbers are generally significantly lower compared to those found in leaf tissue (Blanke, 1998). For example, Blanke

and Lenz (1989) reported that the number of stomata on a mature apple fruit were 30 times less abundant than found on apples leaves. Stomatal numbers are fixed at anthesis and as the fruit expands during growth, they become more dispersed (Hieke *et al.*, 2002, Hetherington and Woodward, 2003). Although it has been reported that stomatal density in fruit typically represents 1-10% of the frequency found in corresponding leaf tissue (Sánchez *et al.*, 2013), these numbers can greatly vary depending on the species. In avocado, stomata per fruit have been observed in the range of 14% of leaf numbers (Blanke, 1992), in green coffee fruit, 13-23% of leaf numbers (Cannell, 1985), whilst in oranges stomatal densities can reach up to 30% of those found on leaves (Moreshet and Green, 1980). To date most studies have focussed on presence of stomata on various fruit tissue but have not fully demonstrated the functionality. However, if functional, the presence and stomatal densities reported above suggest that under certain conditions, in certain plants at least, stomata may play a role in gas exchange and therefore manipulating stomatal numbers through developmental mechanisms or transgenic approaches has the potential to change CO_2 assimilation rates and yield. However, in other plants, the contribution of stomata to assimilation appears to be negligible compared to recycling photosynthesis. In these plants, we cannot rule out that the role of stomata is primarily for evaporative cooling. Although not directly related to CO_2 uptake, this process may help maintain fruit temperature at an optimal level for recycling photosynthesis maximising CO_2 recovery.

Seed and Embryo Photosynthesis

The fruit pericarp is not the only non-foliar green tissue that is capable of photosynthesis. The embryos of many taxa contain significant quantities of chlorophyll, which persists until maturity (Yakovlev and Zhukova, 1980, Simkin *et al.*, 2010, Puthur *et al.*, 2013, Smolikova and Medvedev, 2016). This group includes model species (*Arabidopsis thaliana*) and important crops such as soybean (*Glycine max* L.), peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), oilseed rape (*Brassica napus* L.), broad beans (*Vicia faba L.*), cotton (*Gossypium hirsutum*) and coffee (*Coffea arabica*). These embryos, first referred to as chloroembryos by Palanisamy and Vivekanandan (1986), contain all the photosynthetic complexes of Photosystem I and II, cytochrome b_6f complex and ATP synthase (Weber *et al.*, 2005, Allorent *et al.*, 2015, Kohzuma *et al.*, 2017). Chloroembryos have also been shown to photosynthesise (Smolikova and Medvedev, 2016, Smolikova *et al.*, 2017) and confirmation of carbon fixation is supported by the activity of the CBC enzymes NADP-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the chloroembryo chloroplasts of *Brassica napus* (oil seed rape), and pea (Smith *et al.*, 1990, Eastmond *et al.*, 1996) and fructose-1,6-bisphosphatase (FBPase) in oil seed rape (Kang and Rawsthorne, 1996). Furthermore, Rubisco has also been shown to be active in the seeds of soybean (Allen *et al.*,

2009), oilseed rape (Hills, 2004, Ruuska *et al.*, 2004), *Vicia fabia* (broad bean) (Willmer and Johnston, 1976) and *Trigonella foenum-graecum* (Willmer and Johnston, 1976).

The contribution of photosynthesis in embryos may be different to that described above for fruit as it has been reported that embryo photosynthesis contributes a significant amount of oxygen, which fuels energy generating biochemical pathways including respiration and glycolysis (Ruuska *et al.*, 2004, Borisjuk *et al.*, 2005, Tschiersch *et al.*, 2011, Galili *et al.*, 2014). The role of photosynthesis in chloroembryos has also been associated with the rapid synthesis of ATP and NADPH for the synthesis of complex carbohydrates, fatty acids and proteins (Asokanthan *et al.*, 1997, Wu *et al.*, 2014). It has been reported that a key source of carbon is sucrose, imported from the leaves (Asokanthan *et al.*, 1997), which is respired by the seed, releasing CO_2 (Ruuska *et al.*, 2004, Smolikova and Medvedev, 2016) within chloroembryos, which is subsequently efficiently re-assimilated and thus directly affects the carbon economy of the seed (Puthur et al., 2013).

In oil seed rape, seed photosynthesis plays a role in the accumulation of storage lipids (Eastmond *et al.*, 1996, Ruuska *et al.*, 2004). Interestingly, Rubisco acts in a distinctive context, without the CBC, to increase the carbon use efficiency for the synthesis of oil (Schwender *et al.*, 2004). This unique pathway generates 20% more acetyl-CoA than glycolysis, reducing the loss of CO_2 and increasing the availability of acetyl-CoA for fatty acid biosynthesis (Schwender *et al.*, 2004). In the embryos of legumes, including pea, the main CO_2-refixing enzyme is phosphoenol pyruvate (PEP) carboxylase (Golombek *et al.*, 1999) suggesting that CO_2 is refixed at the site of origin. In the case of *Pisum sativum* (pea), a small spherical seed with a green embryo within a seed-pod, only a fraction of light reaches the photosynthetically active tissue. The light is attenuated by the pod, reflecting or absorbing as much as 75% of the sunlight. Only 32% of the remaining sunlight (approx. 8% of PAR), penetrates the pod and seed coat to reach the surface of the embryo, however, this is enough to drive photosynthesis with the highest electron transport rates in the seed coat (Tschiersch *et al.*, 2011). In addition to seed photosynthesis, pea pods also photosynthesize in two distinct layers. Firstly, the outer layer, comprising of chlorenchyma and mesocarp, assimilates CO_2 from the atmosphere and secondly, the inner epidermis lining of the pod cavity, reassimilates the CO_2 released by the embryonic respiration into the pod cavity (Atkins *et al.*, 1977). Rubisco activity has also been detected in the pod wall of pea embryos, although this activity is 10-100x lower than that detected in the leaf tissue (Hedley *et al.*, 1975).

Importance of photosynthesis in non-foliar cereal organs

In cereals, whilst leaf photosynthesis plays a central role in biomass accumulation and yield formation over the entire growing season (Fischer *et al.*, 1998, Gu *et al.*, 2014), the photosynthetic activity of the ear has been shown to dramatically contribute to the pool of

carbohydrates translocated to the developing grains over the post-anthesis stages (Tambussi *et al.*, 2005, Tambussi *et al.*, 2007, Maydup *et al.*, 2010, Sanchez-Bragado *et al.*, 2014). Although on an area basis, ear CO_2 assimilation rate is lower than that of the flag leaf (Tambussi *et al.*, 2005, Tambussi *et al.*, 2007, Zhou *et al.*, 2016), experimental evidence suggests that in bread and durum wheat, ear photosynthesis can contribute to the individual grain weight yield component by up to 70% in a large range of genotypes (Maydup *et al.*, 2010) and contrasting environments (Sanchez-Bragado *et al.*, 2014). Similarly to wheat, in barley, shading experiments revealed a significant contribution of the ear (up to 50%) to grain weight and therefore yield (Bort *et al.*, 1994). In the next few sections we focus on different aspects of ear photosynthesis and the challenges in assessing photosynthesis in non-foliar organs.

Photosynthetically Active Ear components

The ear bracts (which consist of glume, lemma and palea) contain chlorophyll and possess stomata (**Fig. 3**) and therefore have potential to fix atmospheric CO_2 (Tambussi *et al.*, 2007). Genotypic variation in ear photosynthetic CO_2 assimilation per unit area and contribution of ear photosynthesis to grain weight have been reported in the literature (Maydup *et al.*, 2014; Sanchez-Bragado *et al*., 2014). The exploitation of this variation might be of pivotal importance for cereal improvement. Several ear bracts have been considered putative locations of photosynthetic activity with glumes, lemmas and awns considered the most photosynthetically active (Tambussi *et al.*, 2007, Hu *et al.*, 2019). In particular the floral-derived awns have been targeted as a trait to increase wheat yield owing to their high photosynthetic capacity of between 7 and 35 μmol m^{-2} s^{-1} (Hein *et al.*, 2016) and especially in view of the limited possibility to further increase assimilates partitioning to grains by manipulating the harvest index (Maydup *et al.*, 2014).

The seasonality of the post anthesis stages in cereals are often associated with increases in environmental stresses and severe water deficit conditions leading to reduced yield. Numerous studies provide strong evidence that the ear possesses an elevated drought tolerance when compared to the flag leaf and highlight the ear as the main potential buffer for photoassimilate production under disadvantageous environments (Jia *et al.*, 2015). Additionally, the ear shows lower transpiration rate than the flag leaf and a higher intrinsic water-use efficiency confirmed by less negative δ13C values (Sanchez-Bragado *et al*., 2014; Tambussi *et al*., 2007; Araus *et al*., 1993; Vicente *et al.*, 2018). Xeromorphic characteristics in glumes, lemmas and awns of durum wheat have been observed such as sclerenchymatous tissue and thick walls (Tambussi *et al.*, 2005). The same authors observed a higher osmotic adjustment and relative water content of the

ear compared to the flag leaf under reduced water availability leading to a sustained chlorophyll fluorescence signal. Similarly, in barley (Hein *et al.*, 2016) ear bracts maintained higher relative water content and gas-exchange under water stress rate compared to the leaf as well as greater osmotic adjustment.

Additionally, comparing awned and awnless lines under stress conditions, showed higher ear intrinsic water-use efficiency (mainly driven by a high photosynthetic activity for similar stomatal conductance per unit area) and photosynthetic capacity when awns were present, suggesting that awn photosynthesis also plays an important role when foliar tissue is reduced due to stress (Weyhrich, 1994, Weyhrich *et al.*, 1995). However, no differences in whole plant water-use efficiency and grain weight were found between these lines, therefore, in this investigation, the higher photosynthetic capacity in the awns failed to contribute to yield. In contrast, a multi-location field study on the effect of awns on wheat yield components showed that the presence of awns increased grain size, however this increase was compensated for by a reduction in grain number (Rebetzke *et al.*, 2016), which was mainly attributed to the cost of awn setting. Assimilate partitioning to the floret is decreased in awned varieties due to the allocation to the rapidly growing awns followed by a potential associated reduction in floret fertility (Guo and Schnurbusch, 2016, Rebetzke *et al.*, 2016). It was concluded that awns are mainly useful under terminal drought condition owing to their elevated water stress tolerance that facilitates maintenance of grain weight and a reduced number of shrivelled grains (screenings) compared to awnless lines, thus potentially providing higher economic yield and commercial value under such conditions. This was also confirmed by Maydup et al. (2014), who showed that awned varieties have higher ear photosynthesis, water status and ear water conductance compared to awnless varieties under water stress conditions in the field.

Genotypic variation of ear water stress tolerance has also been shown by Li et al. (2017), where the stress tolerant wheat variety displayed a conservative water-use strategy during post-anthesis by reducing leaf transpiration while maintaining high levels of ear gas-exchange. Vicente et al. (2018) postulated that water stress in wheat reduced the expression of photosynthetic genes (e.g. ATPase) in the flag leaf but not in the ear, and the upregulation of respiration-related genes (e.g. phosphoenolpyruvate carboxylase (PEPCase), 2-oxoglutarate dehydrogenase complex (OGDC), alternative oxidase (AOX), and pyruvate kinase) was associated with the increased re-fixed CO_2 in the ear organs. An observed upregulation of dehydrins (Abebe *et al.*, 2010), increased transcript levels of antioxidant enzymes genes (Vicente *et al.*, 2018) followed by high levels of antioxidants enzymes and low levels of ROS (Kong *et al.*, 2015) confirmed the higher drought tolerance of the ear and its importance as a main contributor to grain weight and, more broadly, grain yield under disadvantageous environmental conditions.

Wheat Endosperm and Pericarp

Caley et al. (1990), followed by Tambussi et al. (2005) also proposed a possible role of the green pericarp in CO_2 re-fixation. Although stomata are almost absent in the growing endosperm, thus suggesting limited gas-exchange capacity, immunohistochemical analysis showed chloroplasts and Rubisco co-localization in the green pericarp with elevated photosynthetic capacity (Kong *et al.*, 2016) that can account for up to 42% of the total photosynthetic activity of the ear (Evans and Rawson, 1970). Recent work reported that genes specific for the C4 pathways such as PEPC, NAD-ME and NADP-MDH are expressed in the cross and tube-cell layer of the pericarp (Rangan *et al.*, 2016), agreeing with earlier studies that had already suggested the presence of C4 or C3-C4 intermediate metabolism in the ear (Ziegler-Jöns, 1989, Imaizumi *et al.*, 1990, Li *et al.*, 2004, Jia *et al.*, 2015), potentially induced under water stressed conditions. However, the following observations suggest limited evidence for a C4 pathway in green pericarp and other ear organs: i) oxygen sensitivity of CO_2 assimilation rate of the ear (increased by up to 45% at 2% O_2 conditions) (Tambussi *et al.*, 2005, Tambussi *et al.*, 2007), ii) high rates of CO_2 assimilation through the CBC rather than conversion into C4 malate or aspartate (Bort *et al.*, 1995) and iii) a lack of the specific C4 anatomy (Tambussi *et al.*, 2005), although future analyses is required to confirm this and it remains a topic of debate.

The importance of stomata for ear photosynthesis

Several studies have demonstrated that stomatal density in the flag leaf of wheat varies between 40 and 90 mm^{-2}, e.g. (Faralli *et al.*, 2019a) and in ear organs stomata density can be either higher than in the leaf (Kong *et al.*, 2015) or drastically lower (Tambussi *et al.*, 2005). Furthermore, different stomatal density and distribution have been reported on both ventral and dorsal side of glume and lemma (**Fig. 3**), with the latter showing variable density depending on the shading area of the neighbouring glume (Tambussi *et al.*, 2005). Since the growing endosperm releases respired CO_2, the presence of stomata in the internal surface of glumes and lemmas is evidence of CO_2 recycling capacity. As reported for fruit (see above), several studies have demonstrated large amounts of re-fixation of respiratory CO_2 in the ear (Bort *et al.*, 1996), which can contribute up to 79% of the sucrose accumulated in bracts (Gebbing and Schnyder, 2001). The re-fixation capacity has several advantages, in particular i) respiratory CO_2 losses are minimized and ii) photosynthetic metabolism is fully independent of the environment.

Genotypic variation in stomatal distribution in glumes and lemmas and on the different sides also exists in current elite bread wheat cultivars (**Fig. 3 and Fig. 4**) which suggests different strategies for atmospheric CO_2 assimilation or CO_2 re-fixation that could be further exploited for

ear gas-exchange optimization. In generally, high stomatal densities are reported on the external side of glumes (up to 32 stomata mm^{-2}) and awns (up to 70 stomata mm^{-2}) with lower numbers found in lemmas (between 20 and 10 stomata mm^{-2}) and absent in paleas (**Fig. 4**). However, the stomatal density on the internal surfaces are comparable for glumes and lemmas (between 20 and 9 stomata mm^{-2}) while again almost absent in paleas. It has been reported however that stomatal functionality may be strongly limited in the ear by i) the mechanical constraint induced by the growing grains inside the florets and ii) by the accumulation of waxes preventing guard cells opening/closing (Araus *et al.*, 1993) hence limiting photosynthetic CO_2 uptake, especially during the late grain filling stage. However, **Fig. 5** shows thermal images from the ear and flag leaf of two wheat varieties, and reveals that although temperature regulation of the ear is significantly lower than the flag leaf (lower transpiration rate), ear stomata are responsive and open when subjected to a low-to-high light transition (**Fig. 5**). In the ear the two cultivars also differ in the magnitude and rapidity of stomatal opening (Faralli *et al.*, 2019b), suggesting potential genotypic variation, driven by either differences in wax accumulation (Araus *et al.*, 1993) or owing to variation in stomatal size, density and distribution as well as functional differences. Indeed, in greenhouse experiments, six recombinant inbred lines grown under heat and water stress conditions showed the presence of cooling capacity in the ear at early anthesis (i.e. before pollen release) (Steinmeyer *et al.*, 2013). Due to the elevated sensitivity of pollen to high temperatures, ear stomatal dynamics and overall evaporative cooling capacity may be an important novel trait for increasing stress tolerance by protecting pollen viability and minimizing floret damage at anthesis. Indeed, at the reproductive stage, stress tolerance in crops is based on both the ability to produce viable pollen and to "shield" the pollen from environmental stresses (i.e. reducing reproductive organs temperature by high transpiration rate) (Steinmeyer *et al.*, 2013). In addition, enhancing stomatal regulation and transpiration may increase assimilate translocation to the developing grains and remobilization of resources and could be considered as an additional target for increasing yield potential.

Challenges associated with measuring photosynthesis in non-foliar tissue.

Further experimental evidence is needed to fully understand the mechanisms involved in photosynthetic activity of the ear and other non-foliar photosynthetic organs. However, there are challenges associated with measuring photosynthesis is non-laminar tissues using standard approaches used for leaves. For example, most leaf level measurements of CO_2 uptake are conducted using Infra-red gas analysis (IRGA), which requires the material to be enclosed in a sealed chamber, with differences in gas fluxes in and out of the chamber assessed. However, using such approaches for non-leaf material represents challenges including; i) the small size of

commercially available leaf gas exchange chambers; ii) the complication of re-fixation of respiratory CO_2 in determining gas differentials; and iii) the complexity of ear architecture in wheat making the normalization of gas-exchange data per unit area particularly difficult and leading to strong uncertainty in the absolute value. New methodologies are needed and should be implemented to assess ear gas-exchange and organ contribution to grain weight. For instance, 3D scanners help refine estimation of the area, in particular in view of the consistent underestimation (and thus gas-exchange overestimation) that occurs with standard techniques (e.g. ruler, **Fig. 6**). Additionally, the design and development of bespoke chambers is required to enclose an entire ear or fruit to allow assessment of whole organ gas exchange. However, such chambers present further challenges that arise from the large volumes required that can lead to slow gas mixing and difficult temperature control. In addition, although saturated light can be provided in large cuvettes for all the surfaces, the shading effects from neighbouring organs, e.g. spikelet morphology and distance between spikelets, may lead to additional sources of error. Chlorophyll fluorescence has been shown to be a good candidate for ear photosynthetic assessment (Tambussi *et al.*, 2005, Maydup *et al.*, 2012) and combined with gas-exchange (McAusland *et al.*, 2013) may help to dissect the amount of photosynthesis relying on re-fixation of respiratory CO_2 from atmospheric CO_2, as well as determining differences in O_2 sensitivity of various genotypes.

Defoliation, inhibition of photosynthesis through shading and herbicide application are some of the most commonly used approaches to evaluate the contribution of ear photosynthesis to yield (Sanchez-Bragado *et al.*, 2016). Whilst these approaches may be useful to evaluate genotypic variation, they are likely to induce compensatory mechanisms (and potentially overestimations). Sanchez-Bragado et al. (2016) suggested carbon isotope discrimination as an alternative for assessing ear photosynthetic traits. In addition, owing to the Rubisco discrimination of ^{13}C and due to the lack of C discrimination of PEPC, the isotopic signature may help to discern potential variation between C3 or C4 pathways (Hu *et al.*, 2019). However, it must be recognised that almost all the approaches outlined above lack the advantage of a high throughput and are generally considered time consuming and laborious and this therefore limits their use for screening large populations or samples for ear photosynthetic phenotypes. There is no doubt that improvement in experimental procedures along with further advances in high throughput approaches for screening ear photosynthesis will increase the knowledge on ear photosynthetic activity and therefore help to design new cereal varieties with elevated yield potential and stability.

Conclusion

Although most studies examining photosynthesis have focused on leaf level measurements, including current approaches to improve photosynthesis, the contribution that other green tissues make to total photoassimilates has largely been ignored. However, as highlighted above, these green tissues contribute significantly to plant development, growth and yield and therefore present novel opportunities for exploitation to improve productivity. The fact that the full spectrum of light harvest, electron transport and CBC proteins and transcripts are found in non-foliar tissues (Barsan *et al.*, 2010, Barsan *et al.*, 2012, Sui *et al.*, 2017, Vicente *et al.*, 2018) offers the potential to manipulate non-foliar photosynthetic pathways to increase rates of photosynthesis using similar approaches to those currently being employed in leaves (see review by (Simkin *et al.*, 2019)). For example, recent experiments in transgenic wheat with increased activity of the CBC enzyme SBPase, driven by a constitutive promotor (Driever *et al.*, 2017) revealed increased gross photosynthesis in the ears of mutant plants relative to the wild type control (**Fig.7**). It is therefore possible that the overall increase in yield of the SBPase overexpressing plants reported by Driever et al. (2017) may have been achieved in part by an increase in ear-derived assimilates, although this would require further investigation. Such studies highlight the potential benefits of improving photosynthesis in organs other than leaves for improving crop productivity and yield. Furthermore, as photosynthesis provides the building blocks for many downstream products and metabolites, modifying photosynthetic processes in fruits for example, offers the potential to alter fruit quality and nutritional value.

A major difference between leaf and non-leaf tissues is the primary source of CO_2 for CBC (atmospheric vs respiratory), therefore manipulation of stomatal density or function presents an additional avenue to manipulate photosynthetic processes in some tissues, e.g. wheat ears. For example increasing stomatal density or aperture could result in increasing assimilation by removing diffusional constraints and increasing the flux of atmospheric CO_2 to the site of carboxylation, however such an approach would also facilitate leakage of respiratory CO_2 (Sui *et al.*, 2017), which has been demonstrated to be of greater importance in some organs. Alternatively, increased SD in wheat ears could improve evaporative cooling, maintaining assimilation rates under elevated temperatures, assuming similar temperature sensitivity of photosynthesis in wheat ears and leaves (Scafaro *et al.*, 2012, Scafaro *et al.*, 2016, Perdomo *et al.*, 2017). On the other hand, this “risky” behaviour might increase the possibility of early ear dehydration under severe terminal stress conditions, and further experimental evidence are required is support this theory. Stomatal behaviour and transpiration in ears may also provide a key role in translocation of photoassimilates to the ear and therefore altering g_s could assist with sink-source relationships. Whilst stomatal behaviour is important for photosynthesis, it should be acknowledged that stomatal pores are also an important component of non-leaf tissues to

facilitate drying, which is essential for dispersal of spores and seeds (e.g. stomata in the spore capsules of moss; (Merced and Renzaglia, 2013, Chater *et al.*, 2016). However, before such novel targets for improved photosynthesis can be exploited, a better understanding of the contribution of non-foliar photosynthesis to yield and quality (particularly under conditions of stress) and the role of stomata in these processes are needed.

Data statement:

Data presented within this review are example data sets that are therefore not publicly available, please contact TL to request access to any data.

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Author Contributions

A.J.S, M.F and T.L all wrote the manuscript and contributed to the figures. Data presented on the SBPase wheat is from work carried out by MF, AJS, TL and Christine Raines.

Conflicts of interest:

There are no conflicts of interest to declare.

References

Abebe, T., Melmaiee, K., Berg, V. and Wise, R.P. (2010) Drought response in the spikes of barley: gene expression in the lemma, palea, awn, and seed. *Functional and Integrative Genomics*, **10**, 191-205.

Allen, D.K., Ohlrogge, J.B. and Shachar-Hill, Y. (2009) The role of light in soybean seed filling metabolism. *The Plant Journal*, **58**, 220-234.

Allorent, G., Osorio, S., Ly Vu, J., Falconet, D., Jouhet, J., Kuntz, M., Fernie, A.R., Lerbs-Mache, S., Macherel, D., Courtois, F. and Finazzi, G. (2015) Adjustments of embryonic photosynthetic activity modulate seed fitness in *Arabidopsis thaliana*. *New Phytologist*, **205**, 707-719.

Aoki, K., Yamamoto, M. and Wada, K. (1998) Photosynthetic and heterotrophic ferredoxin isoproteins are colocalized in fruit plastids of tomato. *Plant physiology*,

118, 439-449.

Aoyagi, K. and Bassham, J.A. (1984) Pyruvate Orthophosphate Dikinase of C3 Seeds and Leaves as Compared to the Enzyme from Maize. *Plant physiology*, **75**, 387-392.

Araújo, W. L., Nunes-Nesi, A., Osorio, S., Usadel, B., Fuentes, D., Nagy, R., ... & Martinoia, E. (2011). Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid–mediated effect on stomatal aperture. The Plant Cell, **23**(2), 600-627.

Araus, J.L., Brown, H.R., Febrero, A., Bort, J. and Serret, M.D. (1993) Ear photosynthesis, carbon isotope discrimination and the contribution of respiratory CO2 to differences in grain mass in durum wheat. *Plant, Cell & Environment*, **16**, 383-392.

Aschan, G. and Pfanz, H. (2003) Non-foliar photosynthesis – a strategy of additional carbon acquisition. *Flora* **198**, 81-97.

Asokanthan, P.S., Johnson, R.W., Griffith, M. and Krol, M. (1997) The photosynthetic potential of canola embryos. *Physiol Plantarum*, **101**, 353-360.

Atkins, C.R., Kuo, J., Pate, J.S., Flinn, A.M. and Steel, T.W. (1977) Photosynthetic pod wall of pea (*Pisum sativum* L.): Distribution of carbon dioxide-fixing enzymes in relation to pod structure. *Plant Physiol.*, **60**, 779-786.

Avila-Lovera, E., Zerpa, A.J. and Santiago, L.S. (2017) Stem photosynthesis and hydraulics are coordinated in desert plant species. *The New phytologist*, **216**, 1119-1129.

Ávila, E., Herrera, A. and Tezara, W. (2014) Contribution of stem CO2 fixation to whole-plant carbon balance in nonsucculent species. *Photosynthetica*, **52**, 3-15.

Barsan, C., Sanchez-Bel, P., Rombaldi, C., Egea, I., Rossignol, M., Kuntz, M., Zouine, M., Latche, A., Bouzayen, M. and Pech, J.C. (2010) Characteristics of the tomato chromoplast revealed by proteomic analysis. *J Exp Bot*, **61**, 2413-2431.

Barsan, C., Zouine, M., Maza, E., Bian, W., Egea, I., Rossignol, M., Bouyssie, D., Pichereaux, C., Purgatto, E., Bouzayen, M., Latche, A. and Pech, J.C. (2012) Proteomic analysis of chloroplast-to-chromoplast transition in tomato reveals metabolic shifts coupled with disrupted thylakoid biogenesis machinery and elevated energy-production components. *Plant Physiol*, **160**, 708-725.

Bassham, J.A. and Calvin, M. (1960) The path of carbon in photosynthesis. In *Die CO2-Assimilation / The Assimilation of Carbon Dioxide: In 2 Teilen / 2 Parts* (Pirson, A. ed. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 884-922.

Biel, K. and Fomina, I. (2015) Benson-Bassham-Calvin cycle contribution to the organic life on our planet. *Photosynthetica*, **53**, 161-167.

Birkhold, K.T., Koch, K.E. and Darnell, R.L. (1992) Carbon and Nitrogen Economy of Developing Rabbiteye Blueberry Fruit. *Journal of the American Society for Horticultural Science*, **117**, 139-145.

Blanke, M.M. (1992) Photosynthesis of Avocado fruit. In *Proc. of Second World Avocado Congress*, pp. 179-189.

Blanke, M.M. (1998) *Fruit Photosynthesis* Dordrecht: Springer, .

Blanke, M.M. and Lenz, F. (1989) Fruit photosynthesis. *Plant, Cell & Environment*, **12**, 31-46.

Borisjuk, L., Nguyen, T.H., Neuberger, T., Rutten, T., Tschiersch, H., Claus, B., Feussner, I., Webb, A.G., Jakob, P., Weber, H., Wobus, U. and Rolletschek, H. (2005) Gradients of lipid storage, photosynthesis and plastid differentiation in developing soybean seeds. *New Phytologist*, **167**, 761-776.

Bort, J., Brown, R.H. and Araus, J.L. (1995) Lack of C4 photosynthetic metabolism in ears of C3 cereals. *Plant, Cell & Environment*, **18**, 697-702.

Bort, J., Brown, R.H. and Araus, J.L. (1996) Refixation of respiratory CO2 in the ears of C3 cereals. *J Exp Bot*, **47**, 1567-1575.

Bort, J., Febrero, A., Amaro, T. and Araus, J.L. (1994) Role of awns in ear water-use efficiency and grain weight in barley. *Agronomie*, **14**, 133-139.

Bravdo, B.-A., Palgi, A., Lurie, S. and Frenkel, C. (1977) Changing ribulose diphosphate carboxylase/oxygenase activity in ripening tomato fruit. *Plant physiology*, **60**, 309-312.

Calderini, D.F., Dreccer, M.F. and Slafer, G.A. (1995) Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. *Plant Breeding*, **114**, 108-112.

Caley, C.Y., Duffus, C.M. and Jeffcoat, B. (1990) Photosynthesis in the Pericarp of Developing Wheat Grains. *J Exp Bot*, **41**, 303-307.

Calvin, M. and Benson, A.A. (1948) The Path of Carbon in Photosynthesis. *Science*,

107, 476-480.

Cannell, M.G.R. (1985) *Physiology of the Coffee Crop* Boston, MA: Springer.

Carrara, S., Pardossi, A., Soldatini, G.F., Tognoni, F. and Guidi, L. (2001) Photosynthetic Activity of Ripening Tomato Fruit. *Photosynthetica*, **39**, 75-78.

Cernusak, L.A. and Hutley, L.B. (2011) Stable Isotopes reveal the contribution of corticular photosynthesis to growth in branches of *Eucalyptus miniata*. *Plant physiology*, **155**, 515-523.

Chater, C.C., Caine, R.S., Tomek, M., Wallace, S., Kamisugi, Y., Cuming, A.C., Lang, D., MacAlister, C.A., Casson, S., Bergmann, D.C., Decker, E.L., Frank, W., Gray, J.E., Fleming, A., Reski, R. and Beerling, D.J. (2016) Origin and function of stomata in the moss Physcomitrella patens. *Nat Plants*, **2**, 16179.

Cheung, A.Y., McNellis, T. and Piekos, B. (1993) Maintenance of Chloroplast Components during Chromoplast Differentiation in the Tomato Mutant Green Flesh. *Plant physiology*, **101**, 1223-1229.

Chida, H., Nakazawa, A., Akazaki, H., Hirano, T., Suruga, K., Ogawa, M., Satoh, T., Kadokura, K., Yamada, S., Hakamata, W., Isobe, K., Ito, T., Ishii, R., Nishio, T., Sonoike, K. and Oku, T. (2007) Expression of the algal cytochrome c_6 gene in Arabidopsis enhances photosynthesis and growth. *Plant & cell physiology*, **48**, 948-957.

Cocaliadis, M.F., Fernández-Muñoz, R., Pons, C., Orzaez, D. and Granell, A. (2014) Increasing tomato fruit quality by enhancing fruit chloroplast function. A double-edged sword? *J Exp Bot*, **65**, 4589-4598.

Driever, S.M., Simkin, A.J., Alotaibi, S., Fisk, S.J., Madgwick, P.J., Sparks, C.A., Jones, H.D., Lawson, T., Parry, M.A.J. and Raines, C.A. (2017) Increased SBPase activity improves photosynthesis and grain yield in wheat grown in greenhouse conditions. *Philosophical Transactions of the Royal Society B*, **372**, 1730.

Eastmond, P., Koláčná, L. and Rawsthorne, S. (1996) Photosynthesis by developing embryos of oilseed rape (*Brassica napus* L.). *J Exp Bot*, **47**, 1763-1769.

Ehleringer, J.R., Comstock, J.P. and Cooper, T.A. (1987) Leaf-twig carbon isotope ratio differences in photosynthetic-twig desert shrubs. *Oecologia*, **71**, 318-320.

Ermakova, M., Lopez-Calcagno, P.E., Raines, C.A., Furbank, R.T. and von

Caemmerer, S. (2019) Overexpression of the Rieske FeS protein of the Cytochrome b6f complex increases C4 photosynthesis. *bioRxiv https://doi.org/10.1101/471680*.

Evans, L.T. and Rawson, H.M. (1970) Photosynthesis and respiration by the flag leaf and components of the ear during grain development in wheat. *Australian Journal of Biological Sciences*, **23**, 245-254.

Evans, T.L. and Fischer, R.A. (1999) Yield potential: its definition, measurement, and significance. *Crop Sci*, **39**, 1544-1551.

FAO (2017) The future of food and agriculture – Trends and challenges. Rome.

Faralli, M., Cockram, J., Ober, E., Wall, S., Galle, A., Van Rie, J., Raines, C. and Lawson, T. (2019a) Genotypic, developmental and environmental effects on the rapidity of gs in Wheat: Impacts on carbon gain and water-use efficiency. *Frontiers in plant science*, **10**.

Faralli, M., Matthews, J. and Lawson, T. (2019b) Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement. *Curr Opin Plant Biol*, **49**, 1-7.

Ferroni, L., Pantaleoni, L., Baldisserotto, C., Aro, E. and Pancaldi, S. (2013) Low photosynthetic activity is linked to changes in the organization of photosystem II in the fruit of Arum italicum. *Plant Physiology and Biochemistry*, **63**, 140-150.

Fischer, R.A., Rees, D., Sayre, K.D., Lu, Z.M., Condon, A.G. and Saavedra, A.L. (1998) Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Science Society of America*, **38**, 1467-1475.

Gale, M.D. and Youssefian, S. (1985) *Dwarfing genes in wheat* London: Butterworth and Co.

Galili, G., Avin-Wittenberg, T., Angelovici, R. and Fernie, A.R. (2014) The role of photosynthesis and amino acid metabolism in the energy status during seed development. *Frontiers in plant science*, **5**.

Gebbing, T. and Schnyder, H. (2001) 13C labeling kinetics of sucrose in glumes indicates significant refixation of respiratory CO2 in the wheat ear. *Functional Plant Biolog*, **28**, 1047-1053.

Glowacka, K., Kromdijk, J., Kucera, K., Xie, J., Cavanagh, A.P., Leonelli, L., Leakey,

A.D.B., Ort, D.R., Niyogi, K.K. and Long, S.P. (2018) Photosystem II subunit S overexpression increases the efficiency of water use in a field-grown crop. *Nat Commun*, **9**, 868.

Golombek, S., Heim, U., Horstmann, C., Wobus, U. and Weber, H. (1999) Phosphoenolpyruvate carboxylase in developing seeds of Vicia faba L.: Gene expression and metabolic regulation. *Planta*, **208**, 66-72.

Gu, J., Yin, X., Stomph, T.J. and Struik, P.C. (2014) Can exploiting natural genetic variation in leaf photosynthesis contribute to increasing rice productivity? *Plant Cell Environ*, **37**, 22-34.

Guo, Z. and Schnurbusch, T. (2016) Costs and benefits of awns. *J Exp Bot*, **67**, 2533-2535.

Harris, W.M. and Spurr, A.R. (1969a) Chromoplasts of tomato fruits: I. Ultrastructure of low-pigment and high-beta mutants: carotene analyses. *American Journal of Botany*, **56**, 369-379.

Harris, W.M. and Spurr, A.R. (1969b) Chromoplasts of tomato fruits: II. The red tomato. *American Journal of Botany*, **56**, 380-389.

Hedley, C.L., Harvey, D.M. and Keely, R.J. (1975) Role of PEP carboxylase during seed development in Pisum sativum. *Nature*, **258**, 352-354.

Hein, J.A., Sherrard, M.E., Manfredi, K.P. and Abebe, T. (2016) The fifth leaf and spike organs of barley (*Hordeum vulgare* L.) display different physiological and metabolic responses to drought stress. *BMC plant biology*, **16**, 248.

Hetherington, A.M. and Woodward, F.I. (2003) The role of stomata in sensing and driving environmental change. *Nature*, **424**, 901-908.

Hetherington, S.E., Smillie, R.M. and Davies, W.J. (1998) Photosynthetic activities of vegetative and fruiting tissues of tomato. *J Exp Bot*, **49**, 1173-1181.

Hibberd, J.M. and Quick, W.P. (2002) Characteristics of C4 photosynthesis in stems and petioles of C3 flowering plants. *Nature*, **415**, 451-454.

Hieke, S., Menzel, C.M. and Ludders, P. (2002) Effects of leaf, shoot and fruit development on photosynthesis of lychee trees (*Litchi chinensis*). *Tree physiology*, **22**, 955-961.

Hills, M.J. (2004) Control of storage-product synthesis in seeds. *Curr Opin Plant Biol*, **7**, 302-308.

Hiratsuka, S., Suzuki, M., Nishimura, H. and Nada, K. (2015) Fruit photosynthesis in Satsuma mandarin. *Plant Sci*, **241**, 65-69.

Hu, Y.-Y., Zhang, Y.-L., Luo, H.-H., Li, W., Oguchi, R., Fan, D.-Y., Chow, W.S. and Zhang, W.-F. (2012) Important photosynthetic contribution from the non-foliar green organs in cotton at the late growth stage. *Planta*, **235**, 325-336.

Hu, Y.-Y., Zhang, Y.-L., Xia, H., Fan, S., Song, J., Lv, X. and Kong, L. (2019) Photosynthetic characteristics of non-foliar organs in main C3 cereals. *Physiol Plantarum*, **166**, 226-239.

Imaizumi, N., Usuda, H., Nakamoto, H. and Ishihara, K. (1990) Changes in the rate of photosynthesis during grain filling and the enzymatic activities associated with the photosynthetic carbon metabolism in rice panicles. *Plant and Cell Physiology*, **31**, 835-844.

Jia, S., Lv, J., Jiang, S., Liang, T., Liu, C., & Jing, Z. (2015). Response of wheat ear photosynthesis and photosynthate carbon distribution to water deficit. Photosynthetica, **53(**1), 95-109.

Kang, F. and Rawsthorne, S. (1996) Metabolism of glucose-6-phosphate and utilization of multiple metabolites for fatty acid synthesis by plastids from developing oilseed rape embryos. *Planta*, **199**, 321-327.

Kohzuma, K., Sato, Y., Ito, H., Okuzaki, A., Watanabe, M., Kobayashi, H., Nakano, M., Yamatani, H., Masuda, Y., Nagashima, Y., Fukuoka, H., Yamada, T., Kanazawa, A., Kitamura, K., Tabei, Y., Ikeuchi, M., Sakamoto, W., Tanaka, A. and Kusaba, M. (2017) The non-mendelian green cotyledon gene in Soybean encodes a small subunit of photosystem II. *Plant physiology*, **173**, 2138-2147.

Kong, L., Sun, M., Xie, Y., Wang, F. and Zhao, Z. (2015) Photochemical and antioxidative responses of the glume and flag leaf to seasonal senescence in wheat. *Frontiers in plant science*, **6**, 358.

Kong, L.A., Xie, Y., Sun, M.Z., Si, J.S. and Hu, L. (2016) Comparison of the photosynthetic characteristics in the pericarp and flag leaves during wheat (*Triticum aestivum L.*) caryopsis development. *Photosynthetica*, **54**, 40-46.

Kromdijk, J., Glowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K. and Long, S.P. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, **354**, 857-861.

Laval-Martin, D., Farineau, J. and Diamond, J. (1977) Light versus Dark Carbon Metabolism in Cherry Tomato Fruits: I. Occurrence of Photosynthesis. Study of the Intermediates. *Plant physiology*, **60**, 872-876.

Lefebvre, S., Lawson, T., Fryer, M., Zakhleniuk, O.V., Lloyd, J.C. and Raines, C.A. (2005) Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. *Plant physiology*, **138**, 451-460.

Lemaire-Chamley, M., Petit, J., Garcia, V., Just, D., Baldet, P., Germain, V., Fagard, M., Mouassite, M., Cheniclet, C. and Rothan, C. (2005) Changes in transcriptional profiles are associated with early fruit tissue specialization in tomato. *Plant physiology*, **139**, 750-769.

Li, X., Hou, J., Bai, K., Yang, X., Lin, J., Li, Z. and Kuang, T. (2004) Activity and distribution of carbonic anhydrase in leaf and ear parts of wheat (*Triticum aestivum* L.). *Plant Sci*, **166**, 627-632.

Li, Y., Li, H., Li, Y. and Zhang, S. (2017) Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought-resistant wheat. *The Crop Journal*, **5**, 231-239.

Livne, A. and Gepstein, S. (1988) Abundance of the Major Chloroplast Polypeptides during Development and Ripening of Tomato Fruits. *An Immunological Study*, **87**, 239-243.

López-Calcagno, P.E., Fisk, S.J., Brown, K., Bull, S.E., P.F., S. and Raines, C.A. (2018) Overexpressing the H-protein of the glycine cleavage system increases biomass yield in glasshouse and field-grown transgenic tobacco plants. *Plant biotechnology journal*, **17**, 141-151.

Lopez, Y., Riaño, N., Mosquera, P., Cadavid, A. and Arcila, J. (2000) Activities of Phosphoenolpyruvate Carboxylase and Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase in Leaves and Fruit Pericarp Tissue of Different Coffee (Coffea sp.) Genotypes. *Photosynthetica*, **38**, 215-220.

Lytovchenko, A., Eickmeier, I., Pons, C., Osorio, S., Szecowka, M., Lehmberg, K., Arrivault, S., Tohge, T., Pineda, B., Anton, M.T., Hedtke, B., Lu, Y., Fisahn, J., Bock, R., Stitt, M., Grimm, B., Granell, A. and Fernie, A.R. (2011) Tomato fruit photosynthesis is seemingly unimportant in primary metabolism and ripening but

plays a considerable role in seed development. *Plant physiology*, **157**, 1650-1663.

Manetas, Y. (2004) Probing corticular photosynthesis through in vivo chlorophyll fluorescence measurements: evidence that high internal CO levels suppress electron flow and increase the risk of photoinhibition. *Physiol Plant*, **120**, 509-517.

Manzara, T., Carrasco, P. and Gruissem, W. (1993) Developmental and organ-specific changes in DNA-protein interactions in the tomato rbcS1, rbcS2 and rbcS3A promoter regions. *Plant molecular biology*, **21**, 69-88.

Maydup, M.L., Antonietta, M., Graciano, C., Guiamet, J.J. and Tambussi, E.A. (2014) The contribution of the awns of bread wheat (Triticum aestivum L.) to grain filling: Responses to water deficit and the effects of awns on ear temperature and hydraulic conductance. *Field Crops Research*, **167**, 102-111.

Maydup, M.L., Antonietta, M., Guiamet, J.J., Graciano, C., Lopez, J.R. and Tambussi, E.A. (2010) The contribution of ear photosynthesis to grain filling in bread wheat. *Field Crops Research*, **119**, 48-58.

Maydup, M.L., Antonietta, M., Guiamet, J.J. and Tambussi, E.A. (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.

McAusland, L., Davey, P.A., Kanwal, N., Baker, N.R. and Lawson, T. (2013) A novel system for spatial and temporal imaging of intrinsic plant water use efficiency. *J Exp Bot*, **64**, 4993-5007.

Merced, A. and Renzaglia, K.S. (2013) Moss stomata in highly elaborated Oedipodium (*Oedipodiaceae*) and highly reduced Ephemerum (*Pottiaceae*) sporophytes are remarkably similar. *Am J Bot*, **100**, 2318-2327.

Millar, A.H., Whelan, J., Soole, K.L. and Day, D.A. (2011) Organization and Regulation of Mitochondrial Respiration in Plants. *Annual Review of Plant Biology*, **62**, 79-104.

Moreshet, S. and Green, G.C. (1980) Photosynthesis and Diffusion Conductance of the Valencia Orange Fruit under Field Conditions. *J Exp Bot*, **31**, 15-27.

Nunes-Nesi, A., Carrari, F., Lytovchenko, A., Smith, A.M.O., Ehlers Loureiro, M., Ratcliffe, R.G., Sweetlove, L.J. and Fernie, A.R. (2005) Enhanced Photosynthetic Performance and Growth as a Consequence of Decreasing Mitochondrial Malate Dehydrogenase Activity in Transgenic Tomato Plants. *Plant*

physiology, **137**, 611-622.

Obiadalla-Ali, H., Fernie, A.R., Lytovchenko, A., Kossmann, J. and Lloyd, J.R. (2004) Inhibition of chloroplastic fructose 1,6-bisphosphatase in tomato fruits leads to decreased fruit size, but only small changes in carbohydrate metabolism. *Planta*, **219**, 533-540.

Osmond, C.B., Smith, S.D., Gui-Ying, B. and Sharkey, T.D. (1987) Stem photosynthesis in a desert ephemeral, Eriogonum inflatum. *Oecologia*, **72**, 542-549.

Palanisamy, K. and Vivekanandan, M. (1986) Photosynthetic Functions and Induction of Etiolation in Chloroembryos of *Dolichos lablab* L. *Journal of plant physiology*, **123**, 395-399.

Parry, M.A., Reynolds, M., Salvucci, M.E., Raines, C., Andralojc, P.J., Zhu, X.G., Price, G.D., Condon, A.G. and Furbank, R.T. (2011) Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *J Exp Bot*, **62**, 453-467.

Pengelly, J.J.L., Kwasny, S., Bala, S., Evans, J.R., Voznesenskaya, E.V., Koteyeva, N.K., Edwards, G.E., Furbank, R.T. and von Caemmerer, S. (2011) Functional Analysis of Corn Husk Photosynthesis. *Plant physiology*, **156**, 503-513.

Perdomo, J.A., Capo-Bauca, S., Carmo-Silva, E. and Galmes, J. (2017) Rubisco and Rubisco Activase Play an Important Role in the Biochemical Limitations of Photosynthesis in Rice, Wheat, and Maize under High Temperature and Water Deficit. *Frontiers in plant science*, **8**, 490.

Pfanz, H., Aschan, G., Langenfeld-Heyser, R., Wittmann, C. and Loose, M. (2002) Ecology and ecophysiology of tree stems: corticular and wood photosynthesis. *Naturwissenschaften*, **89**, 147-162.

Piechulla, B., Glick, R.E., Bahl, H., Melis, A. and Gruissem, W. (1987) Changes in Photosynthetic Capacity and Photosynthetic Protein Pattern during Tomato Fruit Ripening. *Plant physiology*, **84**, 911-917.

Piechulla, B. and Gruissem, W. (1987) Diurnal mRNA fluctuations of nuclear and plastid genes in developing tomato fruits. *The EMBO journal*, **6**, 3593-3599.

Piechulla, B., Pichersky, E., Cashmore, A.R. and Gruissem, W. (1986) Expression of nuclear and plastid genes for photosynthesis-specific proteins during tomato fruit

development and ripening. *Plant molecular biology*, **7**, 367-376.

Puthur, J.T., Shackira, A.M., Saradhi, P.P. and Bartels, D. (2013) Chloroembryos: A unique photosynthesis system. *Journal of plant physiology*, **170**, 1131-1138.

Raines, C.A. (2003) The Calvin cycle revisited. *Photosynthesis research*, **75**, 1-10.

Rangan, P., Furtado, A. and Henry, R.J. (2016) New evidence for grain specific C4 photosynthesis in wheat. *Scientific reports*, **6**, 31721.

Ranjan, S., Singh, R., Soni, D.K., Pathre, U.V. and Shirke, P.A. (2012) Photosynthetic performance of *Jatropha curcas* fruits. *Plant Physiology and Biochemistry*, **52**, 66-76.

Ray, D.K., Mueller, N.D., West, P.C. and Foley, J.A. (2013) Yield trends are insufficient to double global crop production by 2050. *Plos One*, **8**.

Ray, D.K., Ramankutty, N., Mueller, N.D., West, P.C. and Foley, J.A. (2012) Recent patterns of crop yield growth and stagnation. *Nat Commun*, **3**.

Rebetzke, G.J., Bonnett, D.G. and Reynolds, M.P. (2016) Awns reduce grain number to increase grain size and harvestable yield in irrigated and rainfed spring wheat. *J Exp Bot*, **67**, 2573-2586.

Reynolds, M., Foulkes, M.J., Slafer, G.A., Berry, P., Parry, M.A.J., Snape, J.W. and Angus, W.J. (2009) Raising yield potential in wheat. *J Exp Bot*, **60**, 1899-1918.

RSOL (2009) Royal Society of London, Reaping the Benefits: Science and the Sustainable Intensification of Global Agriculture (Royal Society, London, 2009).

Ruuska, S.A., Schwender, J. and Ohlrogge, J.B. (2004) The Capacity of Green Oilseeds to Utilize Photosynthesis to Drive Biosynthetic Processes. *Plant physiology*, **136**, 2700-2709.

Sanchez-Bragado, R., Molero, G., Reynolds, M.P. and Araus, J.L. (2014) Relative contribution of shoot and ear photosynthesis to grain filling in wheat under good agronomical conditions assessed by differential organ δ13C. *J Exp Bot*, **65**, 5401-5413.

Sanchez-Bragado, R., Molero, G., Reynolds, M.P. and Araus, J.L. (2016) Photosynthetic contribution of the ear to grain filling in wheat: a comparison of different methodologies for evaluation. *J Exp Bot*, **67**, 2787-2798.

Sánchez, C., Fischer, G. and Sanjuanelo, D.W. (2013) Stomatal behavior in fruits and leaves of the purple passion fruit (*Passiflora edulis* Sims) and fruits and cladodes

of the yellow pitaya [*Hylocereus megalanthus* (K. Schum. ex Vaupel) Ralf Bauer]. *Agronomía Colombiana*, **31**, 38-47.

Scafaro, A.P., Galle, A., Van Rie, J., Carmo-Silva, E., Salvucci, M.E. and Atwell, B.J. (2016) Heat tolerance in a wild Oryza species is attributed to maintenance of Rubisco activation by a thermally stable Rubisco activase ortholog. *The New phytologist*, **211**, 899-911.

Scafaro, A.P., Yamori, W., Carmo-Silva, A.E., Salvucci, M.E., von Caemmerer, S. and Atwell, B.J. (2012) Rubisco activity is associated with photosynthetic thermotolerance in a wild rice (Oryza meridionalis). *Physiol Plant*, **146**, 99-109.

Schwender, J., Goffman, F., Ohlrogge, J.B. and Shachar-Hill, Y. (2004) Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. *Nature*, **432**, 779-782.

Simbo, D.J., Van den Bilcke, N. and Samson, R. (2013) Contribution of corticular photosynthesis to bud development in African baobab (*Adansonia digitata* L.) and Castor bean (*Ricinus communis* L.) seedlings. *Environ Exp Bot*, **95**, 1-5.

Simkin, A.J., Kuntz, M., Moreau, H. and McCarthy, J. (2010) Carotenoid profiling and the expression of carotenoid biosynthetic genes in developing coffee grain. *Plant physiology and biochemistry : PPB*, **48**, 434-442.

Simkin, A.J., Lopez-Calcagno, P.E., Davey, P.A., Headland, L.R., Lawson, T., Timm, S., Bauwe, H. and Raines, C.A. (2017a) Simultaneous stimulation of sedoheptulose 1,7-bisphosphatase, fructose 1,6-bisphophate aldolase and the photorespiratory glycine decarboxylase H-protein increases CO_2 assimilation, vegetative biomass and seed yield in Arabidopsis. *Plant biotechnology journal*, **15**, 805-816.

Simkin, A.J., Lopez-Calcagno, P.E. and Raines, C.A. (2019) Feeding the world: improving photosynthetic efficiency for sustainable crop production. *Journal of Experimantal Botany*, **70**, 1119-1140.

Simkin, A.J., McAusland, L., Headland, L.R., Lawson, T. and Raines, C.A. (2015) Multigene manipulation of photosynthetic carbon assimilation increases CO_2 fixation and biomass yield in tobacco. *J Exp Bot*, **66**, 4075-4090.

Simkin, A.J., McAusland, L., Lawson, T. and Raines, C.A. (2017b) Over-expression of the RieskeFeS protein increases electron transport rates and biomass yield. *Plant*

physiology, **175**, 134-145.

Smith, A.M., Quinton-Tulloch, J. and Denyer, K. (1990) Characteristics of plastids responsible for starch synthesis in developing pea embryos. *Planta*, **180**, 517-523.

Smolikova, G., Dolgikh, E., Vikhnina, M., Frolov, A. and Medvedev, S. (2017) Genetic and hormonal regulation of chlorophyll degradation during maturation of seeds with green embryos. *International Journal of Molecular Sciences*, **18**, 1993.

Smolikova, G.N. and Medvedev, S.S. (2016) Photosynthesis in the seeds of chloroembryophytes. *Russ J Plant Physl+*, **63**, 1-12.

Steer, B.T. and Pearson, C.J. (1976) Photosynthate translocation in *Capsicum annuum*. *Planta*, **128**, 155.

Steinhauser, M.-C., Steinhauser, D., Koehl, K., Carrari, F., Gibon, Y., Fernie, A.R. and Stitt, M. (2010) Enzyme activity profiles during fruit development in tomato cultivars and *Solanum pennellii*. *Plant physiology*, **153**, 80-98.

Steinmeyer, F.T., Lukac, M., Reynolds, M.P. and Jones, H.E. (2013) Quantifying the relationship between temperature regulation in the ear and floret development stage in wheat (*Triticum aestivum* L.) under heat and drought stress. *Funct Plant Biol*, **40**, 700-707.

Sugita, M. and Gruissem, W. (1987) Developmental, organ-specific, and light-dependent expression of the tomato ribulose-1,5-bisphosphate carboxylase small subunit gene family. *P Natl Acad Sci USA*, **84**, 7104-7108.

Sui, X., Shan, N., Hu, L., Zhang, C., Yu, C., Ren, H., Turgeon, R. and Zhang, Z. (2017) The complex character of photosynthesis in cucumber fruit. *J Exp Bot*, **68**, 1625-1637.

Tambussi, E.A., Bort, J., Guiamet, J.J., Nogués, S. and Araus, J.L. (2007) The photosynthetic role of ears in C3 cereals: metabolism, water use efficiency and contribution to grain yield. *Critical Reviews in Plant Sciences*, **26**, 1-16.

Tambussi, E.A., Nogués, S. and Araus, J.L. (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta*, **221**, 446-458.

Tanaka, A., Fujita, K. and Kikuchi, K. (1974) Nutrio-physiological studies on the tomato plant. *Soil Science and Plant Nutrition*, **20**, 57-68.

Tilman, D. and Clark, M. (2015) Food, agriculture and the environment: Can we feed the world and save the Earth? *Daedalus*, **144**, 8-23.

Timm, S., Florian, A., Arrivault, S., Stitt, M., Fernie, A.R. and Bauwe, H. (2012) Glycine decarboxylase controls photosynthesis and plant growth. *FEBS letters*, **586**, 3692-3697.

Tinoco-Ojanguren, C. (2008) Diurnal and seasonal patterns of gas exchange and carbon gain contribution of leaves and stems of *Justicia californica* in the Sonoran Desert. *Journal of Arid Environments*, **72**, 127-140.

Todd, G.W., Bean, R.C. and Propst, B. (1961) Photosynthesis & respiration in developing fruits . II. Comparative rates at various stages of development. *Plant physiology*, **36**, 69-73.

Tschiersch, H., Borisjuk, L., Rutten, T. and Rolletschek, H. (2011) Gradients of seed photosynthesis and its role for oxygen balancing. *Biosystems*, **103**, 302-308.

Vicente, R., Vergara-Díaz, O., Medina, S., Chairi, F., Kefauver, S.C., Bort, J., Serret, M.D., Aparicio, N. and Araus, J.L. (2018) Durum wheat ears perform better than the flag leaves under water stress: Gene expression and physiological evidence. *Environ Exp Bot*, **153**, 271-285.

Von Groll, U., Berger, D. and Altmann, T. (2002) The subtilisin-like serine protease SDD1 mediates cell-to-cell signaling during Arabidopsis stomatal development. *The Plant cell*, **14**, 1527-1539.

Wang, H., Schauer, N., Usadel, B., Frasse, P., Zouine, M., Hernould, M., Latche, A., Pech, J.C., Fernie, A.R. and Bouzayen, M. (2009) Regulatory features underlying pollination-dependent and -independent tomato fruit set revealed by transcript and primary metabolite profiling. *The Plant cell*, **21**, 1428-1452.

Weber, H., Borisjuk, L. and Wobus, U. (2005) Molecular physiology of legume seed development. *Annual Review of Plant Biology*, **56**, 253-279.

Weyhrich, R.A. (1994) Effects of awn suppression on grain yield and agronomic traits in hard red winter wheat. *Crop Sci*, **v. 34**, pp. 965-969-1994 v.1934 no.1994.

Weyhrich, R.A., Carver, B.F. and Martin, B.C. (1995) Photosynthesis and water-use efficiency of awned and awnletted near-isogenic lines of hard winter wheat. *Crop Sci*, **35**, 172-176.

Willmer, C.M. and Johnston, W.R. (1976) Carbon dioxide assimilation in some aerial plant organs and tissues. *Planta*, **130**, 33-37.

WorldBank (2008) World Development Report 2008: Agriculture for Development ,

World Bank, Washington, DC.

Wu, X.-L., Liu, Z.-H., Hu, Z.-H. and Huang, R.-Z. (2014) BnWRI1 coordinates fatty acid biosynthesis and photosynthesis pathways during oil accumulation in rapeseed. *Journal of Integrative Plant Biology*, **56**, 582-593.

Xu, H., Gauthier, L., Desjardins, Y. and Gosselin, A. (1997) Photosynthesis in leaves, fruits, stem and petioles of greenhouse-grown tomato plants. *Photosynthetica*, **33**, 113-123.

Yadav, S.K., Khatri, K., Rathore, M.S. and Jha, B. (2018) Introgression of UfCyt c_6, a thylakoid lumen protein from a green seaweed *Ulva fasciata* Delile enhanced photosynthesis and growth in tobacco. *Molecular biology reports*, **45(6)**, 1745-1758.

Yakovlev, M.S. and Zhukova, G.Y. (1980) Chlorophyll in embryos of angiosperm seeds, a review. *Bot. Not. Ser*, **133**, 323–336.

Yemm, E.W. and Willis, A.J. (1954) Chlorophyll and Photosynthesis in Stomatal Guard Cells. *Nature*, **173**, 726-726.

Yiotis, C., Psaras, G.K. and Manetas, Y. (2008) Seasonal photosynthetic changes in the green-stemmed Mediterranean shrub Calicotome villosa: a comparison with leaves. *Photosynthetica*, **46**, 262.

Zhou, B., Serret, M.D., Elazab, A., Bort Pie, J., Araus, J.L., Aranjuelo, I. and Sanz-Sáez, Á. (2016) Wheat ear carbon assimilation and nitrogen remobilization contribute significantly to grain yield. *Journal of Integrative Plant Biology*, **58**, 914-926.

Ziegler-Jöns, A. (1989) Gas-exchange of ears of cereals in response to carbon dioxide and light. *Planta*, **178**, 164-117.

Figure legends

Figure 1. Chlorophyll fluorescence (CF) images of PSII operating efficiency (*Fq*'/*Fm*') in green non-leaf was used to demonstrate photosynthetic electron transport. CF images of: (a) wheat ear; (b) sycamore seed pods; (c) tomato fruit ; (d) strawberry fruit; (e) greengage; (f) cherries; (g) apples are illustrated. Colour scale bar represents an *Fq'*/*Fm'* of (a) 0.45-0.75. (b) 0.30- 0.55. (c) 0.50- 0.70. (d) 0.50-0.70. (e) 0.5- 0.75. (f) 0.5- 0.80. (g) 0.45- 0.70.

Figure 2. Example of epidermal impressions taken from tomato (a & b) and cucumber (c & d). Photographs of the fruit are illustrated in a, c and e. Stomata were absent from the epidermis of tomato (b); whilst relatively high stomatal density is illustrated in cucumber (d, with the inset showing a magnified stomatal complex), and large open stomatal found in sycamore (f). **.**

Figure 3. Schematic diagram and Images of epidermal impressions illustrating stomatal anatomy and density in different components of wheat leaves (flag leaf), culm (stem) and ear (i.e. glume, lemma and palea external surface). The insert box provides an example of stomatal density on the awns of Soissons.

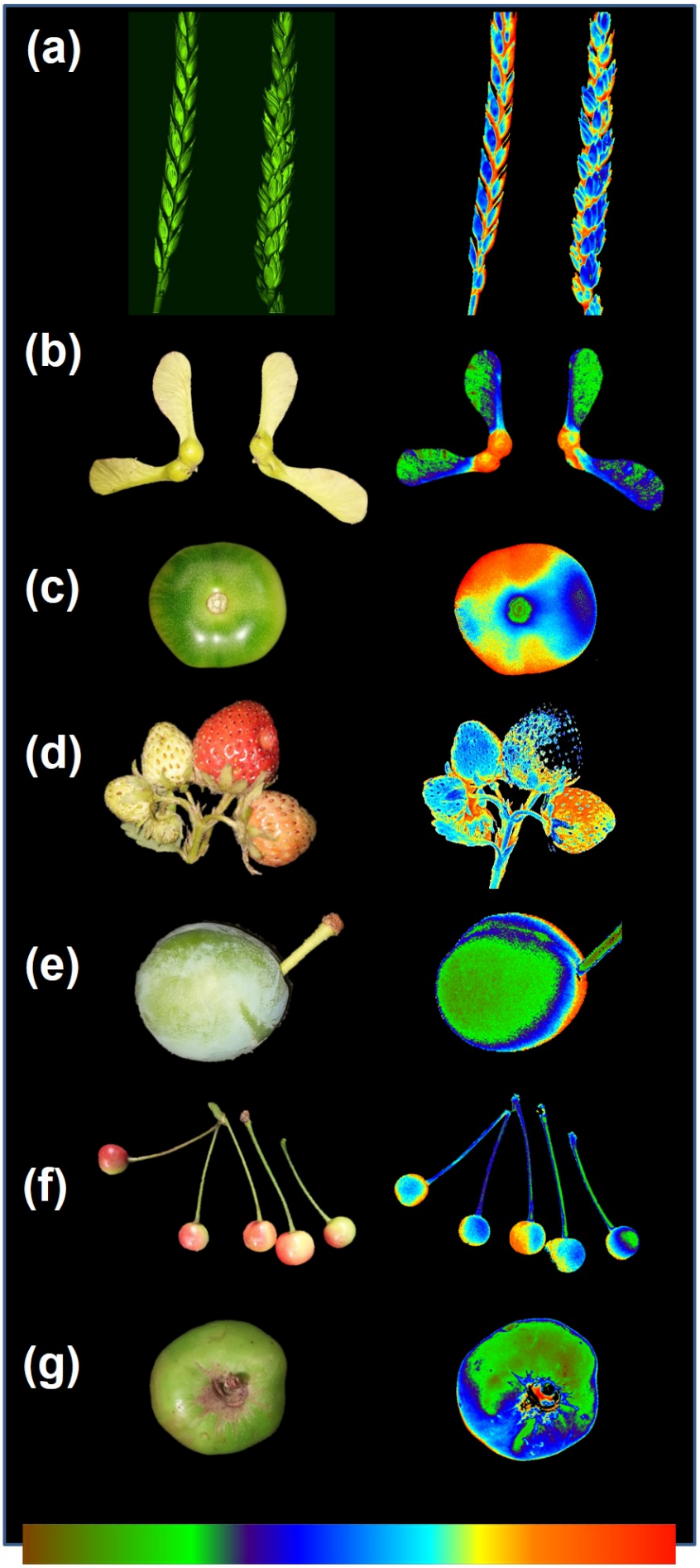
Figure 4. Stomatal density of ear organs (glume, lemma, palea and awns when present) for seven bread wheat elite cultivars collected after anthesis (a and b). Wheat plants were grown in a greenhouse and ears were harvested at GS69. Stomatal analysis were carried out as in Faralli et al. 2019. Data are means ± standard error of the mean (n=2 to 7).

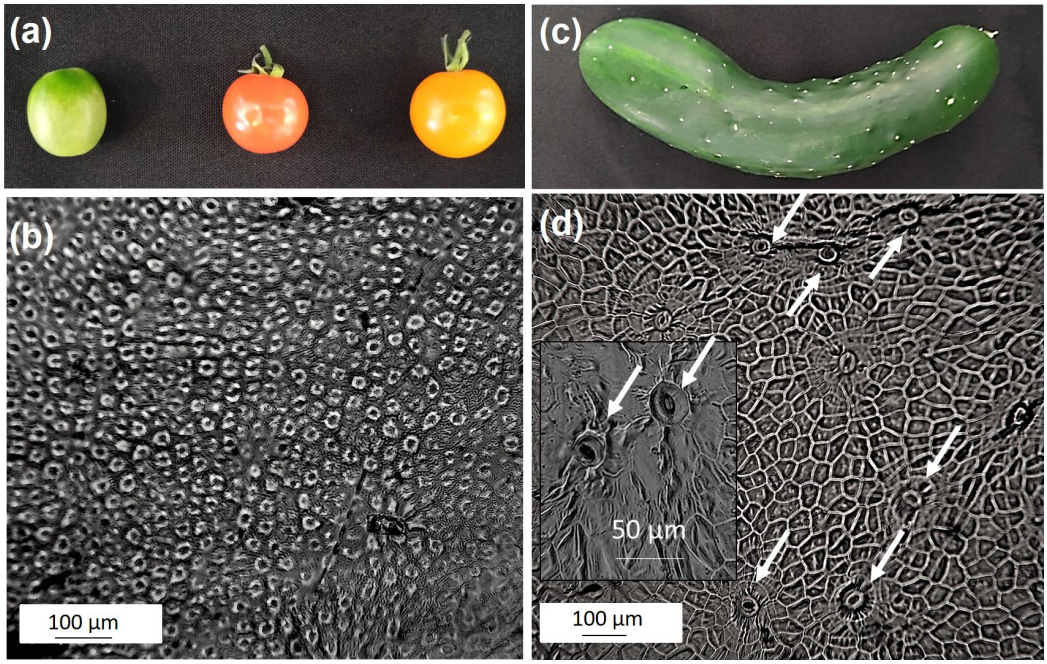
Figure 5. Temperature differential between dry reference and sample for the flag leaf and the ear of two bread wheat varieties grown in greenhouse conditions (n=4 cv. Alchemy and Hereward) subjected to a step change in light (100 to 1000 µmol m^{-2} s^{-1}) and maintained at high light for 30 minutes. Thermal images of a wheat ears (c & d) following the step increase in light intensity shows significant temperature differences in plants subjected to 10 min (c) or 25 min (d) illumination, illustrating stomatal functioning for increase evaporative cooling.

Figure 6. Example of fine assessment of wheat ear area and volume (A) by using 3D scanner approaches and example of ear area underestimation with a ruler based approach compared to a fine 3D scanner estimation (B). In B, wheat plants (cv. Cadenza) were grown in greenhouse and primary and secondary ears were harvested at different timing and over three times after

anthesis. Area was estimated with a ruler by measuring ear length and width of all the four surfaces and then the same ear was assessed with a 3D scanner (n=4 for each harvest).

Figure 7. Gross assimilation rate calculated as the sum of light-saturated assimilation rate and dark respiration of wheat ears (n=5) of control cv Cadenza plants and transgenic plants overexpressing SBPase (Driever et al. 2017). Data were collected post anthesis in greenhouse grown plants with a Licor 6400XT mounted with a bespoke cuvette ensuring saturating light (1000 µmol m^{-2} s^{-1}) and 25°C block temperature.





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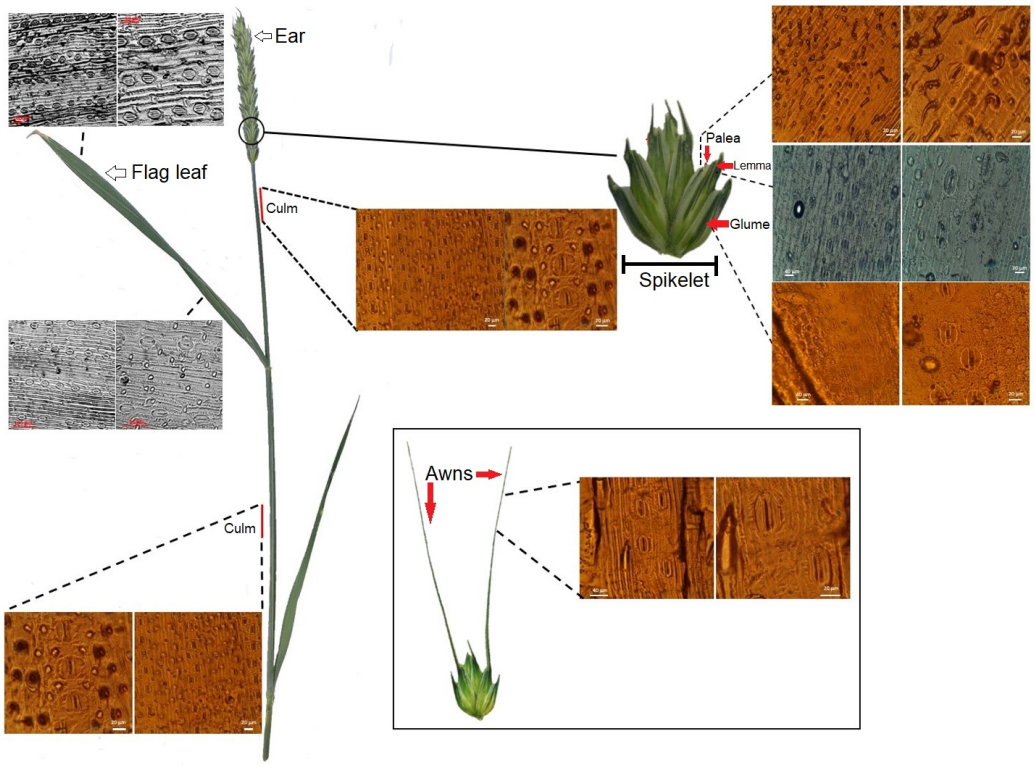

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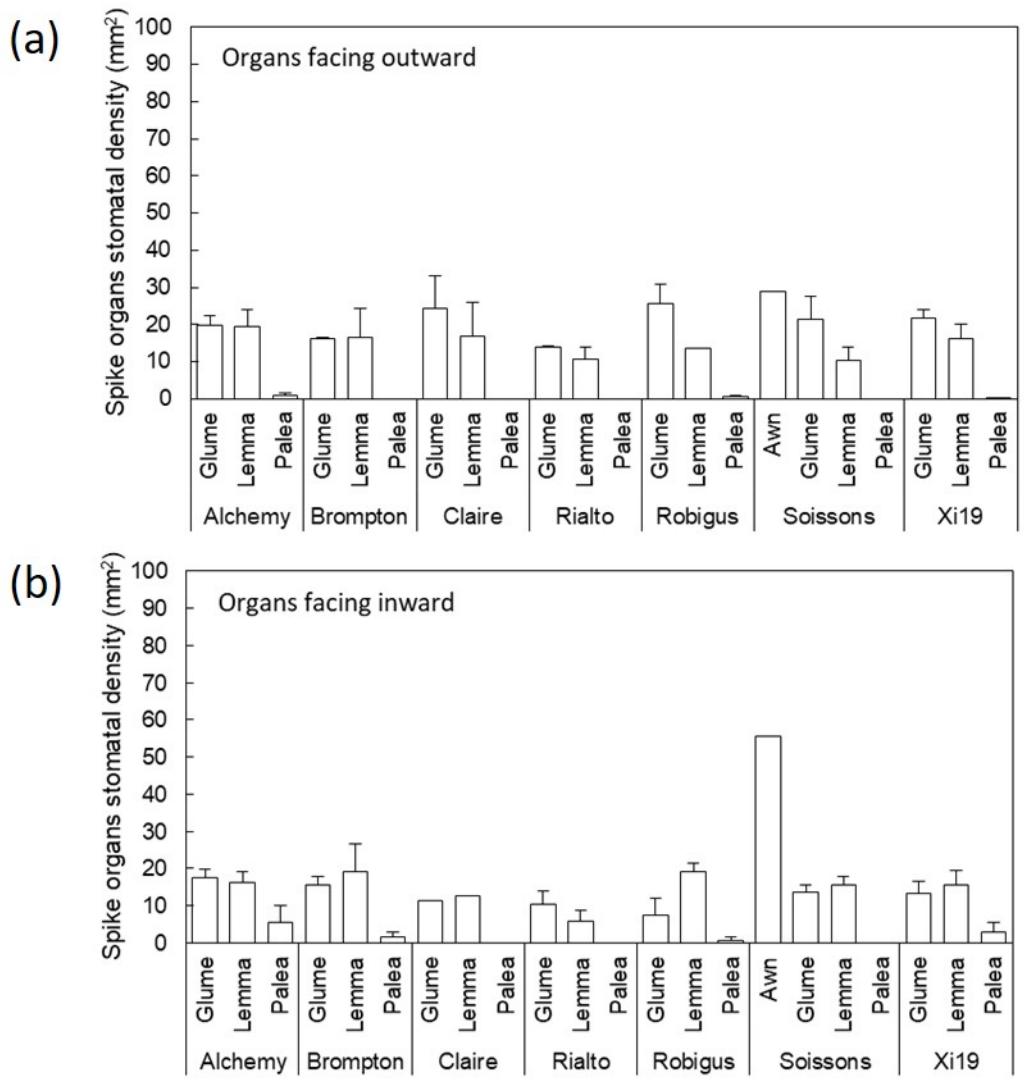

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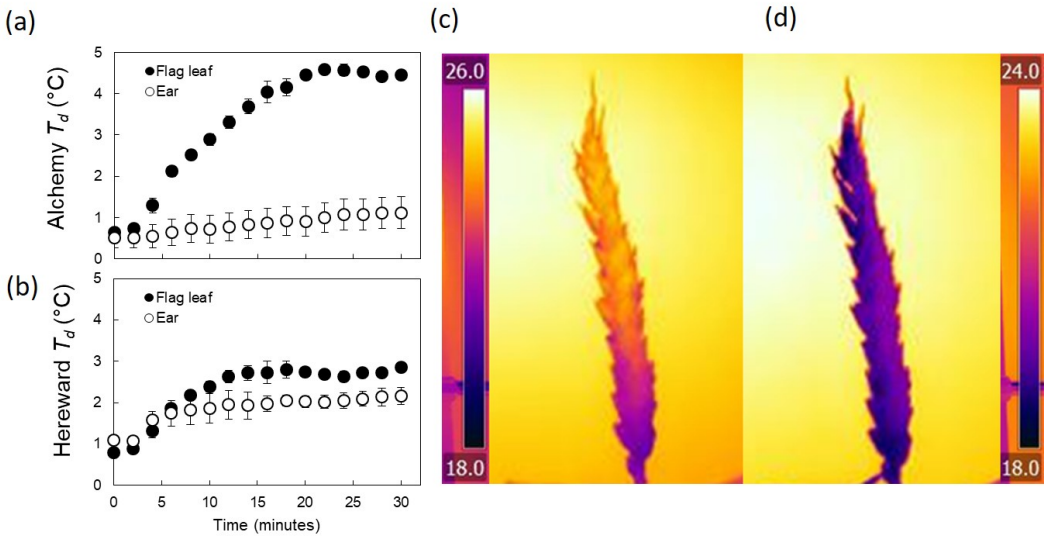

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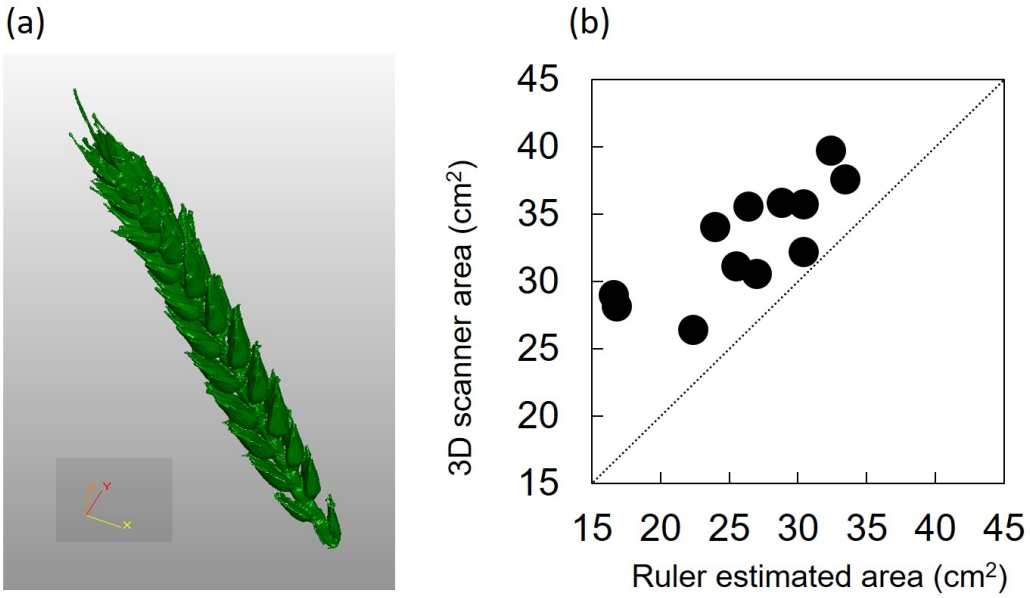

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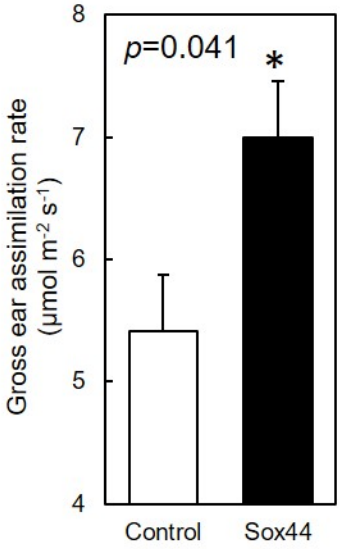

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