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DARWIN REVIEW

Cell-to-cell connectivity: a future target for crop improvement

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Abstract

Boosting crop productivity while enhancing resilience to climate change and disease remains a major challenge. Plasmodesmata (PD), which mediate cell-to-cell connectivity, are crucial for plant growth but remain underutilized as targets for crop improvement. This review focuses on C_4 photosynthesis to demonstrate the importance of enhanced cell-to-cell connectivity to improve productivity. In C_4 plants, connectivity between mesophyll (M) and bundle sheath (BS) cells is essential for building an efficient CO_2-concentrating mechanism. Enhanced PD frequency at the M–BS interface is a key feature of C_4 Kranz leaf anatomy, and thus an important trait to introduce in engineering C_4 photosynthesis into C_3 crops. We propose potential gene targets to engineer PD connectivity, while emphasizing the need for further research to discover new targets that affect PD formation and regulation. We also discuss advances in biotechnological tools that are important for both molecular studies and deploying strategies to manipulate PD in crops. These target genes and tools may ultimately unlock new capabilities to improve crop productivity and resilience by engineering cell-to-cell connectivity within various tissues.

Keywords: C_4 photosynthesis, CO_2-concentrating mechanism, crop improvement, mesophyll-bundle sheath cell interface, plasmodesmata, symplastic transport.

Introduction

By the year 2030, the global population is expected to reach 8.5 billion and urban area expansion will result in a 1.8% to 2.4% loss of global croplands (Bren d'Amour *et al.*, 2017). About 80% of global cropland loss from urban expansion will take place in Asia and Africa, adding more pressure to future food security and threats to livelihood in vulnerable regions (Bren d'Amour *et al.*, 2017; Gerber *et al.*, 2024). Drought, heat, and extreme weather events are predicted to have major impacts on crop production (Ray *et al.*, 2015, 2019; Heino *et al.*, 2023; Yuan *et al.*, 2024). A significant contribution to addressing these challenges could be to improve crop photosynthetic performance, climate-adaptability, and resilience to pests and diseases to potentially boost crop yields despite the future climate challenges (Croce *et al.*, 2024). One promising avenue is to target plasmodesmata, which are central to many important processes in plant biology including growth and development, sugar and nutrient distribution, and stress responses (reviewed in Lu *et al.*, 2018; Tee and Faulkner, 2024; Zanini and Burch-Smith, 2024).

Plasmodesmata (PD) are membrane-lined nanochannels that provide regulated cytoplasmic, endoplasmic, and plasma membrane continuity between plant cells (Sager and Lee, 2014). PD facilitate the transport and signalling of essential molecules, including metabolites, hormones, RNAs, and proteins, between neighbouring cells (Lee and Frank, 2018). These important structures are formed *de novo* either during cytokinesis in an endoplasmic reticulum (ER)-dependent manner (primary PDs), or through insertion in pre-existing cell walls (secondary PDs) (Hepler, 1982; Ehlers and Kollmann, 2001; Faulkner *et al.*, 2008; Z.P. Li *et al.*, 2024).

In this review, we argue that PD present an important and underutilized target to improve crops, particularly in the case of two-celled C_4 photosynthesis. Firstly, we discuss PD in the context of C_4 photosynthesis where enhanced cell-to-cell connectivity is required between distinct cell types to operate a CO_2-concentrating mechanism for more efficient photosynthesis. Secondly, we highlight how chloroplast development and PD biogenesis are interdependent and impact photosynthetic efficiency in C_4 species. Finally, we propose strategies on how enhanced cell-to-cell connectivity could be better engineered towards crop improvement that includes identifying target genes and discussing cutting-edge genetic tools.

Increased plasmodesmata frequency is a hallmark of more efficient two-celled C_4 photosynthesis

Why C_4 photosynthesis is more efficient

Different photosynthesis types have evolved to optimise carbon fixation (Sage *et al.*, 2018). About 95% of plants conduct the ancestral C_3 type of photosynthesis, including most major crops like rice, wheat, barley, oats, potato, canola, and soybean. C_3 plants perform photosynthesis primarily in the leaf mesophyll (M) cells, where atmospheric CO_2 is fixed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) into 3-phosphoglycerate, which is converted into three-carbon sugars via the Calvin–Benson–Bassham cycle (Fig. 1; Farquhar *et al.*, 1980). However, the efficiency of C_3 photosynthesis is compromised when RuBisCO fixes O_2 instead of CO_2, resulting in the formation of a toxic, auto-inhibitory compound, 2-phosphoglycolate (2-PG) (Bowes *et al.*, 1971). Recycling 2-PG in a process called photorespiration is energetically wasteful (Walker *et al.*, 2016). This process is exacerbated under hot, arid, and high light conditions, making C_3 plants more vulnerable to climate change (Badger and Andrews, 1974; Walker *et al.*, 2016). To limit photorespiration, elaborate CO_2-concentrating mechanisms evolved in different plant lineages. Among these, two-celled C_4 photosynthesis evolved multiple times independently in crops such as maize, sorghum, millet, and sugarcane (Sage *et al.*, 2012).

Compared to C_3 plants, C_4 plants require a modified biochemistry and 'Kranz' anatomy to form a spatially separated CO_2-concentrating mechanism between M and bundle sheath (BS) cells (Fig. 1; Hatch, 1987; von Caemmerer and Furbank, 2003; Sage *et al.*, 2012). In C_4 leaves, atmospheric CO_2 is first fixed in M cells into a four-carbon acid (malate or aspartate), which diffuses into BS cells. There, the C_4 acid is decarboxylated, releasing a concentrated stream of CO_2 for fixation by RuBisCO in the BS chloroplasts (Osmond, 1971; Hatch and Osmond, 1976; Stitt and Heldt, 1985; Leegood, 2000). C_4 species can be classified into three subtypes based on their main decarboxylation enzyme: NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME), or PEP carboxykinase (PCK), which can act alone or in combination (Hatch *et al.*, 1975). This CO_2-concentrating mechanism drastically reduces photorespiration (von Caemmerer and Furbank, 2003), making C_4 plants up to 50% more photosynthetically efficient than C_3 plants (Hibberd *et al.*, 2008).

Crucially, this biochemical efficiency confers key physiological advantages. Because the CO_2-concentrating mechanism saturates photosynthesis below ambient CO_2 levels (Ghannoum *et al.*, 2001; von Caemmerer *et al.*, 2012), C_4 plants can operate with more closed stomata (i.e. lower stomatal conductance) than C_3 plants under the same conditions (Huxman and Monson, 2003; Osborne and Sack, 2012). This trait is highly beneficial in hot, arid environments, as it significantly improves water-use efficiency (Lawson and Leakey, 2024). Additionally, since stomata serve as a primary entry point for pathogens, lower stomatal conductance may also reduce the probability of infection in C_4 species (Francesconi and Balestra, 2020; Wu and Liu, 2022; Hou *et al.*, 2024). These combined advantages—enhanced growth, superior water-use efficiency, and potentially greater disease resistance—are likely to give C_4 plants a competitive edge under future climate scenarios. Therefore, engineering C_4 photosynthesis into C_3 crops represents a promising strategy for sustainably increasing yield resilience to climate stress (Sheehy *et al.*, 2008; von Caemmerer *et al.*, 2012; Long *et al.*, 2015).

The role of plasmodesmata in C_4 photosynthesis

The high metabolic flux required for the C_4 CO_2 concentrating mechanism is facilitated by a significantly increased PD frequency at the M–BS cell interface. In monocots, PD frequency at this interface can be up to 12-fold greater in C_4 leaves than in their C_3 counterparts, a trend observed across grass species from seven independent evolutionary lineages (Danila *et al.*, 2018; Ermakova *et al.*, 2020). A similar 8- to 13-fold increase is seen in C_4 eudicots (Schreier *et al.*, 2024). Notably, while PD frequencies at M–M and BS–BS interfaces are also elevated in C_4 leaves, the increase is far less pronounced than at the M–BS interface (Danila *et al.*, 2016, 2018; Schreier *et al.*, 2024; Aleksejeva and Schreier, 2025). This indicates specific, polar enhancement of symplastic connectivity specifically for the metabolite shuttle between M and BS cells during C_4 photosynthesis.

This evolutionary link between PD frequency and photosynthesis is further illustrated by studies of C_3-C_4 intermediate species. In the genus *Flaveria*, M–BS PD frequency increases along the C_3 to C_4 evolutionary gradient (Goodhand, 2019; Aleksejeva and Schreier, 2025). Intermediate species, including

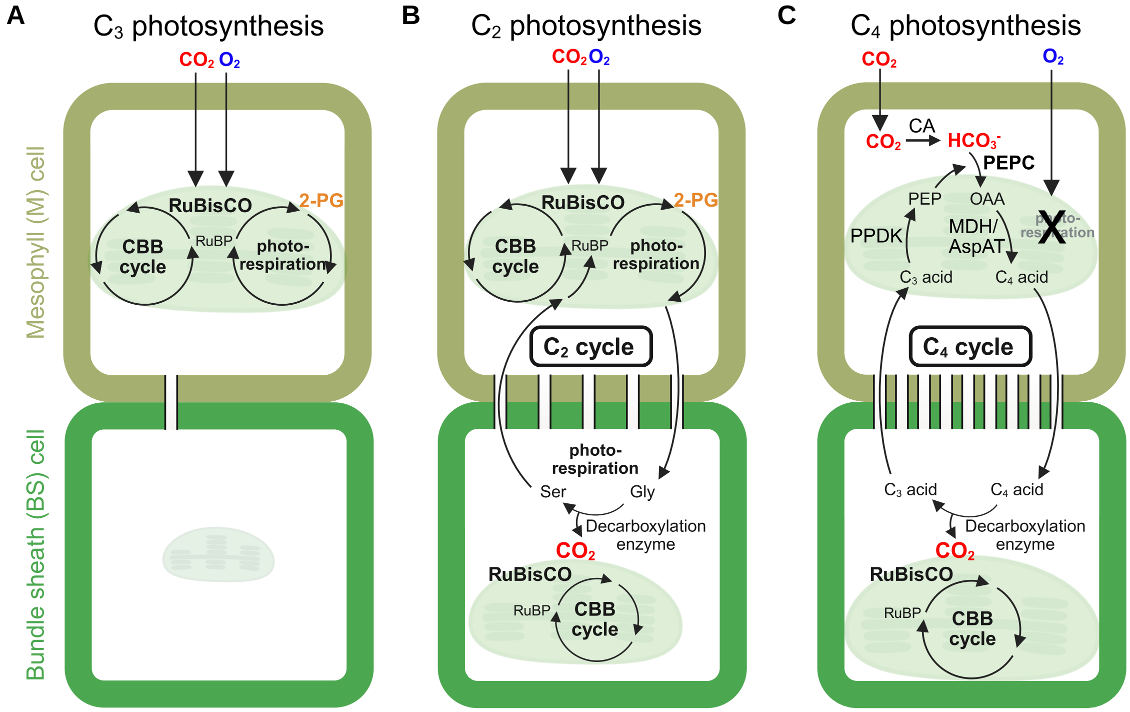

Fig. 1. Overview of cell-autonomous C_3 photosynthesis, and two-celled C_2 and C_4 photosynthesis, highlighting metabolite shuttles between mesophyll and bundle sheath cells facilitated by increased plasmodesmata frequency. (A) In C_3 plants, CO_2 (red) is captured from the atmosphere and incorporated into the Calvin-Benson-Bassham (CBB) cycle by carbon-fixing enzyme RuBisCO in mesophyll (M) cells. However, RuBisCO also reacts with atmospheric O_2 (blue), forming 2-phosphoglycolate (2-PG, orange) and initiating costly photorespiration. (B) Intermediate C_3-C_4 plant species perform a 'C_2-type' of photosynthesis in which part of the photorespiratory pathway is confined to the bundle sheath (BS) cells. This leads to a modest CO_2-concentrating mechanism around BS-localized RuBisCO and relies on shuttling C_2 acid glycine (Gly) from M to BS cell and serine (Ser) from BS to M cells. (C) C_4 photosynthesis minimizes photorespiration by spatially separating phospho*enol*pyruvate carboxylase (PEPC) in M cells from RuBisCO in BS cells, thereby establishing a CO_2-concentrating mechanism. In the C_4 cycle, atmospheric CO_2 is first converted to bicarbonate (HCO_3^-) by carbonic anhydrase (CA) in M cells. Bicarbonate is then fixed by PEPC into the C_4 acid oxaloacetate (OAA) in M cells, which is then converted to either C_4 acids malate (by malate dehydrogenase, MDH) or aspartate (by aspartate aminotransferase, AspAT)–depending on the C_4 subtype–and shuttled to BS cells. There, C_4 acid is decarboxylated by one of three decarboxylation enzymes [chloroplastic NADP-ME, mitochondrial NAD-ME or mitochondrial phospho*enol*pyruvate carboxykinase (PCK) depending on the C_4 subtype], releasing CO_2. This process concentrates CO_2 around RuBisCO in the BS cells. The resulting C_3 acid (pyruvate or alanine) is shuttled back to M cells, where it is either converted to phosphoenolpyruvate (PEP) (by pyruvate orthophosphate dikinase, PPDK) or directly returned as PEP to re-enter the C_4 cycle as a substrate for PEPC. Increased plasmodesmata (PD) connectivity between M and BS cells in C_2 and C_4 leaves is accompanied by functionally specialized BS cells containing more and larger chloroplasts compared to those in C_3 leaf BS cell. PD, which correspond to the white channels at the M–BS cell interface, allow for cytoplasmic continuity and enable metabolite shuttling. Arrows indicate the directionality of metabolite flow. Chloroplasts are schematically depicted in green within the individual cells. Created in BioRender. Danila, F. (2025) https://BioRender.com/n0rd3on.

those performing C_2 photosynthesis (which shuttles glycine instead of C_4 acids, Fig. 1), possess a PD frequency greater than that of C_3 species but lower than that of fully developed C_4 species (Khoshravesh *et al.*, 2020; Aleksejeva and Schreier, 2025). This stepwise increase in symplastic connectivity underscores the importance of enhanced M–BS metabolite exchange as an essential adaptation for the evolution of efficient C_4 photosynthesis from a C_3 ancestor.

Among C_4 plant species, the frequency of PD at the M–BS cell interface varies significantly between biochemical subtypes, a variation linked to their distinct anatomical features (Danila *et al.*, 2018). This variation is proposed to be attributed to several factors including the positioning of BS organelles, presence of agranal BS chloroplasts, and BS cell wall suberization (Fig. 2A; Dengler *et al.*, 1994). For example, NAD-ME subtypes exhibit the highest PD frequency, which could facilitate a greater metabolite flux (Danila *et al.*, 2018). This may compensate for a longer intracellular transport path; in these plants, BS chloroplasts and mitochondria are polarized towards the vasculature, increasing the distance a C_4 acid must travel from the cell wall to the organelles (Fig. 2A; Dengler *et al.*, 1994). This polarized arrangement is thought to create a longer diffusion path that helps prevent CO_2 (released from decarboxylated malate) from leaking backwards into the M cells (Fig. 2A; Hatch and Osmond, 1976; Dengler *et al.*, 1994). Thus, the high PD frequency ensures efficient symplastic transport of metabolites to regenerate CO_2. Conversely, in NADP-ME and PCK subtypes, the BS cell

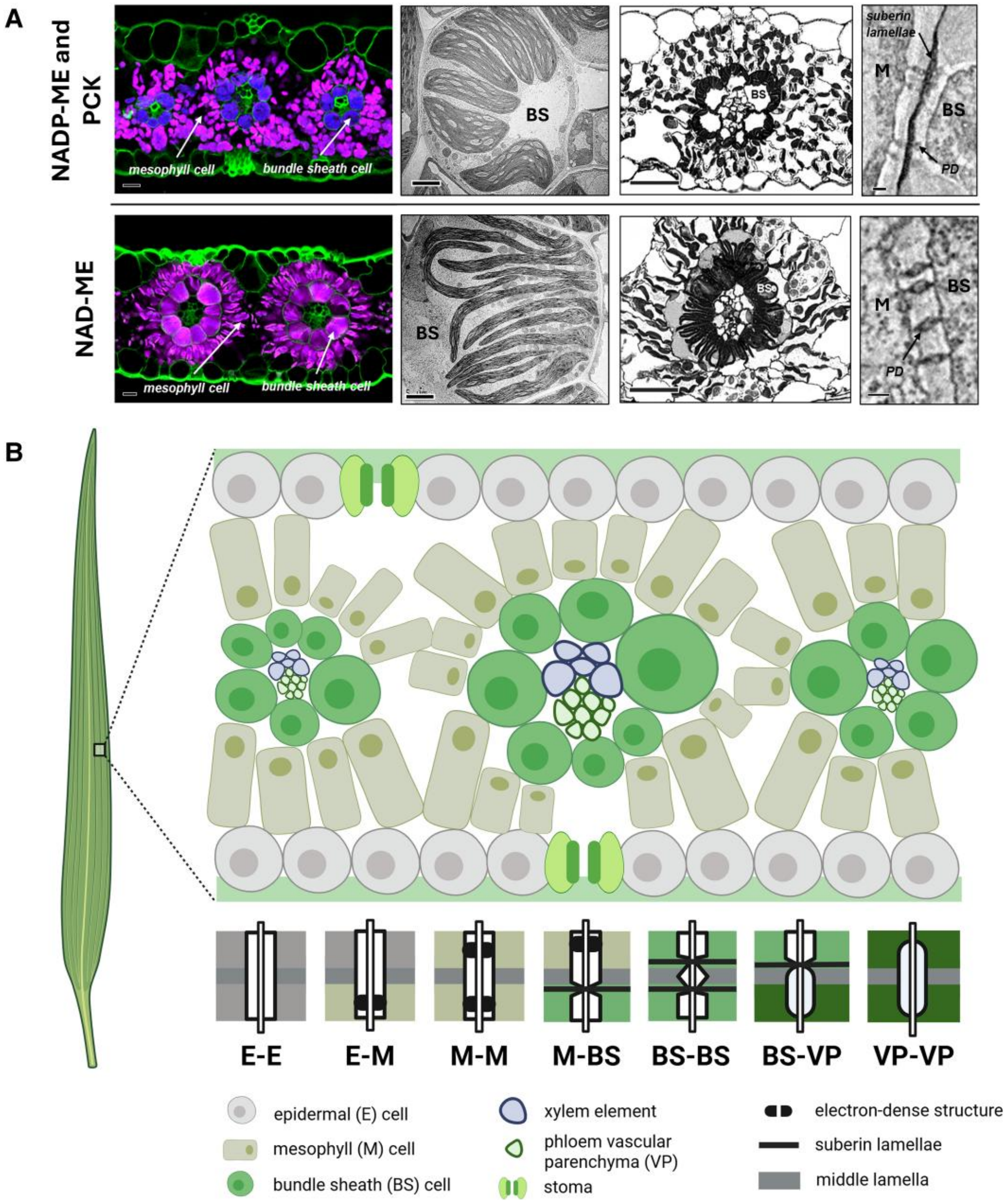

Fig. 2. Plasmodesmata at different cell interfaces in C_4 monocot leaves. (A) In contrast to C_4 eudicots, C_4 monocots can be one or combination of either NADP-ME, NAD-ME, and/or PCK subtype depending on the decarboxylation enzyme present in the bundle sheath (BS). Confocal micrographs (leftmost panel) were obtained using an excitation wavelength at 633 nm and dual emission wavelengths at 650–720 nm (magenta for Photosystem II) and 720–800 nm (blue for Photosystem I) to visualize chloroplasts. Note that BS cells of C_4 NADP-ME and C_4 PCK leaves have agranal chloroplasts (see second panel from left) thus appear pseudo-blue under confocal microscope due to reduced Photosystem II content. The cell wall (green) was visualized at an excitation wavelength of 405 nm and an emission wavelength of 420–480 nm. Presence of suberized BS cell wall in C_4 NADP-ME and C_4 PCK subtypes (rightmost panel) creates a gas barrier preventing CO_2 leakage and enables the centrifugal positioning of the chloroplasts in the BS cells (second panel from right). M, mesophyll cell; PD, plasmodesma. Bars from left to right: 20 µm, 5 µm, 50 µm, 200 nm. (B) In C_4 NADP-ME species like maize, leaf cells are symplastically connected through plasmodesmata (PD) that are generally unbranched. However, PD ultrastructural variations like presence of internal electron-dense structure (on the M cell side) and/or cytoplasmic sleeve constriction due to suberin lamellae (on the BS cell side) could differentially regulate PD transport among cell interfaces. B is based on the work by Evert *et al*. (1977). Created in BioRender. Danila, F. (2025) https://BioRender.com/um6wegm.

wall is suberized, forming a gas-tight barrier that prevents CO_2 leakage (Fig. 2A; von Caemmerer and Furbank, 2003; Danila *et al.*, 2021). This barrier allows BS chloroplasts to remain appressed to the M cells, enabling efficient metabolite exchange despite a lower frequency of PD at M–BS cell wall interface. Similarly, the distinct cellular redox status in BS cells of different C_4 subtypes—driven by the presence of agranal chloroplasts in NADP-ME species versus granal chloroplasts in NAD-ME species—may contribute to variations in their M–BS PD frequency, as redox status is a known regulator of PD biogenesis (Fig. 2A; Benitez-Alfonso *et al.*, 2009; Turkan *et al.*, 2018).

Despite these subtype variations, the trait of increased M–BS PD frequency is universally conserved across diverse C_4 lineages (Danila *et al.*, 2016, 2018; Schreier *et al.*, 2024; Aleksejeva and Schreier, 2025). This conservation, along with its stepwise increase along the C_3 to C_4 evolutionary gradient (Khoshravesh *et al.*, 2020; Aleksejeva and Schreier, 2025), underscores the critical importance of enhanced symplastic connectivity as a fundamental anatomical enabler of two-celled C_4 photosynthetic pathway.

Development of efficient C_4 photosynthesis relies on, and is potentially limited by, the coordinated biogenesis of chloroplasts and plasmodesmata

Photosynthetic efficiency and plasmodesmata frequency in C_4 leaves

Despite the well-established importance of increased PD frequency at the M–BS cell interface for C_4 photosynthesis, the developmental mechanisms controlling this trait remains elusive. Key unanswered questions include the genetic regulators of PD biogenesis in C_4 leaves and the direct impact of PD morphology on photosynthetic performance. However, emerging evidence suggests a strong link between photosynthetic function and PD development. For instance, perturbing C_4 metabolism—by knocking out phosphoenolpyruvate carboxylase (PEPC) in *Setaria viridis* (Alonso-Cantabrana *et al.*, 2018) or mutating pyruvate orthophosphate dikinase (PPDK) and a dicarboxylic acid transporter in *Zea mays* (maize) (Gao *et al.*, 2022)—alters PD frequency and maturation at the M–BS interface. Furthermore, chloroplast and photosynthesis inhibitors modify PD connectivity in the C_4 eudicot *Gynandropsis gynandra* (Schreier *et al.*, 2024), and environmental factors like light intensity and temperature also influence PD frequency in C_4 leaves (Sowiński *et al.*, 2003; Sowiński *et al.*, 2007; Danila *et al.*, 2019). Critically, the up-regulation of PD formation at the M–BS interface coincides precisely with the onset of operational C_4 photosynthesis (Schreier *et al.*, 2024; Tsang *et al.*, 2024), indicating it is a metabolically required adaptation to facilitate a massive metabolite flux.

Plasmodesmata formation in C_4 leaves

Our previous research has identified key anatomical parameters influencing PD frequency in C_4 leaves (Danila *et al.*, 2018; Ermakova *et al.*, 2020). Leaf PD typically appear in clusters within a cell interface referred to as pit fields (Danila *et al.*, 2016). Quantification across 25 grass species (spanning C_3, C_3-C_4, and C_4 phototypes) revealed that the increased PD frequency at the M–BS interface in C_4 species is driven by both a higher density and a larger size of these pit fields (Danila *et al.*, 2018; Ermakova *et al.*, 2020). Pit field size is thought to depend on secondary PD formation, where new PD are inserted adjacent to primary ones in existing cell walls (Faulkner *et al.*, 2008). However, as most studies rely on a single developmental timepoint, the specific contribution of secondary PD formation to this process has been difficult to assess.

A recent developmental study in the monocots *Oryza sativa* (rice, C_3) and *S. viridis* (C_4) used fluorescently tagged PD lines to investigate M–BS PD formation (Tsang *et al.*, 2024). The results correlated the increased M–BS PD frequency in *S. viridis* with the expression of genes related to chloroplastic and mitochondrial signalling, revealing antagonistic expression patterns between the two species. Of particular interest are genes known in the C_3 eudicots Arabidopsis and tobacco to alter symplastic transport by affecting the frequency of branched PD, such as *INCREASED SIZE EXCLUSION LIMIT1* (*ISE1*) (Stonebloom *et al.*, 2009), *ISE2* (Kobayashi *et al.*, 2007), *DECREASED SIZE EXCLUSION LIMIT1* (*DSE1*) (Xu *et al.*, 2012), and *GFP ARRESTED TRAFFICKING1* (*GAT1*) (Benitez-Alfonso *et al.*, 2009). *ISE1* and *ISE2*, which are known to negatively regulate secondary PD formation, were down-regulated in *S. viridis* but up-regulated in rice. Meanwhile, a positive regulator of secondary PD formation *DSE1* was up-regulated in *S. viridis* but down-regulated in rice. Notably, PD in monocots are typically unbranched (Fig. 2B), an observation consistent across both developing and mature leaves (Evert *et al.*, 1977, 1978; Robinson-Beers and Evert, 1991; Evert *et al.*, 1996; Russin *et al.*, 1996; Danila *et al.*, 2016). Given that branching and twinning are hallmarks of secondary PD formation in eudicots, this suggests that monocots may employ a distinct mechanism for secondary PD formation during leaf development.

Developmental link between plasmodesmata, chloroplasts, and mitochondria

The tight developmental links between leaf chloroplasts and PD are well established in eudicots, leading to the organelle–nucleus–PD signalling (ONPS) hypothesis (Burch-Smith *et al.*, 2011). This hypothesis posits that chloroplasts and mitochondria regulate PD formation and function via retrograde signalling to the nucleus, which modulates the expression of PD-associated genes (Azim and Burch-Smith, 2020). In C_3 models like Arabidopsis and tobacco, numerous genes involved in chloroplast and mitochondrial development—including RNA helicases (RH), ribonucleases, and WD-repeat proteins—have been shown to affect leaf PD frequency (Table 1; Benitez-Alfonso *et al.*, 2009; Ganusova *et al.*, 2020). Silencing these genes often represses photosynthetic genes, impairs chloroplast development, and decreases photosynthetic capacity (Ganusova *et al.*, 2020), demonstrating the functional integration of these pathways.

Emerging evidence indicates that this organelle–PD coordination is a conserved feature across angiosperms, including monocots. Notably, orthologues of these genes, such as those encoding chloroplastic DEAD-box helicases (e.g. RH3, RH22) and the WD-repeat protein DSE1, have been identified in the monocots rice (C_3) and *S. viridis* (C_4), where they are associated with secondary PD formation (Tsang *et al.*, 2024). This conservation suggests that mutating these genes in monocots would likely yield similar defects in PD frequency, chloroplast development, and photosynthesis.

Crucially, reverse genetic experiments in monocots provide compelling support for this link. Overexpression of the chloroplast biogenesis transcription factors *GOLDEN-2* and *GOLDEN-2 LIKE* from maize (*ZmG2* or *ZmGLK1*) in rice not only increased chloroplast number and size but also concurrently increased PD frequency (Wang *et al.*, 2017). This finding demonstrates that enhancing organelle biogenesis is sufficient to drive PD formation. A critical next step is to determine whether mutating these newly identified PD-associated genes in monocots—particularly in C_4 species—reciprocally impairs photosynthetic efficiency and chloroplast development, thereby solidifying the mechanistic conservation of the ONPS pathway.

While the influence of chloroplasts on PD is well documented, the role of mitochondria is less understood. Among genes known to alter PD function, only *ISE1* and *ISE3* are mitochondrial, while the majority are chloroplastic (Table 1). *ISE1* encodes a mitochondrial DEAD-box RNA helicase essential for organelle function; its loss in Arabidopsis leads to defective mitochondria and increased symplastic transport due to a higher frequency of branched PD (Stonebloom *et al.*, 2009). Similarly, *ISE3*, involved in mitochondrial glucose-TARGET OF RAPAMYCIN (TOR) signalling, is required for regulating PD transport, though its mechanism remains unclear (Brunkard *et al.*, 2020). These pioneering studies establish that functional mitochondria are critical regulators of cell-to-cell connectivity.

The significance of mitochondria may be particularly pronounced in specific C_4 subtypes. Beyond their universal role in respiration, mitochondria are directly involved in the decarboxylation of C_4 acids in the BS cells of NAD-ME and PCK subtypes (Furbank, 2011; Fan *et al.*, 2022). However, their specific influence on PD development is complex. For instance, in NADP-ME *Neurachne* species, an increase in mitochondrial occupancy in the mestome sheath did not enhance PD frequency (Khoshravesh *et al.*, 2020). In contrast, overexpressing the chloroplast biogenesis factor *ZmG2* in rice increased both chloroplast and mitochondrial numbers and concurrently raised PD frequency (Wang *et al.*, 2017). This correlation between chloroplast content and PD frequency is further supported by observations along the C_3 to C_4 evolutionary gradient in the eudicot *Flaveria* (Aleksejeva and Schreier, 2025).

This distinction highlights an unresolved question: can mitochondria independently influence PD biogenesis? A unique opportunity to address this is presented by C_3-C_4 intermediate species in the *Tribulus* genus, which accumulate mitochondria—but not chloroplasts—in their BS cells (Leung *et al.*, 2024). Studying these species could isolate the specific effect of mitochondrial proliferation on PD development at the M–BS interface, separate from the dominant effects of chloroplast signalling.

How can enhanced cell-to-cell connectivity be engineered in crops to improve photosynthetic performance?

Genes for plasmodesmata formation and structure

To date, no single gene has been identified that independently controls PD structure, frequency, and size exclusion limit (SEL or the maximum size of molecules that can pass through PD). As illustrated in Table 1, mutations in many genes simultaneously affect multiple PD traits, suggesting these processes are developmentally linked. This overlap implies that the genetic mechanisms underlying secondary PD formation and PD maturation (e.g. increased branching during aging) may involve shared players.

For instance, mutations in *CHOLINE TRANSPORTER-LIKE 1* (*CHER1*) alter both PD structure and frequency. CHER1, located in the *trans*-Golgi network, is required for normal PD frequency in the shoot apical meristem and for the formation of complex, branched PD in mature leaves (Kraner *et al.*, 2017). Similarly, mutants of *DSE1* and *ISE1/ISE2* exhibit coordinated changes in PD structure, SEL, and frequency (Table 1, Fig. 3). *dse1* mutants have fewer branched and twinned PDs, resulting in a decreased SEL (Xu *et al.*, 2012). Conversely, *ise1* and *ise2* knockdown mutants exhibit increased symplastic transport due to a higher frequency of these complex PD structures (Burch-Smith *et al.*, 2011); silencing these genes in *N. benthamiana* also increases PD frequency via enhanced secondary PD formation (Burch-Smith and Zambryski, 2010). Given that monocot PD are exclusively simple (Fig. 2B), it would be intriguing to test whether ectopic expression or silencing of these genes could induce more diverse structures.

For other regulators, alterations in PD traits are accompanied by changes in other subcellular properties. This is highly relevant for genes in the ONPS pathway and *ZmG2*, which coordinately affect PD frequency, organelle development, and homeostasis (Wang *et al.*, 2017; Ganusova *et al.*, 2020). Furthermore, recent work identified the pectin acetyltransferase PMR5 as a regulator of cell wall properties that influence secondary PD formation (Fig. 3, Table 1; Jay *et al.*, 2025). The *pmr5* loss-of-function mutant, known for its altered cell wall composition and enhanced fungal resistance (Chiniquy *et al.*, 2019), also showed reduced secondary PD frequency, indicating a direct or indirect role in PD biogenesis during cell expansion.

Genes for plasmodesmata regulation

The most studied mechanism regulating PD aperture is the reversible deposition of callose (β-1,3-glucan) around the pore

Table 1. Summary of genes associated with PD-related phenotypes in overexpression lines or loss-of-function mutants, identified as potential targets for engineering cell-to-cell connectivity in crops

Gene	Species	Gene function	PD phenotype	References
Altered PD structure, size exclusion limit and/or number				
***GOLDEN2* (*G2*)**	*Zea mays*	Nuclear transcription factor essential for chloroplast biogenesis	Increased PD frequency between M–BS and BS-mestome sheath cells after *ZmG2* overexpression under the maize UBI10 promoter in rice	Wang *et al*. (2017)
***DECREASED SIZE EXCLUSION 1* (*DSE1*)**	*Arabidopsis thaliana*	Conserved WD-repeat protein expressed in cytosol and nucleus	Reduced cell-to-cell transport due to decreased frequency of branched and twinned PD in *dse1* mutants	Xu *et al*. (2012)
***INCREASED SIZE EXCLUSION LIMIT 1* (*ISE1*)**	*Arabidopsis thaliana*, *Nicotiana benthamiana*	Nuclear-encoded mitochondrial DEAD-box RNA helicase	Increased cell-to-cell transport due to increased frequency of branched and twinned PD at mid-torpedo stage of embryogenesis in *ise1* and *ise2* mutants	Kobayashi *et al*. (2007); Stonebloom *et al*. (2009); Burch-Smith and Zambryski (2010)
***INCREASED SIZE EXCLUSION LIMIT 2* (*ISE2*)**	*Arabidopsis thaliana*, *Nicotiana benthamiana*	Nuclear-encoded chloroplast RNA helicase		
***INCREASED SIZE EXCLUSION LIMIT 3* (*ISE3*)**	*Arabidopsis thaliana*	Mitochondrial SEL1-like repeat-containing protein involved in the glucose-TARGET OF RAPAMYCIN (TOR) metabolic signalling network	Increased cell-to-cell transport at mid-torpedo stage of embryogenesis in *ise3* mutants and silencing lines	Brunkard *et al*. (2020)
***INCREASED SIZE EXCLUSION LIMIT 4* (*ISE4*)**	*Arabidopsis thaliana*	Involved in the glucose-TOR metabolic signalling network	Increased cell-to-cell transport at mid-torpedo stage of embryogenesis in *ise4* mutants	
***PHLOEM UNLOADING MODULATOR* (*PLM*)**	*Arabidopsis thaliana*	ER-localized putative sphingolipid biosynthesis enzyme	Increased cell-to-cell connectivity due to PD that lack cytoplasmic sleeves at the phloem pole pericycle and endodermal cell interface in *plm* mutants	Yan *et al*. (2019)
***CHOLINE TRANSPORTER-LIKE 1* (*CHER1*)**	*Arabidopsis thaliana*	*trans*-Golgi located choline transporter 1	Reduced numbers of PD in the shoot apical meristem and no complex/branched PD in mature leaves in *cher1* mutants	Kraner *et al*. (2017)
***RNA Helicase 3* (*RH3*)**	*Nicotiana benthamiana*	Chloroplastic DEAD-box helicase	Decreased PD frequency in leaves after virus-induced gene silencing of *RH22*	Ganusova *et al*. (2020)
***RNA Helicase 22* (*RH22*)**	*Nicotiana benthamiana*	Chloroplastic DEAD-box helicase	Increased PD frequency in leaves after virus-induced gene silencing	
***RNA Helicase 39* (*RH39*)**	*Nicotiana benthamiana*	Chloroplastic DEAD-box helicase		
***Ribonuclease J* (*RNase J*)**	*Nicotiana benthamiana*	Essential in maintaining chloroplast RNA quality		
***Polynucleotide Phosphorylase A* (*PNPase A*)**	*Nicotiana benthamiana*	Chloroplast enzyme involved in mRNA 3′-end determination and turnover		

(*continued*)

Table 1. Continued

Gene	Species	Gene function	PD phenotype	References
POWDERY MILDEW RESISTANT 5 (PMR5)	*Arabidopsis thaliana*	Pectin acetyl-transferase	Decreased secondary PD frequency in leaves of *pmr5* mutants	Jay *et al.* (2025)
Callose-dependent alterations in cell-to-cell connectivity				
GFP ARRESTED TRAFFICKING 1 (GAT1)	*Arabidopsis thaliana*	Putative chloroplast thioredoxin	Restricted green fluorescent protein (GFP) transport due to altered PD structures (increased PD branching and PD occlusion through callose deposition) in *gat1* mutants; ectopic expression was sufficient to enhance PD transport in mature tissues	Benitez-Alfonso *et al.* (2009)
***Callose synthase 3* (*CalS3*)/*Glucan synthase-like 12* (*GSL12*)**	*Arabidopsis thaliana*	Callose synthase/glucan synthase-like (converts uridine diphosphate (UDP)-glucose into the beta-1,3-glucan callose)	Drastic increase in PD callose deposition and limited PD permeability from *CalS3/GSL12* gain-of-function mutation	Vatén *et al.* (2011)
***Callose synthase 8* (CalS8)/*Glucan synthase-like 4* (*GSL4*)**	*Arabidopsis thaliana*		No callose deposition in response to hydrogen peroxide treatment and wounding in *cals8/gsl4* mutants	Cui and Lee (2016)
***Callose synthase 10* (*CalS10*)/*Glucan synthase-like 8* (*GSL8*)**	*Arabidopsis thaliana*		Reduced callose deposition at PD and therefore increased PD permeability and auxin movement in *cals10/gsl8* mutants	Guseman *et al.* (2010); Han *et al.* (2014)
β-1,3-glucanase AtBG_ppap	*Arabidopsis thaliana*	Plasmodesmata-localized beta-1,3-glucanases; members of glycosyl hydrolase family	Reduced cell-to-cell connectivity due to increase in callose deposition at PD in *AtBG_ppap* mutants	Levy *et al.* (2007)
***β-1,3-glucanase 1* (*PDBG1*)**	*Arabidopsis thaliana*		Decreased cell-to-cell connectivity in the basal meristem and lateral root-forming zone due to increased callose deposition in *pdbg1 pdbg2* double mutants	Benitez-Alfonso *et al.* (2013)
***β-1,3-glucanase 2* (*PDBG2*)**	*Arabidopsis thaliana*			Benitez-Alfonso *et al.* (2013); Grison *et al.* (2015)
***β-1,3-glucanase 2* (*MtBG2*)**	*Medicago truncatula*		Reduced levels of PD-associated callose and increased nodulation following *MtBG2* overexpression in roots	Gaudioso-Pedraza *et al.* (2018)
***β-1,3-glucanase 6* (*BG6*)**	*Arabidopsis thaliana*		No reduction in callose deposition at PD (and therefore no increase in cell-to-cell connectivity) in response to excess copper in *bg6* mutants, unlike wild-type plants	O'Lexy *et al.* (2018)
***PLASMODESMATA CALLOSE-BINDING PROTEIN 1* (*PDCB1*)**	*Arabidopsis thaliana*	Plasmodesmatal localized protein with callose binding activity	Callose accumulation and reduced cell-to-cell connectivity after *PDCB1* overexpression	Simpson *et al.* (2009); Grison *et al.* (2015)

(*continued*)

Table 1. Continued

Gene	Species	Gene function	PD phenotype	References
***PLASMODESMATA-LOCATED PROTEIN 1* (*PDLP1*)** ***PLASMODESMATA-LOCATED PROTEIN 2* (*PDLP2*)** ***PLASMODESMATA-LOCATED PROTEIN 3* (*PDLP3*)**	*Arabidopsis thaliana* *Arabidopsis thaliana* *Arabidopsis thaliana*	Family of proteins that localize specifically at PD	*pdlp1 pdlp2 pdlp3* triple mutants were unable to significantly increase callose depositions at PD in response to synthetic dsRNA analogue polyinosinic-polycytidilic acid. *pdlp1 pdlp2 pdlp3* triple mutant was also shown to be more susceptible to oomycete *Hyaloperonispora arabidopsidis* while overexpression of *PDLP1* enhances plant resistance (due to increased PD-associated callose deposition). *PDLP1* negatively regulates PD permeability	Thomas *et al*. (2008); Caillaud *et al*. (2014); Huang *et al*. (2023)
***PLASMODESMATA-LOCATED PROTEIN 5* (*PDLP5*)**	*Arabidopsis thaliana*		Increased cell-to-cell connectivity from decreased callose, and increased susceptibility to virulent *Pseudomonas syringae* in *pdlp5* mutants. Four-fold increase in callose levels after *PDLP5* overexpression	Lee *et al*. (2011); Liu *et al*. (2020)
***PLASMODESMATA-LOCATED PROTEIN 6* (*PDLP6*)**	*Arabidopsis thaliana*		Increased callose deposition at PDs in vasculature after *PDLP6* overexpression	Z.P. Li *et al*. (2024)
***PLASMODESMATA-LOCATED PROTEIN 7* (*PDLP7*)**	*Arabidopsis thaliana*		Reduced callose deposition, enlarged PD diameters and faster viral transmission in *pdlp7* mutants	Chen *et al*. (2024)
***NON-RACE-SPECIFIC DISEASE RESISTANCE GENE/ HAIRPIN INDUCED GENE 3* (*NHL3*)**	*Arabidopsis thaliana*	Sequence similarity to tobacco hairpin-induced gene (*HIN1*) and Arabidopsis non-race specific disease resistance gene (*NDR1*), plasma membrane localized	Unable to close PD in response to chitin, *flg22* and salicylic acid through PDLP-mediated activation of callose synthesis in *nhl3* mutants	Tee *et al*. (2023)
***NON-RACE-SPECIFIC DISEASE RESISTANCE GENE/ HAIRPIN INDUCED GENE 12* (*NHL12*)**	*Arabidopsis thaliana*		Low cell-to-cell connectivity in *nhl12* mutants and mutants are unaffected by drought or ABA treatment, unlike wild-type plants	Ayyoub *et al*. (2024)
***LYSIN MOTIF DOMAIN-CONTAINING GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN 2* (*LYM2*)**	*Arabidopsis thaliana*	Chitin receptor-like protein	Cell-to-cell connectivity stays unchanged in the presence of chitin in *lym2* mutants, unlike wild-type plants	Faulkner *et al*. (2013)
***Heavy metal-associated isoprenylated plant proteins* (*HIPPs*)**	*Arabidopsis thaliana*	Metallochaperone-like proteins with heavy metal-associated domain	*HIPP* overexpression or higher-order *hipp* loss-of-function mutations affected callose-mediated responses to biotic and abiotic stresses	Guo *et al*. (2021); Barr *et al*. (2023)

(*continued*)

Table 1. Continued

Gene	Species	Gene function	PD phenotype	References
***FRUIT WEIGHT 2.2* (*FW2.2*)**	*Solanum lycopersicum*	Member of the *CELL NUMBER REGULATOR* (*CNR*) gene family	*FW2.2* overexpression reduced callose deposition at PDs leading to enhanced cell-to-cell connectivity	Beauchet *et al.* (2024)
Callose-independent alterations in cell-to-cell connectivity				
***MULTIPLE C2 DOMAINS AND TRANSMEMBRANE DOMAIN PROTEIN 3* (*MCTP3*)**	*Arabidopsis thaliana*	ER-anchored multiple C2 domains and transmembrane domain proteins (MCTP)	Increased cell-to-cell connectivity independent of callose deposition found in *mctp3 mctp4* double and *mctp3 mctp4 mctp6* triple mutants. *MCTP3*, *4*, and *6* form a tether-complex with plasma membrane-localized phosphatidylinositol 4-phosphate (PI4P) lipid as a binding partner regulating ER-PM distance at PD	Pérez-Sancho *et al.* (2024)
***MULTIPLE C2 DOMAINS AND TRANSMEMBRANE DOMAIN PROTEIN 4* (*MCTP4*)**	*Arabidopsis thaliana*			
***MULTIPLE C2 DOMAINS AND TRANSMEMBRANE DOMAIN PROTEIN 6* (*MCTP6*)**	*Arabidopsis thaliana*			

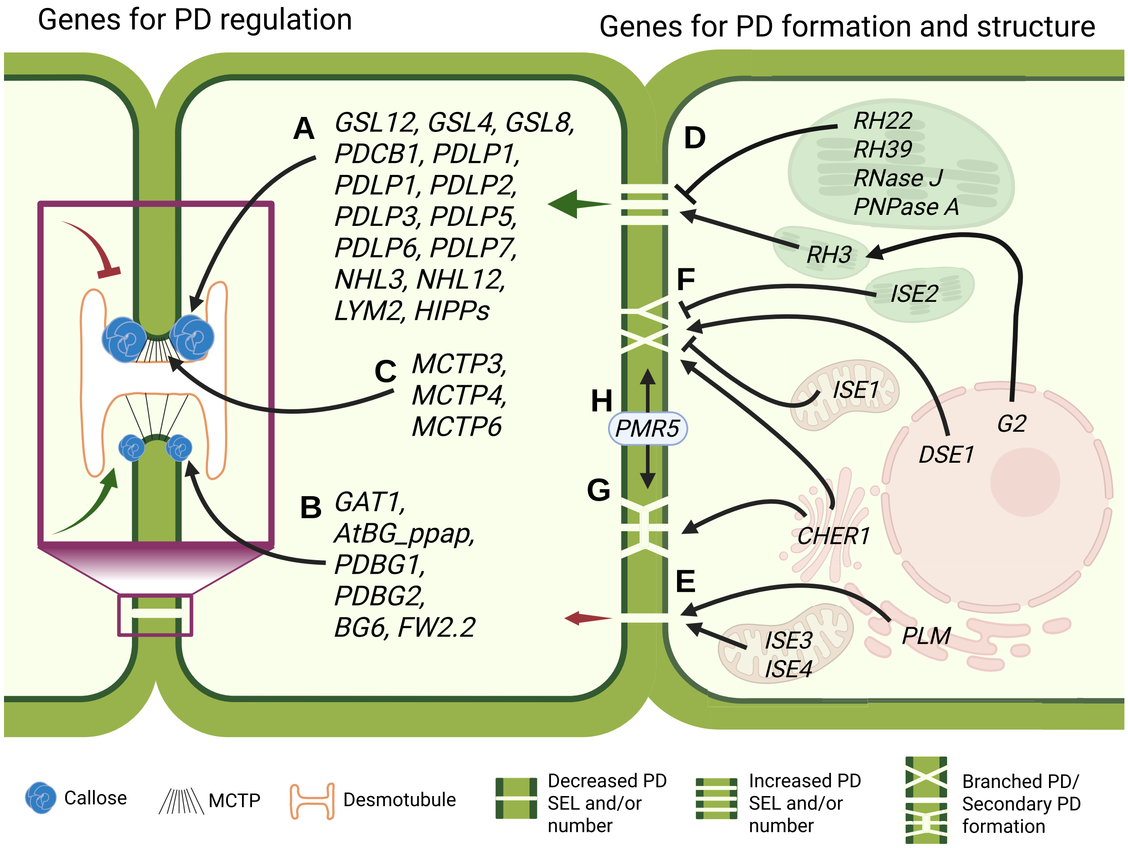

Fig. 3. Potential genetic targets to improve cell-to-cell connectivity in crops. Engineering C_3 crops to incorporate C_4-like cell-to-cell connectivity at the mesophyll-bundle sheath cell interface could lead to improved photosynthesis particularly when combined with tissue-specific overexpression of C_4 metabolism genes such as that of the C_4 Rice Project (Ermakova *et al.*, 2021). Genes involved in callose-dependent (A deposition, B degradation) or callose-independent (C) regulation of plasmodesmatal (PD)-mediated transport, as well as those that increase (D) or decrease (E) size exclusion limit (SEL) or influence PD branching (F), secondary PD formation (G), and cell wall integrity (H) can be utilized to achieve this goal. Details of the genes and corresponding functions can be found in Table 1 including genes for MULTIPLE C2 DOMAINS AND TRANSMEMBRANE DOMAIN PROTEIN (MCTP). Created in BioRender. Danila, F. (2025) https://BioRender.com/u84y825.

neck, which directly restricts the symplastic flow of metabolites (Wu *et al.*, 2018). This is particularly critical at the M–BS cell interface in C_4 plants, where the efficient flux of C_4 acids and photosynthetic intermediates is essential for the CO_2-concentrating mechanism. The amount of callose is determined by the balance between callose synthases (CalS) and β-1,3-glucanases (BGs); a deficiency in either activity alters callose accumulation and consequently, metabolite transport (Radford *et al.*, 1998; Simpson *et al.*, 2009; Guseman *et al.*, 2010). This dynamic regulation of PD permeability via callose is a key cellular response to abiotic and biotic stresses (Zavaliev *et al.*, 2011).

In Arabidopsis, specific CalS isoforms—including CalS3/GSL12, CalS8/GSL4, and CalS10/GSL8—synthesize callose at the PD neck (Fig. 3, Table 1). Mutations in these genes alter callose deposition and symplastic connectivity (Guseman *et al.*, 2010; Vatén *et al.*, 2011; Han *et al.*, 2014; Cui and Lee, 2016). The most widely used tool for manipulating transport is the gain-of-function *icals3m* construct, which, upon estradiol induction, drastically increases callose to limit or abolish symplastic flow (Vatén *et al.*, 2011). This tool could be applied to the C_4 leaf context to experimentally restrict M–BS metabolite exchange and directly quantify its impact on photosynthetic efficiency. It has already proven effective in demonstrating the mobility of transcription factors such as SHORTROOT and SCARECROW, and other mobile signals like auxin between tissue types (Vatén *et al.*, 2011; Wu *et al.*, 2014; Liu *et al.*, 2017; Yu *et al.*, 2017; Paterlini *et al.*, 2021).

The larger family of BGs also contains cell-type specific isoforms that regulate PD callose (Fig. 3, Table 1; Levy *et al.*, 2007; Benitez-Alfonso *et al.*, 2013; Grison *et al.*, 2015; O'Lexy *et al.*, 2018; Perrot *et al.*, 2022). Significantly, comparative transcriptomics of closely related C_3 and C_4 species revealed a notable up-regulation of several BGs in C_4 leaves, including one that is PD-associated (Bräutigam *et al.*, 2011). This suggests an evolutionary adaptation for enhanced symplastic trafficking to support the unique photosynthetic demands of the C_4 pathway. Other BGs play critical roles in development and stress responses (e.g. Benitez-Alfonso *et al.*, 2013; Gaudioso-Pedraza *et al.*, 2018; O'Lexy *et al.*, 2018), making them promising candidates for tissue-specific manipulation of solute flux.

Other PD-localized proteins also influence PD function through callose deposition. These include PLASMODESMATA CALLOSE-BINDING PROTEIN 1 (PDCB1; Simpson *et al.*, 2009; Grison *et al.*, 2015), PLASMODESMATA-LOCATED PROTEINs (PDLPs; Thomas *et al.*, 2008; Lee *et al.*, 2011; Caillaud *et al.*, 2014; Liu *et al.*, 2020; Huang *et al.*, 2023; Chen *et al.*, 2024; Z. Li *et al.*, 2024), various pathogen-response proteins such as NON-RACE SPECIFIC DISEASE RESISTANCE/HAIRPIN-INDUCED-LIKE 3 (NHL3; Tee *et al.*, 2023), NHL12 (Ayyoub *et al.*, 2024), LYSIN MOTIF DOMAIN-CONTAINING GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED PROTEIN 2 (LYM2; Faulkner *et al.*, 2013), and heavy metal-associated isoprenylated plant proteins (HIPPs; Guo *et al.*, 2021; Barr *et al.*, 2023) (Table 1, Fig. 3). However, since many of these proteins have pleiotropic functions, manipulating them could have unintended effects beyond PD permeability. Therefore, targeting CalS and BG enzymes remains the most direct and specific strategy for controlling callose levels and, by extension, the cell-to-cell connectivity that underpins photosynthetic collaboration in complex tissues.

PD function can also be manipulated in a callose-independent manner. For instance, members of the MULTIPLE C2 DOMAINS AND TRANSMEMBRANE REGION PROTEIN (MCTP) family alter cell-to-cell connectivity without affecting callose deposition or PD frequency, instead regulating ER-plasma membrane distance at PD via interactions with lipids (Fig. 3; Pérez-Sancho *et al.*, 2024; Dickmanns *et al.*, 2025). This represents an exciting new avenue for engineering symplastic domains, potentially fine-tuning the metabolite exchange between M and BS cells without triggering stress-related callose responses.

Spatiotemporal drivers to target mesophyll-bundle sheath cell interface

To precisely engineer PD formation at the M–BS interface, reliable cell-type specific promoters are essential for spatial and temporal control of transgene expression. We propose that organelle development in C_4 BS cells is a primary driver of increased PD frequency at this interface (Tsang *et al.*, 2024; Aleksejeva and Schreier, 2025); consequently, BS-specific promoters are a logical starting point. Several C_4-derived promoters, such as *Flaveria trinervia GLYCINE DECARBOXYLASE P-SUBUNIT* (*FtGLDP*) and *Zoysia japonica PCK* (*ZjPCK*), have been used to drive transgene expression specifically in the BS cells of C_3 species (Nomura *et al.*, 2005; Engelmann *et al.*, 2008; Ermakova *et al.*, 2021; Danila *et al.*, 2022). However, their activity is often weak and occurs late in leaf development (Nomura *et al.*, 2005; Lee *et al.*, 2021). The recent identification of the *SULFITE REDUCTASE* (*SIR*) promoter and its synthetic enhancer offers a promising solution, enabling strong, early transgene expression specifically in BS cells (Hua *et al.*, 2025).

Crucially, the choice of promoter must be informed by the developmental timing of PD formation relative to BS cell differentiation. While BS-specific promoters are suitable for stimulating secondary PD formation in existing walls or regulating mature PD, manipulating primary PD requires early intervention, as these structures likely form before BS cell fate is fully established (Gisel *et al.*, 1999). The differentiation of M and BS cells is linked to vein development, with BS specification following procambial initial formation (Langdale *et al.*, 1989; Perico *et al.*, 2022)—a process that can vary between C_3 and C_4 species (Sakaguchi and Fukuda, 2008). Furthermore, M–BS interface formation is spatially and temporally heterogeneous across the leaf (Nelson and Dengler, 1997). Therefore, promoters for genes involved in early vein development—such as *PINFORMED 1a* (*PIN1a*), *SHORTROOT1/2* (*SHR1/2*), and *TOO MANY LATERALS1* (*TML1*) (Perico *et al.*, 2024)—represent key candidates for achieving the precise early expression needed to engineer primary PD biogenesis.

The case of the C_4 Rice Project

Pioneering efforts like the C_4 Rice Project (https://c4rice.com/) aim to harness two-celled C_4 photosynthesis as a blueprint to revolutionize productivity, sustainability, and climate resilience in C_3 crops like rice. Successful installation is predicted to yield 30–50% increases alongside superior water use and nitrogen use efficiencies (Sheehy *et al.*, 2008). However, success hinges not only on cell-specific expression of C_4 enzymes but also on reconstructing intricate leaf anatomical features, including the enhanced cell-to-cell connectivity required to support the rapid metabolic flux of the C_4 cycle (Kajala *et al.*, 2011; Schuler *et al.*, 2016; Ermakova *et al.*, 2020).

Recent progress includes the successful expression of multiple key C_4 enzymes from maize in rice, with efforts ongoing to introduce genes conferring C_4 leaf anatomy (Ermakova *et al.*, 2020; Ermakova *et al.*, 2021). A central anatomical challenge is the functionalization of the BS cell, which involves increasing the number and size of chloroplasts and mitochondria (Sage, 2004). Given the established link between chloroplast biogenesis and PD formation (Azim and Burch-Smith, 2020), enhancing BS organelle content is also expected to increase PD frequency at the M–BS interface to support metabolite shuttling. For instance, ubiquitous overexpression of the chloroplast biogenesis transcription factor *ZmG2* in rice increased chloroplast and mitochondrial numbers and enhanced PD frequency at the M–BS interface (Wang *et al.*, 2017).

However, ubiquitous enhancement of chloroplast biogenesis presents significant trade-offs. Chloroplasts require massive investments in proteins, lipids, and pigments, potentially diverting nitrogen, carbon, and ATP from other critical processes like root development, defence, or reproduction (Herms and Mattson, 1992; Wang *et al.*, 2017). Increased electron transport rates can elevate reactive oxygen species production (e.g. H_2O_2, superoxide; Foyer and Hanke, 2022), and more chloroplasts in guard cells can increase stomatal opening and water loss (Suetsugu *et al.*, 2014). These disadvantages are context-dependent but highlight that boosting photosynthesis must be balanced with a plant's overall resource economy (Woodson, 2016).

Proposed engineering approach

We argue that the greatest advances in engineering C_4 leaf anatomy—including enhanced M–BS PD frequency—will require spatially and temporally controlled modifications. This necessitates using cell-type specific promoters or ectopic expression systems, many of which remain unexplored.

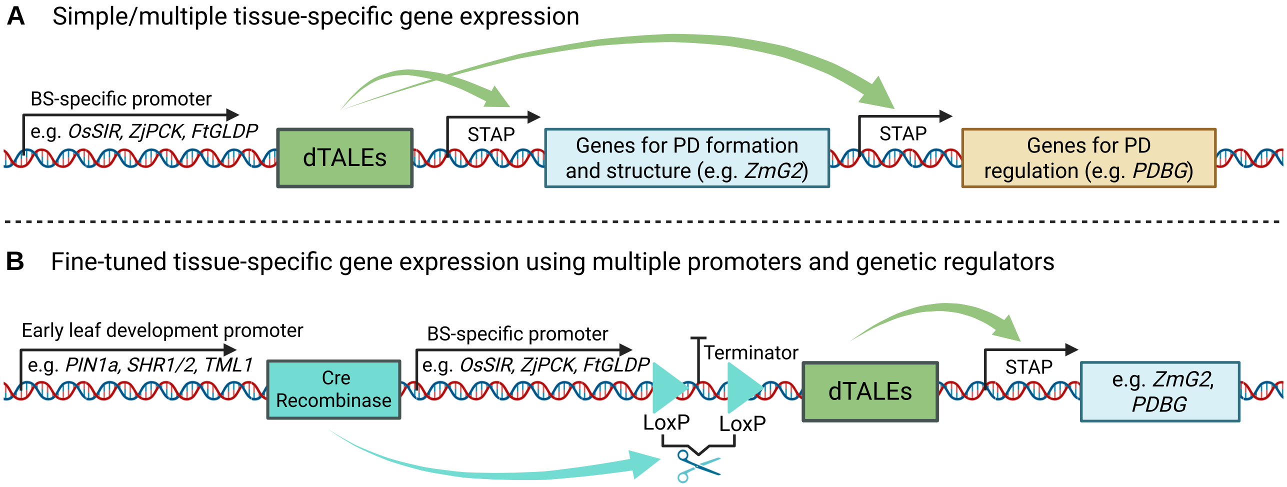

Fig. 4. Proposed engineering strategies to increase cell-to-cell connectivity at the mesophyll-bundle sheath (M–BS) cell wall interface of C_3 crops. Strong and tightly regulated expression of genes known to increase cell-to-cell connectivity through plasmodesmata (PD) formation (e.g. maize *GOLDEN-2*, *ZmG2*) and regulation (e.g. *PLASMODESMAL-ASSOCIATED β-1,3-GLUCANASE*, *PDBG*) at the M–BS cell wall interface would be required. Since BS functionalization is proposed to trigger high PD frequency at the M–BS cell wall interface in C_4 leaves, targeted expression of PD formation and regulation genes at the BS cell would be a logical approach. Known BS-specific promoters such as rice *SULFITE REDUCTASE* (*OsSIR*), *Zoysia japonica PHOSPHOENOLPYRUVATE CARBOXYKINASE* (*ZjPCK*) and *Flaveria trinervia GLYCINE DECARBOXYLASE P-SUBUNIT* (*FtGLDP*) can be used to drive the expression of one or multiple selected PD genes. (A) Designer transcription activator-like effectors (dTALEs) can be used to initiate the expression of one or multiple PD genes through its cognate synthetic TALE-activated promoters (STAPs) for a tuneable gene expression system. (B) Expression in the BS cells can be further restricted to early leaf development using promoters for genes such as *PINFORMED 1a* (*PIN1a*), *SHORTROOT1/2* (*SHR1/2*), and *TOO MANY LATERALS1* (*TML1*). Presence of these early leaf development promoters would lead to irreversible activation of BS-specific promoter by recombinases (e.g. Cre-LoxP) cleaving a terminator signal upstream of dTALEs. Created in BioRender. Danila, F. (2025) https://BioRender.com/ljoyyd3.

Effective engineering may require combining genes to increase both PD frequency and aperture (e.g. *ZmG2* and *PLASMODESMAL-ASSOCIATED β-1,3-GLUCANASE* (*PDBG*), Fig. 4; Bräutigam *et al.*, 2011; Wang *et al.*, 2017), and fine-tuning expression across different developmental stages (Langdale *et al.*, 1989).

Fortunately, recent synthetic biology tools now enable this precision. Designer transcription activator-like effectors (dTALEs) and cognate synthetic TALE-activated promoters (STAPs) allow for tuneable, cell-specific expression of multiple genes from a single promoter (Fig. 4A; Danila *et al.*, 2022). Recombinase-based circuits (e.g. Cre-Lox) offer reversible control in response to environmental cues, while CRISPR-dCas9 and RNA-based systems can create stable, heritable changes in gene expression ideal for early developmental programming (Fig. 4B; Santos-Moreno and Schaerli, 2020; Lloyd *et al.*, 2022; Khan and Lister, 2025). Proof-of-concept studies can be done in rice as these tools can be stacked with existing C_4 metabolic constructs to generate lines with functionalized BS cells (Ermakova *et al.*, 2021).

The combination of C_4 metabolism and enhanced leaf anatomy is expected to yield rice with higher productivity and resource-use efficiency (Sheehy *et al.*, 2008; von Caemmerer *et al.*, 2012; Long *et al.*, 2015). Furthermore, integrating callose-dependent PD regulation could impart resilience to pests and diseases. For example, the rice blast fungus *Magnaporthe oryzae* utilizes PD for cell-to-cell movement (Kankanala *et al.*, 2007). Incorporating a pathogen-inducible callose synthase (e.g. *icals3m*; Vatén *et al.*, 2011) into a dTALE-STAP construct (Fig. 4) could restrict symplastic movement and enhanced blast resistance, adding a valuable layer of climate resilience to C_4 rice.

Conclusions and future perspectives

The importance of PD in plant biology spans development, signalling, and defence, offering a novel opportunity for crop improvement. In this review, we summarized and discussed the critical role PD play in C_4 photosynthesis and highlighted how increased PD frequency at photosynthetic cell interfaces, particularly the M–BS cell interface, is a key anatomical feature that must be included in the design strategies for engineering two-celled C_4 photosynthesis into C_3 crops to enhance yield. We presented a list of potential gene targets and proposed strategies for engineering cell-to-cell connectivity in crops. However, while we have come a long way to better understand the complexity of PD, further research is still required on the fundamentals of PD formation and their regulation before cell-to-cell connectivity can be engineered in a fully predictable and cell-type specific manner. Excitingly, our ongoing efforts to develop new techniques, tools, and resources in tandem with advances in modern biotechnology approaches, such as synthetic promoters, CRISPR, and prime editing, now allow discovery-driven research not only in model plant species, but also directly in relevant crop species. We are hopeful that these efforts will accelerate our understanding of fundamental PD biology and our ability to translate these findings into tangible, agriculturally important crop improvements.

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Author contribution

All authors contributed to the writing of this review.

Conflict of interest

The authors declare no conflict of interest.

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References

Aleksejeva K, Schreier TB. 2025. Stepwise increase in plasmodesmata during C_4 evolution in *Flaveria*. bioRxiv doi: 10.1101/2025.04.18.649591. [Preprint].

Alonso-Cantabrana H, Cousins AB, Danila F, Ryan T, Sharwood RE, von Caemmerer S, Furbank RT. 2018. Diffusion of CO_2 across the mesophyll-bundle sheath cell interface in a C_4 plant with genetically reduced PEP carboxylase activity. Plant Physiology **178**, 72–81.

Ayyoub A, Yu X, Zhang X, Gao C, Li J, Yin S, Chen S, Liesche J. 2024. Drought-dependent regulation of cell coupling in Arabidopsis leaf epidermis requires plasmodesmata protein NHL12. Journal of Experimental Botany **75**, 7019–7030.

Azim MF, Burch-Smith TM. 2020. Organelles-nucleus-plasmodesmata signaling (ONPS): an update on its roles in plant physiology, metabolism and stress responses. Current Opinion in Plant Biology **58**, 48–59.

Badger MR, Andrews TJ. 1974. Effects of CO_2, O_2 and temperature on a high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. Biochemical and Biophysical Research Communications **60**, 204–210.

Barr ZK, Werner T, Tilsner J. 2023. Heavy metal-associated isoprenylated plant proteins (HIPPs) at plasmodesmata: exploring the link between localization and function. Plants **12**, 3015.

Beauchet A, Bollier N, Grison M, Rofidal V, Gévaudant F, Bayer E, Gonzalez N, Chevalier C. 2024. The CELL NUMBER REGULATOR FW2.2 protein regulates cell-to-cell communication in tomato by modulating callose deposition at plasmodesmata. Plant Physiology **196**, 883–901.

Benitez-Alfonso Y, Cilia M, Roman AS, Thomas C, Maule A, Hearn S, Jackson D. 2009. Control of Arabidopsis meristem development by thioredoxin-dependent regulation of intercellular transport. Proceedings of the National Academy of Sciences, USA **106**, 3615–3620.

Benitez-Alfonso Y, Faulkner C, Pendle A, Miyashima S, Helariutta Y, Maule A. 2013. Symplastic intercellular connectivity regulates lateral root patterning. Developmental Cell **26**, 136–147.

Bowes G, Ogren WL, Hageman RH. 1971. Phosphoglycolate production catalyzed by ribulose diphosphate carboxylase. Biochemical and Biophysical Research Communications **45**, 716–722.

Bräutigam A, Kajala K, Wullenweber J, *et al.* 2011. An mRNA blueprint for C_4 photosynthesis derived from comparative transcriptomics of closely related C_3 and C_4 species. Plant Physiology **155**, 142–156.

Bren d'Amour C, Reitsma F, Baiocchi G, Barthel S, Güneralp B, Erb K-H, Haberl H, Creutzig F, Seto KC. 2017. Future urban land expansion and implications for global croplands. Proceedings of the National Academy of Sciences, USA **114**, 8939–8944.

Brunkard JO, Xu M, Scarpin MR, Chatterjee S, Shemyakina EA, Goodman HM, Zambryski P. 2020. TOR dynamically regulates plant cell–cell transport. Proceedings of the National Academy of Sciences, USA **117**, 5049–5058.

Burch-Smith TM, Zambryski PC. 2010. Loss of INCREASED SIZE EXCLUSION LIMIT (ISE)1 or ISE2 increases the formation of secondary plasmodesmata. Current Biology **20**, 989–993.

Burch-Smith TM, Brunkard JO, Choi YG, Zambryski PC. 2011. Organelle–nucleus cross-talk regulates plant intercellular communication via plasmodesmata. Proceedings of the National Academy of Sciences, USA **108**, E1451–E1460.

Caillaud MC, Wirthmueller L, Sklenar J, Findlay K, Piquerez SJ, Jones AM, Robatzek S, Jones JD, Faulkner C. 2014. The plasmodesmal protein PDLP1 localises to haustoria-associated membranes during downy mildew infection and regulates callose deposition. PLoS Pathology **10**, e1004496.

Chen X, Li W-W, Gao J, Wu Z, Du J, Zhang X, Zhu Y-X. 2024. Arabidopsis PDLP7 modulated plasmodesmata function is related to BG10-dependent glucosidase activity required for callose degradation. Science Bulletin **69**, 3075–3088.

Chiniquy D, Underwood W, Corwin J, *et al.* 2019. PMR5, an acetylation protein at the intersection of pectin biosynthesis and defense against fungal pathogens. The Plant Journal **100**, 1022–1035.

Croce R, Carmo-Silva E, Cho YB, *et al.* 2024. Perspectives on improving photosynthesis to increase crop yield. The Plant Cell **36**, 3944–3973.

Cui W, Lee J-Y. 2016. Arabidopsis callose synthases CalS1/8 regulate plasmodesmal permeability during stress. Nature Plants **2**, 16034.

Danila F, Schreiber T, Ermakova M, *et al.* 2022. A single promoter-TALE system for tissue-specific and tuneable expression of multiple genes in rice. Plant Biotechnology Journal **20**, 1786–1806.

Danila FR, Quick WP, White RG, Furbank RT, von Caemmerer S. 2016. The metabolite pathway between bundle sheath and mesophyll: quantification of plasmodesmata in leaves of C_3 and C_4 monocots. The Plant Cell **28**, 1461–1471.

Danila FR, Quick WP, White RG, Kelly S, von Caemmerer S, Furbank RT. 2018. Multiple mechanisms for enhanced plasmodesmata density in disparate subtypes of C_4 grasses. Journal of Experimental Botany **69**, 1135–1145.

Danila FR, Quick WP, White RG, von Caemmerer S, Furbank RT. 2019. Response of plasmodesmata formation in leaves of C_4 grasses to growth irradiance. Plant, Cell, and Environment **42**, 2482–2494.

Danila FR, Thakur V, Chatterjee J, Bala S, Coe RA, Acebron K, Furbank RT, von Caemmerer S, Quick WP. 2021. Bundle sheath suberisation is required for C_4 photosynthesis in a *Setaria viridis* mutant. Communications Biology **4**, 254.

Dengler NG, Dengler RE, Donnelly PM, Hattersley PW. 1994. Quantitative leaf anatomy of C_3 and C_4 grasses (Poaceae): bundle sheath and mesophyll surface area relationships. Annals of Botany **73**, 241–255.

Dickmanns M, Pöge M, Xu P, Gombos S, Miras M, Plitzko JM, Simon R, Schulze WX, Frommer WB, Baumeister W. 2025. *In situ* architecture of plasmodesmata suggests mechanisms controlling intercellular exchange. bioRxiv doi: 10.1101/2025.07.24.666190. [Preprint].

Ehlers K, Kollmann R. 2001. Primary and secondary plasmodesmata: structure, origin, and functioning. Protoplasma **216**, 1–30.

Engelmann S, Wiludda C, Burscheidt J, Gowik U, Schlue U, Koczor M, Streubel M, Cossu R, Bauwe H, Westhoff P. 2008. The gene for the P-subunit of glycine decarboxylase from the C_4 species *Flaveria trinervia*: analysis of transcriptional control in transgenic *Flaveria bidentis* (C_4) and Arabidopsis (C_3). Plant Physiology **146**, 1773–1785.

Ermakova M, Danila FR, Furbank RT, von Caemmerer S. 2020. On the road to C_4 rice: advances and perspectives. The Plant Journal **101**, 940–950.

Ermakova M, Arrivault S, Giuliani R, *et al.* 2021. Installation of C_4 photosynthetic pathway enzymes in rice using a single construct. Plant Biotechnology Journal **19**, 575–588.

Evert RF, Eschrich W, Heyser W. 1977. Distribution and structure of the plasmodesmata in mesophyll and bundle-sheath cells of *Zea mays* L. Planta **136**, 77–89.

Evert RF, Eschrich W, Heyser W. 1978. Leaf structure in relation to solute transport and phloem loading in *Zea mays* L. Planta **138**, 279–294.

Evert RF, Russin WA, Bosabalidis AM. 1996. Anatomical and ultrastructural changes associated with sink-to-source transition in developing maize leaves. International Journal of Plant Sciences **157**, 247–261.

Fan Y, Scafaro AP, Asao S, *et al.* 2022. Dark respiration rates are not determined by differences in mitochondrial capacity, abundance and ultrastructure in C_4 leaves. Plant, Cell & Environment **45**, 1257–1269.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta **149**, 78–90.

Faulkner C, Akman OE, Bell K, Jeffree C, Oparka K. 2008. Peeking into pit fields: a multiple twinning model of secondary plasmodesmata formation in tobacco. The Plant Cell **20**, 1504–1518.

Faulkner C, Petutschnig E, Benitez-Alfonso Y, Beck M, Robatzek S, Lipka V, Maule AJ. 2013. LYM2-dependent chitin perception limits molecular flux via plasmodesmata. Proceedings of the National Academy of Sciences, USA **110**, 9166–9170.

Foyer CH, Hanke G. 2022. ROS production and signalling in chloroplasts: cornerstones and evolving concepts. The Plant Journal **111**, 642–661.

Francesconi S, Balestra GM. 2020. The modulation of stomatal conductance and photosynthetic parameters is involved in Fusarium head blight resistance in wheat. PLoS One **15**, e0235482.

Furbank RT. 2011. Evolution of the C_4 photosynthetic mechanism: are there really three C_4 acid decarboxylation types? Journal of Experimental Botany **62**, 3103–3108.

Ganusova EE, Reagan BC, Fernandez JC, Azim MF, Sankoh AF, Freeman KM, McCray TN, Patterson K, Kim C, Burch-Smith TM. 2020. Chloroplast-to-nucleus retrograde signalling controls intercellular trafficking via plasmodesmata formation. Philosophical Transactions of the Royal Society of London Series B Biological Sciences **375**, 20190408.

Gao P, Wang P, Du B, Li P, Kang B-H. 2022. Accelerated remodeling of the mesophyll-bundle sheath interface in the maize C_4 cycle mutant leaves. Scientific Reports **12**, 5057.

Gaudioso-Pedraza R, Beck M, Frances L, Kirk P, Ripodas C, Niebel A, Oldroyd GED, Benitez-Alfonso Y, de Carvalho-Niebel F. 2018. Callose-regulated symplastic communication coordinates symbiotic root nodule development. Current Biology **28**, 3562–3577.e6.

Gerber JS, Ray DK, Makowski D, *et al.* 2024. Global spatially explicit yield gap time trends reveal regions at risk of future crop yield stagnation. Nature Food **5**, 125–135.

Ghannoum O, von Caemmerer S, Conroy JP. 2001. Plant water use efficiency of 17 Australian NAD-ME and NADP-ME C_4 grasses at ambient and elevated CO_2 partial pressure. Functional Plant Biology **28**, 1207–1217.

Gisel A, Barella S, Hempel FD, Zambryski PC. 1999. Temporal and spatial regulation of symplastic trafficking during development in *Arabidopsis thaliana* apices. Development **126**, 1879–1889.

Goodhand EM. 2019. Plasmodesmatal density and cell wall architecture across the C_3 to C_4 evolutionary gradient in Flaveria. Master's Thesis, University of Toronto.

Grison MS, Brocard L, Fouillen L, *et al.* 2015. Specific membrane lipid composition is important for plasmodesmata function in Arabidopsis. The Plant Cell **27**, 1228–1250.

Guo T, Weber H, Niemann MCE, Theisl L, Leonte G, Novák O, Werner T. 2021. *Arabidopsis* HIPP proteins regulate endoplasmic reticulum-associated degradation of CKX proteins and cytokinin responses. Molecular Plant **14**, 1918–1934.

Guseman JM, Lee JS, Bogenschutz NL, Peterson KM, Virata RE, Xie B, Kanaoka MM, Hong Z, Torii KU. 2010. Dysregulation of cell-to-cell connectivity and stomatal patterning by loss-of-function mutation in *Arabidopsis CHORUS* (*GLUCAN SYNTHASE-LIKE 8*). Development **137**, 1731–1741.

Han X, Hyun Tae K, Zhang M, Kumar R, Koh E, Kang B-H, Lucas William J, Kim J-Y. 2014. Auxin-callose-mediated plasmodesmal gating is essential for tropic auxin gradient formation and signaling. Developmental Cell **28**, 132–146.

Hatch MD, Kagawa T, Craig S. 1975. Subdivision of C_4-pathway species based on differing C_4 acid decarboxylating systems and ultrastructural features. Journal of Functional Plant Biology **2**, 111–128.

Hatch MD, Osmond CB. 1976. Compartmentation and transport in C_4 photosynthesis. In: Stocking CR, Heber U, eds. Transport in plants III. Berlin, Heidelberg: Springer, 144–184.

Hatch MD. 1987. C_4 photosynthesis: a unique blend of modified biochemistry, anatomy and ultrastructure. Biochimica et Biophysica Acta (BBA)–Reviews on Bioenergetics **895**, 81–106.

Heino M, Kinnunen P, Anderson W, Ray DK, Puma MJ, Varis O, Siebert S, Kummu M. 2023. Increased probability of hot and dry weather extremes during the growing season threatens global crop yields. Scientific Reports **13**, 3583.

Hepler PK. 1982. Endoplasmic reticulum in the formation of the cell plate and plasmodesmata. Protoplasma **111**, 121–133.

Herms DA, Mattson WJ. 1992. The dilemma of plants: to grow or defend. The Quarterly Review of Biology **67**, 283–335.

Hibberd JM, Sheehy JE, Langdale JA. 2008. Using C_4 photosynthesis to increase the yield of rice–rationale and feasibility. Current Opinion in Plant Biology **11**, 228–231.

Hou S, Rodrigues O, Liu Z, Shan L, He P. 2024. Small holes, big impact: stomata in plant-pathogen-climate epic trifecta. Molecular Plant **17**, 26–49.

Hua L, Wang N, Stanley S, Donald R, Billakurthi K, Borba AR, Hibberd JM. 2025. A transcription factor ensemble orchestrates bundle sheath expression in rice. Nature Communications 16, 7040.

Huang C, Sede AR, Elvira-González L, Yan Y, Rodriguez ME, Mutterer J, Boutant E, Shan L, Heinlein M. 2023. dsRNA-induced immunity targets plasmodesmata and is suppressed by viral movement proteins. The Plant Cell **35**, 3845–3869.

Huxman TE, Monson RK. 2003. Stomatal responses of C_3, C_3-C_4 and C_4 *Flaveria* species to light and intercellular CO_2 concentration: implications for the evolution of stomatal behaviour. Plant, Cell & Environment **26**, 313–322.

Jay F, Brioudes F, Novaković L, Imboden A, Benitez-Alfonso Y, Voinnet O. 2025. A pectin acetyl-transferase facilitates secondary plasmodesmata formation and RNA silencing movement between plant cells. The Plant Journal **122**, e70194.

Kajala K, Covshoff S, Karki S, *et al.* 2011. Strategies for engineering a two-celled C_4 photosynthetic pathway into rice. Journal of Experimental Botany **62**, 3001–3010.

Kankanala P, Czymmek K, Valent B. 2007. Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. The Plant Cell **19**, 706–724.

Khan A, Lister R. 2025. Synthetic gene circuits in plants: recent advances and challenges. Quantitative Plant Biology **6**, e6.

Khoshravesh R, Stata M, Busch FA, *et al.* 2020. The evolutionary origin of C_4 photosynthesis in the grass subtribe Neurachninae. Plant Physiology **182**, 566–583.

Kobayashi K, Otegui MS, Krishnakumar S, Mindrinos M, Zambryski P. 2007. INCREASED SIZE EXCLUSION LIMIT2 encodes a putative DEVH box RNA helicase involved in plasmodesmata function during Arabidopsis embryogenesis. The Plant Cell **19**, 1885–1897.

Kraner ME, Link K, Melzer M, Ekici AB, Uebe S, Tarazona P, Feussner I, Hofmann J, Sonnewald U. 2017. Choline transporter-like1 (CHER1) is crucial for plasmodesmata maturation in *Arabidopsis thaliana*. The Plant Journal **89**, 394–406.

Langdale JA, Lane B, Freeling M, Nelson T. 1989. Cell lineage analysis of maize bundle sheath and mesophyll cells. Developmental Biology **133**, 128–139.

Lawson T, Leakey ADB. 2024. Stomata: custodians of leaf gaseous exchange. Journal of Experimental Botany **75**, 6677–6682.

Lee D-Y, Hua L, Khoshravesh R, Giuliani R, Kumar I, Cousins A, Sage TL, Hibberd JM, Brutnell TP. 2021. Engineering chloroplast development in rice through cell-specific control of endogenous genetic circuits. Plant Biotechnology Journal **19**, 2291–2303.

Lee J-Y, Wang X, Cui W, *et al.* 2011. A plasmodesmata-localized protein mediates crosstalk between cell-to-cell communication and innate immunity in *Arabidopsis*. The Plant Cell **23**, 3353–3373.

Lee J-Y, Frank M. 2018. Plasmodesmata in phloem: different gateways for different cargoes. Current Opinion in Plant Biology **43**, 119–124.

Leegood RC. 2000. Transport during C_4 photosynthesis. In: Leegood RC, Sharkey TD, von Caemmerer S, eds. Photosynthesis: physiology and metabolism. Dordrecht: Springer Netherlands, 459–469.

Leung A, Patel R, Chirachon V, Stata M, Macfarlane TD, Ludwig M, Busch FA, Sage TL, Sage RF. 2024. *Tribulus* (Zygophyllaceae) as a case study for the evolution of C_2 and C_4 photosynthesis. Plant, Cell & Environment **47**, 3541–3560.

Levy A, Erlanger M, Rosenthal M, Epel BL. 2007. A plasmodesmata-associated β-1, 3-glucanase in Arabidopsis. The Plant Journal **49**, 669–682.

Li Z, Liu S-L, Montes-Serey C, Walley JW, Aung K. 2024. PLASMODESMATA-LOCATED PROTEIN 6 regulates plasmodesmal function in Arabidopsis vasculature. The Plant Cell **36**, 3543–3561.

Li ZP, Moreau H, Petit JD, *et al.* 2024. Plant plasmodesmata bridges form through ER-dependent incomplete cytokinesis. Science **386**, 538–545.

Liu N-J, Zhang T, Liu Z-H, *et al.* 2020. Phytosphinganine affects plasmodesmata permeability via facilitating PDLP5-stimulated callose accumulation in Arabidopsis. Molecular Plant **13**, 128–143.

Liu Y, Xu M, Liang N, Zheng Y, Yu Q, Wu S. 2017. Symplastic communication spatially directs local auxin biosynthesis to maintain root stem cell niche in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA **114**, 4005–4010.

Lloyd JPB, Ly F, Gong P, Pflueger J, Swain T, Pflueger C, Fourie E, Khan MA, Kidd BN, Lister R. 2022. Synthetic memory circuits for stable cell reprogramming in plants. Nature Biotechnology **40**, 1862–1872.

Long SP, Marshall-Colon A, Zhu X-G. 2015. Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell **161**, 56–66.

Lu K-J, Danila FR, Cho Y, Faulkner C. 2018. Peeking at a plant through the holes in the wall–exploring the roles of plasmodesmata. New Phytologist **218**, 1310–1314.

Nelson T, Dengler N. 1997. Leaf vascular pattern formation. The Plant Cell **9**, 1121–1135.

Nomura M, Higuchi T, Ishida Y, Ohta S, Komari T, Imaizumi N, Miyao-Tokutomi M, Matsuoka M, Tajima S. 2005. Differential expression pattern of C_4 bundle sheath expression genes in rice, a C_3 plant. Plant and Cell Physiology **46**, 754–761.

O'Lexy R, Kasai K, Clark N, Fujiwara T, Sozzani R, Gallagher KL. 2018. Exposure to heavy metal stress triggers changes in plasmodesmatal permeability via deposition and breakdown of callose. Journal of Experimental Botany **69**, 3715–3728.

Osborne CP, Sack L. 2012. Evolution of C_4 plants: a new hypothesis for an interaction of CO_2 and water relations mediated by plant hydraulics. Philosophical Transactions of the Royal Society of London Series B Biological Sciences **367**, 583–600.

Osmond C. 1971. Metabolite transport in C_4 photosynthesis. Australian Journal of Biological Sciences **24**, 159–164.

Paterlini A, Dorussen D, Fichtner F, van Rongen M, Delacruz R, Vojnović A, Helariutta Y, Leyser O. 2021. Callose accumulation in specific phloem cell types reduces axillary bud growth in *Arabidopsis thaliana*. New Phytologist **231**, 516–523.

Pérez-Sancho J, Smokvarska M, Dubois G, *et al.* 2024. Plasmodesmata act as unconventional membrane contact sites regulating intercellular molecular exchange in plants. bioRxiv doi: 10.1101/2023.12.18.572149. [Preprint].

Perico C, Tan S, Langdale JA. 2022. Developmental regulation of leaf venation patterns: monocot versus eudicots and the role of auxin. New Phytologist **234**, 783–803.

Perico C, Zaidem M, Sedelnikova O, Bhattacharya S, Korfhage C, Langdale JA. 2024. Multiplexed *in situ* hybridization reveals distinct lineage identities for major and minor vein initiation during maize leaf development. Proceedings of the National Academy of Sciences, USA **121**, e2402514121.

Perrot T, Pauly M, Ramírez V. 2022. Emerging roles of β-glucanases in plant development and adaptative responses. Plants **11**, 1119.

Radford JE, Vesk M, Overall RL. 1998. Callose deposition at plasmodesmata. Protoplasma **201**, 30–37.

Ray DK, Gerber JS, MacDonald GK, West PC. 2015. Climate variation explains a third of global crop yield variability. Nature Communications **6**, 5989.

Ray DK, West PC, Clark M, Gerber JS, Prishchepov AV, Chatterjee S. 2019. Climate change has likely already affected global food production. PLoS One **14**, e0217148.

Robinson-Beers K, Evert R. 1991. Fine structure of plasmodesmata in mature leaves of sugarcane. Planta **184**, 307–318.

Russin WA, Evert RF, Vanderveer PJ, Sharkey TD, Briggs SP. 1996. Modification of a specific class of plasmodesmata and loss of sucrose export ability in the *sucrose export defective1* maize mutant. The Plant Cell **8**, 645–658.

Sage RF. 2004. The evolution of C_4 photosynthesis. New Phytologist **161**, 341–370.

Sage RF, Sage TL, Kocacinar F. 2012. Photorespiration and the evolution of C_4 photosynthesis. Annual Review of Plant Biology **63**, 19–47.

Sage RF, Monson RK, Ehleringer JR, Adachi S, Pearcy RW. 2018. Some like it hot: the physiological ecology of C_4 plant evolution. Oecologia **187**, 941–966.

Sager R, Lee J-Y. 2014. Plasmodesmata in integrated cell signalling: insights from development and environmental signals and stresses. Journal of Experimental Botany **65**, 6337–6358.

Sakaguchi J, Fukuda H. 2008. Cell differentiation in the longitudinal veins and formation of commissural veins in rice (*Oryza sativa*) and maize (*Zea mays*). Journal of Plant Research **121**, 593–602.

Santos-Moreno J, Schaerli Y. 2020. CRISPR-based gene expression control for synthetic gene circuits. Biochemical Society Transactions **48**, 1979–1993.

Schreier TB, Müller KH, Eicke S, Faulkner C, Zeeman SC, Hibberd JM. 2024. Plasmodesmal connectivity in C_4 *Gynandropsis gynandra* is induced by light and dependent on photosynthesis. New Phytologist **241**, 298–313.

Schuler ML, Mantegazza O, Weber APM. 2016. Engineering C_4 photosynthesis into C_3 chassis in the synthetic biology age. The Plant Journal **87**, 51–65.

Sheehy JE, Mitchell PL, Hardy B, eds. 2007. Charting new pathways to C_4 rice. Los Banos, Philippines: International Rice Research Institute.

Simpson C, Thomas C, Findlay K, Bayer E, Maule AJ. 2009. An Arabidopsis GPI-anchor plasmodesmal neck protein with callose binding activity and potential to regulate cell-to-cell trafficking. The Plant Cell **21**, 581–594.

Sowiński P, Rudzińska-Langwald A, Kobus P. 2003. Changes in plasmodesmata frequency in vascular bundles of maize seedling leaf induced by growth at sub-optimal temperatures in relation to photosynthesis and assimilate export. Environmental and Experimental Botany **50**, 183–196.

Sowiński P, Bilska A, Barańska K, Fronk J, Kobus P. 2007. Plasmodesmata density in vascular bundles in leaves of C_4 grasses grown at different light conditions in respect to photosynthesis and photosynthate export efficiency. Environmental and Experimental Botany **61**, 74–84.

Stitt M, Heldt HW. 1985. Generation and maintenance of concentration gradients between the mesophyll and bundle sheath in maize leaves. Biochimica et Biophysica Acta (BBA)–Bioenergetics **808**, 400–414.

Stonebloom S, Burch-Smith T, Kim I, Meinke D, Mindrinos M, Zambryski P. 2009. Loss of the plant DEAD-box protein ISE1 leads to defective mitochondria and increased cell-to-cell transport via plasmodesmata. Proceedings of the National Academy of Sciences, USA **106**, 17229–17234.

Suetsugu N, Takami T, Ebisu Y, Watanabe H, Iiboshi C, Doi M, Shimazaki K-I. 2014. Guard cell chloroplasts are essential for blue light-dependent stomatal opening in Arabidopsis. PLoS One **9**, e108374.

Tee EE, Johnston MG, Papp D, Faulkner C. 2023. A PDLP-NHL3 complex integrates plasmodesmal immune signaling cascades. Proceedings of the National Academy of Sciences, USA **120**, e2216397120.

Tee EE, Faulkner C. 2024. Plasmodesmata and intercellular molecular traffic control. New Phytologist **243**, 32–47.

Thomas CL, Bayer EM, Ritzenthaler C, Fernandez-Calvino L, Maule AJ. 2008. Specific targeting of a plasmodesmal protein affecting cell-to-cell communication. PLoS Biology **6**, e7.

Tsang HT, Ganguly DR, Furbank RT, von Caemmerer S, Danila FR. 2024. Novel resources to investigate leaf plasmodesmata formation in C_3 and C_4 monocots. The Plant Journal **120**, 2207–2225.

Turkan I, Uzilday B, Dietz K-J, Bräutigam A, Ozgur R. 2018. Reactive oxygen species and redox regulation in mesophyll and bundle sheath cells of C_4 plants. Journal of Experimental Botany **69**, 3321–3331.

Vatén A, Dettmer J, Wu S, *et al.* 2011. Callose biosynthesis regulates symplastic trafficking during root development. Developmental Cell **21**, 1144–1155.

von Caemmerer S, Furbank R. 2003. The C_4 pathway: an efficient CO_2 pump. Photosynthesis Research **77**, 191–207.

von Caemmerer S, Quick WP, Furbank RT. 2012. The development of C_4 rice: current progress and future challenges. Science **336**, 1671–1672.

Walker BJ, VanLoocke A, Bernacchi CJ, Ort DR. 2016. The costs of photorespiration to food production now and in the future. Annual Review of Plant Biology **67**, 107–129.

Wang P, Khoshravesh R, Karki S, Tapia R, Balahadia CP, Bandyopadhyay A, Quick WP, Furbank RT, Sage TL, Langdale JA. 2017. Re-creation of a key step in the evolutionary switch from C_3 to C_4 leaf anatomy. Current Biology **27**, 3278–3287.e6.

Woodson JD. 2016. Chloroplast quality control–balancing energy production and stress. New Phytologist **212**, 36–41.

Wu J, Liu Y. 2022. Stomata–pathogen interactions: over a century of research. Trends in Plant Science **27**, 964–967.

Wu S, Lee C-M, Hayashi T, Price S, Divol F, Henry S, Pauluzzi G, Perin C, Gallagher KL. 2014. A plausible mechanism, based upon SHORT-ROOT movement, for regulating the number of cortex cell layers in roots. Proceedings of the National Academy of Sciences, USA **111**, 16184–16189.

Wu S-W, Kumar R, Iswanto ABB, Kim J-Y. 2018. Callose balancing at plasmodesmata. Journal of Experimental Botany **69**, 5325–5339.

Xu M, Cho E, Burch-Smith TM, Zambryski PC. 2012. Plasmodesmata formation and cell-to-cell transport are reduced in *decreased size exclusion limit 1* during embryogenesis in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA **109**, 5098–5103.

Yan D, Yadav SR, Paterlini A, *et al.* 2019. Sphingolipid biosynthesis modulates plasmodesmal ultrastructure and phloem unloading. Nature Plants **5**, 604–615.

Yu Q, Li P, Liang N, Wang H, Xu M, Wu S. 2017. Cell-fate specification in *Arabidopsis* roots requires coordinative action of lineage instruction and positional reprogramming. Plant Physiology **175**, 816–827.

Yuan X, Li S, Chen J, Yu H, Yang T, Wang C, Huang S, Chen H, Ao X. 2024. Impacts of global climate change on agricultural production: a comprehensive review. Agronomy **14**, 1360.

Zanini AA, Burch-Smith TM. 2024. New insights into plasmodesmata: complex 'protoplasmic connecting threads'. Journal of Experimental Botany **75**, 5557–5567.

Zavaliev R, Ueki S, Epel BL, Citovsky V. 2011. Biology of callose (β-1,3-glucan) turnover at plasmodesmata. Protoplasma **248**, 117–130.