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PHOSPHATIDYLETHANOLAMINE-BINDING PROTEINS: the conductors of dual reproduction in plants with vegetative storage organs

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Abstract

Geophytes, the plants that form vegetative storage organs, are characterized by a dual reproduction system, in which vegetative and sexual propagation are tightly regulated to ensure fitness in harsh climatic conditions. Recent findings highlight the role of the *PEBP* (*PHOSPHATIDYLETHANOLAMINE-BINDING PROTEIN*) gene family in geophytes as major players in the molecular cascades underlying both types of reproduction. In this review, we briefly explain the life cycle and reproduction strategies of different geophytes and what is known about the physiological aspects related to these processes. Subsequently, an in-depth overview is provided of the molecular and genetic pathways driving these processes. In the evolution of plants, the *PEBP* gene family has expanded, followed by neo- and subfunctionalization. Careful characterization revealed that differential expression and differential protein complex formation provide the members of this gene family with unique functions, enabling them to mediate the crosstalk between the two reproductive events in geophytes in response to environmental and endogenous cues. Taking all these studies into account, we propose to regard the PEBPs as conductors of geophyte reproductive development.

Keywords: Florigen, flowering, geophyte, PEBP, reproduction, vegetative storage organ.

Introduction

Successful reproduction is key to plant species survival and evolution, but how can sessile organisms accomplish these tasks under continuously fluctuating or extreme climate conditions?

The evolution of an ingenious sexual reproduction strategy optimally adapted to the species' environmental niche appears to be the key. However, the so-called geophytes have evolved an additional way to survive under various climate conditions and to propagate, namely via the formation of specialized

vegetative organs, which for most geophytic species are found underground.

The term 'geophyte' was introduced around 100 years ago and derives from the Greek language. It means 'earth plants' (Raunkiær, 1934), and geophytic species are so named because they produce storage organs such as bulbs, corms, tubers, and rhizomes, which generally are found below ground. These structures act as reservoirs of water and nutrients during periods of unfavourable environmental conditions, protect the dormant meristems, and as such may serve as reproduction vehicles. Geophytes can be found among monocot and dicot taxa and include major ornamentals, such as tulip and lily, and important food crops, for instance potato and onion. They were metaphorically named 'plant computers' because their long-term environment-dependent 'memory' strongly affects phase transitions to flowering and to storage organ formation in their life cycle (Le Nard and De Hertogh, 1993; Duran-Nebreda and Bassel, 2019). The unique dual reproduction strategy has long been recognized and studied at the morphological and physiological levels, but it is only recently that information about the molecular regulation of these processes became available (Kamenetsky and Okubo, 2012).

In this review, we will briefly address and summarize the life cycle and reproduction processes of geophytes and their phenology. Subsequently, the latest insights into the molecular regulation of sexual and vegetative reproduction will be described, with a focus on the most studied plant species belonging to this category: potato, onion, tulip, and garlic. We will discuss in particular the rec3ent findings that suggest a pivotal role for phosphatidylethanolamine-binding proteins (PEBPs) as integrators of various environmental signals and internal 'sink–source' forces, steering the reproduction strategy by initiating development and outgrowth of the different reproductive organs.

Life cycles and reproduction strategies of geophytes

During evolution in regions with restrictive climates, plants developed life strategies for an optimal use of the short annual windows available for growth and reproduction (Werger and Huber, 2006). Geophytes evolved their dual reproduction ability in the areas with high or low temperatures, drought, or an inadequate light level (Howard *et al.*, 2019). It has been proposed that the first geophytes evolved in tropic and subtropic zones where rainfalls became seasonal. Their distribution to new environments resulted in adaptation to xero-mesophytic and mesophytic habitats, and occurred independently in many taxonomic groups. This resulted in a large diversity of geophytic morphological structures and life cycles (Kamenetsky and Okubo, 2012).

Storage organs are reserves of water and nutrients such as carbohydrates, proteins, and mineral salts. Under unfavourable conditions, these organs remain in dormancy (rest, quiescence); however, even during these periods, meristematic activity in

buds continues and environmental conditions are constantly sensed (Kamenetsky and Okubo, 2012). In favourable seasons, fast growth is initiated and new organs emerge, consuming the nutrients available in the storage organs and the available water for rapid cell elongation. Environmental signals, including temperature, moisture, and light, affect all stages of geophyte development such as flower induction, storage organ formation, establishment and release of dormancy, and differentiation of the apical and axillary meristems (Dole, 2003; Erwin, 2006). Since flowering and seed production are not always assured in adverse habitats, vegetative propagation serves as a back-up for plant distribution and species survival, providing an effective alternative to sexual propagation.

The life cycle of geophytes, like that of all perennial plants, includes a long juvenile vegetative stage that can last several years. Only after progressing from juvenile to adult vegetative stage do geophytes become competent to respond to flowering-inducing signals (Kamenetsky and Okubo, 2012). Once geophytes reach the adult stage, their annual cycle consists of several developmental stages (Fig. 1). Currently there is very limited knowledge on the molecular processes orchestrating the juvenile to adult vegetative stage in geophytes. For this reason, we will focus here on the molecular regulation during the annual life cycle of adult plants, in which flowering and the formation of storage organs occur simultaneously or sequentially. In this yearly cycle, flowering and seed production markedly depend on the competition for nutrients with the developing storage organs. For example, in tulip, low temperatures release dormancy of the apical floral meristem inside the bulb (Fig. 1). Subsequently, stem elongation and blooming occur. Concurrently, the process of bulbing also takes place from the axillary buds, resulting in the so-called daughter bulbs that represent the next generation. Flowering is induced in these daughter bulbs by warm temperatures during early summer, followed by summer dormancy. During this summer period, the bulb will remain in the state of dormancy in the sense of 'absence of visible growth'. However, water and sugar contents in the bulb scales allow a constant supply to the developing flowers, and even severe drought will not damage this process.

Hence, understanding of the environmental and internal effects on geophyte development requires a holistic approach which includes the study of flower initiation, stem elongation, blooming, and formation of underground storage organs, together with the interactions between these processes and orchestration of complex sink–source relationships.

PEBPs allow crosstalk in the dual reproduction system of geophytes

Recent molecular studies conducted in different geophyte species revealed that storage organ formation and flower induction are closely connected and regulated by similar genetic networks, in which members of the *PEBP* gene family act as signalling integrators and hubs (Navarro *et al.*, 2011;
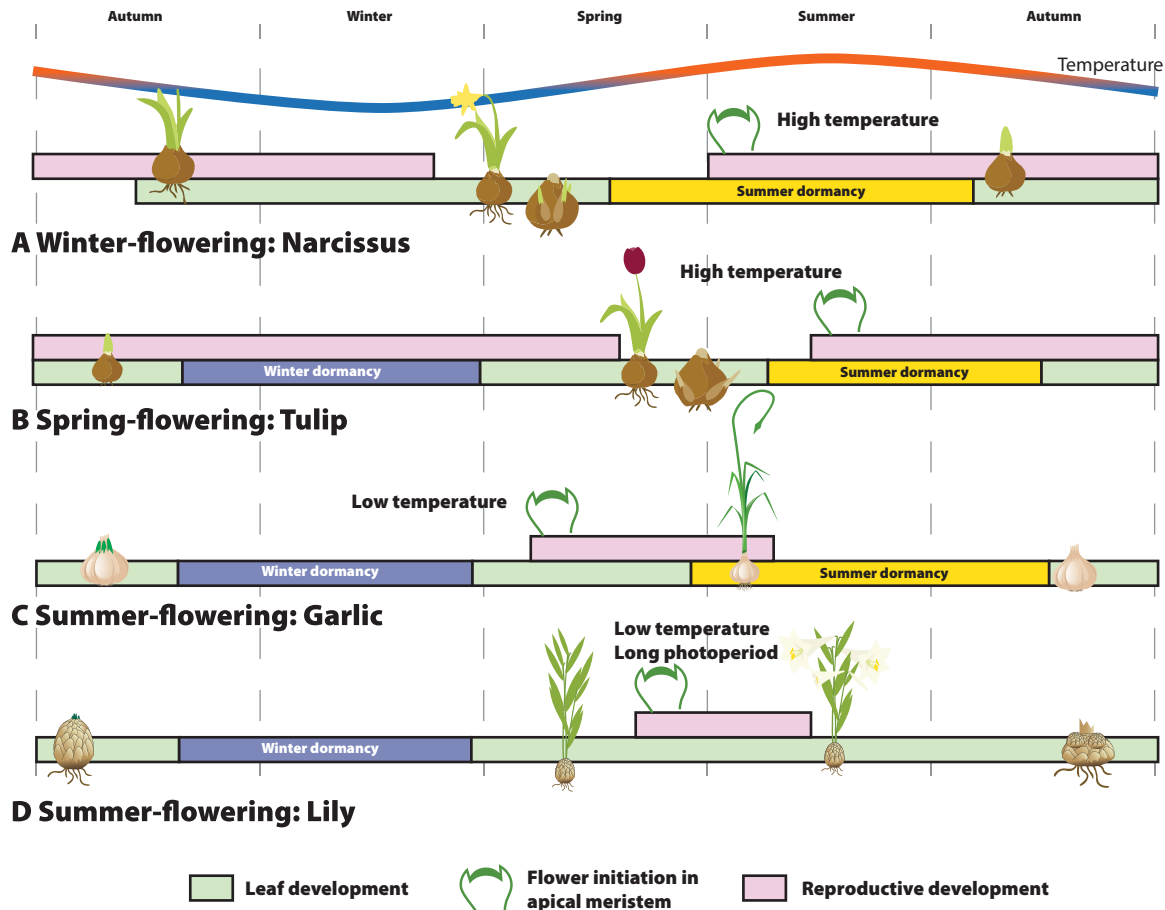
Fig. 1. Schematic representation of annual life cycles in four eco-morphological types of geophytes. For each type, a single species is presented as an example. Note that the nomenclature used is based on open flowers and not on the phase transition to the reproductive stage. (A) Winter-flowering. *Narcissus tazetta* originated from Mediterranean and semi-arid climates. Above-ground growth ceases prior to a hot and dry summer when bulbs form and enter summer dormancy. Flower initiation occurs in dormant bulbs after a period of high temperatures in May–June. In autumn, water availability and decreasing temperatures allow dormancy release; the plants sprout and develop foliage leaves and inflorescences during mild winter (Noy-Porat *et al.*, 2013). (B) Spring-flowering *Tulipa* spp. from the continental thermo-periodic zone cease above-ground growth and produce daughter bulbs in early summer. They experience both summer and winter rest. Flower initiation occurs in early summer, after a period of high temperatures. A further period of low temperatures is required to break dormancy, resulting in leaf and stem elongation and blooming (Le Nard and De Hertogh, 1993). (C) Summer-flowering type I. *Allium sativum* (garlic), originated from continental climates, requires low temperatures for flower initiation and bulbing. A long photoperiod promotes floral stem elongation (Kamenetsky *et al.*, 2004; Ben Michael *et al.*, 2018). (D) Summer-flowering type II. *Lilium* spp. from temperate climates undergo external growth recess as a reaction to short days and low temperatures in autumn. During the winter, however, the apical meristem produces only leaf primordia. For flower induction and bulbing, besides low temperature, a long photoperiod is required (Le Nard and De Hertogh, 1993).

Lee *et al.*, 2013; Leeggangers *et al.*, 2018; Ben Michael *et al.*, 2020). These findings were made possible thanks to the tremendous progress in understanding the molecular mechanisms involved in sensing environmental and endogenous cues to control flowering in the dicot model species *Arabidopsis thaliana* and other model plants (Fornara *et al.*, 2010; Kinoshita and Richter, 2020). Fundamental research has led to the identification of components within signalling pathways that affect flowering and their positioning within molecular hierarchies. Furthermore, distinct signalling pathways have been shown to converge on activation of the same flowering time genes (Wellmer and Riechmann, 2010; Kinoshita and Richter, 2020).

Classical studies demonstrated that a flowering stimulus, known as florigen, is produced in the leaves and acts as a long-distance signal to induce flowering at the shoot apical meristem (SAM; Kardailsky *et al.*, 1999). Subsequently, it was discovered that this mobile flowering stimulus is the FLOWERING LOCUS T (FT) protein (Abe *et al.*, 2005; Wigge *et al.*, 2005; Corbesier *et al.*, 2007; Tamaki *et al.*, 2007). FT shares similarity with RAPIDLY ACCELERATED FIBROSARCOMA (RAF) kinase inhibitors and contains a conserved PEBP domain, on which the name of the family is based. Arabidopsis has six *PEBP* genes; *FT*, *TSF* (*TWIN SISTER OF FT*), *BFT* (*BROTHER OF FT AND TFL1*), *ATC*

(*ARABIDOPSIS THALIANA CENTRORADIALIS*), *MFT* (*MOTHER OF FT AND TFL1*), and *TFL1* (*TERMINAL FLOWER 1*) (Turck *et al.*, 2008; Karlgren *et al.*, 2011; Jin *et al.*, 2021). Among other roles, *FT* and its paralogue *TSF* promote flowering (Kardailsky *et al.*, 1999; Yamaguchi *et al.*, 2005), while *TFL1*, *ATC*, and *BFT* repress flowering (Kobayashi *et al.*, 1999; Yoo *et al.*, 2010; Huang *et al.*, 2012). Finally, *MFT* regulates seed dormancy and germination (Xi *et al.*, 2010).

Members of the *PEBP* gene family have undergone duplication and are subfunctionalized in many dicotyledonous and monocotyledonous plants (Wickland and Hanzawa, 2015; Jin *et al.*, 2021). In geophytes, this phenomenon resulted in multiple *FT* paralogues whose functions are related to both sexual and vegetative reproduction. In potato, the *FT* orthologue *StSP3D* controls flowering, while two other *PEBP* genes, *StSP5G* and *StSP6A*, act as a repressor and inducer of tuber formation, respectively (Navarro *et al.*, 2011; Fig. 2A). Recently it has been shown that *TFL1* in potato acts as a repressor of tuberization by acting against *StSP6A* in stolons (Zhang *et al.*, 2020). Under short days (SDs), tuberization occurs due to up-regulation of *StSP6A*, which also negatively affects flower development (Navarro *et al.*, 2011; Plantenga *et al.*, 2019). The molecular mechanism of *StSP6A* repressive action is not known, but it is proposed that the abortion of floral bud development does not occur due to competition for available assimilates with the underground tuber sink, but rather due to the presence of the tuberization signal itself (Plantenga *et al.*, 2019).

Three different *FT*-like genes control bulb formation and flowering in onion (Lee *et al.*, 2013; Fig. 2B). During the juvenile stage and non-inductive SDs, *AcFT4* acts as a repressor of bulb induction. In post-juvenile plants, inductive long days (LDs) trigger the down-regulation of *AcFT4* and the up-regulation of the bulbing inducer *AcFT1* (Lee *et al.*, 2013). Onion plants overexpressing *AcFT4* never formed bulbs and did not express *AcFT1*, whereas plants overexpressing *AcFT1* formed small bulb-like structures in tissue culture and did not express *AcFT4* (Lee *et al.*, 2013). Altogether, these results provide strong evidence that *AcFT1* acts as an activator and *AcFT4* as a repressor of bulbing, and that they mutually antagonize each other's function and/or expression (Lee *et al.*, 2013) (Fig. 2B). In concert with the two previously mentioned bulbing-related *FT-like* genes, *AcFT2* acts as a flowering signal and is expressed in mature bulbs in response to vernalization (Lee *et al.*, 2013) (Fig. 2B). Recently, another member of the *PEBP* gene family, *AcTFL1*, has been identified in onion; based on its expression pattern, it might be involved in maintaining indeterminate growth of the bulb and inflorescence (Dalvi *et al.*, 2019). Multiple *PEBP* family genes (seven *FT-like* genes and two *TFL1-like* genes) are found in garlic, which might be involved in storage organ formation and flower induction (Kamenetsky *et al.*, 2015; Manoharan *et al.*, 2016; Ben Michael *et al.*, 2020). Low temperatures induce bulbing in garlic, and the expression of *AsFT1* and *AsFT4* correlates with this process. *AsFT2*, the garlic orthologue of onion *AcFT2*, is up-regulated upon vernalization and its overexpression in Arabidopsis leads to early flowering, suggesting its role as the garlic florigen (Rohkin Shalom *et al.*, 2015; Chaturvedi *et al.*, 2018).
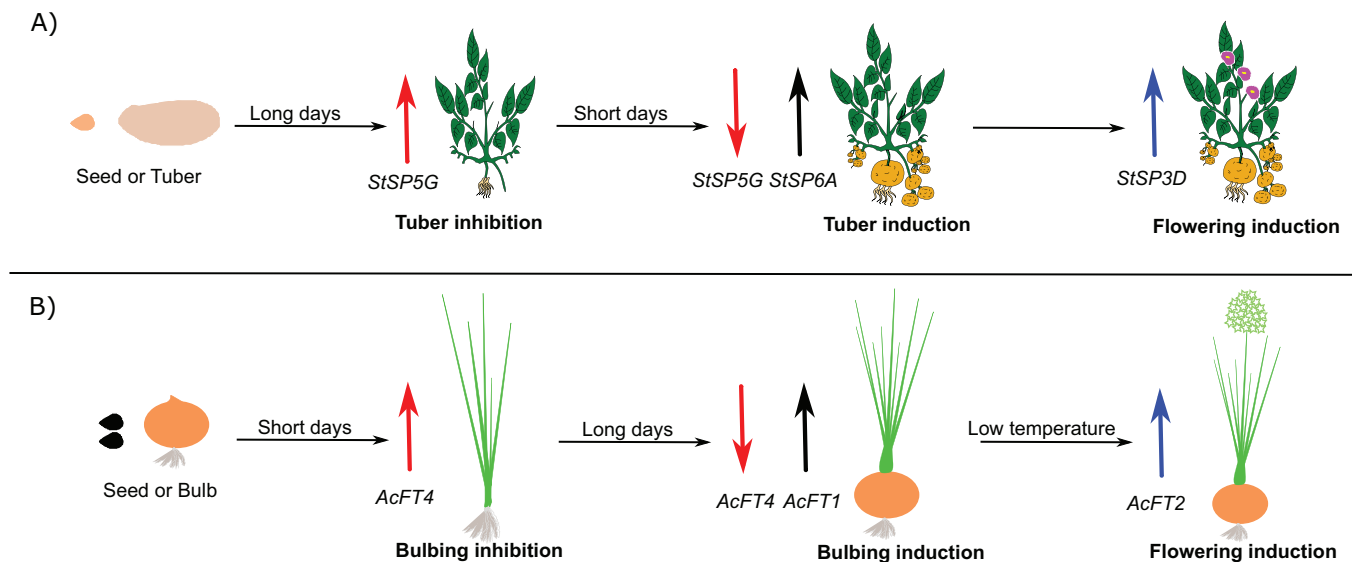

Fig. 2. Role of *PEBP* genes in storage organ formation and flower induction in potato and onion. (A) In potato, a long photoperiod represses tuberization due to *StSP5G* expression, but, under short days, tuberization occurs due to down-regulation of *StSP5G* and up-regulation of *StSP6A*. *StSP3D* promotes flowering. (B) In onion, during the first year, bulbing is inhibited by a short photoperiod due to expression of *AcFT4*. Once the critical long photoperiod is reached in summer, bulbing occurs due to down-regulation of *AcFT4* and up-regulation of *AcFT1*. Overwintering vernalization fulfils cold requirements and leads to up-regulation of *AcFT2* to induce flowering in the second year. However, onion cultivars adapted at different latitudes exhibit extensive variation of vernalization and day length requirement to form flower and bulbs, respectively (Brewster, 2008).

In *Lilium longiflorum*, exposure to low temperatures leads to up-regulation of *LlFT*, whose overexpression causes flowering under non-inductive LDs without low temperature exposure (Leeggangers *et al.*, 2018). Research in Asiatic hybrid lily (*Lilium* hybrid) revealed the presence of four *FT-like* genes: *LhFT1*, *LhFT4*, *LhFT6*, and *LhFT8* (Kurokawa *et al.*, 2020). Of particular interest, the scale-expressed *LhFT1* is thought to have the role of florigen; in fact, it is up-regulated during floral induction and LhFT1 has the capacity to interact with LhFD. In support of this hypothesis, heterologous overexpression of *LhFT1* is able to complement the Arabidopsis *ft-10* mutant (Kurokawa *et al.*, 2020). *LhFT8* is the most similar to *LlFT* based on protein sequence and is able to partially complement the phenotype of the Arabidopsis *ft-10* mutant, but interaction with LhFD was not shown. Based on these findings, the authors hypothesized that LhFT8 plays a role in establishing flowering competence upon vernalization, as was proposed previously for LlFT (Leeggangers *et al.*, 2018; Kurokawa *et al.*, 2020). In Mediterranean *Narcissus tazetta*, *NtFT* is up-regulated after exposure to warm temperature during summer and correlating with flower induction (Noy-Porat *et al.*, 2013). Analysis of transcriptomic data revealed the presence of at least five *PEBP* genes in tulip (Leeggangers *et al.*, 2017, 2018). *TgFT-like* and *TgTFL1* show differential expression in the SAM during the temperature-dependent reproductive transition, while *TgFT1*, *TgFT2*, and *TgFT3* are mainly expressed in the leaves and flower stalk of the mother plant prior to the reproductive switch in the daughter bulbs that have grown from the axillary meristems. Among them, *TgFT2* and *TgTFL1* show the strongest phenotypes in heterologous expression studies in Arabidopsis, resulting in very early and very late flowering, respectively (Leeggangers *et al.*, 2017, 2018). Taking these results and the level of sequence similarity into account, *TgFT2* can be regarded as the best candidate to represent florigen in tulip (Leeggangers *et al.*, 2018). Overall, these studies provide solid evidence that different *FT-like* genes control the formation of asexual (bulb and tuber) and sexual reproductive structures in geophytes.

PEBP genes and sink–source-regulated carbon allocation

Geophytes respond to the perception of carbohydrate availability and sugar metabolism, and, based on various physiological studies, these complex sink–source relationships could be linked to the reproduction strategy (Kondrat'eva *et al.*, 2009; Chaturvedi *et al.*, 2018). Using potato as a model, we propose PEBPs to act as hubs in the still largely unknown molecular cascade that underlies these sink–source relationships.

The physiology of carbon allocation has been extensively studied in plants due to its importance to crop yield (Schulze, 1982; Marcelis, 1993); among the proposed models, sink regulation remained one of the best known. This model assumes that plant heterotrophic organs are 'sinks', each one demanding photoassimilates produced in photosynthetic or 'source' organs and transported through the phloem to sustain their growth. The final success of each sink will depend on its strength, which is a function of the resistance to carbon transport and its utilization (Ho, 1988; Sonnewald and Fernie, 2018). This is especially relevant in geophytes, where growing storage organs are very strong sinks and, once established, they ensure survival in harsh seasonal conditions and become the main carbon source for sustaining the growth of new (aerial) organs. Given that the last might be sexually reproductive organs, flowers and seeds, and that various storage organs represent vegetative reproduction structures (e.g. daughter bulbs in tulip and tubers in potato), the relationship between sink and source organs is intimately interconnected with the choice of a reproduction strategy. Consequently, genetic and sink–source regulation of reproduction are very likely to overlap and should be considered together in the study of geophytic development.

Different classes of sugar transporters such as SWEET ('SUGAR WILL EVENTUALLY BE EXPORTED' TRANSPORTERS) and SUTs (SUCROSE TRANSPORTERS) have been widely described for their involvement in determining sink–source dynamics (Kühn and Grof, 2010; Jeena *et al.*, 2019). SWEETs are passive uniporters responsible for carbon distribution and for apoplastic phloem unloading, allowing movement of different sugars around the plant body (Sonnewald and Fernie, 2018). A recent study provided new insights into the molecular basis of sink dynamics in potato, revealing that StSP6A, the potato PEBP responsible for tuberization, interacts with and blocks the sugar transporter SWEET11 at the cytosolic side of phloem companion cells (CCs). This supposedly prevents sugar leakage in the apoplastic space to favour its symplastic movement into the growing tuber parenchyma and, consequently, stimulating tuber swelling (Abelenda *et al.*, 2019). These findings are in line with past physiological investigations which revealed a local switch in phloem unloading during the process of tuberization (Viola *et al.*, 2001). In fact, by tracking ^{14}C assimilates during the early moments of tuber formation, coinciding with the swelling of the stolon tip, it was possible to observe a clear shift from apoplastic sugar transport in the growing stolon to symplastic transport right after tuber formation is initiated. At a cellular level, symplastic unloading occurs through plasmodesmata between the phloem sieve element (SE)–CCs and the tuber parenchymatic cells. Remarkably, dormant tuber apical buds show symplastic isolation from the phloem until dormancy release, when they initiate fast outgrowth and become strong sinks. This outgrowth is correlated with a decrease in expression of the *PEBP* gene *StCEN* (Morris *et al.*, 2019), providing another association between sink strength and a member of the *PEBP* gene family in geophytes. In parallel, research in Arabidopsis identified SWEET10 as a downstream factor in FT-mediated flower induction, providing an additional linkage

between florigen functioning and sugar transport (Andrés *et al.*, 2020).

In potato, three paralogues of the sucrose transporter genes (SUT or SUC genes) have been identified: *StSUT1*, *StSUT2*, and *StSUT4*, each one belonging to a different sucrose transporter family (the *SUT1*, *SUT2*, and *SUT4* family, respectively). *StSUT1*, expressed in the phloem of sink potato tubers, locally regulates sugar transport and consequently tuber yield and turgor (Kü *et al.*, 2003). Interestingly, StSUT4-RNAi lines showed an early flowering phenotype and were able to produce tubers even in non-inductive LD conditions. Further studies showed a link between *StSUT4* and the potato florigen through the photoperiod pathway (Chincinska *et al.*, 2008, 2013).

Therefore, *PEBP* genes might regulate sink competition which results in fine-tuned dual reproduction. Interestingly, leaf-expressed *StSP6A* tuberigen has a negative effect on flower bud development after flower initiation by *StSP3D* (Plantenga *et al.*, 2019). Although this indicates a stronger molecular imprint over sink–source relationships, the authors cannot rule out that the exact mode of action of StSP6A is through the direct control of assimilates. In parallel, a similar conclusion was obtained from studying StSP6A post-translational regulation using an miRNA-insensitive codon-optimized version of the gene: young potato plants overexpressing this variant formed tubers precociously and exhibited strong growth alterations resulting from an altered sink–source equilibrium favouring underground organs over above-ground structures (Lehretz *et al.*, 2019).

Although detailed knowledge is lacking in other geophytes, a similar link between nutrient availability and reproduction has been observed. During onion bulbing, sugars are transported from leaves to underground leaf sheaths. This phenomenon leads to accumulation of fructans in the bulb. The key enzymes involved in carbohydrate metabolism show a change in activity in leaves and leaf sheaths during the transition to bulbing (Darbyshire and Henry, 1978; Yaguchi *et al.*, 2008). Transcriptome analysis in onion during bulb formation confirmed the importance of carbohydrate metabolism in this process (Zhang *et al.*, 2016). In particular, among the differentially expressed genes, it was possible to find sucrose metabolism-related genes such as those encoding the cell wall invertase, *CWIN*; sucrose transporters, *SUT* genes; the sucrose synthase, *SuSy* genes; and invertases, *INV* genes, suggesting the importance of sugar metabolism and transport during bulbing (Zhang *et al.*, 2016). The role of bulbing-related onion *PEBP* genes (*AcFT1* and *AcFT4*) in carbohydrate metabolism is not known. However, it has been shown that the *SUCROSE:SUCROSE1-FRUCTOSYLTRANSFERASE* (*1-SST*) gene, involved in carbohydrate metabolism, exhibited high expression in bulb-forming *AcFT1*-overexpressing plants and is expressed at low levels in non-bulbing *AcFT4*-overexpressing plants (Lee *et al.*, 2013). This suggests that *AcFT1* and *AcFT4* might be directly or indirectly involved in carbohydrate metabolism changes

during bulbing. In onion and garlic, correlation between the expression of genes coding for acid invertases, a family of enzymes that catalyse sugar hydrolysis, and both vegetative organs and flowering was found (Baldwin *et al.*, 2014; Chaturvedi *et al.*, 2018). In support of the latter, these hydrolytic enzymes are directly controlled by the same environmental factors driving reproductive events, such as temperature and day length (Lercari, 1982; Benkeblia *et al.*, 2004). Taken together, these findings form the basis for understanding sink–source regulation related to reproduction biology at the molecular level. In fact, it is possible that members of the *PEBP* family can integrate environmental-responsive cascades with carbon signalling and promote vegetative and sexual reproduction, setting a potential basis to understand how the dynamic interactions between the two types of reproduction are orchestrated in geophytes.

Molecular mode of action of PEPBs

As discussed, potato StSP6A can physically interact with the plasma membrane-localized SWEET11 protein (Abelenda *et al.*, 2019). However, the best known and studied physical interactions of PEBPs in multiple species involve transcription factors, supporting a role inside the nucleus. In fact, PEBPs lack a DNA-binding domain but can exert a function as transcriptional co-regulators by forming complexes with transcription factors, thus regulating the expression of downstream target genes (Collani *et al.*, 2019). In many species, flowering requires the interaction of an FT-like protein with a bZIP transcription factor (Fig. 3A). In Arabidopsis, this complex is formed with either of the two closely related paralogues, FD and FDP (Abe *et al.*, 2005), initially thought to act redundantly, but recently shown to have partially complementary functions (Romera-Branchat *et al.*, 2020).

Moreover, a study in rice highlighted the presence of a third interacting component in this complex, the scaffold protein 14-3-3. A model was proposed describing the so-called FLORIGEN ACTIVATION COMPLEX (FAC), consisting of two FT-like proteins and two FD-like proteins bridged by two 14-3-3 proteins (Taoka *et al.*, 2011); 14-3-3 acts as a cytosolic receptor for FT and allows its transport to the nucleus, where FD completes a functional FAC (Fig. 3A). Similarly, a FLORIGEN REPRESSION COMPLEX (FRC) is formed with a mobile component of the TFL1 clade in rice (Kaneko-Suzuki *et al.*, 2018). Direct competition between the two PEBPs for complex formation was shown in Arabidopsis (Zhu *et al.*, 2020). In fact, TFL1 and FT compete for interaction with chromatin-bound FD, allowing fine-tuning of many downstream genes, ultimately regulating flower induction and shaping inflorescence development and architecture (Zhu *et al.*, 2020).

Closely related PEBPs can have opposing and diverse functions due to unique molecular determinants. The best-known examples have come from the comparison between Arabidopsis
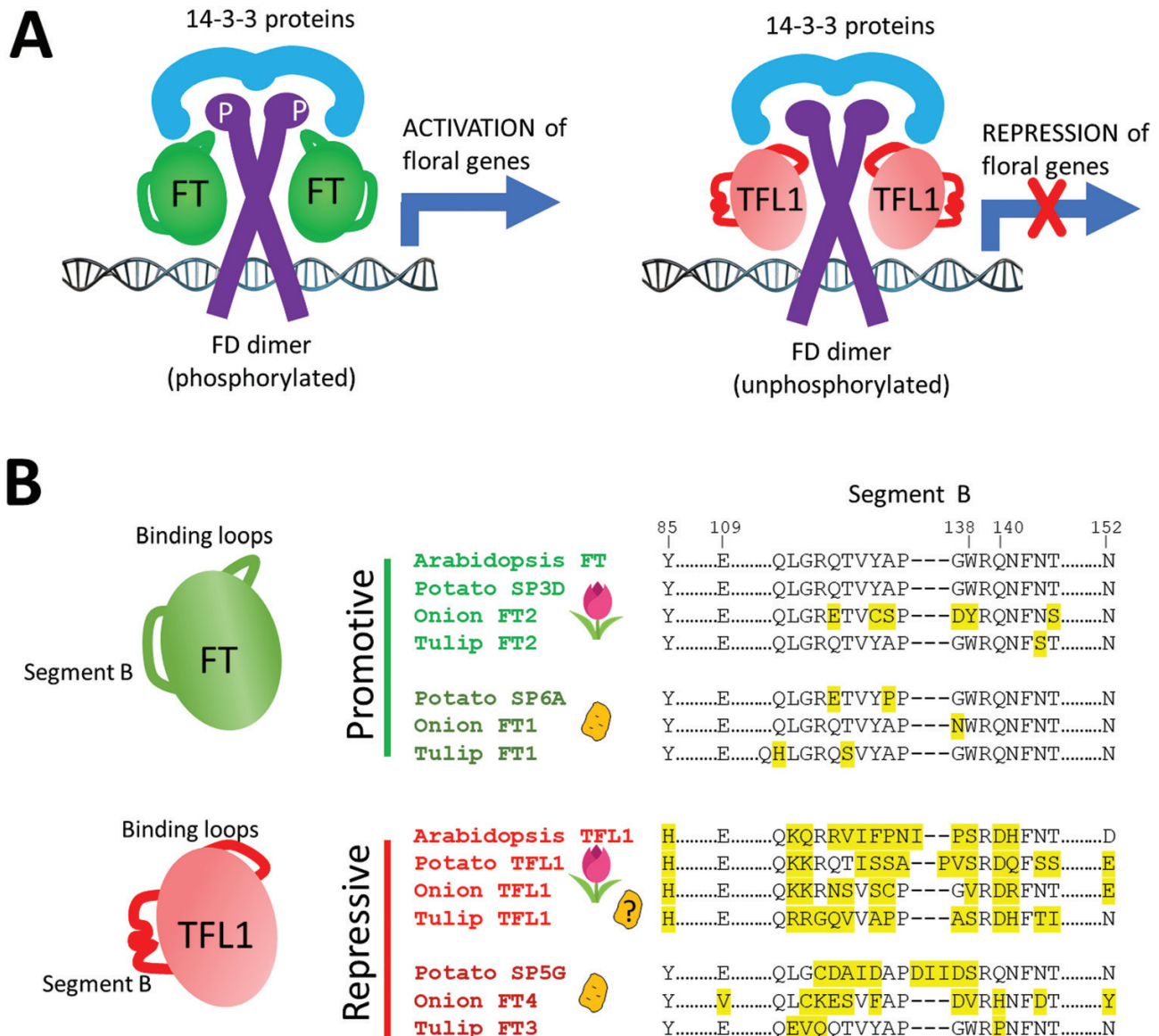
Fig. 3. Sequence variations and functional diversity of PEBPs. (A) Model explaining the opposing activities of FT and TFL1. Structural differences between FT and TFL1 allow FT to form a floral activating complex (FAC) containing phosphorylated FD, whereas TFL1 forms a floral repressive complex (FRC) containing unphosphorylated FD (Collani *et al.*, 2019). (B) Variation in segment B and other critical amino acids probably results in structural changes within binding loops that, in the case of FT/TFL1, determine if they have promotive or repressive activities (Nakamura *et al.*, 2019). The proposed functions of PEBPs in each subclade are indicated by an icon (a tulip for 'florigen'; a tuber for 'tuberigen/bulbigen'). Potato, onion, and probably tulip have both PEBPs that promote flowering and other PEBPs that promote storage organ formation. Subtle sequence differences in segment B are likely to be important for the neofunctionalization of these proteins. More substantial changes within segment B of potato SP5G, onion FT4, and tulip FT3 are probably responsible for their repressive functions. Potato, onion, and tulip also have TFL1-like proteins that might be involved in repressing flowering and/or storage organ formation. The Arabidopsis FT (AT1G65480) sequence was used as reference for the comparison with FT-like and TFL1-like sequences of bulbous geophytes.

FT and TFL1. Surprisingly, only small changes in the amino acid sequence are needed to convert FT into a floral repressor and for TFL to be converted into a floral activator (Hanzawa *et al.*, 2005; Ahn *et al.*, 2006; Pin *et al.*, 2010; Ho and Weigel, 2014). It has been shown that mutations that disrupt or modify segment B, which represents an external loop in the 3D structure of PEBPs and is encoded by the fourth exon, can convert the

Arabidopsis flowering activator FT into a repressor, mimicking TFL1 (Hanzawa *et al.*, 2005; Ahn *et al.*, 2006; Ho and Weigel, 2014) (Fig. 3B). Single amino acid changes at four residues (Glu109, Trp138, Gln140, and Asn152) are sufficient to alter the structure of segment B which in turn alters the FT protein structure, affecting the regions of FT that bind to the 14-3-3 protein (Hanzawa *et al.*, 2005; Ahn *et al.*, 2006; Ho and Weigel,

2014). Similarly, by interchanging residues that affect the segment B structure, TFL1 can acquire FT properties (Hanzawa *et al.*, 2005; Ahn *et al.*, 2006; Pin *et al.*, 2010; Ho and Weigel, 2014). Thus, segment B determines FT's ability to form a FAC complex and TFL1's ability to form a FRC complex.

Functional studies have shown that the amino acid changes responsible for the functional interconversion of FT/TFL1 probably alter the specificity for the phosphorylated or unphosphorylated form of FD. The FD transcription factor can be phosphorylated at Thr282 by calcium-dependent protein kinases (CDKs; in particular, CDK33) that are present within the SAM. FT preferentially interacts with the phosphorylated form of FD, forming a FAC capable of activating genes required for floral transition (Collani *et al.*, 2019) (Fig. 3A). In contrast, TFL1 interacts with the unphosphorylated form of FD, forming a complex that probably represses the transcription of the floral genes. This would explain why subtle changes in protein sequence can have such a dramatic effect on function. Segment B might also be involved in recruiting other transcriptional co-regulators, further explaining how changes in a PEBP could dramatically alter its function (Ho and Weigel, 2014; Wickland and Hanzawa, 2015).

Studies in the geophytes have provided impressive examples of how neofunctionalization of PEBP is likely to involve subtle structural changes that probably alter their ability to form complexes with FD and/or other transcription factors (Navarro *et al.*, 2011; Lee *et al.*, 2013). In onion, tulip, and potato, storage organ formation involves two *FT-like* genes; one which promotes and one which represses the development of storage organs (Navarro *et al.*, 2011; Lee *et al.*, 2013; Leeggangers *et al.*, 2018). As already indicated, specific amino acid residues in segment B distinguish Arabidopsis FT and TFL1. A comparison of these specific positions in the onion activator (AcFT1) and repressor (AcFT4) of bulbing shows that both have a conserved Y85 residue. However, in bulbing activator AcFT1, the key residues affecting B segment structure are conserved with Arabidopsis FT, but in the bulbing repressor, AcFT4, each of them differs (E109V, W138V, Q140H, and N152Y) (Fig. 3B).

In potato, StSP6A induces tuberization and has an FT-like B segment, while StSP5G, which represses tuberization by down-regulating StSP6A expression, has a divergent B segment (Navarro *et al.* 2011). Analogously, in tulip PEBPs, the substitution of the amino acid at position 140 between the proposed flowering inducer TgFT2 and flowering repressor TgFT3 resulted in a swap of function upon ectopic expression in Arabidopsis (Leeggangers *et al.*, 2018). Altogether, these data provide evidence for sub- and neofunctionalization by changes in the conserved B segment.

In the case of onion and tulip, it is yet to be shown whether the FT-like proteins function in a complex involving a 14-3-3 and FD-like proteins. However, for potato, the so-called TUBERIGEN ACTIVATION COMPLEX (TAC) involves the tuber-inducing FT-like StSP6A and a stolon-specific bZIP

transcription factor interacting through a 14-3-3 (Teo *et al.*, 2017). It is not known whether tuber repressor StSP5G binds with FD and 14-3-3 to inhibit tuberization (Teo *et al.*, 2017). Similar to Arabidopsis TFL1, StCEN/StTFL1 has a repressive function and acts as a repressor of tuberization. Furthermore, this protein is able to form a complex with FD/14-3-3, which are components of the TAC. It has been supposed that as such this protein affects StSP6A recruitment into a TAC and inhibits tuberization (Zhang *et al.*, 2020). In potato, expression of Hd3A, the rice florigen, is able to induce tuber formation, but also the development of flower buds at the stolon tip, probably due to the ability of Hd3A to form both a FAC and a TAC (Navarro *et al.*, 2011). This finding confirms the molecular analogy between flower induction and storage organ formation, while also highlighting the key role of PEBP interaction capacity for their subfunctionalization. Further studies are needed to confirm whether analogous protein complexes are present in bulbous geophytes.

FT can also function independently of FD and FAC-like complexes; in particular, a complex with the Arabidopsis Class II TCP transcription factor BRC1 is formed in the axillary meristems to stimulate their outgrowth (Hiraoka *et al.*, 2013) and to delay flowering of the developing axillary shoot (Niwa *et al.*, 2013). Possibly, TB1 and FT sequester and counteract each other (Niwa *et al.*, 2013). This mechanism is conserved in geophytic species such as potato, where StBRC1b binds to the tuberigen StSP6A and inactivates it, preventing aerial tubers from forming in the leaf axils. Interestingly, this tuberization-related function is specific for StBRC1b and not shared with its close paralogue StBRC1a (Nicolas *et al.*, 2020, Preprint). In tulip, TgTB1 was found to be expressed in underground axillary buds (Moreno-Pachon *et al.*, 2018) and associated with the level of bud dormancy set by the hormonal status (Fig. 4). In support of this hypothesis, *TgTB1* expression is consistently lower in tulips that show the so-called 'Springpartij' phenotype, which consists of a full switch to vegetative over sexual reproduction and a complete lack of dormancy, resulting in clusters of equally grown daughter bulbs which are unable to flower.

To conclude, the function of PEBPs as master regulators of dual reproduction in geophytes is controlled at two regulatory levels: (i) systemically, through spatial–temporal *PEBP* gene expression regulation by environmental and endogenous signals, coupled with protein mobility through the phloem, resulting in a quantitative outcome (e.g. in most geophytes storage organ formation and flowers can co-exist); and (ii) locally at the protein level, where competition for complex formation and protein sequestration occurs, which can be considered a true battle with a dichotomous outcome (e.g. growth/dormancy, vegetative/reproductive identity of the various meristems). These two levels form a complex regulatory system, in which a single protein family mediates the crosstalk between sexual and
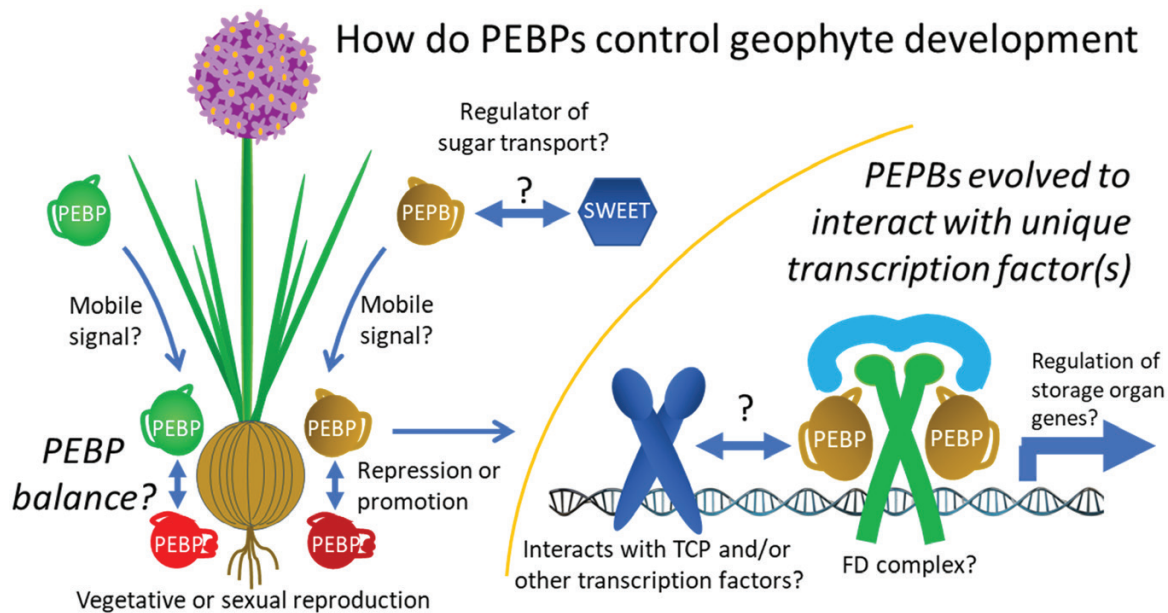
Fig. 4. Summarizing model of how PEBPs are believed to control geophyte development.

vegetative reproduction in geophytes, allowing finely tuned reproductive responses in harmony with the habitat (Fig. 4).

Future outlook

Zooming in on dual reproduction in geophytes makes it clear that 'the battle' between the two reproduction strategies most probably is settled in a relatively small number of undifferentiated cells positioned at different above-ground or underground meristems. Ultimately this does not lead to a single 'winner', flowering and seed set or storage organ formation, but a definition of the survival strategy of the plant with the presence of the different reproductive organs in a particular ratio. This balanced process includes vegetative growth, dormancy, flowering, and storage organ formation, and is steered and fine-tuned by the environmental conditions. The detailed genetic and molecular analyses performed over the last decade point towards *PEBP* gene family members as important conductors of the processes underlying reproduction in geophytes. However, when we take all current cellular, physiological, and molecular knowledge into account, a number of important questions remain unanswered, providing potential future research directions in this field.

(i) How is the integration of signals and PEBP protein activity spatially organized? It is well known that Arabidopsis FT (Corbesier *et al.*, 2007), ACT (Huang *et al.*, 2012), and FT-like proteins of various other species can travel long distances through the phloem, whereas TFL1 is travelling symplastically over short distances (Goretti *et al.*, 2020; Zhang *et al.*, 2020). In light of this, it will be interesting to investigate transport capacity of PEBPs in geophytes. Knowledge of the spatial distribution of the different

PEBPs can reveal how competition for complex formation is regulated.

(ii) What distinguishes a 'bulbing/tuberigen' PEBP from a flowering time controlling PEBP? Previous research identified the differences at key amino acid positions in segment B between flowering-inducing and –repressing PEBPs, but also between PEBPs regulating flowering versus storage organ formation. Whether this is causal and how these differences and others affect, for example, transport capacity or protein–protein interaction capacity and specificity are interesting future research directions in this field. The identification of structural and functional differences between 'florigens' and 'tuberigens/bulbigens' at the amino acid level will provide ways to predict PEBP functioning for applied studies and breeding purposes.

(iii) What is the most important molecular mode of action of different PEBPs? Currently, the focus is on nuclear functions, with PEBPs as co-transcriptional regulators in complexes with bZIP (FACS complexes) or TCP transcription factors. However, recent research in potato shows a function as sugar transport mediators (Abelenda *et al.*, 2019) by interaction with SWEET proteins and, as represented in their name, binding of pholholids appears to be important for PEBP functioning as well (Nakamura *et al.*, 2014). Future research should reveal which specific molecular function is key in specifying the different biological functions of the various PEBPs in geophytes and gain insight into PEBP pleiotropic functioning.

(iv) How did the different functions in the PEBP gene family evolve, and what was the ancestral role of these

proteins? PEBPs are best known for the functioning of FT as florigen; however, the potential to reproduce sexually evolved relatively late in plant evolution and, hence, flowering induction cannot represent the ancestral PEBP function. Different studies point to the importance of the hormone abscisic acid (ABA) in relation to the primary role of *PEBP* genes. In fact, Arabidopsis *MFT*, belonging to the ancient and most conserved phylogenetic clade of the *PEBP* family from which the *FT* and *TFL1* clades evolved (Karlgren *et al.*, 2011; Ospina-Zapata *et al.*, 2020), is a repressor of seed germination through ABA regulation (Xi *et al.*, 2010). This process is likely to be derived from ABA-dependent dormancy regulation in non-vascular plants (Eklund *et al.*, 2018). Accordingly, ABA's effect on multiple *PEBP*-regulated processes has been observed, such as dormancy onset and release (Eklund *et al.*, 2018; Tylewicz *et al.*, 2018), axillary bud outgrowth (Gonzalez-Grandio *et al.*, 2017), stomatal opening (Kinoshita *et al.*, 2011), and resistance to drought (Ryu *et al.*, 2011). Understanding the evolution of *PEBP* function can shed light on the fundamental mechanisms underlying the regulation of geophyte reproductive development.

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Author contributions

JK and FB wrote most of the article together and had an equal contribution. RKG wrote the paragraph on geophyte life cycles and physiology. JK initiated writing of this review, and RKG, RM and RGHI supervised writing. Fig. 1 was made by RKG and RM, Fig. 2 by JK, and Figs 3 and 4 by RM, with input from FB and RGHI. RGHI coordinated writing and completed the manuscript.

Conflict of interest

The authors declare no competing interest.

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