*Annals of Botany* **126**: 1–23, 2020
doi: 10.1093/aob/mcaa063, available online at www.academic.oup.com/aob

ANNALS OF BOTANY
Founded 1887

REVIEW

# Plant science's next top models

**Igor Cesarino[1,‡,a], Raffaele Dello Ioio[2,‡,b], Gwendolyn K. Kirschner[3,†,‡], Michael S. Ogden[4,5,‡,c], Kelsey L. Picard[6,‡,d], Madlen I. Rast-Somssich[7,‡,e] and Marc Somssich[4,*,‡,f]**

*[1]Department of Botany, Institute of Biosciences, University of São Paulo, Rua do Matão 277, 05508-090, Butantã, São Paulo, Brazil, [2]Dipartimento di Biologia e Biotecnologie, Università di Roma La Sapienza, Rome, Italy, [3]University of Bonn, Institute of Crop Science and Resource Conservation (INRES), Division of Crop Functional Genomics, 53113 Bonn, Germany, [4]School of BioSciences, University of Melbourne, Parkville, 3010, VIC, Australia, [5]Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm 14476, Germany, [6]School of Natural Sciences, University of Tasmania, Hobart, 7001, TAS, Australia and [7]School of Biological Sciences, Monash University, Clayton Campus, Melbourne, 3800, VIC, Australia*
*[a]@CesarinoIgor, [b]@DelloRaffaele, [c]@itsMikeOgden, [d]@KelseyOnScience, [e]@MSomssich, [f]@somssichm*
*[†]Present address: King Abdullah University of Science and Technology (KAUST), Biological and Environmental Sciences and Engineering (BESE), Thuwal, 23955-6900, Saudi Arabia.*
**For correspondence. E-mail: marc.somssich@unimelb.edu.au*
*[‡]All authors contributed equally; the order is alphabetical.*

Received: 31 October 2019 Returned for revision: 24 January 2020 Editorial decision: 3 April 2020 Accepted: 8 April 2020
Electronically published: 9 April 2020

• **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 $C_4$ 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.*, 2016*b*). 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.*, 2016*b*). In land plants, both the gametophyte (*n*) and the sporophyte (2*n*) produce multicellular bodies (Bowman *et al.*, 2016*b*). 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 mosre closely resembling the charophycean algae (Bowman *et al.*, 2016*b*). 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.*, 2016*b*). 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.*, 2016*a*).

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,

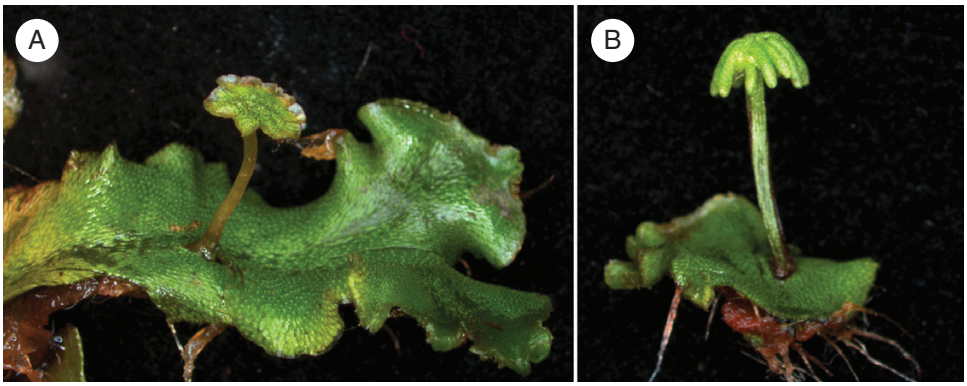


FIG. 1. *Marchantia polymorpha. Marchantia polymorpha* produces a haploid thallus with either (A) a male antheridiophore containing antheridia with flagellated sperm, or (B) a female archegoniophore with archegonia holding an egg. Upon fertilization, the diploid sporophyte undergoes mitosis followed 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 gemmae 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.*, 2013*a*, 2015). In addition, CRISPR/Cas9-based targeted mutagenesis has been demonstrated to work efficiently; however, the haploid dominancy 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 *Cre*/*lox*P 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, 2013*b*; 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.*, 2013*b*, 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 HOMEOBOX* (*KNOXI*) transcription factor (Lee *et al.*, 2008; Bowman *et al.*, 2016*b*; 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.*, 2016*b*; 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).

### *$C_4$ 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 $C_4$ photosynthesis (Schuler *et al.*, 2016). The productivity of several grasses used for food and bioenergy is driven by $C_4$ photosynthesis, which confers improved radiation, nitrogen and water-use efficiencies when compared to $C_3$ photosynthesis, while reducing losses caused by photorespiration (Schuler *et al.*, 2016). Therefore, engineering $C_4$ photosynthesis into $C_3$ 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 $C_4$ 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 bioenergy 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.*, 2015*a*, *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.*, 2015*a*, *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 $C_4$ 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 $C_4$ 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.*, 2016*b*; Packer *et al.*, 2017*a*).

*Phragmites australis* (Fig. 3) is a cosmopolitan perennial grass that spans all continents except Antarctica (Clevering and Lissner, 1999; Packer *et al.*, 2017*b*). 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.*, 2017*b*). 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.*, 2017*b*). 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, 2007*b*; 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, 2003*a*, *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.*, 2016*a*). 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, 2007*a*; Park and Blossey, 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.*, 2007*a*, *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, 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).

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

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 exudated 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 absicic 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 aboveground 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.*, 2018*b*). *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.*, 2018*a*). 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, whuch 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.*, 2018*a*, *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.*, 2018*b*). The number of cortical layers can range from one to several, representing a paradigmatic example of interspecific anatomical variability (di Ruocco *et al.*, 2018*a*). *Cardamine hirsuta* roots have two cortical layers (an outer and an inner one) whereas *A. thaliana* roots have only one (di Ruocco *et al.*, 2018*a*, *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*., 2018*a*; 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*., 2018*a*, *b*). The CEM cells undergo a second asymmetric division, producing the endodermal layer and an inner cortical cell layer (di Ruocco *et al*., 2018*a*, *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 (Smýkal *et al.*, 2012; Kreplak *et al.*, 2019). Due to these developments, *Pi. 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 (Smýkal *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 manipulations easier, and allows for detailed physiological measurements, 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 polyrhiza* 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.

## ACKNOWLEDGEMENTS

The authors would like to thank Staffan Persson, James L. Weller and John L. Bowman for comments on the manuscript, as well as Tom Dierschke and Boubacar Kountche for providing photographs of *M. polymorpha* and *St. hermonthica*, respectively.

## FUNDING

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (BIOEN Young Investigators Awards research grant 2015/02527–1 to I.C.); the SPRINT Project (2016/50189-0 to I.C.); Conselho Nacional de Desenvolvimento Científico e Tecnológico (research fellowship 302927/2018–2 to I.C.); an FIRB (Futuro In Ricerca) research grant (to R.D.I.), the Melbourne-Potsdam PhD Program, and the Natural Sciences and Engineering Research Council of Canada (to M.S.O.); the Australian Government Research Training Program (to K.L.P.); and the Deutsche Forschungsgemeinschaft (DFG) (projects 329720419 to M.I.R-S. and 344523413 to M.S.).

## LITERATURE CITED

**Acharya BR, Roy Choudhury S, Estelle AB, *et al.* 2017.** Optimization of phenotyping assays for the model monocot *Setaria viridis*. *Frontiers in Plant Science* **8**: 2172.

**Achenbach L, Eller F, Nguyen LX, Brix H. 2013.** Differences in salinity tolerance of genetically distinct *Phragmites australis* clones. *AoB PLANTS* **5**: 1–19.

**Albert NW, Thrimawithana AH, McGhie TK, *et al.* 2018.** Genetic analysis of the liverwort *Marchantia polymorpha* reveals that R2R3MYB activation of flavonoid production in response to abiotic stress is an ancient character in land plants. *The New Phytologist* **218**: 554–566.

**Ali NA, Bernal MP, Ater M. 2002.** Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. *Plant and Soil* **239**: 103–111.

**Allen WJ, Young RE, Bhattarai GP, *et al.* 2015.** Multitrophic enemy escape of invasive *Phragmites australis* and its introduced herbivores in North America. *Biological Invasions* **17**: 3419–3432.

**Alonso-Cantabrana H, Cousins AB, Danila F, *et al.* 2018.** Diffusion of $CO_2$ across the mesophyll-bundle sheath cell interface in a C4 plant with genetically reduced PEP carboxylase activity. *Plant Physiology* **178**: 72–81.

**Althoff F, Kopischke S, Zobell O, *et al.* 2014.** Comparison of the MpEF1α and CaMV35 promoters for application in *Marchantia polymorpha* overexpression studies. *Transgenic Research* **23**: 235–244.

**Alves-Carvalho S, Aubert G, Carrère S, *et al.* 2015.** Full-length de novo assembly of RNA-seq data in pea (*Pisum sativum* L.) provides a gene expression atlas and gives insights into root nodulation in this species. *The Plant Journal* **84**: 1–19.

**Ankeny RA, Leonelli S. 2011.** What's so special about model organisms? *Studies in History and Philosophy of Science* **42**: 313–323.

**Balla J, Kalousek P, Reinöhl V, Friml J, Procházka S. 2011.** Competitive canalization of PIN-dependent auxin flow from axillary buds controls pea bud outgrowth. *The Plant Journal* **65**: 571–577.

**Barbier de Reuille P, Routier-Kierzkowska A-L, Kierzkowski D, *et al.* 2015.** MorphoGraphX: a platform for quantifying morphogenesis in 4D. *eLife* **4**: 1–20.

**Barker DG, Bianchi S, Blondon F, *et al.* 1990.** *Medicago truncatula*, a model plant for studying the molecular genetics of theRhizobium-legume symbiosis. *Plant Molecular Biology Reporter* **8**: 40–49.

**Barkoulas M, Hay A, Kougioumoutzi E, Tsiantis M. 2008.** A developmental framework for dissected leaf formation in the Arabidopsis relative *Cardamine hirsuta*. *Nature Genetics* **40**: 1136–1141.

**Bean SJ, Gooding PS, Mullincaux PM, Davies DR. 1997.** A simple system for pea transformation. *Plant Cell Reports* **16**: 513–519.

**Beckie HJ, Brandt SA. 1997.** Nitrogen contribution of field pea in annual cropping systems. 1. Nitrogen residual effect. *Canadian Journal of Plant Science* **77**: 311–322.

**Beilstein MA, Al-Shehbaz IA, Mathews S, Kellogg EA. 2008.** Brassicaceae phylogeny inferred from phytochrome A and ndhF sequence data: tribes and trichomes revisited. *American Journal of Botany* **95**: 1307–1327.

**Beilstein MA, Nagalingum NS, Clements MD, Manchester SR, Mathews S. 2010.** Dated molecular phylogenies indicate a Miocene origin for *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 18724–18728.

**Bennett MD, Leitch IJ, Hanson L. 1998.** DNA amounts in two samples of weeds. *Annals of Botany* **82**: 121–134.

**Bennetzen JL, Schmutz J, Wang H, *et al.* 2012.** Reference genome sequence of the model plant *Setaria*. *Nature Biotechnology* **30**: 555–561.

**Bernasconi G, Antonovics J, Biere A, *et al.* 2009.** Silene as a model system in ecology and evolution. *Heredity* **103**: 5–14.

**Berner DK, Kling JG, Sing BB. 1995.** *Striga* research and control - A perspective from Africa. *Plant Disease* **79**: 652–660.

**Beveridge CA. 2000.** Long-distance signalling and a mutational analysis of branching in pea. *Plant Growth Regulation* **32**: 193–203.

**Beveridge CA, Symons GM, Murfet IC, Ross JJ, Rameau C. 1997.** The rms1 mutant of pea has elevated indole-3-acetic acid levels and reduced root-sap zeatin riboside content but increased branching controlled by graft-transmissible signal(s). *Plant Physiology* **115**: 1251–1258.

**Bewick AJ, Ji L, Niederhuth CE, *et al.* 2016.** On the origin and evolutionary consequences of gene body DNA methylation. *Proceedings of the National Academy of Sciences of the United States of America* **113**: 9111–9116.

**Blein T, Pulido A, Vialette-Guiraud A, *et al.* 2008.** A conserved molecular framework for compound leaf development. *Science (New York, N.Y.)* **322**: 1835–1839.

**Borrill P. 2020.** Blurring the boundaries between cereal crops and model plants. *New Phytologist*. doi:10.1111/nph.16229.

**Bourgeois M, Jacquin F, Savois V, *et al.* 2009.** Dissecting the proteome of pea mature seeds reveals the phenotypic plasticity of seed protein composition. *Proteomics* **9**: 254–271.

**Bowman JL. 2016.** A brief history of marchantia from Greece to genomics. *Plant & Cell Physiology* **57**: 210–229.

**Bowman JL, Araki T, Kohchi T. 2016*a*.** *Marchantia*: past, present and future. *Plant & Cell Physiology* **57**: 205–209.

**Bowman JL, Briginshaw LN, Fisher TJ, Flores-Sandoval E. 2019.** Something ancient and something neofunctionalized-evolution of land plant hormone signaling pathways. *Current Opinion in Plant Biology* **47**: 64–72.

**Bowman JL, Kohchi T, Yamato KT, *et al.* 2017.** Insights into land plant evolution garnered from the *Marchantia polymorpha* genome. *Cell* **171**: 287–304.e15.

**Bowman JL, Sakakibara K, Furumizu C, Dierschke T. 2016*b*.** Evolution in the cycles of life. *Annual Review of Genetics* **50**: 133–154.

**Boyd RA, Gandin A, Cousins AB. 2015.** Temperature response of C4 photosynthesis: biochemical analysis of rubisco, phosphoenolpyruvate carboxylase and carbonic anhydrase in *Setaria viridis*. *Plant Physiology* **169**: 00586.2015.

**Bressan RA, Zhang C, Zhang H, Hasegawa PM, Bohnert HJ, Zhu JK. 2001.** Learning from the Arabidopsis experience. The next gene search paradigm. *Plant Physiology* **127**: 1354–1360.

**Brutnell TP, Bennetzen JL, Vogel JP. 2015.** *Brachypodium distachyon* and *Setaria viridis*: model genetic systems for the grasses. *Annual Review of Plant Biology* **66**: 465–485.

**Brutnell TP, Wang L, Swartwood K, *et al.* 2010.** *Setaria viridis*: a model for C4 photosynthesis. *The Plant Cell* **22**: 2537–2544.

**Carella P, Gogleva A, Hoey DJ, *et al.* 2019.** Conserved biochemical defenses underpin host responses to oomycete infection in an early-divergent land plant lineage. *Current Biology* **29**: 2282–2294.

**Carlsbecker A, Lee JY, Roberts CJ, *et al.* 2010.** Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* **465**: 316–321.

**Cartolano M, Pieper B, Lempe J, *et al.* 2015.** Heterochrony underpins natural variation in *Cardamine hirsuta* leaf form. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 10539–10544.

**Catarino B, Hetherington AJ, Emms DM, Kelly S, Dolan L. 2016.** The stepwise increase in the number of transcription factor families in the Precambrian predated the diversification of plants on land. *Molecular Biology and Evolution* **33**: 2815–2819.

**Chambers RM, Meyerson LA, Saltonstall K. 1999.** Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany* **64**: 261–273.

**Champigny MJ, Sung WW, Catana V, *et al.* 2013.** RNA-Seq effectively monitors gene expression in *Eutrema salsugineum* plants growing in an extreme natural habitat and in controlled growth cabinet conditions. *BMC Genomics* **14**: 578.

**Chang C, Bowman JL, Meyerowitz EM. 2016.** Field guide to plant model systems. *Cell* **167**: 325–339.

**Cissoko M, Boisnard A, Rodenburg J, Press MC, Scholes JD. 2011.** New Rice for Africa (NERICA) cultivars exhibit different levels of post-attachment resistance against the parasitic weeds *Striga hermonthica* and *Striga asiatica*. *The New Phytologist* **192**: 952–963.

**Clayton WA, Albert NW, Thrimawithana AH, *et al.* 2018.** UVR8-mediated induction of flavonoid biosynthesis for UVB tolerance is conserved between the liverwort *Marchantia polymorpha* and flowering plants. *The Plant Journal* **96**: 503–517.

**Clevering OA, Lissner J. 1999.** Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. *Aquatic Botany* **64**: 185–208.

**Clough SJ, Bent AF. 1998.** Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal* **16**: 735–743.

**Considine MJ, Siddique KHM, Foyer CH. 2017.** Nature's pulse power: legumes, food security and climate change. *Journal of Experimental Botany* **68**: 1815–1818.

**Cook DR. 1999.** *Medicago truncatula*–a model in the making! *Current Opinion in Plant Biology* **2**: 301–304.

**Cook CE, Whichard LP, Wall ME, *et al.* 1972.** Germination stimulants. II. Structure of strigol - A potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *Journal of the American Chemical Society* **94**: 6198–6199.

**Courty PE, Smith P, Koegel S, Redecker D, Wipf D. 2015.** Inorganic nitrogen uptake and transport in beneficial plant root-microbe interactions. *Critical Reviews in Plant Sciences* **34**: 4–16.

**Couvreur TL, Franzke A, Al-Shehbaz IA, Bakker FT, Koch MA, Mummenhoff K. 2010.** Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). *Molecular Biology and Evolution* **27**: 55–71.

**Cove D. 2005.** The moss *Physcomitrella patens*. *Annual Review of Genetics* **39**: 339–358.

**Dalmais M, Schmidt J, Le Signor C, *et al.* 2008.** UTILLdb, a *Pisum sativum* in silico forward and reverse genetics tool. *Genome Biology* **9**: R43.

**Danila FR, Quick WP, White RG, Furbank RT, von Caemmerer S. 2016.** The metabolite pathway between bundle sheath and mesophyll: quantification of plasmodesmata in leaves of C3 and C4 monocots. *The Plant Cell* **28**: 1461–1471.

**Dierschke T, Flores-Sondoval E, Rast-Somssich MI, Althoff F, Zachgo S, Bowman JL. 2020.** Gamete-specific expression of TALE class HD genes activates the diploid sporophyte program in Marchantia polymorpha. *BioRxiv* doi:10.1101/2020.04.06.027821.

**Doggett H. 1987.** *Striga*. A parasitic witchweed. *BioEssays* **7**: 135–138.

**Dogra KS, Sood SK, Dobhal PK, Sharma S. 2010.** Alien plant invasion and their impact on indigenous species diversity at global scale: a review. *Journal of Ecology and The Natural Environment* **2**: 175–186.

**Draper J, Mur LA, Jenkins G, *et al.* 2001.** *Brachypodium distachyon*. A new model system for functional genomics in grasses. *Plant Physiology* **127**: 1539–1555.

**Eklund DM, Ishizaki K, Flores-Sandoval E, *et al.* 2015.** Auxin produced by the indole-3-pyruvic acid pathway regulates development and gemmae dormancy in the liverwort *Marchantia polymorpha*. *The Plant Cell* **27**: 1650–1669.

**Eller F, Lambertini C, Nielsen MW, Radutoiu S, Brix H. 2014.** Expression of major photosynthetic and salt-resistance genes in invasive reed lineages grown under elevated $CO_2$ and temperature. *Ecology and Evolution* **4**: 4161–4172.

**Ellis TH, Hofer JM, Timmerman-Vaughan GM, Coyne CJ, Hellens RP. 2011.** Mendel, 150 years on. *Trends in Plant Science* **16**: 590–596.

**Ermawar RA, Collins HM, Byrt CS, *et al.* 2015.** Genetics and physiology of cell wall polysaccharides in the model C4 grass, *Setaria viridis* spp. *BMC Plant Biology* **15**: 236.

**Fernández-Aparicio M, Huang K, Wafula EK, *et al.* 2013.** Application of qRT-PCR and RNA-Seq analysis for the identification of housekeeping genes useful for normalization of gene expression values during *Striga hermonthica* development. *Molecular Biology Reports* **40**: 3395–3407.

**Ferrandiz C, Navarro C, Gomez MD, Canas LA, Beltran JP. 1999.** Flower development in *Pisum sativum*: from the war of the whorls to the battle of the common primordia. *Developmental Genetics* **25**: 280–290.

**Ferreira SS, Simões MS, Carvalho GG, de Lima LGA, Svartman RMA, Cesarino I. 2019.** The lignin toolbox of the model grass *Setaria viridis*. *Plant Molecular Biology* **101**: 235–255.

**Fitter A, Williamson L, Linkohr B, Leyser O. 2002.** Root system architecture determines fitness in an Arabidopsis mutant in competition for immobile phosphate ions but not for nitrate ions. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **269**: 2017–2022.

**Flores-Sandoval E, Dierschke T, Fisher TJ, Bowman JL. 2016.** Efficient and inducible use of artificial MicroRNAs in *Marchantia polymorpha*. *Plant & Cell Physiology* **57**: 281–290.

**Flores-Sandoval E, Eklund DM, Bowman JL. 2015.** A simple auxin transcriptional response system regulates multiple morphogenetic processes in the liverwort *Marchantia polymorpha*. *PLOS Genetics* **11**: e1005207.

**Floyd SK, Bowman JL. 2007.** The ancestral developmental tool kit of land plants. *International Journal of Plant Sciences* **168**: 1–35.

**Frangedakis E, Saint-Marcoux D, Moody LA, Rabbinowitsch E, Langdale JA. 2017.** Nonreciprocal complementation of KNOX gene function in land plants. *The New Phytologist* **216**: 591–604.

**Franssen SU, Shrestha RP, Bräutigam A, Bornberg-Bauer E, Weber AP. 2011.** Comprehensive transcriptome analysis of the highly complex *Pisum sativum* genome using next generation sequencing. *BMC Genomics* **12**: 227.

**Fujioka H, Samejima H, Suzuki H, Mizutani M, Okamoto M, Sugimoto Y. 2019.** Aberrant protein phosphatase 2C leads to abscisic acid insensitivity and high transpiration in parasitic *Striga*. *Nature Plants* **5**: 258–262.

**Gan X, Hay A, Kwantes M, *et al.* 2016.** The *Cardamine hirsuta* genome offers insight into the evolution of morphological diversity. *Nature Plants* **2**: 16167.

**German DA, Koch MA. 2017.** *Eutrema salsugineum* (Cruciferae) new to Mexico: a surprising generic record for the flora of Middle America. *PhytoKeys* **76**: 13–21.

**Ghosh S, Watson A, Gonzalez-Navarro OE, *et al.* 2018.** Speed breeding in growth chambers and glasshouses for crop breeding and model plant research. *Nature Protocols* **13**: 369512.

**Giehl RFH, Wirén N Von. 2014.** Root nutrient foraging. *Plant physiology* **166**: 509–17.

**Gimenez-Ibanez S, Zamarreño AM, García-Mina JM, Solano R. 2019.** An evolutionarily ancient immune system governs the interactions between *Pseudomonas syringae* and an early-diverging land plant lineage. *Current Biology* **29**: 2270–2281.

**Gobena D, Shimels M, Rich PJ, *et al.* 2017.** Mutation in sorghum LOW GERMINATION STIMULANT 1 alters strigolactones and causes *Striga* resistance. *Proceedings of the National Academy of Sciences of the United States of America* **114**: 4471–4476.

**Gomez-Roldan V, Fermas S, Brewer PB, *et al.* 2008.** Strigolactone inhibition of shoot branching. *Nature* **455**: 189–194.

**Graham PH, Vance CP. 2003.** Legumes: importance and constraints to greater use. *Plant Physiology* **131**: 872–877.

**Grant JE, Cooper PA. 2003.** Genetic transformation in pea. In: Jaiwal PK, Singh RP, eds. *Applied Genetics of leguminosae biotechnology. Focus on biotechnology*, Vol. 10B. Dordrecht: Springer, 23–34.

**Grant JE, Cooper PA, Gilpin BJ, *et al.* 1998.** Kanamycin is effective for selecting transformed peas. *Plant Science* **139**: 159–164.

**Guevara DR, Champigny MJ, Tattersall A, *et al.* 2012.** Transcriptomic and metabolomic analysis of Yukon *Thellungiella* plants grown in cabinets and their natural habitat show phenotypic plasticity. *BMC Plant Biology* **12**: 175.

**Guo YL, Bechsgaard JS, Slotte T, *et al.* 2009.** Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 5246–5251.

**Guo X, Hao G, Ma T. 2016.** The complete chloroplast genome of salt cress (*Eutrema salsugineum*). *Mitochondrial DNA* **27**: 2862–2863.

**Guo Y, Wang D, Jia W, Song J, Yang J, Wang B. 2012.** Effects of seed vernalisation and photoperiod on flowering induction in the halophyte *Thellungiella halophila*. *Australian Journal of Botany* **60**: 743.

**Gupta C, Prakash D. 2013.** Duckweed: an effective tool for phyto-remediation. *Toxicological & Environmental Chemistry* **95**: 1256–1266.

**Han GZ. 2017.** Evolution of jasmonate biosynthesis and signaling mechanisms. *Journal of Experimental Botany* **68**: 1323–1331.

**Hansen RM. 1978.** *Shasta* ground sloth food habits, Rampart Cave, Arizona. *Paleobiology* **4**: 302–319.

**Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. 2000.** Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**: 463–499.

**Hasson A, Blein T, Laufs P. 2010.** Leaving the meristem behind: the genetic and molecular control of leaf patterning and morphogenesis. *Comptes Rendus Biologies* **333**: 350–360.

**Hay AS, Pieper B, Cooke E, *et al.* 2014.** *Cardamine hirsuta*: a versatile genetic system for comparative studies. *The Plant Journal* **78**: 1–15.

**Hay A, Tsiantis M. 2006.** The genetic basis for differences in leaf form between *Arabidopsis thaliana* and its wild relative *Cardamine hirsuta*. *Nature Genetics* **38**: 942–947.

**Hay A, Tsiantis M. 2016.** *Cardamine hirsuta*: a comparative view. *Current Opinion in Genetics & Development* **39**: 1–7.

**He R, Kim MJ, Nelson W, *et al.* 2012.** Next-generation sequencing-based transcriptomic and proteomic analysis of the common reed, *Phragmites australis* (Poaceae), reveals genes involved in invasiveness and rhizome specificity. *American Journal of Botany* **99**: 232–247.

**Hecht V, Knowles CL, Vander Schoor JK, *et al.* 2007.** Pea LATE BLOOMER1 is a GIGANTEA ortholog with roles in photoperiodic flowering, deetiolation, and transcriptional regulation of circadian clock gene homologs. *Plant Physiology* **144**: 648–661.

**Hirsch AM. 1992.** Developmental biology of legume nodulation. *New Phytologist* **122**: 211–237.

**Hiwatashi T, Quan KL, Yasui Y, *et al.* 2018.** The RopGEF KARAPPO is essential for the initiation of vegetative reproduction in *Marchantia*. *bioRxiv* doi:10.1101/385682.

**Hofhuis H, Moulton D, Lessinnes T, *et al.* 2016.** morphomechanical innovation drives explosive seed dispersal. *Cell* **166**: 222–233.

**Holdredge C, Bertness MD, Von Wettberg E, Silliman BR. 2010.** Nutrient enrichment enhances hidden differences in phenotype to drive a cryptic plant invasion. *Oikos* **119**: 1776–1784.

**Holmes GD, Hall NE, Gendall AR, Boon PI, James EA. 2016.** Using transcriptomics to identify differential gene expression in response to

salinity among Australian *Phragmites australis* clones. *Frontiers in Plant Science* **7**: 1–12.

**Horst NA, Katz A, Pereman I, Decker EL, Ohad N, Reski R. 2016.** A single homeobox gene triggers phase transition, embryogenesis and asexual reproduction. *Nature Plants* **2**: 15209.

**Hu S, Hu X, Shu J, *et al.* 2018.** Xiaowei, a new rice germplasm for large-scale indoor research. *Molecular Plant* **11**: 1418–1420.

**Hu H, Mauro-Herrera M, Doust AN. 2018.** Domestication and improvement in the model C4 grass, *Setaria*. *Frontiers in Plant Science* **9**: 1–13.

**Huang P, Brutnell TP. 2016.** A synthesis of transcriptomic surveys to dissect the genetic basis of C4 photosynthesis. *Current Opinion in Plant Biology* **31**: 91–99.

**Huang P, Shyu C, Coelho CP, Cao Y, Brutnell TP. 2016.** *Setaria viridis* as a model system to advance millet genetics and genomics. *Frontiers in Plant Science* **7**: 1781.

**Hulme PE. 2009.** Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* **46**: 10–18.

**Hunt VM, Fant JB, Steger L, *et al.* 2017.** PhragNet: crowdsourcing to investigate ecology and management of invasive *Phragmites australis* (common reed) in North America. *Wetlands Ecology and Management* **25**: 607–618.

**Inan G, Zhang Q, Li P, *et al.* 2004.** Salt cress. A halophyte and cryophyte Arabidopsis relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. *Plant Physiology* **135**: 1718–1737.

**Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis CL, Macmillan J. 1984.** Internode length in Pisum. *Planta* **160**: 455–463.

**International Wheat Genome Sequencing Consortium (IWGSC). 2018.** Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* **361**: eaar7191.

**Ishii J, Kadono Y. 2002.** Factors influencing seed production of *Phragmites australis*. *Aquatic Botany* **72**: 129–141.

**Ishizaki K, Chiyoda S, Yamato KT, Kohchi T. 2008.** *Agrobacterium*-mediated transformation of the haploid liverwort *Marchantia polymorpha* L., an emerging model for plant biology. *Plant & Cell Physiology* **49**: 1084–1091.

**Ishizaki K, Johzuka-Hisatomi Y, Ishida S, Iida S, Kohchi T. 2013*a*.** Homologous recombination-mediated gene targeting in the liverwort *Marchantia polymorpha* L. *Scientific Reports* **3**: 1532.

**Ishizaki K, Mizutani M, Shimamura M, Masuda A, Nishihama R, Kohchi T. 2013*b*.** Essential role of the E3 ubiquitin ligase nopperabo1 in schizogenous intercellular space formation in the liverwort *Marchantia polymorpha*. *The Plant Cell* **25**: 4075–4084.

**Ishizaki K, Nishihama R, Ueda M, *et al.* 2015.** Development of gateway binary vector series with four different selection markers for the liverwort *Marchantia polymorpha*. *PLoS One* **10**: e0138876.

**Ishizaki K, Nishihama R, Yamato KT, Kohchi T. 2016.** Molecular genetic tools and techniques for *Marchantia polymorpha* research. *Plant & Cell Physiology* **57**: 262–270.

**Ishizaki K, Nonomura M, Kato H, Yamato KT, Kohchi T. 2012.** Visualization of auxin-mediated transcriptional activation using a common auxin-responsive reporter system in the liverwort *Marchantia polymorpha*. *Journal of Plant Research* **125**: 643–651.

**Jaffe MJ, Galston AW. 1966.** Physiological studies on pea tendrils. I. Growth and coiling following mechanical stimulation. *Plant Physiology* **41**: 1014–1025.

**Jansson S, Douglas CJ. 2007.** *Populus*: a model system for plant biology. *Annual Review of Plant Biology* **58**: 435–458.

**Jiang H, Barbier H, Brutnell T. 2013.** Methods for performing crosses in *Setaria viridis*, a new model system for the grasses. *Journal of Visualized Experiments* doi:10.3791/50527.

**Kabir AH, Paltridge NG, Roessner U, Stangoulis JC. 2013.** Mechanisms associated with Fe-deficiency tolerance and signaling in shoots of *Pisum sativum*. *Physiologia Plantarum* **147**: 381–395.

**Kajikawa M, Yamaoka S, Yamato KT, *et al.* 2003.** Functional analysis of a beta-ketoacyl-CoA synthase gene, MpFAE2, by gene silencing in the liverwort *Marchantia polymorpha* L. *Bioscience, Biotechnology, and Biochemistry* **67**: 605–612.

**de Kathen A, Jacobsen HJ. 1993.** Transformation in pea (*Pisum sativum* L.) Bajaj YPS, ed. *Plant Protoplasts and Genetic Engineering IV. Biotechnology in agriculture and forestry*, Vol. 23. Berlin, Heidelberg: Springer, 331–347.

**Kato H, Ishizaki K, Kouno M, *et al.* 2015.** Auxin-mediated transcriptional system with a minimal set of components is critical for morphogenesis through the life cycle in *Marchantia polymorpha*. *PLoS Genetics* **11**: e1005084.

**Keane RM, Crawley MJ. 2002.** Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **17**: 164–170.

**Kettenring KM, McCormick MK, Baron HM, Whigham DF. 2011.** Mechanisms of *Phragmites australis* invasion: feedbacks among genetic diversity, nutrients, and sexual reproduction. *Journal of Applied Ecology* **48**: 1305–1313.

**Kettenring KM, Whigham DF. 2009.** Seed viability and seed dormancy of non-native *Phragmites australis* in suburbanized and forested watersheds of the Chesapeake Bay, USA. *Aquatic Botany* **91**: 199–204.

**Kierzkowski D, Runions A, Vuolo F, *et al.* 2019.** A growth-based framework for leaf shape development and diversity. *Cell* **177**: 1405–1418.e17.

**Kim Y-G, Sharmin SA, Alam I, *et al.* 2013.** *Agrobacterium*-mediated transformation of reed (*Phragmites communis* Trinius) using mature seed-derived calli. *GCB Bioenergy* **5**: 73–80.

**Kirigia D, Runo S, Alakonya A. 2014.** A virus-induced gene silencing (VIGS) system for functional genomics in the parasitic plant *Striga hermonthica*. *Plant Methods* **10**: 16.

**Kirk H, Paul J, Straka J, Freeland JR. 2011.** Long-distance dispersal and high genetic diversity are implicated in the invasive spread of the common reed, *Phragmites australis* (Poaceae), in northeastern North America. *American Journal of Botany* **98**: 1180–1190.

**van Kleunen M, Weber E, Fischer M. 2010.** A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* **13**: 235–245.

**Knight CA, Molinari NA, Petrov DA. 2005.** The large genome constraint hypothesis: evolution, ecology and phenotype. *Annals of Botany* **95**: 177–190.

**Knott CM. 1987.** A key for stages of development of the pea (*Pisum sativum*). *Annals of Applied Biology* **111**: 233–245.

**Koch MA, German DA. 2013.** Taxonomy and systematics are key to biological information: *Arabidopsis*, *Eutrema* (*Thellungiella*), *Noccaea* and *Schrenkiella* (Brassicaceae) as examples. *Frontiers in Plant Science* **4**: 267.

**Koi S, Hisanaga T, Sato K, *et al.* 2016.** An evolutionarily conserved plant RKD factor controls germ cell differentiation. *Current Biology: CB* **26**: 1775–1781.

**Kolar CS, Lodge DM. 2001.** Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* **16**: 199–204.

**Komatsu A, Terai M, Ishizaki K, *et al.* 2014.** Phototropin encoded by a single-copy gene mediates chloroplast photorelocation movements in the liverwort *Marchantia polymorpha*. *Plant Physiology* **166**: 411–427.

**Koornneef M, Meinke D. 2010.** The development of Arabidopsis as a model plant. *The Plant Journal* **61**: 909–921.

**Kountche BA, Jamil M, Yonli D, *et al.* 2019.** Suicidal germination as a control strategy for *Striga hermonthica* (Benth.) in smallholder farms of sub-Saharan Africa. *Plants, People, Planet* **1**: 107–118.

**Kreplak J, Madoui MA, Cápal P, *et al.* 2019.** A reference genome for pea provides insight into legume genome evolution. *Nature Genetics* **51**: 1411–1422.

**Kroons H de, Hutchings MJ. 1995.** Morphological plasticity in clonal plants: the foraging concept reconsidered. *Society* **83**: 143–152.

**Kubota A, Kita S, Ishizaki K, Nishihama R, Yamato KT, Kohchi T. 2014.** Co-option of a photoperiodic growth-phase transition system during land plant evolution. *Nature Communications* **5**: 3668.

**Kulaeva OA, Zhernakov AI, Afonin AM, *et al.* 2017.** Pea Marker Database (PMD) - A new online database combining known pea (*Pisum sativum* L.) gene-based markers. *PLoS One* **12**: e0186713.

**Lambert AM, Casagrande RA. 2007*a*.** Susceptibility of native and non-native common reed to the non-native mealy plum aphid (Homoptera: Aphididae) in North America. *Environmental Entomology* **36**: 451–457.

**Lambert AM, Casagrande RA. 2007*b*.** Characteristics of a successful estuarine invader: evidence of self-compatibility in native and non-native lineages of *Phragmites australis*. *Marine Ecology Progress Series* **337**: 299–301.

**Lambert AM, Winiarski K, Casagrande RA. 2007.** Distribution and impact of exotic gall flies (*Lipara* sp.) on native and exotic *Phragmites australis*. *Aquatic Botany* **86**: 163–170.

**Lambertini C, Gustafsson MHG, Frydenberg J, Lissner J, Speranza M, Brix H. 2006.** A phylogeographic study of the cosmopolitan genus *Phragmites* (Poaceae) based on AFLPs. *Plant Systematics and Evolution* **258**: 161–182.

**Lambertini C, Gustafsson MHG, Frydenberg J, Speranza M, Brix H. 2008.** Genetic diversity patterns in *Phragmites australis* at the population, regional and continental scales. *Aquatic Botany* **88**: 160–170.

**Lambertini C, Sorrell BK, Riis T, Olesen B, Brix H. 2012.** Exploring the borders of European *Phragmites* within a cosmopolitan genus. *Aob PLANTS* **2012**: 1–18.

**Lampugnani ER, Flores-Sandoval E, Tan QW, Mutwil M, Bowman JL, Persson S. 2019.** Cellulose synthesis – central components and their evolutionary relationships. *Trends in Plant Science* **24**: 402–412.

**Lauzer D, Dallaire S, Vincent G. 2000.** In vitro propagation of reed grass by somatic embryogenesis. *Plant Cell, Tissue and Organ Culture* **60**: 229–234.

**Lee JH, Lin H, Joo S, Goodenough U. 2008.** Early sexual origins of homeoprotein heterodimerization and evolution of the plant KNOX/BELL family. *Cell* **133**: 829–840.

**Lexa M, Cheeseman JM. 1997.** Growth and nitrogen relations in reciprocal grafts of wild-type and nitrate reductase-deficient mutants of pea (*Pisum sativum* L. var. Juneau). *Journal of Experimental Botany* **48**: 1241–1250.

**Li P, Brutnell TP. 2011.** *Setaria viridis* and *Setaria italica*, model genetic systems for the Panicoid grasses. *Journal of Experimental Botany* **62**: 3031–3037.

**Li Y, Sun W, Liu F, *et al.* 2019.** Methods for grafting *Arabidopsis thaliana* and *Eutrema salsugineum*. *Plant Methods* **15**: 93.

**Li M, Zhang D, Gao Q, *et al.* 2019.** Genome structure and evolution of *Antirrhinum majus* L. *Nature Plants*: 443515.

**Lin PC, Lu CW, Shen BN, *et al.* 2016.** Identification of miRNAs and their targets in the liverwort *Marchantia polymorpha* by integrating RNA-Seq and degradome analyses. *Plant & Cell Physiology* **57**: 339–358.

**Liu G, Yang YB, Zhu ZH. 2018.** Elevated nitrogen allows the weak invasive plant *Galinsoga quadriradiata* to become more vigorous with respect to inter-specific competition. *Scientific Reports* **8**: 3136.

**Lugan R, Niogret MF, Leport L, *et al.* 2010.** Metabolome and water homeostasis analysis of *Thellungiella salsuginea* suggests that dehydration tolerance is a key response to osmotic stress in this halophyte. *The Plant Journal* **64**: 215–229.

**Macas J, Neumann P, Navrátilová A. 2007.** Repetitive DNA in the pea (*Pisum sativum* L.) genome: comprehensive characterization using 454 sequencing and comparison to soybean and *Medicago truncatula*. *BMC Genomics* **8**: 427.

**Magee AM, Aspinall S, Rice DW, *et al.* 2010.** Localized hypermutation and associated gene losses in legume chloroplast genomes. *Genome Research* **20**: 1700–1710.

**di Mambro R, Sabatini S, Dello Ioio R. 2018.** Patterning the axes: a lesson from the root. *Plants* **8**: 8.

**Marinos NG. 1970.** Embryogenesis of the pea (*Pisum sativum*). *Protoplasma* **71**: 227–233.

**Marriott PE, Gómez LD, McQueen-Mason SJ. 2016.** Unlocking the potential of lignocellulosic biomass through plant science. *The New Phytologist* **209**: 1366–1381.

**Martins PK, Mafra V, de Souza WR, *et al.* 2016.** Selection of reliable reference genes for RT-qPCR analysis during developmental stages and abiotic stress in *Setaria viridis*. *Scientific Reports* **6**: 28348.

**Martins PK, Nakayama TJ, Ribeiro AP, *et al.* 2015*a*.** *Setaria viridis* floral-dip: a simple and rapid *Agrobacterium*-mediated transformation method. *Biotechnology Reports (Amsterdam, Netherlands)* **6**: 61–63.

**Martins PK, Ribeiro AP, Cunha BADBD, Kobayashi AK, Molinari HBC. 2015*b*.** A simple and highly efficient *Agrobacterium*-mediated transformation protocol for *Setaria viridis*. *Biotechnology Reports (Amsterdam, Netherlands)* **6**: 41–44.

**Matsui H, Iwakawa H, Hyon G-S, *et al.* 2019.** Isolation of natural fungal pathogens from *Marchantia polymorpha* reveals antagonism between salicylic acid and jasmonate during liverwort-fungus interactions. *Plant and Cell Physiology* **0**: 1–11.

**Mbuvi DA, Masiga CW, Kuria E, *et al.* 2017.** Novel sources of Witchweed (*Striga*) resistance from wild sorghum accessions. *Frontiers in Plant Science* **8**: 116.

**McKEE J, Richards AJ. 1996.** Variation in seed production and germinability in common reed (*Phragmites australis*) in Britain and France with respect to climate. *The New Phytologist* **133**: 233–243.

**Meisrimler CN, Wienkoop S, Lyon D, Geilfus CM, Lüthje S. 2016.** Long-term iron deficiency: Tracing changes in the proteome of different pea (*Pisum sativum* L.) cultivars. *Journal of Proteomics* **140**: 13–23.

**Mendel G. 1865.** Experiments in plant hybridization. *Verhandlungen des naturforschenden Vereines in Brünn* **4**: 3–47.

**Meyerson LA, Cronin JT. 2013.** Evidence for multiple introductions of *Phragmites australis* to North America: detection of a new non-native haplotype. *Biological Invasions* **15**: 2605–2608.

**Meyerson LA, Cronin JT, Bhattarai GP, *et al.* 2016*a*.** Do ploidy level and nuclear genome size and latitude of origin modify the expression of *Phragmites australis* traits and interactions with herbivores? *Biological Invasions* **18**: 2531–2549.

**Meyerson LA, Cronin JT, Pyšek P. 2016*b*.** *Phragmites australis* as a model organism for studying plant invasions. *Biological Invasions* **18**: 2421–2431.

**Meyerson LA, Viola D V., Brown RN. 2010.** Hybridization of invasive *Phragmites australis* with a native subspecies in North America. *Biological Invasions* **12**: 103–111.

**Mikić A, Medović A, Jovanović Ž, Stanisavljević N. 2014.** Integrating archaeobotany, paleogenetics and historical linguistics may cast more light onto crop domestication: the case of pea (*Pisum sativum*). *Genetic Resources and Crop Evolution* **61**: 887–892.

**Miller MW, Garber ED, Voth PD. 1962.** Biosynthetic pathways in nutritionally deficient mutants of *Marchantia polymorpha* L. *Nature* **195**: 1220–1221.

**Mishler BD, Churchill SP. 1984.** A cladistic approach to the phylogeny of the 'Bryophytes'. *Brittonia* **36**: 406.

**Mishra JS. 2009.** Biology and management of *Cuscuta* species. *Indian Journal of Weed Sciences* **41**: 1–11.

**Mobini SH, Warkentin TD. 2016.** A simple and efficient method of in vivo rapid generation technology in pea (*Pisum sativum* L.). *In Vitro Cellular & Developmental Biology - Plant* **52**: 530–536.

**Mohamed AH, Housley TL, Ejeta G. 2010.** An in vitro technique for studying specific Striga resistance mechanisms in sorghum. *African Journal of Agricultural Research* **5**: 1868–1875.

**Monniaux M, Pieper B, McKim SM, *et al.* 2018.** The role of APETALA1 in petal number robustness. *eLife* **7**: 1–22.

**Monte I, Ishida S, Zamarreño AM, *et al.* 2018.** Ligand-receptor co-evolution shaped the jasmonate pathway in land plants. *Nature Chemical Biology* **14**: 480–488.

**Monte-Bello CC, Araujo EF, Martins MCM, *et al.* 2018.** A flexible low cost hydroponic system for assessing plant responses to small molecules in sterile conditions. *Journal of Visualized Experiments* **138** (2018 08 25).

**Mozdzer TJ, Megonigal JP. 2012.** Jack-and-master trait responses to elevated $CO_2$ and N: a comparison of native and introduced *Phragmites australis*. *PLoS One* **7**: e42794.

**Mozdzer TJ, Zieman JC, McGlathery KJ. 2010.** Nitrogen uptake by native and invasive temperate coastal macrophytes: importance of dissolved organic nitrogen. *Estuaries and Coasts* **33**: 784–797.

**Musselman LJ. 1980.** The biology of *Striga*, *Orobanche*, and other root-parasitic weeds. *Annual Review of Phytopathology* **18**: 463–489.

**Mutuku JM, Cui S, Hori C, *et al.* 2019.** The structural integrity of lignin is crucial for resistance against *Striga hermonthica* parasitism in rice. *Plant Physiology* **179**: 1796–1809.

**Nature Plants. 2019.** Return of the snapdragon. *Nature Plants* **5**: 121–121.

**Nishihama R, Ishida S, Urawa H, Kamei Y, Kohchi T. 2016.** Conditional gene expression/deletion systems for marchantia polymorpha using its own heat-shock promoter and Cre/loxP-Mediated site-specific recombination. *Plant & Cell Physiology* **57**: 271–280.

**Okada S, Fujisawa M, Sone T, *et al.* 2000.** Construction of male and female PAC genomic libraries suitable for identification of Y-chromosome-specific clones from the liverwort, *Marchantia polymorpha*. *The Plant Journal* **24**: 421–428.

**Osborn HL, Alonso-Cantabrana H, Sharwood RE, *et al.* 2017.** Effects of reduced carbonic anhydrase activity on $CO_2$ assimilation rates in *Setaria viridis*: a transgenic analysis. *Journal of Experimental Botany* **68**: 299–310.

**Packer JG, Meyerson LA, Richardson DM, *et al.* 2017*a*.** Global networks for invasion science: benefits, challenges and guidelines. *Biological Invasions* **19**: 1081–1096.

**Packer JG, Meyerson LA, Skálová H, Pyšek P, Kueffer C. 2017*b*.** Biological Flora of the British Isles: *Phragmites australis*. *Journal of Ecology* **105**: 1123–1162.

**Pandit MK, White SM, Pocock MJ. 2014.** The contrasting effects of genome size, chromosome number and ploidy level on plant invasiveness: a global analysis. *The New Phytologist* **203**: 697–703.

**Pang Q, Chen S, Dai S, Chen Y, Wang Y, Yan X. 2010.** Comparative proteomics of salt tolerance in *Arabidopsis thaliana* and *Thellungiella halophila*. *Journal of Proteome Research* **9**: 2584–2599.

**Park MG, Blossey B. 2008.** Importance of plant traits and herbivory for invasiveness of *Phragmites australis* (Poaceae). *American Journal of Botany* **95**: 1557–1568.

**Petti C, Shearer A, Tateno M, *et al.* 2013.** Comparative feedstock analysis in *Setaria viridis* L. as a model for C4 bioenergy grasses and Panicoid crop species. *Frontiers in Plant Science* **4**: 181.

**Pimentel D, Zuniga R, Morrison D. 2005.** Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**: 273–288.

**Plut K, Paul J, Ciotir C, Major M, Freeland JR. 2011.** Origin of non-native *Phragmites australis* in North America, a common wetland invader. *Fundamental and Applied Limnology* **179**: 121–129.

**Prentis PJ, Wilson JR, Dormontt EE, Richardson DM, Lowe AJ. 2008.** Adaptive evolution in invasive species. *Trends in Plant Science* **13**: 288–294.

**Provart NJ, Alonso J, Assmann SM, *et al.* 2016.** 50 years of Arabidopsis research: highlights and future directions. *The New Phytologist* **209**: 921–944.

**Puonti-Kaerlas J, Eriksson T, Engström P. 1990.** Production of transgenic pea (*Pisum sativum* L.) plants by *Agrobacterium tumefaciens*-mediated gene transfer. *Theoretical and Applied Genetics* **80**: 246–252.

**Pyšek P, Skálová H, Čuda J, *et al.* 2018.** Small genome separates native and invasive populations in an ecologically important cosmopolitan grass. *Ecology* **99**: 79–90.

**Qian W, Yang X, Li J, Luo R, Yan X, Pang Q. 2019.** Genome-wide characterization and expression analysis of aquaporins in salt cress (*Eutrema salsugineum*). *PeerJ* **7**: e7664.

**Qin S, Liu Y, Han Y, *et al.* 2019.** Aquaporins and their function in root water transport under salt stress conditions in *Eutrema salsugineum*. *Plant Science* **287**: 110199.

**Rahman LN, Smith GS, Bamm VV, *et al.* 2011.** Phosphorylation of *Thellungiella salsuginea* dehydrins TsDHN-1 and TsDHN-2 facilitates cation-induced conformational changes and actin assembly. *Biochemistry* **50**: 9587–9604.

**Rast-Somssich MI, Broholm S, Jenkins H, *et al.* 2015.** Alternate wiring of a KNOXI genetic network underlies differences in leaf development of *A. thaliana* and *C. hirsuta*. *Genes & Development* **29**: 2391–2404.

**Reid JB, Ross JJ. 2011.** Mendel's genes: toward a full molecular characterization. *Genetics* **189**: 3–10.

**Renner T, Lan T, Farr KM, *et al.* 2018.** *Carnivorous plant genomes*. Oxford: Oxford University Press.

**Rodenburg J, Demont M, Zwart SJ, Bastiaans L. 2016.** Parasitic weed incidence and related economic losses in rice in Africa. *Agriculture, Ecosystems & Environment* **235**: 306–317.

**Ross JJ, Reid JB. 1991.** Internode length in *Pisum*: le5839 is a less severe allele than Mendel's le. *Pisum Genetics* **23**: 29–34.

**Rowan BA, Weigel D, Koenig D. 2011.** Developmental genetics and new sequencing technologies: the rise of nonmodel organisms. *Developmental Cell* **21**: 65–76.

**Rubio-Somoza I, Zhou CM, Confraria A, *et al.* 2014.** Temporal control of leaf complexity by miRNA-regulated licensing of protein complexes. *Current Biology: CB* **24**: 2714–2719.

**Runo S, Kuria EK. 2018.** Habits of a highly successful cereal killer, *Striga*. *PLOS Pathogens* **14**: e1006731.

**di Ruocco G, Bertolotti G, Pacifici E, *et al.* 2018.** Differential spatial distribution of miR165/6 determines variability in plant root anatomy. *Development* **145**: dev153858.

**di Ruocco G, di Mambro R, Dello Ioio R. 2018.** Building the differences: a case for the ground tissue patterning in plants. *Proceedings of the Royal Society B: Biological Sciences* **285**: 20181746.

**Rushworth CA, Song BH, Lee CR, Mitchell-Olds T. 2011.** *Boechera*, a model system for ecological genomics. *Molecular Ecology* **20**: 4843–4857.

**Safa SB, Jones BMG, Musselman LJ. 1984.** Mechanisms favouring outbreeding in *Striga hermonthica* (Scrophulariaceae). *New Phytologist* **96**: 299–305.

**Saltonstall K. 2002.** Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 2445–2449.

**Saltonstall K. 2003*a*.** A rapid method for identifying the origin of North American *Phragmites* populations using RFLP analysis. *Wetlands* **23**: 1043–1047.

**Saltonstall K. 2003*b*.** Microsatellite variation within and among North American lineages of *Phragmites australis*. *Molecular Ecology* **12**: 1689–1702.

**Saltonstall K, Castillo HE, Blossey B. 2014.** Confirmed field hybridization of native and introduced *Phragmites australis* (Poaceae) in North America. *American Journal of Botany* **101**: 211–215.

**Saltonstall K, Lambert A, Meyerson LA. 2010.** Genetics and reproduction of common (*Phragmites australis*) and giant reed (*Arundo donax*). *Invasive Plant Science and Management* **3**: 495–505.

**Samejima H, Sugimoto Y. 2018.** Recent research progress in combatting root parasitic weeds. *Biotechnology & Biotechnological Equipment* **32**: 221–240.

**Sauer M, Balla J, Luschnig C, *et al.* 2006.** Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. *Genes & Development* **20**: 2902–2911.

**Sauret-Güeto S, Frangedakis E, Silvestri L, *et al.* 2020.** Systematic tools for reprogramming plant gene expression in a simple model, *Marchantia polymorpha*. *ACS Synthetic Biology* **9**: 864–882.

**Scharff AM, Egsgaard H, Hansen PE, Rosendahl L. 2003.** Exploring symbiotic nitrogen fixation and assimilation in pea root nodules by in vivo $^{15}$N nuclear magnetic resonance spectroscopy and liquid chromatography-mass spectrometry. *Plant Physiology* **131**: 367–378.

**Schiltz S. 2004.** Proteome reference maps of vegetative tissues in pea. An investigation of nitrogen mobilization from leaves during seed filling. *Plant Physiology* **135**: 2241–2260.

**Schmid MW, Giraldo-Fonseca A, Rövekamp M, Smetanin D, Bowman JL, Grossniklaus U. 2018.** Extensive epigenetic reprogramming during the life cycle of *Marchantia polymorpha*. *Genome Biology* **19**: 9.

**Schmutz J, Cannon SB, Schlueter J, *et al.* 2010.** Genome sequence of the palaeopolyploid soybean. *Nature* **463**: 178–183.

**Schroeder HE, Schotz AH, Wardley-Richardson T, Spencer D, Higgins T. 1993.** Transformation and regeneration of two cultivars of pea (*Pisum sativum* L.). *Plant Physiology* **101**: 751–757.

**Schuler ML, Mantegazza O, Weber AP. 2016.** Engineering C4 photosynthesis into C3 chassis in the synthetic biology age. *The Plant Journal* **87**: 51–65.

**Schwab R, Ossowski S, Warthmann N, Weigel D. 2010.** Directed gene silencing with artificial MicroRNAs. In: Meyers B, Green P, eds. *Methods in Molecular Biology*, Vol. 592. Totowa, NJ: Humana Press, 71–88.

**Schwarz-Sommer Z, Davies B, Hudson A. 2003.** An everlasting pioneer: the story of *Antirrhinum* research. *Nature Reviews Genetics* **4**: 655–664.

**Sessa EB, Banks JA, Barker MS, *et al.* 2014.** Between two fern genomes. *Gigascience* **3**: 15.

**Shabala S. 2013.** Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops. *Annals of Botany* **112**: 1209–1221.

**Shahul Hameed U, Haider I, Jamil M, *et al.* 2018.** Structural basis for specific inhibition of the highly sensitive ShHTL7 receptor. *EMBO Reports* **19**: 1–14.

**Sharma A, Plaha P, Rathour R, Katoch V, Singh Y, Khalsa GS. 2009.** Induced mutagenesis for improvement of garden pea. *International Journal of Vegetable Science* **16**: 60–72.

**Shimamura M. 2016.** *Marchantia polymorpha*: taxonomy, phylogeny and morphology of a model system. *Plant & Cell Physiology* **57**: 230–256.

**Shull CA, Fisher Stanfield J. 1939.** Thomas Andrew Knight - in memoriam. *Plant Physiology* **14**: 1–8.

**Simões MS, Carvalho GG, Ferreira SS, Hernandes-Lopes J, de Setta N, Cesarino I. 2020.** Genome-wide characterization of the laccase gene family in *Setaria viridis* reveals members potentially involved in lignification. *Planta* **251**: 46.

**Smýkal P, Aubert G, Burstin J, *et al.* 2012.** Pea (*Pisum sativum* L.) in the genomic era. *Agronomy* **2**: 74–115.

**Smýkal P, Kenicer G, Flavell AJ, *et al.* 2011.** Phylogeny, phylogeography and genetic diversity of the *Pisum* genus. *Plant Genetic Resources* **9**: 4–18.

**Somerville C, Koornneef M. 2002.** A fortunate choice: the history of Arabidopsis as a model plant. *Nature Reviews. Genetics* **3**: 883–889.

**Somssich M. 2018.** A short history of *Arabidopsis thaliana* (L.) Heynh. Columbia-0. *PeerJ Preprints* **e26931v3**: 1–7.

**Souza WR de, Martins PK, Freeman J, *et al.* 2018.** Suppression of a single BAHD gene in *Setaria viridis* causes large, stable decreases in cell wall feruloylation and increases biomass digestibility. *New Phytologist* **218**: 81–93.

**Spens AE, Douhovnikoff V. 2016.** Epigenetic variation within *Phragmites australis* among lineages, genotypes, and ramets. *Biological Invasions* **18**: 2457–2462.

**Stougaard J. 2014.** Background and history of the *Lotus japonicus* model legume system. In: Tabata S, Stougaard J, eds. *The Lotus japonicus Genome. Compendium of plant Genomes.* Berlin, Heidelberg: Springer, 3–8.

**Suda J, Meyerson LA, Leitch IJ, Pyšek P. 2015.** The hidden side of plant invasions: the role of genome size. *The New Phytologist* **205**: 994–1007.

**Sudheesh S, Sawbridge TI, Cogan NO, Kennedy P, Forster JW, Kaur S. 2015.** De novo assembly and characterisation of the field pea transcriptome using RNA-Seq. *BMC Genomics* **16**: 611.

**Sugano SS, Nishihama R, Shirakawa M, *et al.* 2018.** Efficient CRISPR/Cas9-based genome editing and its application to conditional genetic analysis in *Marchantia polymorpha*. *PLoS One* **13**: e0205117.

**Sugano SS, Shirakawa M, Takagi J, *et al.* 2014.** CRISPR/Cas9-mediated targeted mutagenesis in the liverwort *Marchantia polymorpha* L. *Plant & Cell Physiology* **55**: 475–481.

**Takahashi R, Nishio T, Ichizen N, Takano T. 2007.** Cloning and functional analysis of the $K^+$ transporter, PhaHAK2, from salt-sensitive and salt-tolerant reed plants. *Biotechnology Letters* **29**: 501–506.

**Takahashi R, Nishio T, Ichizen N, Takano T. 2007.** High-affinity $K^+$ transporter PhaHAK5 is expressed only in salt-sensitive reed plants and shows $Na^+$ permeability under NaCl stress. *Plant Cell Reports* **26**: 1673–1679.

**Takenaka M, Yamaoka S, Hanajiri T, *et al.* 2000.** Direct transformation and plant regeneration of the haploid liverwort *Marchantia polymorpha* L. *Transgenic Research* **9**: 179–185.

**Tang H, Krishnakumar V, Bidwell S, *et al.* 2014.** An improved genome release (version Mt4.0) for the model legume *Medicago truncatula*. *BMC Genomics* **15**: 312.

**Terada R, Urawa H, Inagaki Y, Tsugane K, Iida S. 2002.** Efficient gene targeting by homologous recombination in rice. *Nature Biotechnology* **20**: 1030–1034.

**Todesco M, Rubio-Somoza I, Paz-Ares J, Weigel D. 2010.** A collection of target mimics for comprehensive analysis of microRNA function in *Arabidopsis thaliana*. *PLoS Genetics* **6**: e1001031.

**Togawa T, Adachi T, Harada D, *et al.* 2018.** Cryopreservation of *Marchantia polymorpha* spermatozoa. *Journal of Plant Research* **131**: 1047–1054.

**Triques K, Sturbois B, Gallais S, *et al.* 2007.** Characterization of *Arabidopsis thaliana* mismatch specific endonucleases: application to mutation discovery by TILLING in pea. *The Plant Journal* **51**: 1116–1125.

**Tsuboyama S, Kodama Y. 2014.** AgarTrap: a simplified Agrobacterium-mediated transformation method for sporelings of the liverwort *Marchantia polymorpha* L. *Plant & Cell Physiology* **55**: 229–236.

**Tsuboyama-Tanaka S, Kodama Y. 2015.** AgarTrap-mediated genetic transformation using intact gemmae/gemmalings of the liverwort *Marchantia polymorpha* L. *Journal of Plant Research* **128**: 337–344.

**Tsuchiya Y, Yoshimura M, Sato Y, *et al.* 2015.** PARASITIC PLANTS. probing strigolactone receptors in *Striga hermonthica* with fluorescence. *Science (New York, N.Y.)* **349**: 864–868.

**Tsuzuki M, Nishihama R, Ishizaki K, *et al.* 2016.** Profiling and characterization of small RNAs in the liverwort, *Marchantia polymorpha*, belonging to the first diverged land plants. *Plant & Cell Physiology* **57**: 359–372.

**Ueda M, Kuniyoshi T, Yamamoto H, *et al.* 2012.** Composition and physiological function of the chloroplast NADH dehydrogenase-like complex in *Marchantia polymorpha*. *The Plant Journal* **72**: 683–693.

**Umehara M, Hanada A, Yoshida S, *et al.* 2008.** Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**: 195–200.

**United States Department of Agriculture, Agricultural Research Service, National Plant Germplasm System**. **2019.** *Germplasm Resources Information Network (GRIN-Taxonomy)*. Beltsville, MD: National Germplasm Resources Laboratory.

**Uraguchi D, Kuwata K, Hijikata Y, *et al.* 2018.** A femtomolar-range suicide germination stimulant for the parasitic plant *Striga hermonthica*. *Science (New York, N.Y.)* **362**: 1301–1305.

**Urquhart AA, Joy KW. 1981.** Use of phloem exudate technique in the study of amino acid transport in pea plants. *Plant Physiology* **68**: 750–754.

**Urquhart AA, Joy KW. 1982.** Transport, metabolism, and redistribution of xylem-borne amino acids in developing pea shoots. *Plant Physiology* **69**: 1226–1232.

**Vasquez EA, Glenn EP, Brown JJ, Guntenspergen GR, Nelson SG. 2005.** Salt tolerance underlies the cryptic invasion of North American salt marshes by an introduced haplotype of the common reed *Phragmites australis* (Poaceae). *Marine Ecology Progress Series* **298**: 1–8.

**Velasco VM, Mansbridge J, Bremner S, *et al.* 2016.** Acclimation of the crucifer *Eutrema salsugineum* to phosphate limitation is associated with constitutively high expression of phosphate-starvation genes. *Plant, Cell & Environment* **39**: 1818–1834.

**Vera-Estrella R, Barkla BJ, García-Ramírez L, Pantoja O. 2005.** Salt stress in *Thellungiella halophila* activates $Na^+$ transport mechanisms required for salinity tolerance. *Plant Physiology* **139**: 1507–1517.

**Vignolini S, Moyroud E, Hingant T, *et al.* 2015.** The flower of *Hibiscus trionum* is both visibly and measurably iridescent. *The New Phytologist* **205**: 97–101.

**Vlad D, Kierzkowski D, Rast MI, *et al.* 2014.** Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene. *Science (New York, N.Y.)* **343**: 780–783.

**Vogel A, Schwacke R, Denton AK, *et al.* 2018.** Footprints of parasitism in the genome of the parasitic flowering plant *Cuscuta campestris*. *Nature Communications* **9**: 2515.

**Volkov V, Amtmann A. 2006.** *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, has specific root ion-channel features supporting $K^+/Na^+$ homeostasis under salinity stress. *The Plant Journal* **48**: 342–353.

**Volkov V, Wang B, Dominy PJ, Fricke W, Amtmann A. 2004.** *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, possesses effective mechanisms to discriminate between potassium and sodium. *Plant, Cell and Environment* **27**: 1–14.

**Vretare V, Weisner SEB, Strand JA, Granéli W. 2001.** Phenotypic plasticity in *Phragmites australis* as a functional response to water depth. *Aquatic Botany* **69**: 127–145.

**Vries J de, Archibald JM. 2018.** Plant evolution: landmarks on the path to terrestrial life. *New Phytologist* **217**: 1428–1434.

**Vuolo F, Kierzkowski D, Runions A, *et al.* 2018.** LMI1 homeodomain protein regulates organ proportions by spatial modulation of endoreduplication. *Genes & Development* **32**: 1–6.

**Wang TL, Hedley CL. 1991.** Seed development in peas: knowing your three 'r's' (or four, or five). *Seed Science Research* **1**: 3–14.

**Wang XJ, Shi DC, Wang XY, Wang J, Sun YS, Liu JQ. 2015.** Evolutionary migration of the Disjunct Salt Cress *Eutrema salsugineum* (= *Thellungiella salsuginea*, Brassicaceae) between Asia and North America. *PLoS One* **10**: e0124010.

**Watson A, Ghosh S, Williams MJ, *et al.* 2018.** Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants* **4**: 23–29.

**Weber APM, Bar-Even A. 2019.** Improving the efficiency of photosynthetic carbon reactions. *Plant Physiology* **179**: 803–812.

**Weeden NF. 2018.** Domestication of pea (*Pisum sativum* L.): the case of the Abyssinian Pea. *Frontiers in Plant Science* **9**: 1–11.

**Weeden NF, Brauner S, Przyborowski JA. 2002.** Genetic analysis of pod dehiscence in pea (*Pisum sativum* L.). *Cellular & Molecular Biology Letters* **7**: 657–663.

**Weigel D. 2012.** Natural variation in Arabidopsis: from molecular genetics to ecological genomics. *Plant Physiology* **158**: 2–22.

**Weller JL, Ortega R. 2015.** Genetic control of flowering time in legumes. *Frontiers in Plant Science* **6**: 207.

**Westwood JH, DePamphilis CW, Das M, *et al.* 2012.** The parasitic plant genome project: new tools for understanding the biology of orobanche and striga. *Weed Science* **60**: 295–306.

**Whitewoods CD, Gonçalves B, Cheng J, *et al.* 2020.** Evolution of carnivorous traps from planar leaves through simple shifts in gene expression. *Science (New York, N.Y.)* **367**: 91–96.

**Wilhelmsson PKI, Mühlich C, Ullrich KK, Rensing SA. 2017.** Comprehensive genome-wide classification reveals that many plant-specific transcription factors evolved in streptophyte algae. *Genome Biology and Evolution* **9**: 3384–3397.

**Williams J, Lambert AM, Long R, Saltonstall K. 2019.** Does hybrid *Phragmites australis* differ from native and introduced lineages in reproductive, genetic, and morphological traits? *American Journal of Botany* **106**: 29–41.

**Wong CE, Li Y, Labbe A, *et al.* 2006.** Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in *Thellungiella*, a close relative of Arabidopsis. *Plant Physiology* **140**: 1437–1450.

**Wu HJ, Zhang Z, Wang JY, *et al.* 2012.** Insights into salt tolerance from the genome of *Thellungiella salsuginea*. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 12219–12224.

**Yamaoka S, Nishihama R, Yoshitake Y, *et al.* 2018.** Generative cell specification requires transcription factors evolutionarily conserved in land plants. *Current Biology* **28**: 479–486.e5.

**Yang R, Jarvis DE, Chen H, *et al.* 2013.** The reference genome of the halophytic plant *Eutrema salsugineum*. *Frontiers in Plant Science* **4**: 46.

**Yang Z, Wafula EK, Honaas LA, *et al.* 2015.** Comparative transcriptome analyses reveal core parasitism genes and suggest gene duplication and repurposing as sources of structural novelty. *Molecular Biology and Evolution* **32**: 767–790.

**Yaxley JL, Jablonski W, Reid JB. 2001.** Leaf and flower development in pea (*Pisum sativum* L.): mutants cochleata and unifoliata. *Annals of Botany* **88**: 225–234.

**Yoshida S, Cui S, Ichihashi Y, Shirasu K. 2016.** The haustorium, a specialized invasive organ in parasitic plants. *Annual Review of Plant Biology* **67**: 643–667.

**Yoshida S, Kim S, Wafula EK, *et al.* 2019.** Genome sequence of *Striga asiatica* provides insight into the evolution of plant parasitism. *Current Biology* **29**: 3041–3052.e4.

**Yoshida S, Shirasu K. 2009.** Multiple layers of incompatibility to the parasitic witchweed, *Striga hermonthica*. *The New Phytologist* **183**: 180–189.

**Yuan Y-W. 2018.** Monkeyflowers (*Mimulus*): new model for plant developmental genetics and evo-devo. *New Phytologist* **222**: 694–700.

**Zedler JB, Kercher S. 2004.** Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Reviews in Plant Sciences* **23**: 431–452.

**Zhang Y, Lai J, Sun S, *et al.* 2008.** Comparison analysis of transcripts from the halophyte *Thellungiella halophila*. *Journal of Integrative Plant Biology* **50**: 1327–1335.

**Zhang Y, Li Y, Lai J, *et al.* 2012.** Ectopic expression of a LEA protein gene TsLEA1 from *Thellungiella salsuginea* confers salt-tolerance in yeast and Arabidopsis. *Molecular Biology Reports* **39**: 4627–4633.

**Zhang Q, Zhao C, Li M, *et al.* 2013.** Genome-wide identification of *Thellungiella salsuginea* microRNAs with putative roles in the salt stress response. *BMC Plant Biology* **13**: 180.

**Zhou C, Ma ZY, Zhu L, Guo JS, Zhu J, Wang JF. 2015.** Overexpression of EsMcsu1 from the halophytic plant *Eutrema salsugineum* promotes abscisic acid biosynthesis and increases drought resistance in alfalfa (*Medicago sativa* L.). *Genetics and Molecular Research: GMR* **14**: 17204–17218.

**Zhu JK. 2001.** Plant salt tolerance. *Trends in Plant Science* **6**: 66–71.

**Zhu C, Yang J, Shyu C. 2017.** *Setaria* comes of age: meeting report on the second International Setaria Genetics conference. *Frontiers in Plant Science* **8**: 854–857.