

REVIEW

Plant science's next top models

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- **Background** Model organisms are at the core of life science research. Notable examples include the mouse as a model for humans, baker's yeast for eukaryotic unicellular life and simple genetics, or the enterobacteria phage λ in virology. Plant research was an exception to this rule, with researchers relying on a variety of non-model plants until the eventual adoption of *Arabidopsis thaliana* as primary plant model in the 1980s. This proved to be an unprecedented success, and several secondary plant models have since been established. Currently, we are experiencing another wave of expansion in the set of plant models.
- **Scope** Since the 2000s, new model plants have been established to study numerous aspects of plant biology, such as the evolution of land plants, grasses, invasive and parasitic plant life, adaptation to environmental challenges, and the development of morphological diversity. Concurrent with the establishment of new plant models, the advent of the 'omics' era in biology has led to a resurgence of the more complex non-model plants. With this review, we introduce some of the new and fascinating plant models, outline why they are interesting subjects to study, the questions they will help to answer, and the molecular tools that have been established and are available to researchers.
- **Conclusions** Understanding the molecular mechanisms underlying all aspects of plant biology can only be achieved with the adoption of a comprehensive set of models, each of which allows the assessment of at least one aspect of plant life. The model plants described here represent a step forward towards our goal to explore and comprehend the diversity of plant form and function. Still, several questions remain unanswered, but the constant development of novel technologies in molecular biology and bioinformatics is already paving the way for the next generation of plant models.

Key words: Plant biology, model organisms, plant models, non-model plant models, *Cardamine hirsuta*, *Eutrema salsugineum*, *Marchantia polymorpha*, *Phragmites australis*, *Pisum sativum*, *Setaria viridis*, *Striga hermonthica*.

INTRODUCTION

Model organisms (MOs) are used in research to study certain scientific questions (Ankeny and Leonelli, 2011). They can either function as a representative for a whole group of organisms (such as plants, mammalians or prokaryotes), or act as a 'stand-in' for specific organisms of interest that cannot be easily studied, such as mice instead of humans for example for ethical reasons. There are two main reasons to use MOs. First, they are typically simple, both biologically and in handling. MOs are generally small, can be easily grown in a lab, have short life cycles, produce sufficient

offspring, have small and simple genomes, and can be easily transformed, mutated and crossed. Second, to study every aspect of a given organism's life, sophisticated methods, techniques and equipments are typically required (Ankeny and Leonelli, 2011). Their development, production, acquisition and maintenance can be expensive, time-consuming and laborious. Therefore, it is more practical to focus on specific MOs for the initial development and production of such technologies, instead of studying countless different organisms, each with individual requirements. Eventually, knowledge gained with an MO can be extrapolated to the actual organisms of interest, allowing researchers to limit

experimentation on these organisms to a few targeted and well-established final tests.

In the plant field, *Arabidopsis thaliana* was only established as a universal MO in the 1980s (Somssich, 2018). One reason for this relatively late adoption of a plant MO was that plant-specific aspects of development, morphology and physiology were typically studied directly in established crops, thereby eliminating the usual final step of extrapolating the knowledge from the model to the crops (Koornneef and Meinke, 2010). These plants are now considered part of the ‘non-model plant models’ group, meaning that they are established MOs, without actually carrying the typical characteristics of MOs. Relying on such non-model plant models in plant research became problematic with the advent of modern genetics and molecular biology (Koornneef and Meinke, 2010). When these fields became more important, the work with non-model plant models became technically impractical, slow and inefficient. As a result, plant biologists eventually recognized the need to adopt one specific model as a means of advancing the plant science field, resulting in *A. thaliana* becoming the universal MO (Koornneef and Meinke, 2010). Since then, the field of plant biology, and specifically plant molecular biology and genetics, has expanded enormously and produced a wealth of knowledge and understanding of plant biology (Somerville and Koornneef, 2002).

In a first wave of expansion, mostly in the late 1990s, the plant community adopted a set of ‘second-generation’ plant models. These were chosen to represent individual groups of plants that were too distantly related to *A. thaliana* to be studied in this primary model (Chang et al., 2016). Among those adopted, *Brachypodium distachyon* was chosen as a grass (monocot) model, *Physcomitrella patens* to represent the mosses, *Medicago truncatula* to cover the legumes and *Populus trichocarpa* to study trees (Cook, 1999; Draper et al., 2001; Cove, 2005; Jansson and Douglas, 2007; Chang et al., 2016). At the same time, *A. thaliana* is still far from being ‘solved’, and *A. thaliana* research will remain at the forefront of plant science (Provart et al., 2016). As such, it will continue to produce new insights at an ever-increasing molecular detail, while providing a basis for the development of new techniques (Provart et al., 2016). Notably, the field of *A. thaliana* research has seen its own expansion with the emerging research area of natural variation (Weigel, 2012).

More recently, some third-generation model plants have been proposed to cover research areas such as the early evolution of land plants from aquatic ancestors, plant parasitism, the formation of complex organs, tissue forms and shapes, and specific adaptations to environmental conditions. Concurrently, the group of non-model plant models is also experiencing a resurgence since plant science entered the genomics (or generally ‘omics’) era (Rowan et al., 2011). New genomics techniques such as high-throughput whole genome sequencing, the CRISPR/Cas9 system for precise genome editing, new cloning techniques that make it easier than ever to clone and express genes of interest, *de novo* gene synthesis, or the modern high- and super-resolution fluorescence microscopy techniques have advanced molecular biology research even for these highly complex plants, allowing them a comeback into modern molecular biology labs (Rowan et al., 2011; Borrill, 2020). Philippa Borrill has recently written an insightful article on the

‘blurring of the boundaries between cereal crops and model plants’ (Borrill, 2020).

With this review, we will introduce some of these emerging third-generation plant models. More precisely, we will discuss *Marchantia polymorpha* as a model to study land plant evolution, *Setaria viridis* as a model for C₄ photosynthesis and biomass recalcitrance, *Phragmites australis* for invasive plants, *Striga hermonthica* for plant parasitism, *Eutrema salsugineum* for salt tolerance and *Cardamine hirsuta* for comparative developmental studies. Furthermore, we will discuss *Pisum sativum*, a member of the non-model plant model group that is currently experiencing a resurgence as a model for legume crops. The scientific and biological relevance of these species are discussed, and the tools and resources available for the scientific community are highlighted.

THE NEW PLANT MODELS TO STUDY

Land plant evolution: introducing Marchantia polymorpha
(common liverwort)

The conquering of land by plants ~470 million years ago was a major step in evolution (Bowman et al., 2016b). Fossil and phylogenetic evidence suggest that land plants evolved from a common charophycean algal ancestor with a haplobiontic life cycle, meaning a dominant multicellular gametophyte (*n*), while the diploid phase only includes a fertilized unicellular zygote that immediately undergoes meiosis (Bowman et al., 2016b). In land plants, both the gametophyte (*n*) and the sporophyte (2*n*) produce multicellular bodies (Bowman et al., 2016b). The relative dominance of these two multicellular phases has shifted during land plant evolution: the haploid phase is dominant in basal land plants while the sporophyte is only short lived and determinate, thereby more closely resembling the charophycean algae (Bowman et al., 2016b). In vascular plants, the diploid phase became dominant over the haploid phase, causing morphological diversity of vascular plants to reside in the sporophyte, while the gametophyte was reduced to a few cells that produce male and female gametes (Bowman et al., 2016b). In this context it is an open question whether the genetic programme underlying the development of two multicellular bodies, and the genetic programme that enabled the increasing complexity of the sporophyte, already pre-existed in the algal ancestor, or if they evolved *de novo*. To address this, it is of major importance to study the development of a basal land plant, as well as the relationships of this basal plant to its ancestors, charophycean algae and its descendants, vascular plants. Bryophytes are a group of basal land plants that include the non-vascular liverworts, mosses and hornworts (Mishler and Churchill, 1984). *Marchantia polymorpha* (Fig. 1) is a complex thalloid liverwort with a well-studied taxonomy and morphology (Bowman, 2016). Liverworts have experienced a low rate of chromosomal and molecular evolution, and thus the genetic makeup of *M. polymorpha* is probably more similar to that of the common ancestor of all land plants, making it a versatile model to study land plant origin and evolution (Bowman et al., 2016a).

The predominant and persisting generation of the *M. polymorpha* life cycle is the gametophyte. This haploid dominance makes genetic analysis faster compared to diploid-dominant plants, as it eliminates the need of heterozygosity,

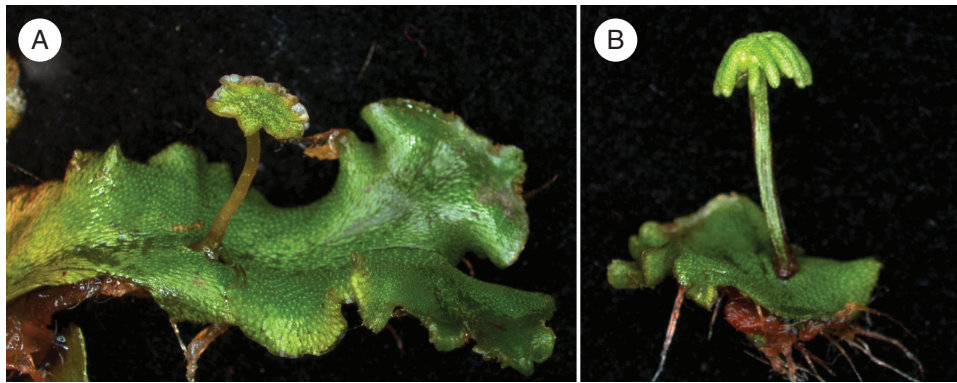


FIG. 1. *Marchantia polymorpha*. *Marchantia polymorpha* produces a haploid thallus with either (A) a male antheridiophore containing antheridia with flagellated sperm, or (B) a female archegoniophore with archegonia holding an egg. Upon fertilization, the diploid sporophyte undergoes mitosis followed by meiotic divisions of the sporogenous tissues to produce haploid spores. Photo credit Tom Dierschke (Monash University).

allowing mutant and transgenic phenotypes to be studied in their isolated generation. *Marchantia polymorpha* can reproduce sexually through flagellated sperm and egg cells, which are produced in the gametophores (antheridia and archegonia) (Fig. 1A, B) (Shimamura, 2016). Antheridia produce sperm, while archegonia produce eggs (Shimamura, 2016). *Marchantia polymorpha* can also reproduce asexually via small, disc-shaped propagules called gemmae that are formed in gemma cups on the dorsal side of the haploid thallus and remain dormant until dispersed (Eklund et al., 2015). These two modes of reproduction allow genetic crossings and the establishment and propagation of individual isogenic lines from a spore or gemma, derived from a single cell (Ishizaki et al., 2016). The gametophytic generation can be cultured and maintained under sterile conditions or stored at ultra-low temperatures, and cryopreservation of fertile *M. polymorpha* spermatozoa has been reported (Ishizaki et al., 2016; Togawa et al., 2018). These techniques provide the opportunity to reliably preserve *M. polymorpha* lines. Further advantages include a short generation time of ~3 months, 2–3 weeks for asexual reproduction and a small genome size (225.8 Mb, nine chromosomes) (Ishizaki et al., 2016; Bowman et al., 2017). The apparent absence of ancient polyploidization and the lack of gene duplication also account for a low functional redundancy (Ishizaki et al., 2016; Bowman et al., 2017).

An assembled *M. polymorpha* genome sequence was generated using the natural accessions Takaragaike-1 (Tak-1, male) and Takaragaike-2 (Tak-2, female), which were isolated in Kyoto, Japan (Okada et al., 2000; Ishizaki et al., 2008; Bowman et al., 2017). However, as *M. polymorpha* is a cosmopolitan species distributed globally from tropical to arctic climates, other natural accessions have been collected as laboratory strains and used for experimental research. At present, no comprehensive collection of all accessions exists in the research community. The first genetic transformations of *M. polymorpha* were achieved by particle bombardment, but practical high-frequency *Agrobacterium*-mediated transformation protocols are now available as well (Takenaka et al., 2000; Ishizaki et al., 2008; Tsuboyama and Kodama, 2014; Tsuboyama-Tanaka and Kodama, 2015). Common binary vectors such as pCambia, pPZP and pGWBs can be used for transformations, and a gene

targeting procedure via homologous recombination has also been adopted for *M. polymorpha* (Terada et al., 2002; Ishizaki et al., 2013a, 2015). In addition, CRISPR/Cas9-based targeted mutagenesis has been demonstrated to work efficiently; however, the haploid dominance of the *M. polymorpha* life cycle limits the ability to isolate mutants of essential genes, and as such, null mutations are potentially lethal (Sugano et al., 2018). To overcome this issue, knockdown strategies such as inducible artificial microRNA (amiR)-mediated gene silencing or the *CrelloxP* site-specific recombination system, combined with heat-shock- and DEX-controlled gene expression, were established (Flores-Sandoval et al., 2016; Nishihama et al., 2016). Constitutive overexpression can be achieved using the *CaMV 35S* or the endogenous *ELONGATION FACTOR 1a* (*MpEF1a*) promoter (Althoff et al., 2014). Both are capable of driving strong expression, but there are significant differences in terms of spatial distribution (Kajikawa et al., 2003; Althoff et al., 2014; Kubota et al., 2014; Sugano et al., 2014; Eklund et al., 2015; Flores-Sandoval et al., 2015; Kato et al., 2015). For gene expression studies, RNA *in situ* hybridization protocols and reporter genes such as β -glucuronidase (GUS) and fluorescent proteins have been tested and used successfully (Ishizaki et al., 2012, 2013b; Althoff et al., 2014; Komatsu et al., 2014; Kubota et al., 2014). Due to its low genetic redundancy, *M. polymorpha* is also highly suitable for forward genetic approaches. For instance, a T-DNA tagging strategy to generate mutants has been successfully employed, as has physical mutagenesis using X-ray irradiation (Miller et al., 1962; Ueda et al., 2012; Ishizaki et al., 2013b, 2016). In addition to the nuclear and organelle genome sequences, microRNA (miRNA) profiles and their targets, as well as DNA methylation profiles for different developmental stages and tissues are available (Lin et al., 2016; Tsuzuki et al., 2016; Bowman et al., 2017; Schmid et al., 2018). Recently, a whole suite of molecular biology and genetics tools, protocols and resources for the work with *M. polymorpha* has been made available as the OpenPlant toolkit (Sauret-Güeto et al., 2020).

Recent findings have shed some light on the basic principle of how sporophyte-specific gene expression is initiated in land plants. The core regulatory network controlling this genetic switch involves the interaction, translocation and subsequent regulatory action of a *BELL-LIKE* (*BELL*) and a *KNOTTED 1*

LIKE HOMEBOX (KNOXI) transcription factor (Lee *et al.*, 2008; Bowman *et al.*, 2016b; Dierschke *et al.*, 2020). This mechanism probably evolved in unicellular green algae, such as *Chlamydomonas reinhardtii*, and then diversified to activate sporophytic gene expression in land plants (Floyd and Bowman, 2007; Bowman *et al.*, 2016b; Horst *et al.*, 2016; Frangedakis *et al.*, 2017). However, less is known about the genetic programmes that enabled sporophytic multicellularity and three-dimensional (3-D) growth. One underlying feature of multicellular life is the network of signalling pathways by which cells communicate (Bowman *et al.*, 2017). Analysis of the *M. polymorpha* genome demonstrated that all necessary components for most land-plant signalling pathways are also encoded in the *M. polymorpha* genome, but reduced to the minimum number of components (Bowman *et al.*, 2017). In the case of the auxin signalling pathway, the network in *M. polymorpha* is simple but functional, with all components existing as single orthologues (Kato *et al.*, 2015). Phylogenetic analyses suggest that this feature is shared with the last common ancestor of land plants, and that *M. polymorpha* has probably retained this ancestral condition (Flores-Sandoval *et al.*, 2015). Studies have also shown that auxin is required for cell patterning during transition from 2-D to 3-D growth in the *M. polymorpha* gametophyte (Flores-Sandoval *et al.*, 2015). Similarly, it was found that the jasmonate signalling network in *M. polymorpha* consists of some ancient components and others that arose through duplication and neofunctionalization of algal genes (Han, 2017; Monte *et al.*, 2018; Bowman *et al.*, 2019). More recently, *M. polymorpha* has also been adopted as a model for evolutionary molecular plant–microbe interaction studies, with the first bacterial, fungal and oomycete pathogens being described (Carella *et al.*, 2019; Gimenez-Ibanez *et al.*, 2019; Matsui *et al.*, 2019). In all cases, infection with the pathogen results in the activation of typical hallmarks of plant immunity, demonstrating that the plant is also a suitable model to study defensive mechanisms without the redundancy present in vascular plants (Carella *et al.*, 2019; Gimenez-Ibanez *et al.*, 2019; Matsui *et al.*, 2019). With regard to cell-wall biology, the evolution of the highly complex cellulose synthesis machinery has recently been analysed (Lampugnani *et al.*, 2019). At the core of this machinery are the members of the CELLULOSE SYNTHASE (CesA) family, which form multimeric complexes in vascular plants, making it complicated to study their function (Lampugnani *et al.*, 2019). It was found that these key components already exist in *M. polymorpha*, but that this early land plant only has two *CesA* genes, compared to ten in *A. thaliana* (Lampugnani *et al.*, 2019).

These studies have revealed that components of the different pathways often consist of a combination of pre-existing algal genes and/or genes that have undergone neofunctionalization (Bowman *et al.*, 2017). Accordingly, genetic regulators that were considered specific to land plants have since been found in the charophycean algae (Catarino *et al.*, 2016; Bowman *et al.*, 2017; Wilhelmsson *et al.*, 2017; Vries and Archibald, 2018). Hence, a number of developmental innovations relevant to land plant evolution can be traced back to the common ancestor of land plants. This includes UVB-tolerance through UVR8-mediated flavonoid induction and flavonoid production in response to abiotic stress, the genetic control of vegetative reproduction, photoperiodic control for the transition

from vegetative to reproductive growth, and germ cell differentiation (Kubota *et al.*, 2014; Koi *et al.*, 2016; Albert *et al.*, 2018; Clayton *et al.*, 2018; Hiwatashi *et al.*, 2018; Yamaoka *et al.*, 2018). Moreover, these basic mechanisms were first acquired in the gametophytic generation, then co-opted between the generations, and finally diversified to pattern the sporophyte (Bowman *et al.*, 2019).

C₄ photosynthesis and biomass recalcitrance: introducing Setaria viridis (green foxtail)

Historically, most research on grass genetics and genomics has been carried out in agriculturally important crops, such as maize, wheat or rice. These plants are not ideal MOs because of particular intrinsic difficulties, such as long life cycles, large plant size and lack of efficient transformation protocols (Li and Brutnell, 2011; Brutnell *et al.*, 2015). The temperate grass *Brachypodium distachyon* was later adopted as a model grass at a remarkably rapid rate due to several biological attributes, such as small stature, short life cycle, simple growth requirements and amenability to genetic transformation. Despite its major contributions to research, *B. distachyon* lacks perhaps one of the most economically important traits generally found in grasses: the mechanism of *C₄* photosynthesis (Schuler *et al.*, 2016). The productivity of several grasses used for food and bioenergy is driven by *C₄* photosynthesis, which confers improved radiation, nitrogen and water-use efficiencies when compared to *C₃* photosynthesis, while reducing losses caused by photorespiration (Schuler *et al.*, 2016). Therefore, engineering *C₄* photosynthesis into *C₃* crops is a major objective for crop improvement, but such a strategy has been hampered by the lack of a complete list of genes and their corresponding functions required to support the trait (Weber and Bar-Even, 2019).

Setaria viridis (Fig. 2) is a *C₄* grass belonging to the subfamily Panicoideae, tribe Paniceae, which is sister to the tribe Andropogoneae, one of the most economically important groups of plants that includes maize, sorghum and sugarcane (Huang *et al.*, 2016). *Setaria viridis* shows several desirable attributes for an MO, such as a short life cycle (6–8 weeks), self-fertility, small stature (15–30 cm), large seed yield (~13 000 seeds per plant), simple growth requirements and a small diploid genome (510 Mb). Boosted by its remarkable capacity to invade, colonize and adapt to local environments, *Se. viridis* has spread from its centre of origin in Eurasia to a wide range of habitats, becoming the most widely distributed weed in the world (Li and Brutnell, 2011; Zhu *et al.*, 2017). Significant phenotypic variation is observed among different natural populations, including differences in inflorescence architecture, plant height, seed morphology and flowering time (Li and Brutnell, 2011). However, the genetic diversity underlying those traits is apparently low and distributed in subpopulations, suggesting strong local adaptation (Li and Brutnell, 2011; Brutnell *et al.*, 2015). It is largely accepted that *Se. viridis* is the wild ancestor of the cereal crop *Setaria italica* (common name: foxtail millet) (Li and Brutnell, 2011; Brutnell *et al.*, 2015; Huang *et al.*, 2016). Although genetically similar, *Se. italica* shows distinct morphological and physiological traits compared to its wild ancestor, including larger stature, enlarged inflorescence,



FIG. 2. *Setaria viridis*. Representative picture of *Se. viridis*, an emerging model for C_4 grasses.

reduced vegetative branching, synchrony of flowering and loss of seed dormancy. These differences are thought to be part of the ‘domestication syndrome’ caused by artificial selection made by humans during foxtail millet domestication (Li and Brutnell, 2011). Regarding its photosynthetic apparatus, *Se. viridis* is a C_4 plant employing an NADP-dependent malic enzyme (NADP-ME subtype) as the decarboxylating enzyme located in the bundle sheath, similar to important food and bio-energy crops such as maize, sorghum and sugarcane (Danila et al., 2016). These facts make *Se. viridis* an excellent model for studying plant domestication, to understand the molecular mechanisms underlying several aspects of C_4 photosynthesis and to validate biotechnological strategies aimed at boosting plant yields.

A wider adoption of *Se. viridis* as a universal grass model will be facilitated by the continuing development of novel resources and protocols. The foundation for genetic, genomic and functional studies has already been created with its published genome (Bennetzen et al., 2012), which is in its second annotated version and available at Phytozome (<https://phytozome.jgi.doe.gov>). To further exploit the genetics of *S. viridis*, an optimized protocol for genetic crosses has been developed, involving panicle pruning followed by emasculation using hot water treatment to kill viable pollen (Jiang et al., 2013). This method was reported to yield one to seven cross-pollinated seeds per panicle (Jiang et al., 2013). Another major breakthrough was the establishment of various *Agrobacterium tumefaciens*-mediated transformation protocols, including tissue culture-based and floral-dip methods (Martins et al., 2015a, b). Although the latter method is more straightforward, it typically shows very low transformation efficiencies (~0.6–0.8%), whereas the more laborious and time-consuming tissue culture-based method

presents a transformation frequency as high as 29% (Martins et al., 2015a, b). These methods provide a valuable tool for gene discovery and functional studies, and for rapid validation of biotechnological strategies before their translation to dedicated crops. Reliable reference genes for expression analysis via quantitative real-time PCR have been identified and validated for a wide range of experimental conditions, including different plant developmental stages and diverse stress conditions (Martins et al., 2016). Standard phenotyping protocols have also been developed, with detailed descriptions of multiple growth and developmental assays under controlled conditions, and in response to phytohormone treatment and abiotic stresses (Acharya et al., 2017). These assays will be particularly important for mutant screens, for large-scale phenotyping, and for the characterization of transgenic lines during functional studies. Growth in a hydroponic system allows the uniform production of robust seedlings that can be used to assess plant responses to a wide range of chemicals in highly reproducible experiments (Monte-Bello et al., 2018).

Setaria viridis is being employed in efforts to address three major ‘biological problems’: (1) plant domestication, (2) C_4 photosynthesis and (3) biomass recalcitrance. Although some genes involved in plant domestication might be conserved among species, no differences were found in the coding sequences of candidate domestication genes between *Se. viridis* and its cereal crop descendant, *Se. italica*, suggesting that a different set of genes or regulatory mechanisms was involved in foxtail millet domestication (Bennetzen et al., 2012). Still, several quantitative trait loci of key domestication traits were mapped and partially characterized (with candidate genes often identified), such as those related to shattering, plant height, plant branching, flowering time and photoperiod sensitivity. These data are nicely compiled and discussed in the excellent review from H. Hu et al. (2018). *Setaria viridis* has also been widely employed to study C_4 photosynthesis (Brutnell et al., 2015; Huang and Brutnell, 2016; Zhu et al., 2017). In addition to its use in comparative transcriptomic studies with C_3 and intermediate C_3 – C_4 species to select novel candidate genes related to the C_4 mechanism, the availability of efficient transformation approaches has allowed *Se. viridis* to become a platform to functionally validate those targets, and consequently, to provide new insights into several aspects of C_4 photosynthesis (Boyd et al., 2015; Alonso-Cantabrana et al., 2018). Transgenic *Se. viridis* depleted in carbonic anhydrase (CA) was generated to address the role of CA in C_4 photosynthesis (Osborn et al., 2017). CA is localized in the cytosol of mesophyll cells, where it catalyses the hydration of CO_2 to HCO_3^- , which is further used by phosphoenolpyruvate carboxylase (PEPC) in the first step of C_4 photosynthesis (Osborn et al., 2017). It was shown that under normal atmospheric conditions, CA activity was not rate-limiting for C_4 photosynthesis in *Se. viridis*, whereas under conditions that result in lower intercellular CO_2 concentrations (such as drought), mesophyll conductance may pose a greater limitation than CA activity (Osborn et al., 2017). Therefore, increasing mesophyll conductance may be an interesting strategy to boost CO_2 assimilation in a scenario of global warming and limited water availability. Silencing of PEPC in *Se. viridis* resulted in reduced cell wall thickness and increased plasmodesmata (PD) density at the mesophyll–bundle sheath interface, leading to an intriguing speculation that PD development might be responsive to changes in C_4 photosynthetic

flux (Alonso-Cantabrana *et al.*, 2018). These are only a few examples demonstrating the potential of *Se. viridis* as a model for molecular manipulation of the C₄ photosynthetic pathway.

Setaria viridis has also been suggested as a model for lignocellulosic biomass crops, based on its phylogenetic proximity to potential feedstock, such as sugarcane, *Miscanthus* spp. and switchgrass (Brutnell *et al.*, 2010, 2015; Li and Brutnell, 2011). Plant biomass is mainly composed of secondary cell walls (SCWs), whose major components are polysaccharides that can potentially be converted into fermentable sugars for the production of biofuels and biomaterials. However, the complex chemical compositions and rigid structure of SCWs hinder the efficient processing of plant biomass in biorefineries, an issue known as biomass recalcitrance (Marriott *et al.*, 2016). Therefore, the production of optimized bioenergy crops with reduced recalcitrance requires a deep characterization of several aspects of SCW deposition. Above-ground biomass of *Se. viridis* was shown to be similar to that of other panicoid bioenergy crops in terms of cellulose and lignin content and cell wall polysaccharide composition (Petti *et al.*, 2013). In addition, the characteristics of the CELLULOSE SYNTHASE gene superfamily and the accumulation and distribution of (1,3;1,4)- β -glucans, polysaccharides that are typical of grass cell walls, were shown to be similar between *Se. viridis* and other C₄ grasses (Ermawar *et al.*, 2015). The core set of biosynthetic genes potentially involved in developmental lignification and lignin-related laccases were identified using a combination of comparative phylogenetic studies, high-throughput expression analysis and quantitative RT-PCR analysis (Ferreira *et al.*, 2019; Simões *et al.*, 2020). Regarding gene discovery, only one SCW-related gene has been functionally characterized in *Se. viridis*. Souza *et al.* (2018) showed that the BAHD acyl-CoA transferase SvBAHD01 has a key role in arabinoxylan (AX) feruloylation in *Se. viridis*, as down-regulation of this gene resulted in a 60% decrease in AX feruloylation in stems without affecting biomass accumulation. Notably, biomass saccharification efficiency was increased by ~40–60%, which not only demonstrates that AX feruloylation is a promising target for reducing biomass recalcitrance, but also confirms *Se. viridis* as a platform to validate biotechnological strategies.

The development of diverse resources and tools for *Se. viridis* is rapidly advancing, although various challenges and opportunities are predicted for the *Setaria* community. Despite an efficient tissue culture-based transformation protocol being available, a more robust spike dip protocol is urgently needed to boost functional studies and gene discovery efforts. The use of CRISPR/Cas9 technology to generate null mutants will also greatly increase the possibilities for functional studies. The close genetic relationship between *Se. italica* and *Se. viridis*, in addition to the continuing development of new genome technologies, will probably facilitate the identification of the set of genes responsible for the phenotypic variation occurring during the domestication process. Finally, deeper knowledge on SCW biology in *Se. viridis* is essential to understand the molecular basis of biomass recalcitrance prior to the development of biotechnological strategies to generate optimized crops for biorefineries.

Invasive plants: introducing Phragmites australis (common reed)

Exploration and globalization have rapidly increased since the industrial revolution, and natural barriers that typically restrict

species' ranges have largely dissolved (Hulme, 2009). Many species previously confined to certain geographical regions have been introduced to non-native locations through human activity, leading to invasion events, where the introduced species establishes itself in a new habitat and outcompetes native species due to diverse ecological factors (Kolar and Lodge, 2001). Examples of such factors include a lack of natural predators, exploitation of eutrophic conditions and plant-specific characteristics, such as shoot and leaf-area allocation, fitness, growth rate and size (Keane and Crawley, 2002; van Kleunen *et al.*, 2010; Mozdzer and Megonigal, 2012; Liu *et al.*, 2018). Invasive species negatively impact global species' diversity and ecosystem health and pose a global economic burden. Invasive plants cost the USA ~\$35 billion annually, mainly associated with negative impacts to agricultural operations (Pimentel *et al.*, 2005; Dogra *et al.*, 2010). The scientific community would benefit from an established plant model to continue driving research in the field of invasion biology. The well-studied species *Phragmites australis* (common reed) is emerging as a promising model candidate (Meyerson *et al.*, 2016b; Packer *et al.*, 2017a).

Phragmites australis (Fig. 3) is a cosmopolitan perennial grass that spans all continents except Antarctica (Clevering and Lissner, 1999; Packer *et al.*, 2017b). It is associated with widespread growth in wetland habitats, particularly in marshes and along the shores of freshwater and brackish water bodies (Chambers *et al.*, 1999; Packer *et al.*, 2017b). Often found growing in dense patches (so-called 'stands'; Fig. 3), it typically propagates vegetatively through rhizome and stolon growth (Lambertini *et al.*, 2008). *Phragmites australis* displays a high degree of phenotypic plasticity, a range of salt tolerances and, in some cases, the ability to grow in arid environments (Saltonstall *et al.*, 2010; Achenbach *et al.*, 2013; Holmes *et al.*, 2016; Packer *et al.*, 2017b). Sexual reproduction is facilitated through wind pollination of its inflorescences, which bear thousands of hermaphrodite florets capable of producing ~1000 seeds with long hairs to facilitate wind dispersal (McKee and Richards, 1996; Saltonstall *et al.*, 2010). Cross-pollination by hand drastically increases seed set, which is typically quite low due to partial self-incompatibility (Ishii and Kadono, 2002; Lambert and Casagrande, 2007b; Kettenring *et al.*, 2011). Although flowering can take several years following germination, vegetative propagation via a rhizome is a simple, rapid way to produce genetic clones (Ali *et al.*, 2002; Saltonstall *et al.*, 2010). *Phragmites australis* has been present in North America for at least 40 000 years, and during the 1800s it was an uncommon plant with distribution gaps across the continent; however, it now spans the entirety of the USA and into Canada (Hansen, 1978; Saltonstall, 2002). Anthropogenic habitat disturbance and seed dispersal are probably major promoters of the surge in population and range. Recently, chloroplast DNA sequencing has revealed that an invasive European haplotype heavily contributed to this expansion (Saltonstall, 2002; Meyerson and Cronin, 2013). This rapid, cryptic colonization has been the subject of many studies surrounding invasion biology, including phenotypic plasticity, genetic diversity, hybridization with native plants, predation and nutrient foraging, among others (Clevering and Lissner, 1999; Vretare *et al.*, 2001; Meyerson *et al.*, 2010; Mozdzer *et al.*, 2010; Saltonstall *et al.*, 2014; Allen *et al.*, 2015).

Global phylogenetic analyses using conserved chloroplast DNA sequences have identified 27 haplotypes, with 11 being



FIG. 3. *Phragmites australis*. A mature, flowering stand of *Ph. australis* (left) growing next to immature plants (right).

native to North America (Saltonstall, 2002). Furthermore, by analysing variable nuclear markers, high rates of genetic diversity were found among the invasive European ‘M’ haplotype across North America, suggesting a series of multiple introductions from Europe (Kirk *et al.*, 2011; Plut *et al.*, 2011). A second European haplotype, referred to as ‘L1’, was identified among two stands in Quebec, Canada, although whether it is invasive was not confirmed (Meyerson and Cronin, 2013). As there are 14 European haplotypes, it is important to investigate the differing traits between M and other haplotypes, to understand the factors that give rise to its invasive nature.

There is no genome sequence available for *Ph. australis*; however, a full plastid genome is available on the NCBI website (accession PRJNA174737), and a transcriptome dataset can be found at the NCBI Sequence Read Archive (accessions SRR3233385–SRR3233398, GenBank BioProject accession PRJNA314710, Shotgun Assembly accession GEKX00000000) (Holmes *et al.*, 2016). Transcriptomics-based studies have aimed to identify genes involved in salinity tolerance and rhizome growth, with 124 450 unique transcripts assembled and 1280 non-redundant proteins identified using mass spectrometry (He *et al.*, 2012; Eller *et al.*, 2014; Holmes *et al.*, 2016). Tools have been developed to determine chloroplast haplotypes and nuclear genotypes, including chloroplast DNA markers, restriction fragment length polymorphisms, amplified fragment length polymorphisms and microsatellite DNA markers (Saltonstall 2002, 2003a, b; Lambertini *et al.*, 2006). Denmark hosts a garden containing 188 genotypes of *Ph. australis*, acting as a living library of plants that can be used for physiological and genetic analyses (Lambertini *et al.*, 2012). PhragNet is a network of individuals overseeing the management of 209 *Ph. australis* stands from 16 states spanning the USA and the Canadian province of Ontario, established to crowdsource

ecological and genetic investigations of native and non-native haplotypes (Hunt *et al.*, 2017). *Phragmites australis* can grow under standard glasshouse conditions in soil or hydroponics, with rhizome cuttings growing up to 2 m within 5 months, allowing rapid tissue production for growth assays and sampling (Ali *et al.*, 2002; Vasquez *et al.*, 2005). As seeds exhibit varying degrees of dormancy, protocols have been developed to increase germination efficiency, involving diurnal temperature fluctuations and high-intensity lighting (Kettenring and Whigham, 2009; Saltonstall *et al.*, 2010). For genetic manipulations, an optimized protocol has been established to generate stable *Ph. australis* transformants using agrobacterium-mediated transformation of callus tissue (Kim *et al.*, 2013). Additionally, *Ph. australis* has been successfully propagated by somatic embryogenesis (Lauzer *et al.*, 2000).

The ‘large genome constraint hypothesis’ suggests that species with smaller genome sizes are more amenable to adapting a larger range of physiological traits and expanded ecological distributions, allowing them to exploit more extreme environments (Knight *et al.*, 2005). Invasive species often have smaller genome sizes compared to non-invasive species; indeed, the genome size of the invasive European haplotype is 6.9 % smaller than native North American *Ph. australis* (Bennett *et al.*, 1998; Pandit *et al.*, 2014; Pyšek *et al.*, 2018). The European haplotype displays traits favouring invasive species characteristics, including resistance to aphid predation, low C : N ratio, long rhizomes, and an abundance of early emerging shoots, which may be linked to its smaller genome (Pyšek *et al.*, 2018). *Phragmites australis* exhibits a diversity of karyotypes, with cells containing 12 chromosomes and individuals displaying a range of euploidy and aneuploidy (Clevering and Lissner, 1999). Compared to octoploids, *Ph. australis* tetraploids grow taller with an increased abundance of stems, exhibit stronger

chemical defence mechanisms, increased water content in leaves, and support more aphids (Meyerson *et al.*, 2016a). By taking advantage of variations in ploidy and genome sizes, *Ph. australis* has been proposed as a model to study the relationship between genome size, ploidy and invasion potential, whereas these variations, coupled with long flowering periods, make it less suitable for classical genetics and more so for population genetics (Suda *et al.*, 2015).

The ‘enemy-release hypothesis’ suggests species introduced outside of their native range are less vulnerable to predation due to lack of co-evolved natural enemies (Keane and Crawley, 2002). Native North American *Ph. australis* exhibits significantly higher rates of herbivory by gallflies and aphids compared to the invasive haplotype, leading to delayed flowering time, stem chlorosis and, in some cases, whole plant death (Lambert *et al.*, 2007; Lambert and Casagrande, 2007a; Park and Blosssey, 2008; Allen *et al.*, 2015). The preference for herbivores to target native *Ph. australis* is interesting from a genetics perspective to investigate potential genes that may influence herbivory and defence.

The invasive European haplotype resists higher salinity levels compared to native *Ph. australis*, which may be linked to its invasive nature (Vasquez *et al.*, 2005). It was shown that ploidy does not affect salinity tolerance, but rather there exists a partial correlation with geographical origin, suggesting localized adaptation (Achenbach *et al.*, 2013). Furthermore, transcriptomics studies on salinity tolerance identified numerous differentially regulated genes, including the *HIGH AFFINITY K⁺ TRANSPORTER (HAK/HAT)* gene family expressed in salt-tolerant reed plants (Eller *et al.*, 2014; Holmes *et al.*, 2016). Yeast that express the *Ph. australis* gene *PhaHAK2* or *PhaHAK5* exhibit decreased potassium uptake in the presence of sodium chloride, and increased sodium permeability (Takahashi *et al.*, 2007a, b), suggesting an importance in potassium/sodium balance for salinity tolerance.

Differences in nutrient requirements, nutrient use efficiencies and biomass allocation may give invasive species competitive advantages over native species (Kroons and Hutchings, 1995; Zedler and Kercher, 2004). As nutrients are absorbed at the root–soil interface, root morphology and root system architecture play important roles in nutrient foraging, absorption and transport (Fitter *et al.*, 2002; Giehl and Wirén, 2014). Native North American *Ph. australis* develops thin, compact rhizomes with an abundance of lateral roots, whereas the invasive haplotype develops thick, long rhizomes with fewer lateral roots but increased root hair abundance (Holdredge *et al.*, 2010). Under nutrient-limited conditions, both native and invasive *Ph. australis* develop the same above-ground and below-ground biomass; however, under nutrient-rich/eutrophic conditions, the invasive haplotype grows significantly faster, with a doubling of rhizome biomass and length, and significant increase in above-ground biomass (Holdredge *et al.*, 2010). The invasive haplotype is associated with growth in soils containing higher nitrite/nitrate and ammonium, and in the native haplotypes, nitrogen assimilation is decreased at a higher rate under increasing salinity (Mozdzer *et al.*, 2010; Hunt *et al.*, 2017). These findings support the hypothesis that invasive *Ph. australis* is capable of exploiting eutrophic habitats under a wider range of environmental conditions compared to native haplotypes (Mozdzer

et al., 2010). Investigations into plant nutrition between haplotypes using molecular biology approaches may elucidate important genetic determinants for classifying and predicting invasive species.

Phragmites australis has been studied in thousands of publications from physiology and ecology perspectives; however, advances in technology and techniques now facilitate molecular and genetic investigations. It will be important to sequence and assemble the genomes of native, invasive and ancestral European haplotypes to undertake broader ‘omics’ studies and to study single genes. The establishment of mutant libraries would greatly benefit the study of *Ph. australis*, and researchers could take advantage of natural variation among the many haplotypes. The influence of epigenetics is increasingly being investigated in the field of invasion biology, including in *Ph. australis*, to help understand the invasion rates of species with low genetic diversity and monoclonal growth (Prentis *et al.*, 2008; Spens and Douhovnikoff, 2016). In North America, it was confirmed that introduced *Ph. australis* has interbred with native haplotypes, producing hybrids that maintain invasive traits (Meyerson *et al.*, 2010; Williams *et al.*, 2019). This provides another area of research into how introgression influences species invasion.

Plant parasitism: introducing Striga hermonthica (witchweed)

The other species discussed in this review are green, autotrophic plants that produce carbohydrates through photosynthesis, although parasitic plants live heterotrophically and survive from water and nutrients from host plants (Musselman, 1980). Parasites of the genus *Striga* (witchweed) cause annual losses of 293 000 tons (the equivalent of US\$117 million) in milled rice production (Rodenburg *et al.*, 2016). The dodder plant *Cuscuta* is another species that is increasingly being used to study plant parasitism (Vogel *et al.*, 2018). While *Cuscuta* infects eudicotyledonous crop plants, such as sugar beet, potato and tomato, *Striga* targets monocotyledonous plants, including the main cereal crops, such as sorghum, millet, rice and maize; therefore, we will focus our review on this parasite (Musselman, 1980; Mishra, 2009; Vogel *et al.*, 2018). *Striga* crop infestation occurs worldwide, but primarily in Africa, India, China, Indonesia and the USA (Musselman, 1980; Doggett 1987). To date, these root parasitic plants are mainly controlled by herbicide applications and by breeding host plants for resistance (Samejima and Sugimoto, 2018). However, to efficiently combat crop infestation by parasitic plants and to reduce the use of herbicides and other harmful chemicals, a better understanding of the development and physiology of parasitic plants, and especially their host plant invasion mechanisms, is urgently needed. Nevertheless, research in this area is hampered by the lack of an established model organism.

Striga’s trivial name ‘witchweed’ originated from the belief of early farmers that a *Striga*-infested host plant must be ‘bewitched’ when it exhibited drought-like symptoms for no apparent reason, as the *Striga* plant was still below ground and therefore invisible (Musselman, 1980; Runo and Kuria, 2018). The most studied *Striga* species are those with highest economic importance, including *Striga asiatica*, *Striga*

gesneroides and *Striga hermonthica*, which are the focus of this chapter (Fig. 4 shows *St. hermonthica*) (Musselman, 1980). Recently, the life cycle and parasitic characteristics have been reviewed by Runo and Kuria (2018). *Striga* seeds in the soil only germinate in the presence of a potential host plant, which they sense through a germination-inducing signal secreted by host roots (Musselman, 1980). This germination stimulant has been identified as strigolactones: a group of terpenoid lactones considered phytohormones (Cook et al., 1972; Umehara et al., 2008). Germination is first visible by the outgrowth of the radicle, often referred to as the ‘germ tube’ (Musselman, 1980). A specialized organ is then formed at the tip of the radicle, known as the haustorium, which attaches to and penetrates the host root, subsequently forming a xylem connection with the host plant (Yoshida et al., 2016). After attachment, the *Striga* seedling grows below ground and develops its first leaves. During this time, most of the damage to the host plant occurs, resulting in symptoms that resemble drought and nutrient deficiency and ultimately cause severe stunting of the host plant (Berner et al., 1995). After emerging from the soil, *Striga* produces chlorophyll and begins to photosynthesize, completing its life cycle with flowering and the production of new seeds (Fig. 4) (Berner et al., 1995). *Striga hermonthica* exhibits several favourable characteristics of a model plant. It can be grown in growth chambers and glasshouses due to its low height of around 30 cm, and has a short life cycle of 3–4 months,



FIG. 4. *Striga hermonthica*. Mature *St. hermonthica* (white arrowhead) next to its host plant (grey arrowhead). Photo credit Boubacar Kountche (KAUST).

consisting of 4–7 weeks below ground, 4 weeks from emergence to flowering, and 4 weeks to seed maturation. *Striga hermonthica* produces a high number of seeds (up to 42 000 per plant) that remain viable for over 20 years, and its attachment to host plants can be carried out in the lab in rhizotrons, as well as on agar plates (Doggett, 1987; Berner et al., 1995; Yoshida and Shirasu, 2009; Mohamed et al., 2010). Furthermore, because *St. hermonthica* is able to invade established plant models, such as rice, maize and sorghum, mutant and natural variation collections from these crops can be exploited to analyse potential mechanisms of resistance (Cissoko et al., 2011; Mbuvi et al., 2017). The model eudicot *A. thaliana* is also susceptible to *St. hermonthica*, but the parasite fails to invade the vessel elements; therefore, it may be used to study vessel element resistance and help to differentiate between attack and actual infection (Yoshida and Shirasu, 2009). Furthermore, as some *Striga* species, such as *St. hermonthica*, are cross-fertilizing, whereas others are self-fertilizing (e.g. *St. asiatica*), parasitic invasion strategies can also be studied in a context of adaptation and speciation (Safa et al., 1984).

A reference genome for *St. asiatica* is published, and the parasitic plant genome project has generated large-scale transcriptomic datasets for *St. hermonthica*, providing a comprehensive developmental expression atlas (Westwood et al., 2012; Yang et al., 2015; Yoshida et al., 2019). This includes expression data for different developmental stages, during host plant attack, and from different tissues of the adult plant. Additionally, housekeeping genes for quantitative PCR experiments have been established (Fernández-Aparicio et al., 2013). These tools can aid to identify targets for putative herbicides. To genetically manipulate *St. hermonthica*, a virus-induced gene silencing system was established, in which *Agrobacterium* transformation is used to introduce a virus-based T-DNA that activates post-transcriptional gene silencing in order to reduce expression of a target gene (Kirigia et al., 2014). For *Agrobacterium* transformation, both leaf transformation and the *Agro*-drench method can be used, which involves applying the *Agrobacterium* solution directly onto the soil adjacent to the crown part of the 3- to 4-week-old *St. hermonthica* seedling (Kirigia et al., 2014).

The recent sequencing of the *St. asiatica* genome has contributed to understanding the evolution of parasitic plants (Yoshida et al., 2019). One of the main insights supports the hypothesis that transition from autotrophic to parasitic life includes three stages: (1) Neofunctionalization of existing genes and pathways to develop the distinct parasitic organs. *Striga asiatica* has undergone at least two whole-genome duplications, allowing for the recruitment of genes for new functions, where genes for lateral root development were recruited for haustorium formation, which could be specifically useful for the formation of new xylem connections. (2) The establishment of host-dependence, which goes along with a loss of gene functions involved in photosynthesis and hormone responses. (3) The establishment of a cellular transport machinery that facilitates the transport of host resources to the parasite (Yoshida et al., 2019). Next to these findings, genome sequencing has also uncovered evidence for horizontal gene transfer, specifically of retrotransposons, indicating gene flow from hosts to the parasite (Yoshida et al., 2019).

Another milestone in understanding *Striga* infection was the discovery of the substance exuded by host plants that induces *Striga* germination, which was later found to be the phytohormone strigolactone (Cook *et al.*, 1972; Umehara *et al.*, 2008). Genome analysis revealed that the family of strigolactone receptors is highly expanded in the *Striga* genome, and many of the receptors were found to be highly expressed at the seedling stage, probably to facilitate the detection of host plants (Yoshida *et al.*, 2019). A useful tool to identify or test strigolactone receptors is the fluorescent substrate Yoshimulactone Green (YLG) (Tsuchiya *et al.*, 2015). In several plants, including *A. thaliana*, rice, and petunia, α/β -hydrolase-fold enzymes have been identified as strigolactone receptors. These proteins bind strigolactones and subsequently hydrolyse them into two fragments. YLG takes advantage of this by structurally mimicking a strigolactone, but its breakdown products include one fragment that becomes fluorescent following cleavage. This visible readout can be used to further test the putative strigolactone receptors identified in the genome, which can then be targeted by blocking agents that bind to *Striga* but not to the host's strigolactone receptors, thereby suppressing *Striga* germination (Tsuchiya *et al.*, 2015; Shahul Hameed *et al.*, 2018). Synthetic strigolactones can also be potentially utilized for so-called 'suicidal germination', in which germination stimulants are applied to the soil before planting the target crop. This causes the parasitic plant's seeds to germinate and die due to the lack of nutrients before crops are planted (Uraguchi *et al.*, 2018; Kountche *et al.*, 2019). Conversely, the engineering of crops with reduced strigolactone exudation should impair *Striga* germination, thereby reducing infection efficiency. Indeed, mutations at the *LOW GERMINATION STIMULANT 1* (*LGS1*) locus in sorghum caused a reduction in exudation of a highly active form of strigolactone, resulting in lower germination rates of *Striga* in proximity to the host plant (Gobena *et al.*, 2017).

Besides manipulating strigolactone exudation, host plants also form mechanical barriers to block the formation of a vascular connection between host and parasite (Yoshida and Shirasu, 2009). Some plants can inhibit cell wall degradation by the parasite prior to haustorium attack on the root, while others prevent penetration by accumulating blocking substances, such as the deposition of lignin that was found in the *St. hermonthica*-resistant rice cultivar 'Nipponbare' (Mutuku *et al.*, 2019). Finally, establishment of the vascular connection fails in various plant species, but the mechanism remains unknown (Yoshida and Shirasu, 2009).

A better understanding of the growth and development, as well as the physiology and invasion strategies, of parasitic plants would aid in developing better strategies for combating these agricultural pests to reduce yield losses. Because *A. thaliana* is resistant to *St. hermonthica* invasion, the library of established marker lines available for *A. thaliana* could be tested for their role in *St. hermonthica* resistance, including markers for developmental genes, resistance genes or genes involved in cell wall integrity sensing. Furthermore, natural variation among *Arabidopsis*, but also susceptible host crops, can be exploited to find accessions with enhanced or reduced tolerance, which might be correlated with changes in the genome or epigenome, and could help to get a better understanding of naturally evolved resistance. From a developmental perspective, it

will be exciting to analyse the organ formation of the parasite, because it is unclear how the transition from a root-like organ to a haustorium takes place. This transition is crucial for the xylem connection to the host plant that provides water and nutrients to the parasite. Interestingly, *St. hermonthica* does not respond to the phytohormone abscisic acid (ABA), which controls stomata closure, and is thereby able to maintain a high transpiration rate also under drought conditions, favouring its parasitic behaviour (Fujioka *et al.*, 2019). Because other members of the Orobanchaceae are sensitive to ABA, this attribute makes *Striga* outstanding even among other root parasitic plants and interesting as a subject to study physiological questions.

Salt tolerance: introducing Eutrema salsugineum (salt cress)

Soil salinity, the contamination of otherwise fertile soil with salt cations, is a major problem for agriculture worldwide (Shabala, 2013). Soil salinity is now estimated to affect ~50 % of irrigated land, resulting in massive losses in agricultural production (Shabala, 2013). To combat this problem, research has focused on improving the salt tolerance of crop plants; however, most research in understanding the molecular basis of salt tolerance is conducted on the model plant *A. thaliana*, which is a glycophyte (meaning that it is salt-sensitive; Bressan *et al.*, 2001). To fully understand salt tolerance, a halophyte model is needed (a plant that has already evolved salt tolerance) allowing researchers to study and learn from this plant's adaptation to saline environments (Bressan *et al.*, 2001). To this end, the salt cress *Eutrema salsugineum* (formerly *Thellungiella salsuginea* or *Thellungiella halophila*) was suggested as a new model plant (Bressan *et al.*, 2001).

Eutrema salsugineum is thought to have originated in the Shandong province of China, from where it spread to north-east Asia, across the Bering Strait to north-west Canada, and then along the Rocky Mountains into the USA (Wang *et al.*, 2015; German and Koch, 2017). In the lab, work has been done with plants originating from Yukon, Canada (Fig. 5A), and Shandong, China (Fig. 5B) (Koch and German, 2013). *Eutrema salsugineum* was identified through its ability to thrive under extreme conditions, such as drought, salinity and frost, as well as by its morphological similarity to *A. thaliana* (Fig. 5) (Bressan *et al.*, 2001). It has a short life cycle of ~2–3 months, is self-fertile, produces around 4000–8000 seeds, can be efficiently transformed using the floral-dip method, and can be ethyl methanesulfonate (EMS)-mutagenized (Bressan *et al.*, 2001; Inan *et al.*, 2004). It also has a small genome (~260 Mb, double that of *A. thaliana*), consisting of seven chromosomes with an average coding sequence identity of ~92 % to *A. thaliana* (Bressan *et al.*, 2001; Inan *et al.*, 2004; Wu *et al.*, 2012). However, in contrast to *A. thaliana*, *E. salsugineum* supposedly has an obligate vernalization requirement of ~3 weeks in order to flower, which was confirmed for the Yukon accession, whereas the Shandong accession flowered without a vernalization step (Bressan *et al.*, 2001; Guo *et al.*, 2012; M. Somssich *et al.*, unpubl. data). Furthermore, *E. salsugineum* is able to withstand a salinity shock of up to 500 mM NaCl, whereas *A. thaliana* is already sensitive to 100 mM (Bressan *et al.*, 2001; Inan *et al.*, 2004).



FIG. 5. *Eutrema salsugineum*. *Eutrema salsugineum* plants of the Yukon (A) and Shandong (B) natural accessions.

To do so, *E. salsugineum* has evolved several morpho-physiological mechanisms: stomata in *E. salsugineum* leaves are present at higher density when compared to those of *A. thaliana*, but their conductance is lower and they respond to salt stress by closing more tightly, leading to lower transpiration rates (Inan et al., 2004). The leaves are also more succulent-like, with a second layer of palisade mesophyll cells, and they are frequently shed during extreme salt stress (Inan et al., 2004). The roots develop additional layers of endodermis and cortex cells in order to restrict ion movement towards the vasculature, thereby limiting salt uptake during salt exposure (Inan et al., 2004). Curiously, germination is actually impaired in *E. salsugineum* when grown on high-salt medium, compared to *A. thaliana*, probably to delay germination during unfavourable conditions (Inan et al., 2004). In addition to this increased salt tolerance, *E. salsugineum* also has a higher cold tolerance, being able to survive a cold shock of -15°C , and is also more tolerant to phosphate starvation (Inan et al., 2004; Velasco et al., 2016).

Because *E. salsugineum* was suggested as a potential model for salt tolerance, several labs have focused on ‘omics’ approaches to characterize the plant, resulting in several datasets that are now available to the community. Two draft genomes (using Sanger and Illumina sequencing) and the chloroplast genome are available (Wu et al., 2012; Yang et al., 2013; Guo et al., 2016). Microarray and expressed sequence tag transcriptomes, and proteome datasets from non-stressed plants and plants that were exposed to cold, drought and salt stress have also been published (Wong et al., 2006; Zhang et al., 2008; Pang et al., 2010). Genome-wide characterization of miRNAs was performed using high-throughput sequencing, and genes differentially regulated after salt stress were identified (Zhang et al., 2013). Metabolomic datasets are available for control plants and plants that were exposed to osmotic stress alongside an *A. thaliana* metabolome for comparison (Lugan et al., 2010). Metabolomics and transcriptomics data were also generated for the Yukon accession with salt-stressed plants grown in growth chambers under a controlled environment or in their natural habitat (Guevara et al., 2012). RNA-sequencing datasets were generated for a comparative study of the Yukon and Shandong accessions (Champigny et al., 2013). Two studies describe the identification and expression analysis of aquaporin family proteins that regulate water conductivity and could be important for the salt tolerance of *E. salsugineum* (Qian et al., 2019; Qin et al., 2019). To identify shoot- or root-derived signals that are important for salt tolerance, grafting experiments between *A. thaliana* and *E. salsugineum* have also been successfully performed (Y. Li et al., 2019). Finally, the methylome of *E. salsugineum* is also available (Bewick et al., 2016). Several of these resources, especially protocols and genome datasets, were made available early on the thellungiella.org webpage and via the plant genomics portal Phytozome.

Salt stress is a combination of ionic and osmotic stress (Lugan et al., 2010). Successful adaptation to these conditions involves four interacting basic signal perception–response systems: ion homeostasis, osmotic adjustments, injury avoidance and growth changes (Zhu, 2001). Data for *E. salsugineum* give some indications on how this species has adapted to such conditions. On the genetic level, several candidate genes potentially involved in salt stress adaptation were identified. Interestingly, some EMS mutants of *E. salsugineum* with decreased salt tolerance follow a single-locus genetic segregation pattern, indicating that individual loci can contribute significantly to salt tolerance (Inan et al., 2004). Two examples that were studied in closer detail are the *LATE EMBRYOGENESIS ABUNDANT PROTEIN 1 (LEA1)* and the *MOLYBDENUM COFACOR SULFURASE 1 (Mcsu1)* genes (Zhang et al., 2012; Zhou et al., 2015). *LEA1* was upregulated under salt stress conditions, and ectopic overexpression of the *E. salsugineum LEA1* gene in *A. thaliana* and yeast was shown to increase the salt tolerance of both organisms (Zhang et al., 2012). Similarly, overexpression of *E. salsugineum Mcsu1* increased drought tolerance in transgenic alfalfa plants in an ABA-dependent manner (Zhou et al., 2015). Several genes that are known to be salt stress-associated in *A. thaliana* are constitutively expressed at higher levels in *E. salsugineum*, and are further induced under stress (Inan et al., 2004). Interestingly, when comparing the transcriptomes of Yukon *E. salsugineum* plants grown in

their natural Yukon habitat or under controlled conditions in a growth chamber, there was a difference in both gene expression and phenotype (Guevara *et al.*, 2012). Furthermore, there was comparatively little overlap in gene activation in response to natural occurring drought and drought treatment (Guevara *et al.*, 2012). The transcriptomes of the Yukon and Shandong accessions grown in their natural environment did not display drastic differences; however, among the differentially regulated genes were several stress-related genes, which could help to differentiate between genes involved in salt- and cold-stress adaptation, because the latter would be required primarily in Yukon plants (Champigny *et al.*, 2013). Concerning osmotic stress, a comparison of the metabolomes of *A. thaliana* and *E. salsugineum* did not reveal any major differences in activated pathways, but rather quantitative differences (Lugan *et al.*, 2010). Overall, *E. salsugineum* seems to cope better with dehydration, for example through stabilization of the shoot to soil water gradient, or through adjustments in water solubility and polarity of their metabolites (Lugan *et al.*, 2010). Furthermore, two proteins of the dehydrin family were implicated to be involved in cytoskeleton-stabilization during drought stress, to improve dehydration tolerance (Rahman *et al.*, 2011).

Regarding ion homeostasis under salt stress conditions, halophytes are typically classified as either ion excluders or accumulators (Hasegawa *et al.*, 2000); however, the ability to tightly regulate the uptake and distribution of salt ions within the plant seems to be a key attribute of halophytes (Hasegawa *et al.*, 2000). Importantly, *E. salsugineum* can discriminate between sodium and potassium ions during salt stress, and has two barriers to control salt uptake: one at the root–soil interface, and another particularly strong one, at the site of xylem-loading, preventing salt entry and transport into the shoot and above-ground organs (Volkov *et al.*, 2004; Volkov and Amtmann, 2006). At the site of xylem-loading, sodium and potassium translocation is negatively correlated in several plants, meaning loading of sodium into the xylem was paralleled by unloading of potassium (Volkov *et al.*, 2004). This connection seems to be lost in *E. salsugineum*, where potassium can be translocated independently of sodium (Volkov *et al.* 2004). One of the main sites for sodium deposition under salt stress conditions are the old leaves of *E. salsugineum*, which appear to act as a salt sink (Vera-Estrella *et al.*, 2005).

The large amount of ‘omics’ data available for *E. salsugineum* provide several starting points for new research projects, and the close relationship to *A. thaliana* should allow the use of standard molecular tools, such as fluorescent reporters. One tool that is lacking is a mutant plant collection, such as the T-DNA collections for *A. thaliana*, although the two draft genomes in combination with the CRISPR/Cas9 system may allow the generation and study of specific mutants. Accordingly, interesting candidate genes, identified by mining of the available ‘omics’ datasets, could be easily tested. Such candidates could then be expressed in *A. thaliana* to test if they can improve the salt tolerance of this glycophyte, before moving on to crop plants. However, with these large-scale datasets readily available, it appears that an integrated systems biology approach would be an especially interesting way to characterize salt tolerance on a whole system level. While manipulating individual genes can already cause specific effects, to really engineer salt-tolerant

crop plants it must be assumed that the plant has to be comprehensively reprogrammed.

Comparative development: introducing Cardamine hirsuta (hairy bittercress)

Over the course of the last two decades, comparative development studies between different Brassica species have become a useful tool to uncover molecular mechanisms underlying morphological variability. While the success of *A. thaliana* as the main model system to research plant development is apparent, the study of developmental mechanisms governing morphological traits, such as compound leaf development, formation of multiple cortical cell layers or explosive pod shattering, cannot be performed in this species. Therefore, close relatives of *A. thaliana* that have evolved these distinct morphological or ecological features have been adopted as new models to allow for comparative analyses (Hay and Tsiantis, 2016). *Cardamine hirsuta* was among the earliest plants adopted for this reason (Fig. 6) (Hay and Tsiantis, 2016). Initially chosen to uncover the molecular mechanisms controlling leaf shape variability, and more precisely the evolution of complex leaves from simple leaves, *C. hirsuta* has since proven to be an interesting model for several developmental processes, thereby making it a complementary development model next to *A. thaliana* (Hay and Tsiantis, 2016; di Ruocco *et al.*, 2018b). *Cardamine hirsuta* is endemic to Europe and North Africa, but several populations are also found on Atlantic islands and in North America, although these populations were only recently introduced (Hay *et al.*, 2014). The genus name *Cardamine* is derived from the Greek ‘Kardamon’ (*Nasturtium*), owing to its similar taste, whereas the species name *hirsuta* is derived from the Latin word for ‘hairy’, due to the massive presence of trichomes and root hairs on the plant. *Cardamine hirsuta* is a close relative of *A. thaliana*, but it exhibits morphologically divergent traits from its famous relative, such as compound leaves, pod shattering, and altered root anatomy and trichome morphology. Studies on fossils estimated that the lineages of *C. hirsuta* and *A. thaliana* diverged roughly 14 million years ago, a moderately short time in terms of species divergence (Beilstein *et al.*, 2008, 2010; Couvreur *et al.*, 2010). Among the *A. thaliana* relatives, *C. hirsuta* stands out because it shows the important characteristics of a model system, including a small diploid genome (196 Mb) on eight chromosomes, being self-compatible, possible clonal propagation, a short life cycle of 3–4 months, and with abundant seed set (Hay *et al.*, 2014). Furthermore, the availability of the complete genome sequence allows studies on large-scale genomic rearrangements, which have driven the evolution of specific traits (Monniaux *et al.*, 2018). Production of transgenics is also simple, as *C. hirsuta* can be transformed by the *Agrobacterium tumefaciens*-based floral dip method, albeit with a lower efficiency (~35 %) when compared to *A. thaliana* (Clough and Bent, 1998; Hay *et al.*, 2014). All of these characteristics make *C. hirsuta* a suitable counterpart to *A. thaliana* for exhaustive and unbiased parallel genetic studies of intraspecific phenotypic variability. The *C. hirsuta* genome has recently been sequenced and annotated, simplifying genetic analysis, genome-wide characterization studies and cloning (Hay and Tsiantis, 2006;



Fig. 6. *Cardamine hirsute*. Four-week-old *C. hirsuta* plant. Scale bar = 1 cm.

Barkoulas *et al.*, 2008; Gan *et al.*, 2016). In conjunction with this, it is now possible to easily perform tissue/organ-specific RNA sequencing (Gan *et al.*, 2016). Transcriptome data of leaf, fruit and simulated shade-treated plants are available on the *C. hirsuta* genome assembly website (<http://chi.mpipz.mpg.de/assembly.html>). The ease of genetic tractability in *C. hirsuta* enables agile genetic screens and gene expression analyses. Several mutant lines for genes involved in root, leaf and flower development are available, as well as fluorescent markers, such as the auxin signalling marker *DR5::3XVENUS* and the cortical marker *CO2::3xVENUS* (di Ruocco *et al.*, 2018a). Moreover, the use of artificial miRNA or engineered nucleic molecules targeting endogenous miRNA have also been established in *C. hirsuta* to knock down gene activity or miRNAs, respectively (Schwab *et al.*, 2010; Todesco *et al.*, 2010; Rubio-Somoza *et al.*, 2014). The relative recent divergence of *A. thaliana* and *C. hirsuta* not only allows the utilization of most molecular biology and genetics tools developed for *A. thaliana*, but also permits clonal analysis experiments in a comparative context. Methodologies to acquire high-resolution images of cellular organization in *C. hirsuta* organs have been developed for *in silico* analysis, cell tracking and growth quantification via specialized software such as Morphographix (Vlad *et al.*, 2014; Barbier de Reuille *et al.*, 2015; Kierzkowski *et al.*, 2019). Furthermore, the availability of several different natural *C. hirsuta* accessions permits quantitative trait loci analyses, an important tool to study the basis of intraspecific morphological diversity (Hay *et al.*, 2014; Cartolano *et al.*, 2015). While there are several morphologically divergent traits separating *C. hirsuta* and *A. thaliana*, research has predominantly focused on leaf shape, pod shattering and root anatomy. While *A. thaliana* exhibits

simple leaf morphology, *C. hirsuta* carries compound leaves, which develop a lamina dissected into discrete units called leaflets (Fig. 6) (Hay and Tsiantis, 2016). It was found that several *C. hirsuta* orthologues of meristem-specific *A. thaliana* genes are expressed in *C. hirsuta* leaves (Blein *et al.*, 2008; Hasson *et al.*, 2010; Rast-Somssich *et al.*, 2015). This includes members of the Class I and II *KNOX*, *PLETHORA* and *CUP SHAPED COTYLEDON* gene families (Blein *et al.*, 2008; Hasson *et al.*, 2010; Rast-Somssich *et al.*, 2015). Indeed, knock down of those genes in *C. hirsuta* leads to leaf simplification, whereas their ectopic expression in *A. thaliana* leaves enhances leaf complexity (Hay and Tsiantis, 2006; Blein *et al.*, 2008; Rast-Somssich *et al.*, 2015; Gan *et al.*, 2016). Another fundamental regulator for compound leaf development identified in *C. hirsuta* is the *REDUCED LEAF COMPLEXITY* (*RCO*) transcription factor, which is a paralogue of the *A. thaliana* *LATE MERISTEM IDENTITY* (*LMI*) (Vlad *et al.*, 2014). *RCO* is derived from an *LMI* gene duplication event in an ancestor of *C. hirsuta* and is conserved in all brassicas with compound leaves (Vlad *et al.*, 2014). The *RCO* gene was lost in more recent species with simple leaves, such as *A. thaliana* (Vlad *et al.*, 2014). *LMI* and *RCO* show complementary expression domains in *A. thaliana* and *C. hirsuta*, where *LMI* is expressed in terminal and lateral leaflet margins. Conversely, *RCO* is expressed only at the base of terminal and lateral leaflets, where it locally represses growth, thereby dissecting the leaf and allowing the leaflet to form (Vlad *et al.*, 2014; Vuolo *et al.*, 2018; Kierzkowski *et al.*, 2019). It was recently shown in *A. thaliana* that *LMI* controls leaf growth via regulation of endoreduplication timing. In the future, it will be interesting to understand whether *RCO* represses growth at the margin of the leaflet, controlling cell endoreduplication via *LMI1*, or whether *RCO* controls other pathways to repress growth.

More recently, *C. hirsuta* was adopted to study the genetic differences underlying seed dispersal mechanisms (Hofhuis *et al.*, 2016). *Cardamine hirsuta* disperses its seeds through explosive pod shattering, a mechanism used by some angiosperm species to launch seeds far from the parent (Hofhuis *et al.*, 2016). Using *C. hirsuta* as a model, it was shown that explosive pod shattering depends on the asymmetrical deposition of lignin in the secondary walls of cells in the silique's endocarp, in combination with an increase in turgor pressure (Hofhuis *et al.*, 2016). Rapid expansion of the exocarp cells, followed by an increase in turgor, and the inflexibility of the endocarp cells induce a coiling of the valves and launching of the seeds (Hofhuis *et al.*, 2016).

Work on *C. hirsuta* has also expanded our understanding of the genetic basis underlying the differences in root anatomy (di Ruocco *et al.*, 2018a, b). The cortex is a fundamental root tissue for plant life as its secondary growth helps plants to cope with different environmental conditions, such as wet lands or cold weather (di Ruocco *et al.*, 2018b). The number of cortical layers can range from one to several, representing a paradigmatic example of interspecific anatomical variability (di Ruocco *et al.*, 2018a). *Cardamine hirsuta* roots have two cortical layers (an outer and an inner one) whereas *A. thaliana* roots have only one (di Ruocco *et al.*, 2018a, b). Comparing cortical development of *A. thaliana* and *C. hirsuta* allows for studying the basis of these anatomical differences. The cortex

and endodermis of *A. thaliana* roots emerge through an asymmetric cell division of a stem cell daughter, called the cortex and endodermis initial (CEI) (di Ruocco *et al.*, 2018a; di Mambro *et al.*, 2018). This patterning mechanism is partially based on the miRNA165- and miRNA166- (miR165/6) dependent exclusion of *HOMEODOMAIN LEUCINE ZIPPER III* (*HD-ZIPIII*) transcription factor expression in the CEI, cortex and endodermis cells (Carlsbecker *et al.*, 2010). In *C. hirsuta*, miR165/6 activity is confined to the cortex and endodermis, but is absent from the CEI, resulting in the CEI giving rise to a cortex cell and a cell with mixed cortex and endodermis identity, called CEM (di Ruocco *et al.*, 2018a, b). The CEM cells undergo a second asymmetric division, producing the endodermal layer and an inner cortical cell layer (di Ruocco *et al.*, 2018a, b). Hence, a differential distribution of miR165/6 activity underlies the variability of cortical cell layers between *A. thaliana* and *C. hirsuta*. It will now be interesting to understand how this diverse distribution of miR165/6 is generated and how HD-ZIPIIIs regulate the asymmetric cell divisions.

Cardamine hirsuta has been useful in shedding light on developmental questions that could not be answered utilizing only *A. thaliana* as the sole plant development model. The use of the CRISPR/Cas9 system, together with the generation of *ad hoc* suppressor screens, will probably allow the discovery of additional genetic networks underlying the development of species-specific morphological traits. Nowadays, several molecular mechanisms governing characteristic morphological traits of *C. hirsuta* are starting to be unveiled. In the future, it will be interesting to understand whether the knowledge acquired from *C. hirsuta* can be extrapolated to phylogenetically distant species having similar morphological traits.

Legume crops: reintroducing Pisum sativum (pea)

The broad genetic diversity within the family Fabaceae offers a wealth of material to optimize crops for the changing climate. Intraspecific (gene pool) diversity allows optimization through breeding, whereas diversity in environmental tolerances between species may help by giving options for alternative crop traits. However, compared with cereals, legumes have been largely neglected by gene technology (Considine *et al.*, 2017). *Pisum sativum* (pea) is the oldest ‘model’ legume, but comparatively little investment has been made toward pea research. This is expected to change due to the recently published genome, which will bring pea into the genomic era (Kreplak *et al.*, 2019). To some degree, this recent lack of investment has to do with pea being part of the non-model plant model group, which does not carry the typical characteristics of a good model system. Due to pea’s agricultural importance and the fact that humans have been optimizing it for centuries through breeding and research makes it more applicably relevant than traditional model plants. Pea and several other classical models became problematic to work with once the era of molecular genetics arrived, for several reasons. The pea genome is large (~4.45 Gb) and highly complex, with up to 97 % being repetitive DNA composed of transposable elements (Macas *et al.*, 2007; Kreplak *et al.*, 2019). This presented too great a challenge for early genome sequencing and assembly approaches

for pea, and eventually resulted in the adoption of *Medicago truncatula* and *Lotus japonica* as model legume species (Barker *et al.*, 1990; Cook, 1999; Stougaard, 2014). There has long been a battle between the two systems to be the universally accepted legume model. Work on both persists (especially for symbiosis genetics); however, they both have their practical disadvantages and have proven to be difficult plants to work with in the lab. *Medicago truncatula* and *L. japonica* do not have the century-old background of research that pea possesses, and unlike pea, are not seed-crop plants. With the advent of ‘next-generation’ techniques, such as advanced whole-genome sequencing approaches and modern cloning techniques, the problems that hampered pea research since the emergence of molecular biology and genetics in the 1980s have now been overcome (Smykal *et al.*, 2012; Kreplak *et al.*, 2019). Due to these developments, *P. sativum*, one of the first plants studied by geneticists, has finally arrived in the genome era of plant science, and has become the most well-characterized legume used in plant biochemistry and physiology (Meisrimler *et al.*, 2016).

Pea (Fig. 7) has a long history of scientific investigation that dates back to its use by Thomas Andrew Knight in the 1790s, and more famously by Gregor Johann Mendel in the 1860s in early studies of inheritance (Mendel, 1865; Shull and Fisher Stanfield, 1939). Ellis *et al.* (2011) nicely illustrate the molecular nature of some of Mendel’s results (also reviewed by Reid and Ross, 2011). Pea was prominent early on as a genetic biochemical model, particularly for seed embryo biology and hormonal control of plant growth, differentiation, and plant architecture, due to its predictable, well-characterized growth habit and developmental staging (Marinos, 1970; Knott, 1987; Wang and Hedley, 1991; Sauer *et al.*, 2006; Gomez-Roldan *et al.*, 2008; Balla *et al.*, 2011). In more recent times, pea has proven valuable for studying morphological and developmental processes, such as flowering time control and circadian rhythms (Hecht *et al.*, 2007; Weller and Ortega, 2015). In addition, the high agronomical relevance of nitrogen-fixation in the root nodules of legumes is an area of great interest due to the reduction in fertilizer requirement (Hirsch, 1992; Beckie and Brandt, 1997; Scharff *et al.*, 2003). The pea diploid genome is roughly 10× larger than that of *Medicago truncatula*, but when discounting the repetitive DNA sequences, the exomic component of the pea genome is actually smaller than that of *Medicago truncatula*, with an estimated 45 000 and 62 000 genes, respectively (Macas *et al.*, 2007; Tang *et al.*, 2014; Sudheesh *et al.*, 2015; Kreplak *et al.*, 2019). The large structure of the pea flower makes for easy emasculation and crossing without the magnification aid required for *A. thaliana* or *Medicago truncatula*. Flowers remain closed, and efficient self-fertilization thus occurs without the need for a pollinator species. This also makes the flowers ideal for controlled, manual cross-pollination, as the unopened flower buds have receptive stigmas and undehisced anthers that are easy to remove. Newly opened flowers provide an abundance of brightly coloured, self-adhering pollen for crossings. Dwarf varieties can be employed in a research setting for cultivation in small cabinets and glasshouses, using only simple tying or staking to manage individuals (Ross and Reid, 1991). The pea life cycle from germination to harvest takes from 8 to 12 weeks (Mobini and Warkentin, 2016). Most common laboratory varieties are domesticated forms that have indehiscent



FIG. 7. *Pisum sativum*. *Pisum sativum* can be easily maintained and studied in a controlled, laboratory or glasshouse setting. Simple tying and twisting of the plants as they grow allows for easy comparison of their physiology.

Pods, allowing fruit and seed to be left to desiccate on the plant and easily collected (Weeden *et al.*, 2002). Pea has a significant advantage over typical lab models such as *A. thaliana*, or field-suitable models such as maize, as pea is suitable for growth in the field, glasshouse, growth chambers and tissue culture environments.

A fully annotated and assemble genome sequence for *Pi. sativum* ‘Caméor’ has been published recently, thanks to the rapid evolution of next-generation sequencing technologies, bridging the gap between classical ‘model’ plants and crop plants (Kreplak *et al.*, 2019). A large number of pea gene-based molecular markers have been designed and a comprehensive map of key trait-associated genes in the pea genome has been constructed using molecular markers and cDNA cloned for comparative mapping studies. Kulaeva *et al.* (2017) have combined the molecular pea markers into one user-friendly online tool: the Pea Marker Database (PMD). With the published genome, opportunities for gene-discovery, characterization of known and unknown mutants, and genomic-assisted crop improvement are now immense. Pea seeds are amenable to EMS mutagenesis, and extensive collections of TILLING mutants of both ‘Caméor’ and ‘Terese’ *Pi. sativum* cultivars with phenotypic and sequence data are available through UTILLdb (Triques *et al.*, 2007; Dalmais *et al.*, 2008; Sharma *et al.*, 2009). Pea transcriptomes and proteomes are published and annotated using the genomes of *Medicago truncatula* and other sequenced model species (Schiltz, 2004; Bourgeois *et al.*, 2009; Franssen *et al.*, 2011; Alves-Carvalho *et al.*, 2015). The pea chloroplast genome has also been sequenced, which provides information that can be used for both evolutionary and transgenic applications (Magee *et al.*, 2010). Worldwide germplasm collections provide a wealth of diverse genetic material for crop breeding and optimization, with over 6000 accessions being listed on the USDA National Plant Germplasm System (Smykal *et al.*, 2011; United States Department of Agriculture *et al.*, 2019). Pea is

amenable to genetic transformation using *Agrobacterium*-mediated transformation of different sources of initial explants, such as protoplasts, lateral cotyledonary meristems, or segments of nodes, epicotyls and embryonic axis, but like many other legumes, optimization of transformation efficiency remains a challenge due to recalcitrance to post-transformation regeneration (Puonti-Kaerlas *et al.*, 1990; Kathen and Jacobsen, 1993; Schroeder *et al.*, 1993; Bean *et al.*, 1997; Grant *et al.*, 1998; Grant and Cooper, 2003). With pea growing in interest as a favourable legume research species, more research should be invested in improving transformation as a genetic tool. Particular interest is being paid to reduce the length of the breeding cycle in pea (Mobini and Warkentin, 2016). Termed ‘speed breeding’, this research aims to overcome the longer life cycles of typical crop plants through the manipulation of growth conditions and hormonal application, and has been proven to work efficiently for pea (Watson *et al.*, 2018).

Pea was one of the first plants to be domesticated. This brings the benefits of thousands of years of selection for favourable traits of a crop plant, which also benefits its candidature as a strong model plant (Mikić *et al.*, 2014). For example, beneficial traits include high-yielding seed pods that all mature around the same time and do not shatter, and a predictable growth habit and determinate growth (Weeden, 2018). Pea provides biological information not accessible with other models such as *A. thaliana*. The well-characterized life-cycle stages and caulescent habit (cf. the rosette of *A. thaliana*) can make many types of physiological measurements easier, and allows for detailed physiological manipulations, such as studying shoot branching, axillary bud formation, compound leaf development and coiling of tendrils (Jaffe and Galston, 1966; Ingram *et al.*, 1984; Knott, 1987; Beveridge, 2000; Yaxley *et al.*, 2001). Pea produces a compound inflorescence consisting of lateral secondary inflorescences, making it an interesting plant from a floral development perspective (Ferrándiz *et al.*, 1999). The ability to graft

and extract phloem and xylem sap provides a platform to study whole-plant physiological processes, such as nutrient uptake, long-distance communication through hormones, mRNA and protein signals, and even epigenetic control (Urquhart and Joy, 1981, 1982; Lexa and Cheeseman, 1997; Beveridge *et al.*, 1997; Kabir *et al.*, 2013). Pea provides the benefits of researching a legume seed crop and having direct agronomic application for seed crops, without the issues of genome duplication that has occurred in soybean (Schmutz *et al.*, 2010). Pea offers insight into the 18 000+ other legume species, many of which we rely on for food and pasture (Graham and Vance, 2003). Finally, legumes play a pivotal role in crop rotation, with the symbiotic bacteria in the nitrogen-fixing root nodules providing bio-available nitrogen, thereby minimizing fertilizer requirements and the associated cost and environmental impact (Courty *et al.*, 2015). The wealth of historical research, combined with the recently published genome waiting to be fully utilized, means pea promises a breadth of information vital for key biological processes that have applications for yield, fruit set and low-input farming systems, thereby contributing to food security and improving sustainable agricultural practices.

OUTLOOK AND CONCLUSIONS

The new models discussed here have been around for several years and are well established. There are, however, several more plant species that have either already been established or were proposed as new models to answer even more specific scientific questions. In the final part of this work, we would like to give a brief mention to some of the fascinating plants that were not included here, mainly due to space constraints, but that could be part of a future wave of plant models. *Boechera* (rockcress) and *Erythranthe guttata* (yellow monkeyflower) allow the study of genotypic and phenotypic trait variations among natural populations, while *Silene latifolia* (white campion) is an interesting model to study the evolution of sexual plant systems (Bernasconi *et al.*, 2009; Rushworth *et al.*, 2011; Yuan, 2018). *Azolla* and *Ceratopteris* have been suggested as model ferns, as have the duckweeds *Lemna minor* and *Spirodela polyrrhiza* for aquatic plant life and phytoremediation (Gupta and Prakash, 2013; Sessa *et al.*, 2014). *Capsella rubella* has also been studied for some time, and is used to investigate plant reproductive biology (Guo *et al.*, 2009). *Hibiscus trionum* (Venice mallow) is an interesting new model to study pollinator attraction, while *Utricularia gibba* (floating bladderwort) is an interesting model for the evolution of carnivorous plant life and three-dimensional plant form, as well as genome biology (Vignolini *et al.*, 2015; Renner *et al.*, 2018; Whitewoods *et al.*, 2020).

There are also some very interesting recent developments regarding other non-model plant models. Similar to the case of *Pi. sativum* that we have described in this paper, research on *Triticum aestivum* has also been hampered by the enormous complexity of the plant's hexaploid genome. On top of that, the space and time required to grow wheat over multiple generations have proven to be significantly problematic in carrying out research. The past year has seen two giant leaps taken to improve these conditions. First, the speed breeding technique has accelerated plant growth speed, thereby decreasing generation time and accelerating research (Ghosh *et al.*, 2018; Watson *et al.*, 2018). Additionally, the publication of the annotated wheat genome has provided the basis

for full genetic and genomic work [International Wheat Genome Sequencing Consortium (IWGSC) 2018]. Of all the non-model plant models, rice is probably the most developed one to date, although a major problem that persists is the propagation of such a big plant in the confined space of a research laboratory. Publication of the 'Xiaowei' germplasm now aims to eliminate this issue (S. Hu *et al.*, 2018). 'Xiaowei' is a dwarf mutant of the *japonica* and *indica* rice varieties, which is 30 % smaller than the wild type varieties and exhibits a shorter growth period, lower biomass and improved space utilization (S. Hu *et al.*, 2018). As such, it should be suitable for large-scale indoor experiments before moving on to the standard rice varieties and field studies. Finally, *Nature Plants* has recently announced the return of the snapdragon, referring to the genus *Antirrhinum*, which has been a very important plant model throughout the 20th century to specifically study flower development (Schwarz-Sommer *et al.*, 2003; *Nature Plants*, 2019). The recent publication of its genome might reignite interest to study *Antirrhinum majus* as a model for flower development and genome architecture (Schwarz-Sommer *et al.*, 2003; M. Li *et al.*, 2019).

In conclusion, the expansions in the set of available plant models represents a paradigm shift in plant research. The 19th and 20th centuries were mostly defined by the use of non-model plant models to study agriculturally relevant or phenotypically interesting traits. Following the adoption of *A. thaliana* as the primary plant model, plant science entered the era of molecular biology and genetics, in which traits could be studied at the molecular level. With the availability of new 'omics' tools, new plant models are added to our collection at an unprecedented speed, and old non-model plant models are, in many regards, elevated to proper model system status. With these recent developments, we will draw closer to eventually understanding plant life with all its different aspects and facets.

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