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 (Brassicaceae) 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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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].

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Bioinformatics data/ resources</th>
<th>Link</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis thaliana</td>
<td>Central access point for Arabidopsis data; large set of visualization and analysis</td>
<td>TAIR</td>
</tr>
<tr>
<td></td>
<td>Tools</td>
<td><a href="http://www.arabidopsis.org/index.jsp">http://www.arabidopsis.org/index.jsp</a> (Lamesch et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>gene mapping tool</td>
<td><a href="http://signal.salk.edu/cgi-bin/tdnaexpress">http://signal.salk.edu/cgi-bin/tdnaexpress</a> (Lee et al., 2003)</td>
</tr>
<tr>
<td>Medicago truncatula</td>
<td>Gene Expression Atlas: expression profile of all major organs and under many treatments</td>
<td><a href="http://mtgea.noble.org/v2/MGESA">http://mtgea.noble.org/v2/MGESA</a> V2</td>
</tr>
<tr>
<td>Legumes</td>
<td>LugeumTFDB, comprehensive database for transcription factors</td>
<td><a href="http://legumetfdb.psc.riken.jp/">http://legumetfdb.psc.riken.jp/</a></td>
</tr>
<tr>
<td>Populus trichocarpa</td>
<td>Overview, various links, functional genomics tools</td>
<td><a href="http://www.ornl.gov/sci/ipgc/">http://www.ornl.gov/sci/ipgc/</a></td>
</tr>
<tr>
<td>(black cottonwood)</td>
<td></td>
<td><a href="http://grassius.org/">http://grassius.org/</a></td>
</tr>
<tr>
<td>Brachypodium distachyon</td>
<td>EST sequence database, links to homology-based annotations</td>
<td><a href="http://www.brachypodium.org">http://www.brachypodium.org</a></td>
</tr>
<tr>
<td>Oryza sativa (rice)</td>
<td>functional genomics database, identify mutants in gene of interest</td>
<td><a href="http://signal.salk.edu/cgi-bin/RiceGE">http://signal.salk.edu/cgi-bin/RiceGE</a></td>
</tr>
<tr>
<td></td>
<td>Obtain full-length cDNA clones</td>
<td>(<a href="http://cdna01.dna.affrc.go.jp/cDNA/">http://cdna01.dna.affrc.go.jp/cDNA/</a>)</td>
</tr>
<tr>
<td>Monocots</td>
<td>GRAMENE for comparative genome analysis to study cross-species homology</td>
<td><a href="http://www.gramene.org/">http://www.gramene.org/</a></td>
</tr>
<tr>
<td></td>
<td>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</td>
<td><a href="http://grassius.org/">http://grassius.org/</a></td>
</tr>
<tr>
<td>most model organisms</td>
<td></td>
<td><a href="http://pntfdb.bio.uni-potsdam.de/v3.0/">http://pntfdb.bio.uni-potsdam.de/v3.0/</a></td>
</tr>
<tr>
<td></td>
<td>tools and resources for plant genomics</td>
<td><a href="http://plantgdb.org/">http://plantgdb.org/</a></td>
</tr>
</tbody>
</table>

Table 2: Bioinformatic resources and tools for research in model plants and selected crops.
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 monocolot 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 and protein families of interest (specific amino acid residues, peptide motifs, calculated three-dimensional structures). Ultimately, these resources provide a major source of food for humans and domestic animals.

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 hardness 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 overexpressing poplar. This transgenic line and its use were patented in 2013.

Transgenesis: A plant’s value as a model organism 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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Transformation principle, time required (co-cultivation to the planting of transformants into pots)</th>
<th>Protocol references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis thaliana</td>
<td>floral dip</td>
<td>(Weigel and Glazebrook, 2006)</td>
</tr>
<tr>
<td>Arabidopsis lyrata</td>
<td>root explants, 4 months</td>
<td>(Fobis-Loisy et al. 2007)</td>
</tr>
<tr>
<td>Arabidopsis halleri</td>
<td>root explants</td>
<td>(Hankikene et al. 2008)</td>
</tr>
<tr>
<td>Brassica napus (rapeseed)</td>
<td>seedling explants, 2.5-3.5 months</td>
<td>(Bhalla and Singh, 2008)</td>
</tr>
<tr>
<td>Lotus japonicus</td>
<td>root explants, 4 months</td>
<td>(Lombardi et al. 2003)</td>
</tr>
<tr>
<td>Medicago truncatula</td>
<td>root explants, 4 months</td>
<td>(Crane et al. 2006)</td>
</tr>
<tr>
<td>Glycine max (soybean)</td>
<td>hypocotylia</td>
<td>(Oesterhaus et al. 2008)</td>
</tr>
<tr>
<td>Brachypodium distachyon</td>
<td>immature embryos, 8 months</td>
<td>(Pacurar et al. 2008; Vain et al. 2008; Vogel and Hill, 2008; Alves et al. 2009; Thole and Vain, 2012)</td>
</tr>
<tr>
<td>Oryza sativa ssp. indica</td>
<td>explants from mature seeds, 2 months</td>
<td>(Sahoo et al. 2011)</td>
</tr>
<tr>
<td>Oryza sativa ssp. japonica</td>
<td>embryos (scutellum), 2-3 months</td>
<td>(Duan et al. 2012)</td>
</tr>
<tr>
<td>Triticum (wheat)</td>
<td>additional advance: non-antibiotic selectable agent</td>
<td>(RabG3b oxerexpressing poplar. This transgenic line and its use were patented in 2013.</td>
</tr>
<tr>
<td>Triticosecale ssp. (triticale)</td>
<td>in-planta transformation of germ cells (agrobacterial incubation of pistil filaments during artificial pollination)</td>
<td>(Mamontova et al. 2010)</td>
</tr>
<tr>
<td>Zea mays (maize)</td>
<td>floral dip (pre-anthesis spikes with clipped florets)</td>
<td>(Agarwal et al., 2009; Zale et al. 2009)</td>
</tr>
<tr>
<td>Populus trichocarpa (black cotton wood)</td>
<td>in vitro-prepared nano-complex consisting of transferred DNA, agrobacterial virulence protein D2, and recombination protein A delivered to triticale microspores, 2 months</td>
<td>(Ziemienowicz et al. 2012)</td>
</tr>
</tbody>
</table>

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 transformatants 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, wr1 (encoding an AP2/EREBP transcription factor) was found to be differentially expressed in B. napus lines with different oil content. Constitutive overexpression of Bnwr1 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 Bnwr1 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.

<table>
<thead>
<tr>
<th>Source of transgene</th>
<th>Constitutive expression, promoter description</th>
<th>Stress-related phenotype in transgenic crops</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TaNAC69</td>
<td>Stress-responsive transcription factor from wheat</td>
<td>ectopic expression (drought-inducible HvDHn4s promoter)</td>
<td>enhanced tolerance to moderate salt and drought stress in barley</td>
</tr>
<tr>
<td>OsNAC1</td>
<td>Stress-responsive transcription factor from rice</td>
<td>overexpression (maize ubiquitin promoter)</td>
<td>enhanced tolerance to salt and drought stress in wheat</td>
</tr>
<tr>
<td>ZmPPTF1</td>
<td>low-phosphate-inducible bHLH-type transcription factor from maize</td>
<td>overexpression</td>
<td>enhanced tolerance to phosphate deficiency in maize</td>
</tr>
<tr>
<td>OsNAC5, NAC transcription factor from rice</td>
<td>root-specific promoter</td>
<td>enhanced drought tolerance and increased grain yield in rice</td>
<td>(Jeong et al. 2013)</td>
</tr>
<tr>
<td>LOSS/ABA3</td>
<td>molybdenum cofactor (ubiquinase) indirectly controls abscisic acid biosynthesis in Arabidopsis</td>
<td>constitutive overexpression</td>
<td>enhanced drought tolerance, antioxidative enzyme activities and proline accumulation in soybean</td>
</tr>
<tr>
<td>TiVP, proton-translocating PPase from Thellungiella halophila</td>
<td>constitutive overexpression (CaMV35S promoter)</td>
<td>enhanced osmotic+drought stress tolerance and higher seed yield in cotton</td>
<td>(Lv et al. 2009)</td>
</tr>
<tr>
<td>TiVP, proton-translocating PPase from Thellungiella halophila</td>
<td>constitutive overexpression (ubiquitin promoter)</td>
<td>enhanced tolerance to phosphate deficiency in maize</td>
<td>(Pei et al. 2012)</td>
</tr>
<tr>
<td>ANRDK2, Nudix-sive 2phosphate kinase 2 from Arabidopsis</td>
<td>oxidative stress-inducible SWPA2 promoter from sweet potato</td>
<td>enhanced tolerance to cold, salinity and oxidative stress in potato and sweetpotato</td>
<td>(Tang et al. 2008; Kim et al. 2009)</td>
</tr>
<tr>
<td>enhanced tolerance to oxidative stress, antioxidative enzyme activities and growth in poplar</td>
<td>(Kim et al. 2011)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.
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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**References**
