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From Bench to Barn: Plant Model Research and its Applications in Agriculture

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Abstract

In view of the increasing world population and climate change, solutions are needed for improving agricultural productivity and energy supply. Biologists recognized the merits of employing model organisms as representatives of their species/subspecies to investigate molecular principles and pathways in great depth. For most model organisms, full genome sequence information and numerous bioinformatic resources are available. Protocols for transgenesis of model plants and various crops have been established. It is this jewelry of data and tools that helps to improve plant properties in a highly targeted manner.

Combining transgenic approaches with traditional plant breeding is a promising strategy to enhance agricultural productivity and the nutritional value of crops, to clean up contaminated environments and to improve efficiency in biofuel production. This review article provides an update on plant model organisms, including monocots and dicots; legumes and trees. It summarises current bioinformatic resources and transgenesis protocols. Recent achievements in genetic engineering are highlighted and theoretic scenarios for further optimization of plants are discussed.

Keywords: Plant model organisms; Symbiosis; Sustainable agriculture; Transgenesis; Bioinformatic resources

Plant Models and their Merits

The diversity of living species on our planet is immense. It is impossible to understand each of them in detail. Phylogenetically related species display strong similarities in their genetic set-up, physiology and behaviour. Moreover, at the molecular level, principle cellular signaling pathways are highly conserved. Biologists recognised the merits of employing model organisms as representatives of their species/subspecies to investigate the phenomena and mechanisms of development in great depth. The focused and extensive study in these experimental models has aided progress in microbiology, animal and plant science enormously [1].

As a result of these concerted efforts, for most model organisms full genome sequencing is accomplished and various omics data are available. The strategic use of bioinformatic resources makes the value of these data to disentangle biological complexity, to unravel networks of molecular interactions and finally to predict mechanisms in distantly related species indefinite [2].

Arabidopsis thaliana

Arabidopsis thaliana has emerged as the model organism of choice in plant biology [3]. Almost 30 years later, this modest weed is the by far best-studied plant. It was the first plant to be fully sequenced (Arabidopsis Genome Initiative, 2000), followed by rice [4], poplar [5] and others (Table 1). As a member of the mustard (Brassicacea) family, *Arabidopsis* is closely related to cultivated species, including oilseed rape and cabbages. *A. thaliana* offers important advantages for basic research in genetics and molecular biology: It is small in size and can be easily cultivated in restricted space.

The life cycle is short (6-8 weeks). *A. thaliana* has a small genome, comprising 119 Mb DNA and 26,000 genes. Extensive genetic and physical maps of all 5 chromosomes as well as a large number of mutant lines and genomic resources are available (Table 2). The plant is easily accessible to genetic manipulation (see below). The vast information arising from efforts of an international research community is collected and made accessible by The Arabidopsis Information Resource (TAIR).

Arabidopsis halleri and *Arabidopsis lyrata*

Two species in the *Arabidopsis* genus have attracted the attention of plant scientists, *Arabidopsis lyrata* and *Arabidopsis halleri*. The *A. lyrata* genome is approximately 65% larger genome than that of *A. thaliana* [6]. Full genome sequencing was accomplished in 2011 [6]. *A. lyrata* and *A. thaliana*, which are phenotypically very similar, can now be compared systematically at the genome level. Such comparison will advance our understanding of mutation and selection in plants. Surprisingly, despite its much smaller size, the *A. thaliana* genome only contains 17% fewer genes than *A. lyrata* [6].

Since the divergence of these two species, approximately 10 million years ago, numerous small deletions, mostly in non-coding DNA and transposes have occurred in *A. thaliana* [6]. *A. lyrata* is very closely related to *A. halleri* is of interest both for nutritional (biofortification) and ecological (phytoremediation) reasons. A recent study has documented the usability of *A. halleri* for phytoextraction of cadmium-polluted soil [7].

Genome sequencing of *A. halleri* is in progress (the Joint Genome Initiative). Comprehensive analyses of the genetic set-up and transcription profiles of *A. lyrata* vs. *A. halleri* will facilitate the identification of factors and pathways involved in metal binding. Individual factors or even entire pathways that mediate metal binding in *A. halleri* can be revealed and functionally characterized in a more systematic manner.

Upon further optimization (e.g. replacing particular amino acid residues to enhance enzyme activities), such factors or pathways

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Organism	Importance, research area	(haploid) genome size	Reference
Arabidopsis thaliana	general model plant	5 chromosomes, diploid, 119 Mb	(Kaul et al. 2000)
Arabidopsis lyrata	close relative of *A. thaliana*	5 chromosomes, diploid, 207 Mb	(Hu et al. 2011)
comparison *A. thaliana* vs. *A. lyrata*	functional, evolutionary and ecological studies		
Arabidopsis halleri	metal-binding model organism	8 chromosomes,diploid, estimated size 255 Mb	(Johnston et al. 2005)
comparison *A. lyrata* vs. *A. halleri*	identification of crucial factors in metal binding/ metal accumulation. biotechnological relevance: phytoremediation, bio-fortification		
Lotus japonicus	model legumesnitrogen fixation,	6 chromosomes, diploid, 470 Mb	
Medicago truncatula	root nodule symbiosis and arbuscular mycorrhzia, incl. overlap (common symbiosis pathway)	8 chromosomes, diploid, 240 Mb	(Young et al., 2011)
Populus trichocarpa **(poplar, black cottonwood)**	model tree, carbon sequestration forest tree growth and development phytoremediationdifficulties: no self-pollination long generation interval (4-8 years)	19 chromosomes, diploid, 550 Mb	(Tuskan et al. 2006)
Brachypodium distachyon	model monocot	5 chromosomes, diploid, 227 Mb	(InternationalBrachy podiumInitiative, 2010)
Oryza sativa **ssp. japonica**	model monocot; food crop	12 chromosomes, diploid, 420 Mb	(Goff et al. 2002)
Oryza sativa **ssp. indica**	model monocot; food crop	12 chromosomes, diploid, 466 Mb	(Yu et al. 2002)
Comparison O.s. japonica vs. O.s. **indica**	Identification of single nucleotide polymorphisms, transcripts and proteins related to altered biotic and abiotic stress tolerance levels in rice		e.g. (Lee et al. 2003; Ventelon-Debout et al. 2004; Liu et al. 2007)

Table 1: Model plants and their importance for main aspects in plant research.

Organism	Bioinformatics data/ resources	Link
Arabidopsis thaliana	Central access point for Arabidopsis data; large set of visualization and analysis	TAIR
	Tools	http://www.arabidopsis.org/index.jsp (Lamesch et al., 2012)
	gene mapping tool	http://signal.salk.edu/cgi-bin/tdnaexpress
	AtTFDB, comprehensive database for transcription factors	http://arabidopsis.med.ohio-state.edu/AtTFDB/
Lotus japonicus	various links/tools for functional genomics	http://www.kazusa.or.jp/lotus/
Medicago truncatula	various links/tools for functional genomics	http://www.medicago.org/
	Gene Expression Atlas: expression profile of all major organs and under many treatments	http://mtgea.noble.org/v2/ MtGEA V2
	Medicago hapmap, sequence information of 330 inbred *M. truncatula* accessions. For discovering single nucleotide polymorphisms insertions/ deletions and copy number variants.	http://www.medicagohapmap.org/home/view
legumes	LegumeTFDB, comprehensive database for transcription factors	http://legumetfdb.psc.riken.jp/
Populus trichocarpa **(black cotton wood)**	Overview, various links, functional genomics tools	http://www.ornl.gov/sci/ipgc/ http://popgenie.org/
Brachypodium distachyon	EST sequence database, links to homology-based annotations	www.brachypodium.org
Oryza sativa **(rice)**	functional genomics database, identify mutants in gene of interest /mapping tool	http://signal.salk.edu/cgi-bin/RiceGE
	Obtain full-length cDNA clones	(http://cdna01.dna.affrc.go.jp/cDNA/)
Monocots	GRAMENE for comparative genome analysis to study cross-species homology	http://www.gramene.org/
	GRASSIUS, collection of databases, computational and experimental resources that relate to the control of gene expression in the grasses, and their relationship with agronomic traits	http://grassius.org/
various species, incl. most model organisms	extensive set of genomic, gene expression, protein and mapping tools	BAR–Bio-Analytic Resource for Plant Biology http://bar.utoronto.ca/welcome.htm
	transcription factor database	http://plntfdb.bio.uni-potsdam.de/v3.0/
	PLACE, database of cis-acting regulatory DNA elements	
	tools and resources for plant genomics	http://plantgdb.org/

Table 2: Bioinformatic resources and tools for research in model plants and selected crops.

may be engineered to confer metal-binding properties on transgenic target plants. Potentially, this strategy presents a means of large-scale phytoremediation. Which target species to be used is determined by the specific characteristics of the affected area. Obviously, such target species must be accessible to genetic manipulation. Two recent studies in tomato and tobacco have shown the principle applicability of this approach [8,9].

Symbiosis research and model legumes

Not all key questions in plant science can be addressed in *Arabidopsis*. Most land plants form symbiotic associations with *arbuscular mycorrhizal* (AM) fungi. *Arabidopsis* is one exemption to this rule. Many agricultural crops are mycorrhizal, and despite marked differences in the organization of monocot and dicot root systems, the morphology of fungal colonization is similar. Endosymbioses (i.e. microbe lives within plant cells) are of imminent agricultural importance, because the host plant´s benefits from these associations directly correlate with harvest yield parameters. Benefits associated with AM symbiosis formation include improved phosphate nutrition, enhanced resistance to soil-borne pests and disease, improved resistance to drought, tolerance to heavy metals and better soil structure [10,11].

The second major type of endosymbiosis is the association of leguminous plants with nitrogen-fixing bacteria. This so-called root nodule (RN) symbiosis is much less abundant than AM and is restricted to some species belonging to the rosid I clade [12]. All legumes are found in one branch of this clade. RN symbiosis has a high economic and ecologic value and presents a solution towards sustainable agriculture. Biologically fixed nitrogen offers a number of benefits over the use of nitrogen fertilizer as the primary source of nitrogen input into crop production systems [13,14]. These benefits include: improved soil physical conditions, less potential for environmental degradation and lower costs. Crop rotation and intercropping of legumes with cereals are known to contribute to soil fertility [15,16]. In addition, legumes themselves provide a major source of food for humans and domestic animals.

The above paragraphs document the agricultural meaning of plant symbioses and indicate how important it is to understand these plant-microbe associations in detail. Two leguminous plants, *Lotus japonicus* [17] and *Medicago truncatula* [18], have emerged as equal-ranking model organisms to study both arbuscular mycorrhiza and root nodule symbiosis. Research in these model legumes has furthermore disclosed some striking similarities in cellular signaling of symbiotic bacterial invasion and pathogen attack [19]. Genome sequence data of *L. japonicus* [20] and *M. truncatula* [21] are available.

The most important food legume, soybean *Glycine max*, is now also fully sequenced [22]. Comprehensive bioinformatic resources (Table 2) will assist functional genomics studies in *L. japonicus, M. truncatula* and *G. max*. These tools can be employed intensively for collaborative analyses to direct identification of e.g. conserved features of proteins and protein families of interest (specific amino acid residues, peptide motifs, calculated three-dimensional structures). Ultimately, potentially promising traits, such as enhanced stress tolerance, or higher nutritional value can be optimised prior to introduction into soybean, in a very targeted manner.

The ability of *L. japonicus* (Sato et al. [20]) and *M. truncatula* to engage in the two main types of symbiosis has allowed to define overlaps and unique features in the plant´s response to *arbuscular mycorrhizal* fungi and nitrogen-fixing rhizobacteria [12]. The establishment of these symbioses involves calcium oscillations in plant root cells. A distinct set of genes encoding host factors non-specifically required for the formation of *Arbuscular mycorrhiza* and root nodules could be identified.

This provided insight into the evolution of plant-microbe interactions. Using forward genetic screens in legumes, a signaling pathway, the common symbiosis pathway, was uncovered (reviewed by Parniske [23]). As mentioned above, AM symbiosis is widely distributed in the plant kingdom. The evolutionary much younger legume root nodule symbiosis has likely evolved from the ancient AM symbiosis. Common symbiosis pathway genes may therefore be functionally conserved between legumes and non-nodulating plants [23]. In fact, the orthologs of three common symbiosis genes from model legumes were also found in rice, *OsPollux, OsCastor* and *OsCCaMK* [24].

Castor and *Pollux* encode nuclear porins. In legumes, these proteins likely act as ion channels to modulate the nuclear membrane potential, thereby contributing to the perinuclear calcium spiking induced during legume symbioses [25]. *CCaMK* encodes a calcium/calmodulin-dependent protein kinase, which is essential in the interpretation of calcium oscillations in plant root cells for the establishment of AM and root nodule symbiosis [26-28]. *OsPollux* and *OsCCaMK* were shown to be indispensable for rice AM symbiosis [24]. What is more, overexpression of either *OsCastor* or *OsCCaMK* could fully complement AM and RN symbiosis defects in the corresponding *Lotus japonicus* mutant lines [24].

From a biotechnological perspective, the straight-forward question is: "What is the "minimal legume code?" Can RN symbiosis be transferred to other crops, thus minimizing the need for exogenous nitrogen supply?" A recent break-through technology for multiple gene transfer via *Agrobacterium tumefaciens*-mediated transformation (see below), was the development of the binary vector pHUGE-Red. In a case study, eight genes essential for *Medicago truncatula* to establish a symbiosis with rhizobia bacteria were transferred as one 74 kb T-DNA into four non-leguminous species (strawberry, poplar, tomato and tobacco) [29]. Intriguingly, the vector was also equipped with an inducible recombination system allowing subsequent removal of the selection markers in the newly generated transgenic plants. It appears feasible to imprint not only single functions but entire pathways onto plants.

Model monocots

The above-described model plants are all dicots and thus only distantly related to the most important plants for human subsistence, the domesticated grass crops rice and wheat.

Oryza sativa: Oryza sativa (rice), whose genome was the second plant genome to be fully sequenced [4], has served as reference monocot for many years. Rice is the cereal with the smallest genome, consisting of 420-466 Mb across 12 chromosomes (Table 1). The two major subspecies, *japonica* and *indica* differ in their growth requirements and grain texture. While ssp. *japonica*, which has short and sticky grains, is grown in dry fields, ssp. *indica* produces non-sticky long grains and is grown mostly submerged. Rice molecular biologists have benefited from the fact that both subspecies are fully sequenced. A comparison between ten varieties of *japonica* and *indica* revealed a higher salinity tolerance in the *indica* varieties [30].

Furthermore, in viral infection experiments with *japonica* and *indica* cells, followed by transcriptome and proteome analysis a certain overlap between virus-induced and abiotic stress-related responses was noted. A number of transcripts/proteins differentially expressed in the partially resistant *japonica* cultivar were identified [31,32]. Altered transcriptome profiles as well as substantial sequence divergence between *indica* and *japonica* subspecies have also been observed in a more recent large-scale study [33]. The rice research community has provided resource to numerous bioinformatic tools and data sets. GRAMENE and GRASIUS furthermore facilitate cross-species comparisons within the monocot group (Table 2).

Brachypodium distachyon: Unfortunately, unlike *Arabidopsis* (petite, rapid growth, small genome, self-compatible), rice has less convenient characteristics. It is demanding in its growth requirements; the rice genome is approximately four times larger than that of *Arabidopsis*. An alternative species was proposed as model plant for functional genomics in monocots: *Brachypodium distachyon* [34]. In terms of plant size (15-20 cm), generation time (8-12 weeks) and growth requirements (simple), *Brachypodium distachyon* is similar to *Arabidopsis* (Table 1).

These desirable features were the driving force for establishing *Brachypodium distachyon* as grass *Arabidopsis* [35]. As a result, key resources including genetic stocks, EST collections, BAC libraries and transformation protocols have become available [36] (Table 2). Synergistic efforts of the International Brachypodium Initiative allowed accomplishing full sequencing and annotation of the Brachypodium genome (International Brachypodium Initiative, 2010). A large collection of T-DNA insertion lines can be employed for reverse genetics approaches. Thus, in terms of research progress, *Brachypodium* will keep pace with *Arabidopsis*. Both species are excellent models for monocots and dicots, respectively. Their value further correlates with the availability of comprehensive bioinformatics resources (Table 2).

Since its introduction as model monocot, the value of *Brachypodium* for addressing diverse areas of plant biology has been amply substantiated. To name a few, these reports include studies on vernalisation [37] and flowering time pathways [38] and seed storage proteins [39], as well as responses to drought stress [40,41] and pathogens [42,43]. The roles of microRNAs involved in drought and cold stress responses have also been addressed [44,45]. The number of reports on comparative genetic analysis of *Brachypodium* cultivated crop species is growing [38,46-48].

One highlight demonstrating the biotechnological potential of *Brachypodium* is found in a very recent report [49]. The authors characterized a cinnamoyl alcohol dehydrogenase gene, *BdCAD1*, involved in lignification. Disruption of the gene alters the branching of lignin; saccharification yields are improved in *Bdcad1* mutant lines, while biomass yield is not compromised. With respect to optimizing the cellulose-to-ethanol conversion process, knocking out or silencing *BdCAD1* homologs in other grasses is a promising approach for the fuel industry. An equally promising strategy is the overexpression of recombinant cellulolytic enzymes in sugarcane [50].

The simplicity of *A. thaliana* and *B. distachyon* on the one hand and their taxonomic distance on the other hand shall assist identification of universal factors. If a particular *Arabidopsis* gene (characterised, documented positive effect on e.g. stress resistance) has similar effects when heterologously overexpressed in *B. distachyon* (and vice versa), it may be functional in most plants (monocots and dicots). Reciprocally, a *B. distachyon* gene that confers desired characteristics to transgenic *A. thaliana* may also act universally upon heterologous overexpression in other target species (Figure 1).

Model trees and biofuel: Forest trees have tremendous economic and ecological value. Wood is an important source for fuel. It also represents the major form of structural material in industry. Forests are unique ecosystems; they prevent soil erosion and play an irreplaceable role in carbon sequestration. A number of anatomical and physiological features solely occur in trees. These include e.g. their perennial growth habit, extensive formation of secondary xylem (i.e. wood), seasonal reallocation of nutrients, cold hardiness and longevity [51].

Research in very large and long-lived organisms is a difficult and challenging undertaking. From these considerations, the demand for a model system for forest trees became apparent. In 2000, poplar (*Populus*) was proposed as model forest tree [51]. Now, black cottonwood (*Populus trichocarpa*) is the established model organism in tree research. Poplar combines several advantages, including rapid growth, prolific sexual reproduction, ease of cloning and accessibility to genetic transformation (Table 3). The *Populus trichocarpa* genome is comparatively small (550 Mb); it was the first tree genome to be fully sequenced [5] (Table 1). Poplars are bred on detached female branches with pollen that can be stored for several years Seeds germinate (within 24 h) and grow rapidly (1-2 m in the first year) [51].

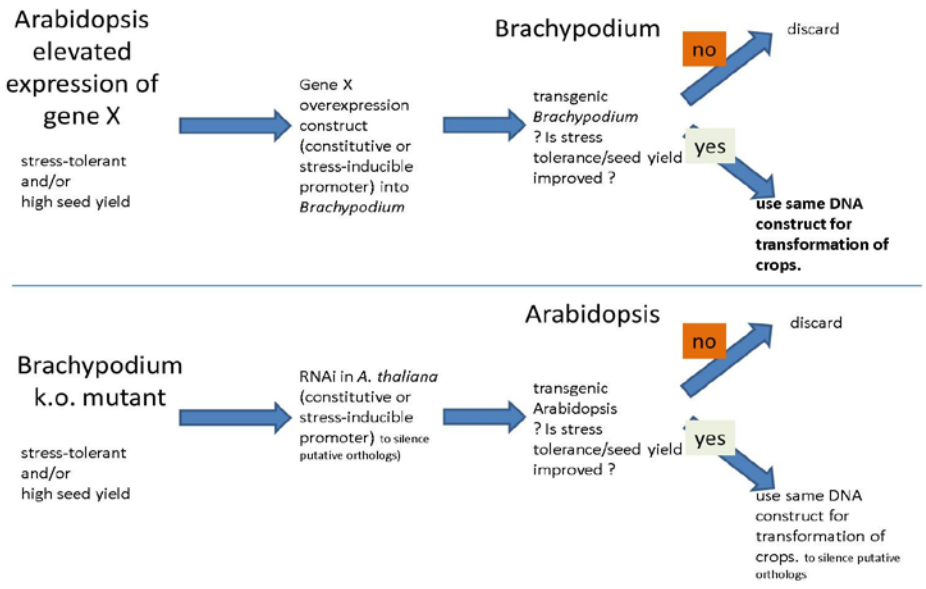

Figure 1: Employing simplicity and taxonomic distance of Arabidopsis and brachypodium genetics to test candidates "universal" genes. Strategy 1 (top) starts with a plant over expressing an advantageous gene (of known sequence). Strategy 2 (bottom) starts with a mutant lacking a disadvantageous gene (of known sequence).

Unlike many other model organisms, poplar is of direct economic and ecologic importance: Besides its value as source of wood and fuel, poplars can be utilised for phytoremediation of environmental toxins [52-54] as well as for long-term biomonitoring of soil contamination [55]. Poplar is also considered suitable for large-scale carbon sequestration [56-58]. Poplar plantations might thus be used to partially offset increasing atmospheric carbon dioxide concentrations.

The accessibility of poplar to transgenesis (Table 3) on the one hand and the rich pool of bioinformatic resources on the other hand (Table 3) renders this tree a prime candidate for the development of transgenic energy crops. A recent study exemplifies the value of combinatorial bioinformatic and transgenic approaches: Transgenic poplar, in which the *RabG3bCA* gene (encoding a GTPase) from *Arabidopsis* was overexpressed, were taller and thicker and had a higher cellulose content and enhanced enzymatic digestibility (saccharification) compared to wild- type poplar [59]. Furthermore, fiber cell length, which is the most important factor determining paper and pulp quality, was increased in RAbG3b oxerexpressing poplar. This transgenic line and its use were patented in 2013.

Transgenesis: A plant´s value as a model organisms and its usability for functional genomics strongly depends on its accessibility to genetic manipulation, i.e., transformation with tailor-made DNA constructs. The major plant transgenesis technologies include agrobacterium-mediated transformation, microprojectile bombardment, electroporation, pollen tube pathway transformation and silicon-carbide-whiskers-mediated transformation. A comprehensive overview of these methods has recently been published by Barampuram and Zhang [60]. Agrobacterium-mediated transformation, the most predominantly employed strategy, will be addressed in the following paragraphs. A contemporary review on novel nanotechnology-based strategies can be found in Rafsanjani et al. [61].

The transfer of designed DNA cassettes into plant cells with the aid of *Agrobacterium tumefaciens*, is a standard method for plant transformation [62-64]. An overview about alternative, less often employed strategies can be found in Ziemienowicz [65]. The soil-borne pathogen *A. tumefaciens* is the only organism capable of inter-kingdom DNA transfer. Under natural conditions, it causes tumor growth in infected host plants. However, so-called "disarmed" agrobacterial strains, deprived of their tumorigenicity, can be employed as "DNA shuttles" for the delivery of engineered transfer DNA (T-DNA) into plant cells [62-64].

Depending on whether the nuclear-targeted T-DNA is degraded or incorporated into the host´s chromosomal DNA, agrobacterial infection leads to transient (lasting several days) or stable (inherited) transgene expression [66]. In the latter case, whole transgenic plants can be obtained. The regeneration of stable transgenic plants mostly involves the rather tedious procedure of tissue culturing. Briefly, explants from various organs are incubated with agrobacteria, resulting in the formation of non- differentiated cells (calli), from which whole plants can be regenerated.

Protocols are being developed and modified frequently to simplify and shorten the transformation procedure and/or to enhance transformation rates. Table 3 provides an overview on current transformation protocols, with a focus on model plants and selected

Organism	**Transformation principle,** time required (co-cultivation to the planting of transformants into pots)	**Protocol references**
Arabidopsis thaliana	floral dip	(Weigel and Glazebrook, 2006)
Arabidopsis lyrata	root explants, 4 months	(Fobis-Loisy et al. 2007)
Arabidopsis halleri	root explants	(Hanikenne et al. 2008)
***Brassica napus* (rapeseed)**	seedling explants, 2.5-3.5 months	(Bhalla and Singh, 2008)
Lotus japonicus	root explants, 4 months	(Lombari et al. 2003)
Medicago truncatula	root explants, 4 months	(Crane et al. 2006)
	alternative: Agrobacterium rhizogenes-mediated transformation via hairy roots	(Deng et al. 2011) (Crane et al. 2006)
***Glycine max* (soybean)**	hypocotyls	(Wang and Xu, 2008)
Brachypodium distachyon	immature embryos, 8 months	(Pacurar et al. 2008; Vain et al. 2008; Vogel and Hill, 2008; Alves et al. 2009; Thole and Vain, 2012)
***Oryza sativa* ssp. indica**	explants from mature seeds, 2 months	(Sahoo et al. 2011)
***Oryza sativa* ssp. japonica**	embryos (scutellum), 2-3 months	(Duan et al. 2012)
	additional advance: non-antibiotic selectable agent	
***Triticum* (wheat)**	floral dip (pre-anthesis spikes with clipped florets)	(Agarwal et al., 2009; Zale et al. 2009)
	in-planta transformation of germ cells (agrobacterial incubation of pistil filaments during artificial pollination)	(Mamontova et al. 2010)
***Triticosecale ssp.* (triticale)**	in vitro-prepared nano-complex consisting of transferred DNA, agrobacterial virulence protein D2, and recombination protein A delivered to triticale microspores, 2 months	(Ziemienowicz et al. 2012)
***Zea mays* (maize)**	embryos, 3 months	(Ishida et al. 2007; Sidorov and Duncan, 2009; Mamontova et al. 2010)
***Populus trichocarpa* (black cotton wood)**	stem explants, 5 months	(Song et al. 2006)
Transient expression systems		
Nicotiana benthamiana ***Nlcotiana tabacum***	leaf infiltration	(Cevik and Kazan, 2013) (Persak and Pitzschke, 2013)
Tropaeolum majus	leaf infiltration	(Pitzschke, 2013)

Table 3: Model plants and current transgenesis protocols via Agrobacterium-mediated transformation. For an overview on alternative transformation strategies the reader is referred to a recent review (Barampuram and Zhang, 2011) and references therein.

crop species. Table 4 lists a number of recent examples of transgenic crops with improved stress tolerance. This list is not saturating but shall provide a glimpse on the diverse possibilities. *Arabidopsis thaliana* stable transformants are easily obtained via the "floral dip" method [67]. Plants at the early-flowering stage are dipped into agrobacterial suspension and allowed to set seeds. Normally, 0.1-1% of seeds, representing the next generation, harbor one or multiple copies of the transgene [68]. Floral dipping may also be applicable to transgenesis of wheat [69,70].

The simplicity of *Arabidopsis* floral dipping and its versatility as heterologous expression system has facilitated characterization of genes from various plant species. For biotechnological applications, a fast and informative insight into factors from *Arabidopsis*` close relative, rapeseed (*Brassica napus*), is particularly interesting. Integrative molecular biology studies involving *Arabidopsis* and rapeseed aim at enhancing bio-diesel production. For example, *wri1* (encoding an AP2/EREBP transcription factor) was found to be differentially expressed in *B. napus* lines with different oil content. Constitutive overexpression of *Bnwri1* in *A. thaliana* resulted in highly elevated seed oil content and enlarged seed size and mass [71]. Similar effects were observed in transgenic *Arabidopsis* overexpressing *BnGRF2* (growth-regulating factor2, a transcription factor) [72].

The authors concluded that *Bnwri1* and *BnGRF2* have potential applications in food and oil industries and in rapeseed breeding. Reciprocally, overexpression of AtNHX1, a vacuolar Na(+)/H(+) antiport protein from *Arabidopsis*, conferred enhanced salt tolerance to rapeseed [73]. These examples show the interchangeability of genetic factors between *Arabidopsis* and rapeseed and suggest that desired properties in crops can be achieved through the modification of a single trait.

Agrobacterium-mediated transformation for transient expression studies

Stable transformation is the only means to maintain transgene expression in the next generations. However, for various questions in molecular biology, transient expression systems are the method of choice. They provide rapid answers on e.g. subcellular localization, stability and posttranslational modification of recombinant proteins. Agrobacterial infiltration into *Nicotiana* leaves (*N. benthamiana, N. tabacum*) is the most popular system for transient expression studies in plant tissue [74].

Chimeric promoter-reporter gene constructs can be utilized to assess promoter activities and their responsiveness to environmental stimuli. This approach can also be employed for identification of regulatory cis-elements. Moreover, transient expression directed by heterologous promoters provides some (limited) information on gene regulation in other species. Recent advances in that direction include the identification of a novel moderate constitutive promoter from poplar [75] and the characterization of an AP2-family transcription factor-activated promoter from chickpea involved in growth regulation and stress tolerance [76].

Because multiple transgenes can be delivered at once, transient expression in *Nicotiana* is a convenient system for studying protein-protein interactions and promoter/effector (transcription factor) relations–a comparatively simple approach towards the deciphering of signaling pathways [77]. Agrobacterial infiltration of *Nicotiana* yields transformation efficiencies (i.e. fraction of transgene-expressing cells) of up to 100% (own data). However, recombinant protein accumulation may vary significantly depending on leaf age and leaf position, thus complicating quantitative comparisons. A recently developed protocol circumvents this problem: Randomised pools of leaf discs rather than leaves of intact *N. tabacum* plants are subjected to agrobacterial treatment [78].

Unfortunately, most plants are poorly, or not at all, accessible to transient transformation by Agrobacteria. In *Arabidopsis*, transformation efficiency is low; it strictly depends on growth conditions and has yielded satisfactory results only in young seedlings [79]. Since *Nicotiana* (order Solanales) is only distantly related to *Arabidopsis* (order Brassicales), data from heterologous expression in *Nicotiana* may not fully reflect the situation in *Arabidopsis*. Clearly, an expression system combining the simplicity of leaf infiltration and the detailed knowledge of *Arabidopsis* genomics would be desirable.

Source of transgene	Constitutive expression, promoter desription	Stress-related phenotype in transgenic crops	Reference
TaNAC69, Stress-responsive transcription factor from wheat	ectopic expression (drought-inducible HvDhn4s promoter)	enhanced tolerance to moderate **salt** and **drought** stress in **barley**	(Xue et al. 2011)
OsNAC1, stress-responsive transcription factor from rice	overexpression (maize ubiquitin promoter)	enhanced tolerance to salt and **drought** stress in **wheat**	(Saad et al. 2013)
ZmPTF1, low-phosphate-inducible bHLH-type transcription factor from maize	overexpression	enhanced tolerance to **phosphate deficiency** in **maize**	(Li et al. 2011)
OsNAC5, NAC transcription factor from rice	root-specific promoter RCc3:OsNAC5	enhanced **drought** tolerance and increased **grain yield** in **rice**	(Jeong et al. 2013)
LOS5/ABA3 (molybdenum cofactor sulphurase) indirectly controls abscisic acid biosynthesis in *Arabidopsis*	constitutive overexpression	enhanced **drought** tolerance, **antixodative** enzyme activities and **proline** accumulation in **soybean**	(Li et al. 2013)
TsVP, proton-translocating PPase from *Thellungiella halophila*	constitutive overexpression (CaMV35S promoter)	enhanced **osmotic+drought** stress tolerance and higher **seed yield** in **cotton**	(Lv et al. 2009)
TsVP, proton-translocating PPase from *Thellungiella halophila*	constitutive overexpression (ubiquitin promoter)	enhanced tolerance to **phosphate deficiency** in **maize**	(Pei et al. 2012)
AtNDPK2, Nucleoside diphosphate kinase 2 from *Arabidopsis*	oxidative stress-inducible SWPA2 promoter from sweet potato	enhanced tolerance to **cold, salinity** and **oxidative stress** in **potato** and **sweetpotato**	(Tang et al. 2008; Kim et al. 2009)
		enhanced tolerance to **oxidative stress, antioxidant** enzyme activities and growth in poplar	(Kim et al. 2011)

Table 4: Examples of transgenic crops exhibiting enhanced stress tolerance. It should be noted that in many cases the transgene employed for crop transformation originates from a model plant.

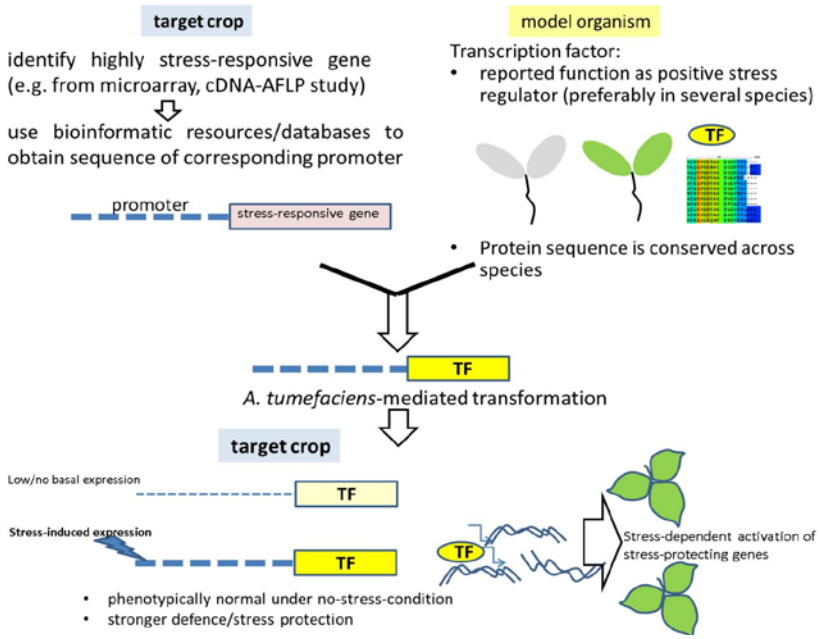

Figure 2: Fine-tuning gene expression for improving stress tolerance in crops. A chimeric DNA construct, consisting of a stress-responsive promoter from a crop species and a functionally-conserved well-characterised transcription factor gene from a model plant is employed. See main manuscript for details.

The development of such expression system is part of my current research. As reported very recently, nasturtium (*Tropaeolum majus*), which belongs to the same order as *Arabidopsis*, i.e., Brassicales, is easily accessible to agroinfiltration, yielding high transformation efficiencies. This new expression system may provide the *Arabidopsis* community with a tool to study subcellular localisation, protein-protein interactions and reporter gene activities in a genetic background that is closely related to their model organism [80]. An additional plus is the fact that *Tropaeolum majus* can be colonized by *arbuscular mycorrhizal* fungi.

Fine-tuning gene expression for improving stress tolerance

Transcription factors (TFs) are key components of signal transduction pathways. TFs translate information received from their upstream regulators (e.g. stress-activated kinases) into altered expression of target genes. Since TFs usually have numerous target genes, they can initiate substantial re-programming of a plant´s transcriptome. TFs are particularly promising candidates for genetic engineering of stress-tolerant crops. However, improved stress resistance through overexpression of TFs often comes at the expense of retarded growth or other developmental abnormalities [81,82].

The main reasons for these undesired side effects are: i) the introduced transcription factor regulates additional genes besides the desired target(s); and ii) permanent hyper-activation of stress response pathways is costly; and energy resources are withdrawn from "maintenance programmes". These side effects may be circumvented or reduced, by placing candidate transcription factors under the control of stress-responsive promoters. Transcriptome analysis (employing bioinformatic resources) to identify genes displaying low basal but strongly stress-induced expression levels. Isolate promoter of such gene. Select a transcription factor. Criteria: Documented positive regulator of stress in at least one species, well-conserved protein sequence.

This can for instance be a TF from *Arabidopsis* or another model plant. Place this transcription factor under control of above-mentioned stress-responsive promoter and introduce the resulting construct into the species of interest. Combining the knowledge about conserved stress-responsive promoters plus functional characterization of candidate TF proteins will allow activation of self-defense mechanisms in engineered plants on demand (Figure 2). Ideally, only as long as a certain stressor is present, will a TF be produced, thereby minimizing undesired side effects.

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