Title: Transient reprogramming of crop plants for agronomic performance

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Dedicated to Dmitri Ivanovsky (1864-1920), the discoverer of viruses.

Abstract: The development of a new crop variety is a time-consuming and costly process due to plant breeding's reliance on gene shuffling to introduce desired genes into elite germplasm followed by backcrossing. We propose alternative technology that transiently targets various regulatory circuits within a plant, leading to operator-specified alterations of agronomic traits, such as time of flowering, vernalization requirement, plant height or drought tolerance. We redesigned techniques of gene delivery, amplification and expression around RNA viral transfection methods that can be implemented on an industrial scale and with multiple crop plants. The process does not involve genetic modification of the plant genome and is thus limited to a single plant generation, is broadly applicable, fast, tunable, versatile, and can be used throughout much of the crop cultivation cycle. The RNA-based reprogramming may be especially useful in case of major plant pathogen pandemics, but also for commercial seed production and for rapid adaptation of orphan crops.

Modern plant breeding relies on recombination to introduce novel useful genes/alleles into elite germplasm. Development of a new variety is time-consuming and expensive, even with a use of most advanced technologies such as genome editing. We sought to design a flexible, rapid and industrially scalable alternative platform to alter hormonal and other regulatory circuits within a plant, by rebuilding the known techniques of transient gene expression around gene delivery methods that can be performed on an industrial scale, and that can be practiced with multiple crop plants. Our approach focused on two types of vectors commonly used in laboratory science; namely, *Agrobacterium* as the primary DNA vector, and RNA viral amplicons as secondary/primary vectors and amplifiers of information molecules. We and others have successfully used *Agrobacterium*-based transfection to design industrial-scale manufacturing processes for producing recombinant proteins in plants[1-4], including biopharmaceuticals, vaccines and biomaterials[5]. This earlier-generation transient reprogramming focused on a single plant species, *Nicotiana benthamiana*. The method required vacuum-assisted infiltration of bacteria into the intercellular leaf space and, by design, ignored the general agronomic performance of the plant other than the high-level expression of heterologous recombinant proteins that were almost exclusively of non-plant origin. A few attempts to modify agronomic traits, namely viral induction of flowering, were also previously reported, but were limited to research-scale experiments[6-11].

We report here that multiple economically important crop plants can be induced to exhibit desirable agronomic performance traits, by simply spraying them with agrobacteria carrying viral replicons to express plant genes. Moreover, we also demonstrate that most of the agronomic traits can also be engineered by spraying plants with packaged RNA viral vectors thus

eliminating DNA release into environment altogether. In our studies, manipulation of traits was based on expression or silencing of specific plant regulatory genes that are components of hormonal circuits, such as flowering control, gibberellin, abscisic acid, ethylene pathways and several others. Using appropriate molecular techniques, we evaluated several viral vectors, tested a large set of plant genes and characterized molecular events linked to plant phenotypes. We show that proposed transfection generates a temporary cascade of new information in the plant and enables the controlled alteration of agronomic performance in multiple useful ways.

Results

Agrobacterium and packaged RNA viral vectors as tools for industrially scalable transfection

Based on processes widely practiced in agronomy, namely spraying plants with solutions or suspensions to deliver agrochemicals, we evaluated delivery of agrobacteria and viral particles. These vectors were found effective when applied using standard conditions typical for industrial sprayers (1-3 bar pressure; 1-4 mm atomizer nozzles) and were unaffected by these spraying conditions. The delivery of agrobacteria required use of surfactants such as Silwet® L-77, Silwet® Gold, Triton™ X-100 or Tween® 20 to induce 'stomatal flooding' and allow bacteria to enter the intercellular space of the leaf[4]. We also explored the use of abrasives such as silicon carbide F800 or diatomaceous earth as described in ref. [12,13], which allowed for improved transfection of several plant species. By applying a suspension of agrobacteria of approximately 10^6 cfu per ml (10^3 dilution of the overnight culture OD_{600} = 1.5) mixed with Silwet® L-77 at a concentration of 0.1% to the test plant *N. benthamiana* (**Fig. 1a**), the frequency of transfection by agrobacteria was as high as 10^{-2} per leaf cell (**Fig. 1b**, and ref. [4]). Agrobacteria were efficient in

the delivery of viral vectors with either localized or systemic movement as well as movement-disabled ones (**Fig.1c,d**). Subsequent studies revealed that for most traits to be efficiently delivered, the viral vector should be able to move locally or systemically through the phloem. In the proof-of-principle experiments using dipping of *Nicotiana benthamiana* leaves into the suspension of agrobacteria, we also showed that it is possible to achieve subsequent transfections by treating the same plant several times (**Fig. 1e**).

Using *Agrobacterium* carrying viral vectors with a *GFP* cargo (reporter) gene, we evaluated representatives of 28 plant species from 6 plant families (**Supplementary Table 1**) and found that leaves of many dicotyledonous plants of practical interest such as tomato, potato, pepper, sugar beet, spinach, soybean, and monocotyledonous plants maize, wheat, etc., can be efficiently transfected using our standard spray technique. *Potato virus X* (PVX)-based replicons performed efficiently in most crop plants we tested (**Fig. 1f**). In a few cases, we used other viral backbones (**Supplementary Fig. 1**) developed by us or by other laboratories, such as *Tobacco mosaic virus* (TMV), *Tobacco rattle virus* (TRV), or *Clover yellow vein virus* (ClYVV)[14-17] (**Fig. 1g**). As an example of a monocotyledonous species, maize was transfected by *Maize streak virus* (MSV) (**Fig. 1g**). It should be mentioned that the plant viral vectors used in our work had generally only mild effects on the plant phenotypes such as slightly delayed growth and occasionally leaf mosaic.

We also tested the efficacy of spray-based delivery of RNA virus particles (**Fig. 1h**) and found that the process is less efficient, with a frequency of transfection in *N. benthamiana* of less than 10^{-4}-10^{-5} per cell. Nevertheless, even at this low frequency, viral particle spraying was sufficient to achieve the results described below.

Transient manipulation of the flowering regulatory pathway

Underlying mechanisms of the transition from vegetative to flowering state in plants have been the subject of century-long studies, with the concept of a 'principle' or inducer moving from leaves to apical meristem and causing flowering ('florigen') first postulated by Mikhail Chailakhyan in 1936[18]. The molecular basis of the process, however, has been clarified only during the last two decades[19]. In the core of the process is the so called Flowering Locus T mobile protein (FT) whose expression is induced by external light intensity/day length via the phytochrome machinery; FT then moves from leaves through phloem to apical meristem and interacts with transcription factors that trigger the transition to flowering differentiation of the meristem[20,21]. Since the primary delivery site for our transfection treatment is the plant leaf, we decided to hijack the process by transiently producing additional FT protein in the leaves.

Most of these experiments relied on *Agrobacterium*-based delivery of PVX vectors harboring one of the multiple genes known to be involved in flowering control (**Supplementary Table 2**). In agreement with their central and universal role in flowering control, expression of genes encoding mobile *Arabidopsis* FT or its orthologs from various species such as tobacco, tomato, rice, and others promoted flowering, shortening floral transition in multiple plant species (*Arabidopsis thaliana*, tobacco, tomato, pepper, and wheat) (**Fig. 2a-j**) whereas flowering repressors of the same family delayed flowering (**Supplementary Fig. 3**). The flowering induction results were most dramatically detectable in the tobacco variety 'Maryland Mammoth', which does not naturally flower under long-day field conditions (flowering occurs close to December in the Northern hemisphere), leading to high accumulation of vegetative biomass. A limited number of genes controlling upstream (*PhyB*) or downstream (*SOC1*) components of the

flowering regulatory cascade that we tested didn't result in any visible phenotypic changes (data not shown).

It would be practically useful to control vernalization, the requirement for prolonged seasonal cold (e.g., winter) for successful flowering[22,23]. A tighter control of vernalization (to avoid 'bolting' in crops such as sugar beet), or an easy way of lifting the vernalization block, would be of importance for seed production and breeding of crops such as winter wheat, barley, rapeseed and others. In *Arabidopsis*, there are numerous biennial ecotypes that do not flower during the first year. We evaluated the effect of transient *FT* expression or antisense silencing of the flowering repressor *FLC* in proof-of-principle experiments. We found that both of those treatments were effective in promoting flowering in vernalization-dependent ecotypes such as Tul-0, Tamm-2, Lov-5 and others, without any vernalization treatment (**Fig. 2j, k**).

We subsequently evaluated the version of the transient delivery cascade that relies on spraying plants with PVX viral particles produced in *N. benthamiana*, rather than *Agrobacterium* cells, thus obviating the need to release DNA-based vectors into the environment altogether. Transition to flowering was demonstrated with tobacco and tomato (**Fig. 2l,m** and **Supplementary Fig. 3n,o**). Due to practical considerations and performance, as well as compliance with environmental and regulatory constraints (no DNA release in the field; see also below and Discussion), we view the application to crops of self-limited RNA virus particles as the more promising version of the transient agronomic trait modification platform.

Transient manipulation of the gibberellin regulatory pathway: dwarfism

Traits such as dwarfism and semi-dwarfism have been the basis of the 'green revolution' in plant breeding during 1960s-70s, and the underlying molecular mechanisms of these traits are well

understood[24,25]; recently reviewed by Eshed and Lippman[26]. Many of the agronomically important 'semi-dwarfism' genes belong to the gibberellin regulatory pathway, the best characterized of which being gibberellic acid (GA) oxidases; the others are dehydration-responsive element binding (DREB) proteins. To effect dwarfism using transient regulatory interference, we evaluated multiple GA oxidases as well as relevant DREB and DELLA-motif proteins[27-29] transiently expressed in a number of important model and crop plants including tobacco, tomato, pepper, pea, broad beans and wheat. Being delivered by *Agrobacterium*, PVX vectors harboring *GA2-oxidase* or *DREB1-type* genes consistently suppressed stem elongation and plant height in tobacco, tomato, pepper and *Nicotiana benthamiana* (**Fig. 3a-d** and **Supplementary Fig. 4c-f**), while PVX-delivery of GA20-oxidase enhanced stem length (**Fig. 3g,h** and **Supplementary Fig. 4c-f**). Similarly, *Agrobacterium*-delivered ClYVV vectors carrying *GA2-oxidase* gene significantly reduced the stem length in pea and broad bean (**Fig. 3e** and **Supplementary Fig. 4**). Notably, plant height responded to the dose of *Agrobacterium* (**Supplementary Fig. 4a**) and was reversal to external GA application (**Supplementary Fig. 4b**). Viral particle-based transfection with GA2-oxidase or GA20-oxidase was similarly effective in modulating plant height in wheat and tomato (**Fig. 3i-l**). Both *Agrobacterium*-based as well as viral particle-based transfection allowed the control of plant height, and the effects of the various genes on plant height are in line with the current understanding of the role of those genes in gibberellin metabolism[30]. Various genes and their homologues from different species yielded different levels of dwarfism. Up to 40% reduction in height was obtained with *GA2ox8* from soybean and *DREB1A* from *Arabidopsis*. Detailed analyses of individual gibberellins in leaves and stems of transfected plants confirmed significant changes in active and inactive gibberellins in *Nicotiana benthamiana* and tomato which were in line with the phenotypes observed

(**Supplementary Figs. 4 and 5**). We also overexpressed DELLA proteins which contained mutations known to impair GA-promoted protein degradation and plant growth[30], but no significant phenotypic effect was detected.

We also performed a limited set of open field experiments under permit No. 15-041-101r from the US Department of Agriculture (USDA). In those studies, tomato plants were sprayed with *Agrobacterium* carrying the PVX-based gene *GA2ox1*. As anticipated, the transfected plants exhibited significantly reduced height (**Fig. 3m,n**).

Transient reprogramming of other agronomic traits

Drought tolerance is among the most economically important agronomic traits, and a potential to manipulate those responses rapidly and only when the stress factor is present ('trait on demand' concept) would be very useful. We therefore evaluated transient expression as a rapid-response intervention to induce drought tolerance by using the well-characterized *notabilis* mutant of tomato, which is deficient in 9-cis-epoxycarotenoid dioxygenase (NCED3), a central component of the abscisic acid (ABA) biosynthetic pathway[31]. Due to the lack of ABA, this mutant is highly sensitive to drought, but we show that the sensitivity is significantly reduced as a result of transfection with a functional *NCED3* gene (**Fig. 4a**). The transfected plants also showed increased water retention ability as well as significantly elevated concentrations of abscisic acid and of its biologically inactive but reversible glucose conjugate (**Fig. 4b**), and phaseic acid and dihydrophaseic acid (abscisic acid catabolites)[32] (**Supplementary Fig. 6**). In another experiment, wild-type tomato plants were transfected with DREB transcription factors that are mediating stress tolerance[33]; again, higher drought tolerance along with higher water retention was demonstrated (**Fig. 4c,d**).

In an attempt to effect transiently the trait of insect resistance that has been the basis of modern transgenic insect-resistant crops (corn, soybean, cotton)[34], we expressed the *Bacillus thuringiensis* gene *cry2Ab* in tobacco using a PVX vector with subsequent infestation of the plants with tobacco-adapted hornworm *Manduca sexta*. Cry2Ab-transfected plants demonstrated high toxicity to hornworms, concomitant with the presence of relevant levels of Bt toxin in plant leaves (**Supplementary Fig. 6**).

One important feature of the transient reprogramming concept would be the ability to spray plant leaves to manipulate traits in distal organs such as flowers, seeds and roots. Efficient development of fruits and overall productivity are of special interest in fruit-bearing crops such as tomato. In a proof-of-principle study, we conducted expression experiments with a tomato *sft* ('*single flower truss*') mutant deficient in flower organ formation, which results in development of a single flower per flower truss[35]. *SP3D*, the corresponding gene controlling the fate of meristem in inflorescence, was expressed using *Agrobacterium* delivery of PVX viral vectors. The treatment effectively restored multi-flower truss structure of inflorescence and restored the number of flowers/fruits per truss (**Fig. 4e,f**).

In yet another demonstration of the usefulness of the proposed technology, we delayed fruit ripening in tomato by spraying plants with *Agrobacterium* carrying PVX vector containing antisense fragment of the *DML2* gene for DEMETER-like DNA demethylase regulating the transcription of genes involved in fruit ripening[36] (**Fig. 4i,j**).

Similarly, to demonstrate the ability of transient delivery to modulate another useful agronomic trait, production of a pigment with reported human health benefits, we expressed in tomato plants the *ANT1* gene encoding a transcription factor controlling anthocyanin synthesis[37,38]. Although

fruits of the resultant transfected plants were only sectorially transfected (**Fig. 4g**), the fruits nevertheless accumulated high levels of anthocyanin in the pericarp (**Fig. 4h**).

Tunable control of agronomic trait expression

Results of our experiments demonstrate that the amplitude of expression of multiple agronomic traits can be modulated by more than one mechanism; for example, by inducing expression of different proteins participating in the same specific regulatory circuit, or by using protein orthologs from various plant species. We also explored additional approaches to vector optimization with the ultimate goal of achieving flexible control of trait expression. These studies were done using PVX, because in our hands it represents the more flexible and broadly effective viral platform.

PVX-based vectors used in these studies achieved systemic movement within the plant and exhibited the ability to move and transfect organs other than primary transfected leaves. The latter feature was dependent on various factors, the most obvious one being the length of the heterologous gene insert. We also found that the GC content of the insert is another important factor in this process. Genes with higher GC content were more stable and were not eliminated as quickly from the vector relative to genes with lower GC content[39]. Consequently, using inserts optimized for GC content resulted in much more stable vectors that better delivered the genes of interest to distal parts of the plant (**Fig. 5a**).

Another way of controlling trait expression is to design vectors that provide higher expression of the cargo gene. A solution that we applied in these studies was to place the gene of interest (GOI) in the distal 3' end of the RNA genome, which is where the most highly expressed gene (viral coat protein) typically resides[40]. This solution somewhat compromises overall efficacy of

the viral vector but provides for higher expression of the gene of interest. Among the new empirical approaches, we chose to engineer our PVX backbone by inserting known modulators of viral pathogenicity[41,42]. We found that small viral genes of cysteine rich proteins (CRP), which are believed to interfere with the plant gene-silencing machinery[42], inserted between the STOP-codon of gene of interest and 3'UTR of PVX, can dramatically increase GOI expression (**Fig. 5b,g** and **Supplementary Fig. 7**). This in turn provides for broader expression range of the specific agronomic traits described above (**Fig. 5h,k**). The effect was particularly pronounced for floral repression; it was stably achieved only with the new vector containing CRP.

Even limited levels of viral vector replication within the plant may impose some penalties on overall crop performance. In our experiments, we typically included two negative controls: plants that were not transfected and plants that were transfected with the GFP gene. The latter sometimes resulted in statistically measurable although very limited in terms of phenotype and practical consequences effects on the parameters under investigation, for example somewhat lower height, altered time to flowering, etc. (e.g. **Figs. 2-4**). An additional control consisting of empty viral vectors devoid of heterologous genes was found to be less practical because these vectors were in some cases phytotoxic, likely due to higher aggressiveness of the vector, and therefore confounding interpretation of results.

Safety and regulation-compliance aspects of transient expression technologies

We evaluated the stability of gene inserts upon systemic movement of RNA vectors using PVX-based constructs, and found that the heterologous inserts are invariably and relatively rapidly lost during systemic movement of the vector in the plant (**Fig. 6a**). The loss is more rapid if the inserts are large genes and if the GC content of the insert is lower[38]. This sensitivity of the

vectors to the GC content of the insert allowed us to engineer vectors having either higher stability, resulting in higher expression of the trait due to the longer time that the vector is intact (discussed above, **Fig. 5h,k**), or lower stability, leading to more rapid loss of the cargo gene from the RNA virus backbone. To illustrate, we rewrote the GFP gene (61.4% GC) by designing a sequence with lower GC content (40.3%) by altering the codon usage. The viral vector carrying this synthetic gene demonstrated lower overall GFP fluorescence in infected leaves and a more rapid loss of the insert (**Fig. 6b,c**).

Plant viruses fall into two general categories: (a) transmissible viruses that can be inherited by the progeny upon sexual reproduction of the plant, albeit with low frequency, and (b) non-transmissible ones that cannot be transmitted to the progeny. The main viruses used as vector backbones in this study, namely PVX and TMV, are known to be non-transmissible, and our own studies confirmed this for PVX-based viral vectors (**Fig. 6d**).

In a limited set of experiments under permit No. 13-323-101r from the USDA, we released disarmed non-auxotrophic *Agrobacterium* strain NMX021 carrying a binary vector encoding a PVX-driven GFP gene into an open field environment by spraying tobacco (**Fig. 6e**) and tomato plants, resulting in a release of approximately 10^{11} bacterial CFU. We then followed the fate of released agrobacteria in transfected plants and in the soil surrounding the plant roots, and found that within 90 days after spraying the number of detectable bacteria in a plant drops by four logs, from $>10^5$ to less than 10 cells/gram of plant biomass (**Fig. 6g**). Similarly, the number of detectable agrobacteria in the soil falls from initially 10^4 cells per gram of soil to undetectable levels 11 months later (**Fig. 6f**). It should be mentioned than during spring and summer time, the natural agrobacterial population in a rich soil can be as high as 10^7 cells per cm^3 of soil[43].

Discussion

We demonstrate here in a range of various crop species a transient and practically useful alteration of the major regulatory circuits that have been the basis of crop domestication and past agricultural revolutions. These include vegetative/reproductive changes and short/tall stature control[27] and several others. The procedure results in industrially scalable delivery of genetic information in the form of self-replicating RNA vectors. The practice does not involve permanent genetic modification of the crop. The fundamental differences between this technology on the one hand, and the current breeding methods including those based on genetic transformation on the other, are that our approach does not involve genetic modification of the plant genome, is broadly applicable, fast, tunable, versatile, limited to one plant generation, and can be used throughout most of the crop cultivation cycle.

The major differences between our approach and the other emerging transient technology, namely treatment of plants with short double-stranded RNAs (in its present form, limited to RNA interference-based control of plant insects[44]) are that the vectors described here are capable of limited self-replication and movement within a plant, thus providing virtually endless applicability and, additionally, the vector products can be made at very low manufacturing cost. The described interventions involving replication of viral constructs within a host can result in certain penalties, but those can be minimized through further tuning and weighted against the obvious (and demonstrated) benefits. Plant viral vectors used in this study had generally only mild phenotypic effects such as slightly delayed growth and occasionally leaf mosaic. In this regard, it should be mentioned that selection of the optimal final result is also a requirement of any conventional crop breeding program. Importantly, however, the trait development based on transient approach is faster and allows for higher throughput.

We hope we have provided here substantial and broad evidence that practically useful phenotype changes can be generated through fast transient transfection of crops. This general proof of principle addressing multiple crops and multiple traits needs further development steps in future; those should include defining and testing most important traits/crop combinations, conducting field trials and generating data for regulatory approvals, and scale up. Judging from our results, a single universal viral vector effective across all important crops is unrealistic; vectors' efficacy will be determined primarily by virus host spectrum: species-specific to multi-species or even multi-family specific. Currently, PVX vector backbone shows the broadest applicability, but this backbone is not optimal for families such as beans, or for monocots.

Open field trials would need to be conducted using industrial-size equipment and an optimal control of the spraying under open field conditions (day time, wind, humidity) needs to be developed.

Early studies on the safety of proposed technology indicate that the initial vector organisms, *Agrobacterium* or packaged RNA virus particles, are self-limiting, and it should be possible to improve their environmental containment further by introducing additional safety locks. For example, *Agrobacterium* can be made multiply auxotrophic, suicidal or otherwise disabled, or alternatively it can be re-coded[45] making it unable to interact with other bacteria or survive after release. The technology based on release of packaged RNA viral vectors is arguably safer than *Agrobacterium* vector, as no DNA is released into environment, and remaining RNA degradation products are all already present in plants and in soil due to the ubiquitous nature of plant viruses. A precision spraying would probably allow more economical delivery with minimal undesired release to the environment.

Continuing emergence of multidrug- and pandrug-resistant bacteria and of novel viral pathogens repeatedly demonstrates power and technology potential inherent in the molecular machinery of microbes and viruses. The number of approved (and thus allowed to be released into environment) recombinant bacteria (*Salmonella* as oral vaccine, *Agrobacterium* for control of its pathogenic species) and viruses (influenza virus vaccine, adeno-associated viruses for treatment of spinal muscular atrophy) illustrates the potency and safety of such technical solutions.

The introduction of new technologies is always a challenge. We expect that the technology described herein will initially gain regulatory approval and commercial recognition in certain niche areas before garnering attention for mainstream application on large-acreage major crops. One such application area is the production of commercial seed, where production can be made simpler and more efficient through acceleration of flowering time or control of sterility. The other would be a more rapid deployment of orphan crops (e.g., millet, amaranth, buckwheat, cowpea, quinoa, cassava, etc.) for flowering control, drought tolerance improvement etc. Current swift approval and adoption of RNA-based viral vaccines for human health shows that the speed of acceptance of RNA transfection-based agriculture will be greatly facilitated by its expected efficacy during unavoidable major plant pathogen outbreaks.

Methods

Bacterial strains and growth conditions

Escherichia coli DH10B cells were cultivated at 37 °C in LB medium. For ClYVV-based constructs, *Escherichia coli* DH5α cells were used. *Agrobacterium tumefaciens* ICF320 (auxotrophic derivative (DcysK$_a$, DcysK$_b$, DthiG) of *Agrobacterium tumefaciens* strain C58) cells[46] or NMX021 cells were cultivated at 28 °C in LBS medium (modified LB medium

containing 1% soya peptone (Duchefa)). The NMX021 strain was a modified and fully disarmed version of CryX strain[47], wherein a Ti plasmid region was deleted (including the Amp resistance gene with its flanking left and right region - about 30 Kb deleted, from nucleotide 30499 to 60264) and replaced with the *LacZ* gene to facilitate monitoring for the presence of this strain.

Plasmid constructs and viral vectors

TMV-based assembled vectors were described in ref. [2]. TMV vectors used in these studies lack a coat protein (CP) gene (ΔCP); those further modified by removal of the movement protein (MP) gene are indicated as TMVΔMP. The PVX viral vectors used here with the CP coding sequence placed between the polymerase and the triple gene block ORFs were based on the ones first described in ref. [3]. PVX vectors lacking CP are indicated as PVXΔCP. ClYVV viral vectors were developed based on ref. [16,17] with T27I mutation to obtain a less aggressive virus[48]. We received pClYVV-GFP as a gift from Dr. Takeshi Matsumura (Hokkaido University, Japan) and modified it to be used with our T-DNA binary vector for *Agrobacterium*. Bipartite TRV vectors[15] were obtained from Arabidopsis Biological Resource Center (ABRC), Ohio State University, Columbus, OH, USA): pTRV1 (AF406990, stock #CD3-1039) and pTRV2-MCS (AF406991, stock #CD3-1040). We modified the pTRV2-MCS to be used with our T-DNA binary vector for *Agrobacterium*. SMV vector was designed based on ref. [49]. TMVcg viral vectors were developed based on sequence D38444 and modified similarly as in ref. [2]. MSV viral vectors were developed based on a viral sequence deposited in NCBI as Y00514. *Foxtail mosaic virus* (FoMV) viral vectors are described in ref. [50].

Specifically modified PVX-based RNA amplicons including cysteine rich proteins (CRP) from Carlaviruses were created by insertion of one CRP from *Cowpea mild mottle virus* (called NABP)[41] or one CRP from *Chrysanthemum virus B* (called CVB-CRP)[42]. Those proteins are

pathogenicity determinants believed to be RNA silencing suppressors. They were inserted 2 bp downstream of the gene of interest in the PVX backbone, similarly as is found in the *Cowpea mild mottle virus*, downstream of the coat protein ORF. The presence of the specific CRP in the vector backbone is indicated as subscript (e.g. PVX-GFP_{NABP} , PVX-$GFP_{CVB-CRP}$).

Cloning of specific genes into the viral vectors was achieved either by inserting PCR products (PCR performed with KOD hot start DNA polymerase, Merck KGaA) or fragments synthesized by external providers (Eurofins Genomics). Synthesized fragments were for *BnA2FT, BnC6FTb, SlSP5G, OsGA2ox1, AtGA20ox1, AtDREB1B, AtNCED3, SlANT1, BtCry2Ab, IPT*.

Plant species and growth conditions

Plants and varieties used in the experiments described included: *Nicotiana benthamiana*; tobacco (*Nicotiana tabacum L.*) 'Samsun' and 'Maryland Mammoth'; tomato (*Solanum lycopersicum*) 'Balcony Red', 'Tamina' and 'Ailsa Craig', tomato 'Ailsa Craig' mutants *notabilis* (LA3614) and *sft* MSU100 (LA2460) (both obtained from TGRC - Tomato Genetics Resource Center at University of California); pepper (*Capsicum annuum*) 'Early California Wonder' ('ECW'); *Arabidopsis thaliana* ecotype Col-0, and the ecotypes responding to vernalization Bla-2, Bla-11, Can-0, Co-4, Lov-5, Sf-2, Tamm-2, Te-0, Tul-0 (all obtained from Prof. Marcel Quint, Martin Luther University Halle-Wittenberg, Germany), *Arabidopsis* mutant *ft-10* (obtained from Nottingham Arabidopsis Stock Centre (NASC stock), as GABI-Kat T-DNA insertion library code 290E08; http://www.gabi-kat.de/). In *ft-10*, the T-DNA is inserted into the first intron; broad bean (*Vicia faba*) 'Dreifach Weiße'; pea (*Pisum sativum*) 'Dinga'; potato (*Solanum tuberosum*) 'Elfe'; spinach (*Spinacia oleracea*) 'Frühes Riesenblatt'; red beet (*Beta vulgaris*) 'Moulin Rouge'; soybean (*Glycine max*) 'Blyskavytsya'; maize (*Zea mays*) 'Sturdi Z'; wheat

(*Triticum aestivum*) cultivar 'Cadenza'. Other plants were tested only for transfection and they are included in **Supplementary Table 1**.

Plants were grown in the greenhouse (day and night temperatures of 19-23 °C and 17-20 °C, respectively, with long day condition as 12 h light / 12 h dark, and 35-70% humidity). *Arabidopsis thaliana* plants in short day conditions (8 h light / 16 h dark) were grown in growth chambers (Model AR-22L, Percival Scientific), equipped with fluorescent lamps (4 lamps, 100 – 130 µmoles / m^2 x s). Wheat was grown in controlled-environment rooms with day/night temperatures of 26.7°C/21.1°C at around 65% relative humidity and a 16-h photoperiod with light intensity of approximately 220 µmoles / m^2 x s.

Agrobacterium-mediated transfection of plants

Plants were inoculated with diluted cultures of *Agrobacterium tumefaciens* using one of three procedures: 1) infiltration of plant leaves using a needleless syringe (agroinfiltration), 2) spraying of aerial parts of plants using a sprayer (agrospray), and 3) dipping of aerial parts of plants into an agrobacterial suspension (agrodip).

Agroinfiltration procedure. Saturated *Agrobacterium* overnight cultures were adjusted to OD_{600} = 1.5 (approximately 10^9 cfu/mL) with *Agrobacterium* inoculation buffer (AIB: 10 mM MES pH 5.5, 10 mM $MgSO_4$), and further diluted with same solution to reach the desired dilution of the *Agrobacterium* suspension. Inoculation of individual leaf sectors was performed using a syringe (syringe infiltration). For inoculation of entire plants, a vessel containing the infiltration solution was placed in a vacuum chamber with the aerial parts of a plant dipped into the solution. A vacuum was applied for 5 min using a ME 8 NT pump (Vacuubrand), with pressure ranging from 0.1 to 0.2 bar (vacuum infiltration).

Agrospray procedure. Saturated *Agrobacterium* overnight cultures were adjusted to OD_{600} = 1.5 with AIB, and further diluted with same solution supplemented with a surfactant to OD_{600} = 0.015 (1:100 dilution). In some cases, carborundum (silicon carbide SiC) F800 (Mineraliengrosshandel Hausen) used as an abrasive was added to agrobacterial suspensions. The surfactants used were: Silwet® L-77 (Kurt Obermeier), Silwet® Gold (Arysta LifeScience), Tween® 20 (Carl Roth) and Triton™ X-100 (AppliChem). Plants were sprayed using High-performance sprayer 405 TK Profiline (Gloria Haus- & Gartengeraete), with 3 bar pressure, and plastic hand sprayers for routine use (Carl Roth). Some test experiments were performed with the compressor sprayer Einhell® BT-AC 200/24 OF (Einhell). Spraying solution composition depended on plants species. AIB supplemented with 0.1% (v/v) Silwet® L-77 was used for spraying transfection of *Nicotiana* species, tomato, pepper, potato, red beet, and spinach. AIB containing 0.05% (v/v) Tween® 20 was used for *Arabidopsis thaliana* and broad bean. For soybean, AIB supplemented with 0.05% (v/v) Tween® 20, 1 mM DTT, and 0.3% (w/v)) silicon carbide F800 was used. Maize plants were sprayed using AIB supplemented with 0.1% (v/v) Silwet® Gold, 0.3% (w/v) silicon carbide F800 and 5% (w/v) sucrose.

Agrodip procedure. Saturated *Agrobacterium tumefaciens* ICF320 overnight cultures of OD_{600}= 4 were diluted with AIB supplemented with 0.1% (v/v) Silwet® L-77 to OD_{600} = 0.004 (dilution 1:1000). Aerial parts of *Nicotiana benthamiana* plants were dipped upside down into the agrobacterial suspension for 20 seconds.

Generation of viral particles and spraying solution

In order to obtain viral particles (VPs) for plant infection, we inoculated *Nicotiana benthamiana* plants with a suspension of agrobacteria carrying a PVX construct using either syringe or vacuum infiltration. If performed with a syringe, systemic leaves of inoculated plants were

collected two weeks post infiltration; if performed with a vacuum, all leaves were collected one week post infiltration. To recover sap containing VPs, plant material was further ground in liquid nitrogen and extracted using PBS buffer in a 5:1 (v/w) buffer:biomass ratio. The extract was filtered using Miracloth followed by centrifugation of the filtrate for 15 min at 4500 rpm at 4°C. For syringe infiltration, this supernatant solution was used directly for spraying. For vacuum-infiltrated plants, the supernatant solution was first filtered to remove agrobacterial cells using sequential filtrations with filters of 8-12 μm and 0.22 μm pore size. Spraying solution included 0.3% (w/v) silicon carbide F800. Plant species sprayed were *Nicotiana benthamiana*, tobacco and tomato.

Protoplast isolation

Protoplasts were isolated as described in ref. [3].

Immunocytochemistry

Tomato (*Solanum lycopersicum*) 'Tamina' plants were grown for 24 days and then Agro-infiltrated. Agrobacteria carried several PVX constructs (PVX-empty vector, PVX-GFP and PVXΔCP-GFP). Small leaf discs were collected at 10 dpi for immune-staining. Segments of leaves were fixed with 3 % para-formaldehyde/0.05 % Triton™ X-100 in PBS for 3 hours at room temperature and subsequently embedded in PEG 1500 as described in (*51*). GFP was labelled in 3 μm sections with a polyclonal antibody from goat (# 600-101-215; Rockland; diluted 1:500 in PBS containing 5% bovine serum albumin) detected with a donkey-anti-goat-Alexa 488 secondary antibody (# A-11055, Thermo Fisher Scientific; diluted 1:500 in PBS containing 5% bovine serum albumin).

Protein analysis

About 100 mg fresh weight plant leaf material was ground in liquid nitrogen, and crude protein extracts were prepared with 5 volumes of 2 x Laemmli buffer. Total soluble protein (TSP) was extracted from approximately 100 mg fresh weight plant material ground in liquid nitrogen and dissolved in 500 µl 1 x PBS and incubated for 30 min at room temperature. After centrifugation 40 µl SDS sample buffer were added to 10 µl supernatant solution. Sample aliquots (15 µl) were resolved by SDS-PAGE (12% polyacrylamide gel) and Coomassie-stained using PageBlue™ Protein Staining Solution (Thermo Fisher Scientific). Protein extracts were denatured at 95°C for 5 minutes before loading.

For immunoblot analysis, sample aliquots (15 µl) were resolved by SDS-PAGE (12% polyacrylamide gel) and subsequently blotted on a PVDF membrane. FT protein was detected using FT-specific antibodies (Agrisera; diluted 1/1000); GA2ox8H or GA20ox1H (*H*: His_6-tag) were detected using Tetra His™ (Qiagen) mouse monoclonal IgG1 anti-His antibody as the primary antibody (diluted 1/2500). GFP was detected using anti-GFP rabbit polyclonal antibody (Thermo Fisher Scientific; diluted 1/5000). Secondary antibodies were IgG (whole molecule) peroxidase affinity isolated antibody (Sigma-Aldrich), anti-mouse (diluted 1/5000) or anti-rabbit (diluted 1/10000).

Genes used in the studies described

A list of genes with their GenBank accession numbers is provided in **Supplementary Table 3**.

Experiments to manipulate flowering

Arabidopsis thaliana plants of wild-type ecotypes and the null mutant allele *ft-10* were induced to flower by spraying with agrobacteria carrying TMV vectors with genes inducing flowering (from *FT* gene family) or TRV vectors to silence the *FLC* gene. Plants were sprayed at approximately 4 weeks of age. For Col-0 ecotype, plants were kept under short day conditions

(see above) to avoid induction of flowering by day length. For the other ecotypes, plants were kept under long day conditions in a greenhouse (see above). For each plant, days to flowering were counted from the day of spray until the day of bolting (around 1 cm of bolt appearing from the rosette leaves) as days post spraying (dps).

Tobacco (*Nicotiana tabacum*) plants were sprayed with agrobacteria harboring TMV and PVX vectors (expressing genes of the FT-family, either inducers or repressors) when they were 3 to 4 weeks of age. The cultivar 'Maryland Mammoth' was kept under long day conditions (see above) in the greenhouse, which does not induce flowering. The cultivar 'Samsun' is unresponsive to day length; therefore, it flowers normally under the standard greenhouse conditions described. For each plant, days to flowering were counted from the day of spray until the day of bolting (floral buds visible arising from the plant) as days post spraying (dps).

Tomato (*Solanum lycopersicum*) 'Balcony Red' and pepper (*Capsicum annuum* 'ECW') plants 3 to 4 weeks of age were sprayed with agrobacteria carrying PXV vectors harboring genes of the FT-family. Both plant species flower independently of day length (day-neutral plant species) and the constructs described were used to modulate their time to flowering.

Wheat (*Triticum aestivum*) cultivar 'Cadenza' plants at 2 weeks of age were rub-inoculated using FoMV vectors virus particles prepared from infected *Nicotiana benthamiana* plants as described in ref. [50].

Experiments to manipulate gibberellin content

For all sets of experiments, *Nicotiana benthamiana*, tobacco (*Nicotiana tabacum*) 'Samsun', tomato (*Solanum lycopersicum*) 'Tamina' and pepper (*Capsicum annuum*) 'ECW' plants were used for agrospray when they were in the range of 3 to 4 weeks of age. Agrobacteria were applied to plants to introduce PVX constructs expressing genes that affect gibberellin

metabolism. Plant height was scored at the day of spray (day 0) to ensure that no significant difference was present at the beginning of the experiment, and later at several time points during a time course. Broad bean (*Vicia faba*) 'Dreifach Weiße' plants were used for agrospray when they were 2 to 3 weeks of age. For each time point (days post spraying; dps), plant height was scored as length between the soil level and the last apical inflorescence (end of the stem). The same conditions were used when spraying the plants with viral particles (VPs).

Wheat (*Triticum aestivum*) cultivar 'Cadenza' plants at 2 weeks of age were rub-inoculated as described above.

For the field trials, tomato (*Solanum lycopersicum*) 'Tamina' plants (28 days-old) were sprayed with suspensions of the *Agrobacterium* strain NMX021 harboring PVX constructs (single spray ca. 10^7 bacteria/ml or ca. 2-3 x 10^8 bacteria per sq ft).

External application of active gibberellins via spray

The gibberellins GA_3 and GA_4 (Sigma-Aldrich) were dissolved in ethanol to 1 mM concentration and diluted 100 times with water supplemented with 0.02% (v/v) Silwet® L-77 for spray application. *Nicotiana benthamiana* plants, previously inoculated with PVX constructs PVX-GFP and PVX-GA2ox8, were sprayed at two different time points (13 dps and 27 dps) either with a 1 % ethanolic solution supplemented with 0.02% (v/v) Silwet® L-77 with 10 µM of GA_3, GA_4 or no gibberellins (mock).

Gibberellin analysis

Analysis of gibberellins content in *Nicotiana benthamiana* and tomato 'Tamina' plants was performed as follows. Plants were sampled at the indicated dps and separated into leaf and stem material. Individual gibberellins were identified and quantified using UPLC-MSMS as described

518 in ref. [52]. The mass spectrometry data of individual gibberellins were processed by using
519 TargetLynx V4.1 SCN 904 (Waters Corporation).

520 ***Experiments on drought tolerance***

521 *Experiment with mutant tomato variety.* The tomato (*Solanum lycopersicum*) 'Ailsa Craig' mutant
522 *notabilis* variety was used for these experiments. Six pots, each with five 19 days-old plants,
523 were sprayed with agrobacteria harboring PVX vectors expressing either GFP or the NCED
524 gene. At 9 dps, each three pots per treatment were not watered for three days. Watered (*no*
525 *drought*) and stressed (*drought*) plants were photo documented after 12 dps. At this time point
526 watered plants were harvested and ground and samples were used for HPLC analysis to
527 determine the content of abscisic acid (*ABA*), its derivative abscisic acid-glucose ester *(ABA-*
528 *Glc*), phaseic acid (*PA*) and dihydrophaseic acid (*DHPA*), in relation to the treatments.
529 *Experiment with wild-type tomato plants.* Three pots, each with five 19 days-old tomato
530 (*Solanum lycopersicum*) 'Tamina' plants, were sprayed with agrobacteria carrying PVX
531 expressing GFP, DREB1 or DREB-LP1. After 9 dps, plants were not watered for 7 days and
532 subsequently analyzed regarding the relative water content (RWC). To determine the relative
533 water contents (RWC %), immediately after drought stress all plants were cut directly over the
534 soil, weighted separately and watered overnight in glasses filled with 100 ml tap water. After
535 weighting the plants again, the plant material was dried separately at 60°C for 24h to determine
536 the dry weight per plant. The relative water content per plant was calculated using the formula
537 RWC% = ((desiccated weight-dry weight) / (fresh weight-dry weight)) x 100.

538 ***Experiments on anthocyanin production***

In order to induce anthocyanin production in tomato fruits, 6-week-old tomato (*Solanum lycopersicum*) 'Balcony Red' plants were sprayed with agrobacteria carrying PVX-ANT1 constructs.

At fruit maturation, fruits were ground to a fine powder in liquid nitrogen. To extract anthocyanins, 1.5 g samples of ground fruit material were mixed with 3 ml methanol supplemented with 1% HCl and further incubated overnight at 4°C in the dark. At the next day, first 2 ml sterile water were added and the samples intensively mixed, and subsequently 5 ml chloroform were added and again the samples were intensively mixed. The samples were then centrifuged at 4500 rpm for 10 min at room temperature. Lastly, the supernatant solution was collected and each sample was supplemented with 4 ml of a 60% methanol solution plus 1% HCl. The concentration of anthocyanin pigment (in cyanidin-3-glucoside equivalents) in the extracts was subsequently determined by measuring of OD_{520} and OD_{700} in a 1:2 dilution of a KCl buffer (0.025 mM KCl, pH 1.0) and a Na-acetate buffer (0.4 M Na-acetate, pH 4.5) relative to a standard curve[53].

Experiments on fruit ripening

Tomato (*Solanum lycopersicum*) 'Balcony Red' plants (31 days-old) were sprayed with agrobacteria harboring PVX vectors with an antisense sequence (Fragment 1-480 bp) of the gene *DEMETER-like DNA demethylase 2* from tomato (*DML2*). Because the absolute time of fruit maturation can be masked by changes in several parameters (principally the flowering time), the duration of fruit ripening procedure was measured, for each developing fruit, as the number of days to anthesis: the time between the appearance of a flower and the maturation of the fruit developed from this specific flower. We scored the fruit as mature once it becomes completely red.

Experiments on insect resistance

Tobacco (*Nicotiana tabacum*) 'Samsun' plants were sprayed with agrobacteria carrying PVX-Cry2Ab vectors. To determine the concentration of expressed Cry2Ab toxin, leaf material from each plant was collected at 7 dps, extracted in PBST buffer and analyzed using a Cry2Ab specific ELISA Kit (Cry2Ab #KBA010-10, KRISHGEN Biosystems) following the manufacturer´s instructions. At 7 dps, 3 larvae of the hornworm (*Manduca sexta*) were placed on each plant for feeding. Plant shapes were photo documented at the beginning of insect feeding and 28 days later. In a parallel experiment, tobacco plants were syringe-infiltrated with agrobacteria harboring PVX-Cry2Ab vectors. At 5 dpi, infiltrated leaves were detached, and one hornworm larva was placed on each detached leaf. Individual leaves were photographed at the beginning of insect feeding and 5 days later.

Experiments on plant senescence

Tobacco (*Nicotiana tabacum*) 'Samsun' plants (62 days-old) were sprayed with agrobacteria harboring PVX-IPT vectors. At 39 days post spraying, the four oldest leaves of sprayed and control plants were collected and photo documented.

Detection of PVX in the progeny of agrosprayed plants

Seeds were collected from tomato (*Solanum lycopersicum*) 'Balcony Red' plants sprayed with the constructs PVX-GFP and PVX-GA2ox1 (*Oryza sativa*) and germinated on filter paper (*pr*). Total RNA was isolated from pooled seedlings as well as from leaves of PVX-GFP sprayed plant used as a positive control (*sp*), and used to generate cDNA. The presence of PVX and cystatin control was determined by PCR.

Detection of Agrobacterium in leaves and soil

584 *Nicotiana tabacum* 'Maryland Mammoth' plants (63 days-old) were sprayed with a suspension of
585 *Agrobacterium tumefaciens* (strain NMX021) cells harboring PVX-GFP (single spray, ca. 10^6
586 bacteria/ml or ca. 2-3 x 10^7 bacteria per sq ft). At different time points after spraying, 12 samples
587 of soil within a constant distance from the plants were collected and analyzed for the presence of
588 agrobacteria. For this purpose 1 g soil per sample was suspended in 5 ml of sterile SCP broth
589 (0.43% (w/v) NaCl, 0.1% (w/v) peptone, 0.36% KH_2PO_4, 0.58% Na_2HPO_4, pH 7.0), shaken for
590 ca. 15 min on an orbital shaker (120 rpm), filtered through 2 layers of Miracloth and washed
591 with 2 volumes water. 250 µl of soil extract were plated on LB (rif, cyc, X-gal), incubated for 3
592 days at 28°C and used for enumeration of colonies. Colony forming unites (cfu) per g soil were
593 calculated. At different time points after spraying, 12 samples of leaves were collected and
594 analyzed for the presence of agrobacteria. For this purpose 1 g leaf material per sample was
595 ground in liquid nitrogen, admixed with 5 volumes of sterile SCP broth, shaken for ca. 15 min on
596 an orbital shaker (120 rpm), and filtered through 2 layers of Miracloth. A volume of 250 µl of
597 leaf extract was plated on LB (rif, cyc, X-gal), incubated for 3 days at 28°C for colony
598 enumeration. Colony forming unites (cfu) per g leaf material were calculated.

599 ***Detection of T-DNA in plants transfected with Agrobacterium***

600 Seeds were collected from untreated *Nicotiana benthamiana* plants and from those sprayed with
601 agrobacteria carrying PVX-GFP vectors. Genomic DNA (gDNA, 100 ng) was isolated using
602 NucleoSpin RNA Plant II kit (Macherey-Nagel) from 100 mg seeds. The PVX-GFP plasmid
603 DNA spike-in (0.2 pg) was used as a positive control. The presence of PVX and NbSO
604 (housekeeping gene control) was determined by using KAPA3G-PCR Kit (Sigma-Aldrich) with
605 primers specific for PVX, GFP and NbSO. For PCR analysis, 10^{-1}, 10^{-2} and 10^{-3} dilutions of
606 gDNA were used.

Field trials

Experiments with field release of agrobacteria were performed at Kentucky Bioprocessing, Inc. facilities (Owensboro, KY, USA) in 2014 and 2015. These studies were conducted under permits from the US Department of Agriculture (USDA) No. 13-323-101r and No. 15-041-101r. Publicly available information can be accessed at URL: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/sa_permits/status-update/release-permits.

RT-PCR

RNA was isolated from plant material using NucleoSpin RNA Plant kit (Macherey-Nagel) and used to generate cDNA by reverse transcriptase reaction with PrimeScript RT Reagent kit (Takara Bio). PCR was performed using Taq-polymerase (Thermo Fisher Scientific) and target-specific oligos (synthesized by Thermo Fisher Scientific).

Codon optimization and relative sequences

Various ORFs from plant genes were inserted into PVX constructs for expression of specific protein products. For some of these genes, the protein sequence was kept as the original but the sequence was changed by means of a different codon-usage (defined here as codon optimization). We used the algorithm from GENEius software designed and developed for Eurofins Genomics and let the company synthesize the ORFs (Eurofins Genomics). With this program, it is possible to choose for codon-optimization based on specific organisms. For the selected genes, PVX-constructs with the wild-type and the codon optimized sequence of the gene were compared for their stability to keep the insert in the vector with time (as in ref. [39]). The ORFs shown are relative to *SlTAGL1*, *SlOVATE*, *SlANT1* (all from *Solanum lycopersicum*) and GFP. All the sequences are reported in **Supplementary Table 4**.

Statistics and reproducibility

Statistical parameters are reported in the figures and corresponding figure legends. Statistical significance was evaluated by one-way ANOVA test using GraphPad Prism v. 8.0.2. 'n' value corresponds to the number of samples for each column, where the type of sample is indicated in the figure legends. In cases where 'n' value is not the same for each column, it is indicated in the legends. Full datasets are reported in the **Source data**. Experiments shown in the graphs were repeated at least two times, with equivalent results. Experiments showing expression of a reporter gene in different plant species and generated with different methods were repeated at least three times with equivalent results. Experiments performed on field trials were performed one time for each type of experiment. Analysis of gibberellin content was performed one time for each plant species tested.

Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files). All materials are available for research purpose upon request from the corresponding author under a material transfer agreement with Nomad Bioscience. The following sequences of codon optimized genes have been deposited in NCBI as GenBank accession numbers: MT877076 (SlTAGL1, codon-optimized for rice), MT877077 (SlOVATE, codon-optimized for rice), MT877078 (SlANT1, codon-optimized for Bifidobacterium longum), MT877079 (GFP, codon-optimized for tobacco).

References

1. Marillonnet S., Giritch A., Gils M., Kandzia R., Klimyuk V. & Gleba Y. In planta engineering of viral RNA replicons: efficient assembly by recombination of DNA modules delivered by *Agrobacterium*. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 6852-6857 (2004).

2. Marillonnet S., Thoeringer C., Kandzia R., Klimyuk V. & Gleba Y. Systemic *Agrobacterium tumefaciens*-mediated transfection of viral replicons for efficient transient expression in plants. *Nat. Biotechnol.* **23**, 718-723 (2005).

3. Giritch A., Marillonnet S., Engler C., van Eldik G., Botterman J., Klimyuk V. & Gleba Y. Rapid high-yield expression of full-size IgG antibodies in plants coinfected with noncompeting viral vectors. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 14701-14706 (2006).

4. Hahn S., Giritch A., Bartels D., Bortesi L. & Gleba Y. A novel and fully scalable *Agrobacterium* spray-based process for manufacturing cellulases and other cost-sensitive proteins in plants. *Plant Biotechnol. J.* **13**, 708-716 (2015).

5. Gleba Y. Y., Tusé D. & Giritch A. Plant viral vectors for delivery by *Agrobacterium*. *Curr. Top. Microbiol. Immunol.* **375**, 155-192 (2014).

6. Lin M. K., Belanger H., Lee Y. J., Varkonyi-Gasic E., Taoka K., Miura E., Xoconostle-Cázares B., Gendler K., Jorgensen R. A., Phinney B., Lough T. J. & Lucas W. J. FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. *Plant Cell.* **19**, 1488-1506 (2007).

7. McGarry R. C. & Ayre B. G. Geminivirus-mediated delivery of florigen promotes determinate growth in aerial organs and uncouples flowering from photoperiod in cotton. *PLoS ONE.* **7**, e36746 (2012).

8. Yamagishi N., Kishigami R. & Yoshikawa N. Reduced generation time of apple seedlings to within a year by means of a plant virus vector: a new plant-breeding technique with no transmission of genetic modification to the next generation. *Plant Biotechnol. J.* **12**, 60-68 (2014).

9. Velázquez K., Agüero J., Vives M. C., Aleza P., Pina J. A., Moreno P., Navarro L. & Guerri J. Precocious flowering of juvenile citrus induced by a viral vector based on *Citrus leaf blotch virus*: a new tool for genetics and breeding. *Plant Biotechnol. J.* **14**, 1976-1985 (2016).

10. Yuan C., Li H., Qin C., Zhang X., Chen Q., Zhang P., Xu X., He M., Zhang X., Tör M., Xue D., Wang H., Jackson S., He Y., Liu Y., Shi N. & Hong Y. Foxtail mosaic virus-induced flowering assays in monocot crops. *J Exp Bot.* **71**, 3012-3023 (2020)

11. Pasin F., Menzel W. & Darós J. A. Harnessed viruses in the age of metagenomics and synthetic biology: an update on infectious clone assembly and biotechnologies of plant viruses. *Plant Biotechnol. J.* **17**, 1010-1026 (2019).

12. Turpen T. H., Reinl S. J., Charoenvit Y., Hoffman S. L., Fallarme V. & Grill L. K. Malarial epitopes expressed on the surface of recombinant *Tobacco mosaic virus*. *Biotechnology* **13**, 53-57 (1995).

13. Pogue G. P., Lindbo J. A., Garger S. J. & Fitzmaurice W.P. Making an ally from an enemy: plant virology and the new agriculture. *Annu. Rev. Phytopathol.* **40**, 45-74 (2002).

14. Yamanaka T., Komatani H., Meshi T., Naito S., Ishikawa M. & Ohno T. Complete nucleotide sequence of the genomic RNA of *Tobacco mosaic virus* strain Cg. *Virus Genes* **16**, 173-176 (1998).

15. Liu Y., Schiff M., Marathe R. & Dinesh-Kumar S.P. Tobacco Rar1, EDS1 and NPR1/NIM1 like genes are required for N-mediated resistance to *Tobacco mosaic virus*. *Plant J.* **30**, 415-429 (2002).

16. Masuta C., Yamana T., Tacahashi Y., Uyeda I., Sato M., Ueda S. & Matsumura T. Development of *Clover yellow vein virus* as an efficient, stable gene-expression system for legume species. *Plant J.* **23**, 539-546 (2000).

17. Wang D., Ueda S., Uyeda I., Yagihashi H., Sekiguchi H., Tacahashi Y., Sato M., Ohya K., Sugimoto C. & Matsumura T. Positional effect of gene insertion on genetic stability of a *Clover yellow vein virus*-based expression vector. *J. Gen. Plant Pathol.* **69**, 327-334 (2003).

18. Chailakhyan M. Kh. New facts in support of the hormonal theory of plant development. *Compt Rend (Dokl.) Acad. Sci. URSS.* **13**, 79-83 (1936).

19. Andrés F. & Coupland G. The genetic basis of flowering responses to seasonal cues. *Nat. Rev. Genet.* **13**, 627-639 (2012).

20. Corbesier L., Vincent C., Jang S., Fornara F., Fan Q., Searle I., Giakountis A., Farrona S., Gissot L., Turnbull C. & Coupland G. FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* **316**, 1030-1033 (2007).

21. Tamaki S., Matsuo S., Wong H. L., Yokoi S. & Shimamoto K. Hd3a protein is a mobile flowering signal in rice. *Science* **316**, 1033-1036 (2007).

22. Ream T. S., Woods D. P. & Amasino R. M. The molecular basis of vernalization in different plant groups. *Cold Spring Harb. Symp. Quant. Biol.* **77**, 105-115 (2012).

23. Luo X. & He Y. Experiencing winter for spring flowering: a molecular epigenetic perspective on vernalization. *J. Integr. Plant Biol.* **62**, 104-117 (2020).

24. Peng J., Richards D. E., Hartley N. M., Murphy G. P., Devos K. M., Flintham J. E., Beales J., Fish L. J., Worland A. J., Pelica F., Sudhakar D., Christou P., Snape J. W., Gale M. D. & Harberd N. P. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400**, 256-261 (1999).

25. Sasaki A., Ashikari M., Ueguchi-Tanaka M., Itoh H., Nishimura A., Swapan D., Ishiyama K., Saito T., Kobayashi M., Khush G. S., Kitano H. & Matsuoka M. Green revolution: a mutant gibberellin-synthesis gene in rice. *Nature* **416**, 701-702 (2002).

26. Eshed Y. & Lippman Z. B. Revolutions in agriculture chart a course for targeted breeding of old and new crops. *Science* **366**, eaax0025 (2019).

27. Sakamoto T., Morinaka Y., Ishiyama K., Kobayashi M., Itoh H., Kayano T., Iwahori S., Matsuoka M. & Tanaka H. Genetic manipulation of gibberellin metabolism in transgenic rice. *Nat. Biotechnol.* **21**, 909-913 (2003).

28. Suo H., Ma Q., Ye K., Yang C., Tang Y., Hao J., Zhang Z. J., Chen M., Feng Y. & Nian H. Overexpression of AtDREB1A causes a severe dwarf phenotype by decreasing endogenous gibberellin levels in soybean [Glycine max (L.) Merr.]. *PLoS ONE* **7**, e45568 (2012).

29. Achard P., Gong F., Cheminant S., Alioua M., Hedden P. & Genschik P. The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. *Plant Cell* **20**, 2117-2129 (2008).

30. Hedden P. & Sponsel V. A century of gibberellin research. *J. Plant Growth Regul.* **34**, 740-760 (2015).

31. Thomson J., Thorne E. T., Burbidge A., Jackson A. C., Sharp R. E. & Taylor I. B. Complementation of *notabilis*, an abscisic acid-deficient mutant of tomato: importance of sequence context and utility of partial complementation. *Plant, Cell Environ.* **27**, 459-471 (2004).

32. Sah S. K., Reddy K. R. & Li J. Abscisic acid and abiotic stress tolerance in crop plants. *Front. Plant Sci.* **7**, 571 (2016).

33. Kasuga M., Liu Q., Miura S., Yamaguchi-Shinozaki K. & Shinozaki K. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.* **17**, 287-291 (1999).

34. Vaek M., Reynaerts A., Hofte A., Jansens S., De Beuckeleer M., Dean C., Zabeau M., Van Montagu M. & Leemans J. Transgenic plants protected from insects. *Nature* **325**, 33-37 (1987).

35. Lifschitz E., Eviatar T., Rozman A., Shalit A., Goldshmidt A., Amsellem Z., Alvarez J. P. & Eshed Y. The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 6398-6403 (2006).

36. Liu R., How-Kit A., Stammitti L., Teyssier E., Rolin D., Mortain-Bertrand A., Halle S., Liu M., Kong J., Wu C., Degraeve-Guibault C., Chapman N. H., Maucourt M., Hodgman T. C., Tost J., Bouzayen M., Hong Y., Seymour G. B., Giovannoni J. J. & Gallusci P.,A DEMETER-like DNA demethylase governs tomato fruit ripening. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 10804-10809 (2015).

37. Mathews H., Clendennen S. K., Caldwell C. G., Liu X. L., Connors K., Matheis N., Schuster D. K., Menasco D. J., Wagoner W., Lightner J. & Wagner D. R. Activation tagging in tomato identifies a transcriptional regulator of anthocyanin biosynthesis, modification, and transport. *Plant Cell* **15**, 1689-1703 (2003).

38. Butelli E., Titta L., Giorgio M., Mock H. P., Matros A., Peterek S., Schijlen E. G., Hall R. D., Bovy A. G., Luo J. & Martin C. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nat. Biotechnol.* **26**, 1301-1308 (2008).

39. Torti S., Schlesier R., Giritch A. & Gleba Y. Method of improving potexviral vector stability. European Patent EP3456829 A1 (2019).

40. Marillonnet S., Engler C., Klimyuk V. & Gleba Y. Potexvirus-derived replicon. European Patent EP2061890 B1 (2012).

41. Carvalho S. L., Nagata T., Junqueira B. R., Zanardo L. G., Paiva A. C. S. & Carvalho C. M. Construction of a full-length infectious cDNA clone of *Cowpea mild mottle virus*. *Virus Genes.* **53**, 137-140 (2017).

42. Fujita N., Komatsu K., Ayukawa Y., Matsuo Y., Hashimoto M., Netsu O., Teraoka T., Yamaji Y., Namba S. & Arie T. N-terminal region of cysteine-rich protein (CRP) in carlaviruses is involved in the determination of symptom types. *Mol. Plant Pathol.* **19**, 180-190 (2018).

43. Krimi Z., Petit A., Mougel C., Dessaux Y. & Nesme X. Seasonal fluctuations and long-term persistence of pathogenic populations of *Agrobacterium* spp. in soils. *Appl. Environ. Microbiol.* **68**, 3358–3365 (2002).

44. Cagliari D., Dias N. P., Galdeano D. M., dos Santos E. Á., Smagghe G. & Zotti M. J. Management of pest insects and plant diseases by non-transformative RNAi. *Front. Plant Sci.* **10**, 1319 (2019).

45. Mandell D. J., Lajoie M. J., Mee M. T., Takeuchi R., Kuznetsov G., Norville J. E., Gregg C. J., Stoddard B. L. & Church G. M. Biocontainment of genetically modified organisms by synthetic protein design. *Nature* **518**, 55-60 (2015).

46. Bendandi M., Marillonnet S., Kandzia R., Thieme F., Nickstadt A., Herz S., Fröde R., Inogés S., Lòpez-Dìaz de Cerio A., Soria E., Villanueva H., Vancanneyt G., McCormick A., Tusé D., Lenz J., Butler-Ransohoff J.-E., Klimyuk V. & Gleba Y. Rapid, High-yield production in plants of individualized idiotype vaccines for non-Hodgkin's lymphoma. *Ann. Oncol.* **21**, 2420-2427 (2010).

47. Roemer P., Bortesi L., Tiede D., Giritch A. & Gleba Y. *Agrobacterium* for transient transfection of whole plants. European Patent EP2834362 B1 (2018).

48. Yambao M.L., Yagihashi H., Sekiguchi H., Sasaki T., Sato M., Atsumi G., Tacahashi Y., Nakahara K. S. & Uyeda I. Point mutations in helper component protease of clover yellow vein virus are associated with the attenuation of RNA-silencing suppression activity and symptom expression in broad bean. *Arch Virol.* **153**, 105-115 (2008)

49. Seo J., Lee H. & Kim K. Systemic gene delivery into soybean by simple rub-inoculation with plasmid DNA of a *Soybean mosaic virus*-based vector. *Arch Virol* **154**, 1575 (2009).

50. Bouton C., King R. C., Chen H., Azhakanandam K., Bieri S., Hammond-Kosack K. E. & Kanyuka K. *Foxtail mosaic virus:* a viral vector for protein expression in cereals. *Plant Physiol.* **177**, 1352–1367 (2018).

51. Bergau N., Bennewitz S., Syrowatka F., House G. & Tissier A. The development of type VI glandular trichomes in the cultivated tomato *Solanum lycopersicum* and a related wild species *S. habrochaites*. *BMC Plant Biol.* **15**, 289 (2015).

52. Eggert K. & von Wirén N. Response of the plant hormone network to boron deficiency. *New Phytol.* **216**, 868-881 (2017).

53. Lee J., Durst R. W. & Wrolstad R. E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J. AOAC Int.* **88**, 1269-1278 (2005).

54. Yamaguchi S., Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* **59**, 225-251 (2008).

55. Regnault T., Davière J.-M., Wild M., Sakvarelidze-Achard L., Heintz D., Carrera Bergua E., Diaz I. L., Gong F., Hedden P. & Achard P. The gibberellin precursor GA_{12} acts as a long-distance growth signal in *Arabidopsis*. *Nat Plants* **1**, 15073 (2015).

56. Lo S.-F., Yang S.-Y., Chen K.-T., Hsing Y.-I., Zeevaart J. A. D., Chen L.-J. & Yu S.-M. A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice. *Plant Cell* **20**, 2603-2618 (2008).

57. Kobayashi Y., Kaya H., Goto K., Iwabuchi M. & Araki T. A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286**, 1960-1962 (1999).

58. Harig L., Beinecke F. A., Oltmanns J., Muth J., Müller O., Rüping B., Twyman R. M., Fischer R., Prüfer D. & Noll G. A. Proteins from the FLOWERING LOCUS T-like subclade of the PEBP family act antagonistically to regulate floral initiation in tobacco. *Plant J.* **72**, 908-921 (2012).

59. Cao K., Cui L., Zhou X., Ye L., Zou Z. & Deng S. Four tomato FLOWERING LOCUS T-like proteins act antagonistically to regulate floral initiation. *Front Plant Sci*. **6**, 1213 (2016).

60. Pin P. A., Benlloch R., Bonnet D., Wremerth-Weich E., Kraft T., Gielen J. J. L. & Nilsson O. An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. *Science* **330**, 1397-400 (2010).

61. Zhai H., Lü S., Liang S., Wu H., Zhang X., Liu B., Kong F., Yuan X., Li J. & Xia Z. GmFT4, a homolog of FLOWERING LOCUS T, is positively regulated by E1 and functions as a flowering repressor in soybean. *PLoS One* **9**, e89030 (2014).

62. Kong F., Liu B., Xia Z., Sato S., Kim B. M., Watanabe S., Yamada T., Tabata S., Kanazawa A., Harada K. & Abe J. Two coordinately regulated homologs of FLOWERING LOCUS T are involved in the control of photoperiodic flowering in soybean. *Plant Physiol.* **154**, 1220-31 (2010).

63. Kojima S., Takahashi Y., Kobayashi Y., Monna L., Sasaki T., Araki T. & Yano M. Hd3a, a rice ortholog of the *Arabidopsis* FT gene, promotes transition to flowering downstream of Hd1 under short-day conditions. *Plant Cell Physiol.* **43**, 1096-1105 (2002).

64. Meng X., Muszynski M. G. & Danilevskaya O. N. The FT-like ZCN8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize. *Plant Cell* **23**, 942-960 (2011).

65. Yan L., Fu D., Li C., Blechl A., Tranquilli G., Bonafede M., Sanchez A., Valarik M., Yasuda S. & Dubcovsky J. The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19581–19586 (2006).

66. An H., Roussot C., Suárez-López P., Corbesier L., Vincent C., Piñeiro M., Hepworth S., Mouradov A., Justin S., Turnbull C. & Coupland G. CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. *Development* **131**, 3615-3626 (2004).

67. Lee H., Suh S.S., Park E., Cho E., Ahn J.H., Kim S.G., Lee J.S., Kwon Y.M. & Lee I. The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. *Genes Dev*. **14**, 2366-2376 (2000).

68. Wang J. W., Czech B. & Weigel D. miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. *Cell* **138**, 738-749 (2009).

69. Seo E., Yu J., Ryu K.H., Lee M.M. & Lee I. WEREWOLF, a regulator of root hair pattern formation, controls flowering time through the regulation of FT mRNA stability. *Plant Physiol.* **156**, 1867-1877 (2011).

70. Halliday K. J., Salter M. G., Thingnaes E. & Whitelam G. C. Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT. *Plant J*. **33**, 875–885 (2003).

71. Michaels S. D. & Amasino R. M. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11**, 949-956 (1999).

72. Swiezewski S., Liu F., Magusin A. & Dean C. Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. *Nature* **462**, 799–802 (2009).

73. Heo J. B. & Sung S. Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. *Science* **331**, 76-79 (2011).

Acknowledgments

We thank Eva-Maria Franken (Bayer CropScience) for encouragement and valuable advices on experiments and Hugh Haydon (Kentucky BioProcessing) for support in conducting field trials. We would also like to thank our colleagues at Nomad Bioscience and Icon Genetics Carola Engler, Katharina Havranek, Tim-Martin Ehnert, Victor Klimyuk, Frank Thieme, Romy Kandzia, Anja Nickstadt, Yuri Symonenko, Elsbeth Stegemann-Oelerich for their valuable help. We are grateful to Ernie Hiatt, Jennifer Poole, John W. Shepherd, Ethan Blandford (Kentucky BioProcessing) for their help in conducting field trials. We would like to thank Dr. Takeshi Matsumura and Dr. Ichiro Uyeda (Hokkaido University) for the plasmid pClYVV-GFP received as a gift, Prof. Marcel Quint (Martin Luther University Halle-Wittenberg) for providing several *Arabidopsis* ecotypes, Dr. Margret Köck (Martin Luther University Halle-Wittenberg) for providing of climatic cabinets for plant growth. We thank Kai Eggert and Barbara Kettig (IPK-Gatersleben) for phytohormone analysis and Anke Steppuhn (Free University Berlin) for providing hornworm eggs. We thank Daniel Tusé (DT/Consulting Group) and Nikolaus Amrhein (ETH Zürich) for critical reading of manuscript. **Funding:** Part of this work has been financially supported by Bayer CropScience. KK and VP would like to acknowledge financial support by the Institute Strategic Program Grant ‘Designing Future Wheat’ (BB/P016855/1) from the Biotechnology and Biological Sciences Research Council of the UK (BBSRC).

Author contributions

Y.G. conceptualized and supervised research. Y.G. and A.G. directed the research. Y.G., A.G., S.T., R.S., A.T., P.R., S.W., and K.K. designed research. S.T., R.S., A.T., D.B., P.R., B.K., S.W.,

V.P., and G.H. performed research. Y.G., A.G., S.T., R.S., A.T., P.R., B.K., S.W., V.P., K.K., J.D.G.J., N.v.W., and G.H. analyzed data. Y.G., A.G., S.T., R.S. wrote the paper. All authors read and approved the manuscript.

Competing interests

Y.G. has shares in Nomad Bioscience. S.T., R.S., A.T., D.B., P.R., B.K., A.G., and Y.G. are employed by Nomad Bioscience. S.W. has been employed by Nomad Bioscience. V.P. and K.K. are affiliated with Rothamsted Research, Harpenden, United Kingdom; N.v.W. is affiliated with Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany; J.D.G.J. is affiliated with The Sainsbury Laboratory, University of East Anglia, Norwich, United Kingdom; and G.H. is affiliated with Martin Luther University of Halle-Wittenberg, Halle, Germany. A.G., D.B., P.R., and Y.G. are inventors on the patent application entitled “Process of transfecting plants”; P.R., D.B., A.G., and Y.G. are inventors on the patent application “Agrobacterium for transient transfection of whole plant”; Y.G. is an inventor on the patent application “Potexvirus-derived replicon”; A.T., D.B., A.G., and Y.G. are inventors on the patent application “Process of providing plants with abiotic stress resistance”; and S.T, R.S., A.G., and Y.G. are inventors on the patent application “Method of improving potexviral vector stability”. The ownership of the patents resides with Nomad Bioscience. The authors declare that they have no other competing interests.

Figure legends:

Fig. 1: Transfection of various crop plants with viral vectors delivered using *Agrobacterium* or as viral particles. (**a**) Spraying of *N. benthamiana* plants with *Agrobacterium tumefaciens* strain ICF320 carrying PVX-GFP vector using industrial sprayer (top panel). All plants showed intensive green fluorescence at 21 days post spraying (dps) (bottom panel). Inserts show an untreated *N. benthamiana* control plant under UV-light (bottom left) as well as GFP fluorescence at 16 dps in seedlings sprayed with the same agrobacterial suspension (top right). (**b**) Microscopic image of protoplasts isolated from plants sprayed with *Agrobacterium* harboring cell-to-cell-movement-disabled PVXΔCP-GFP vector visualizes the efficiency of agrobacterial transfection based on GFP fluorescence (CP: coat protein). Scale bar, 100 µm. (**c**) TMV vectors without own CP can still move cell-to-cell in tobacco using own MP (movement protein). Scale bar for both images, 50 µm. (**d**) Co-infiltration of agrobacteria strain ICF320 carrying PVXΔCP-GFP and TMV-dsRED vectors, providing green and red fluorescence, respectively. Only the TMV construct shows systemic movement, as indicated by dsRED expression in leaf veins of *N. benthamiana* at 36 days post infiltration (dpi). (**e**) Transient delivery can be used repeatedly. *N. benthamiana* plants were dipped successively with one week interval in diluted agrobacterial cultures harboring viral vectors TMVΔMP-GFP (day 0), PVX-dsRED (day 7), and TMV-GFP (day 14) (top panel). For controls (bottom panel) *N. benthamiana* plants were dipped in different *Agrobacterium* suspensions separately. Photos were taken at 21 days post dipping (dpd). Dipping was used here for proof-of-principle in order to get more uniform distribution of the transfection spots for better visualization. Same concept applies for spray (see main text). (**f**) GFP fluorescence in leaves of multiple plant species sprayed with agrobacteria carrying PVX-GFP: *N. benthamiana*, tobacco 'Samsun', tomato 'Balcony Red', potato 'Elfe', pepper 'Early California

927 Wonder‘, red beet 'Moulin Rouge'. (**g**) GFP fluorescence in leaves of plant species sprayed with
928 *agrobacteria* harboring TMVcg-GFP, ClYVVT27I-GFP, and MSV-GFP viral vectors: spinach
929 'Frühes Riesenblatt', broad bean 'Dreifach Weiße', maize 'Sturdi Z'. (**h**) GFP fluorescence in
930 leaves of tomato 'Tamina‘, tobacco 'Samsun‘, soybean 'Blyskavytsya', and wheat ‘Cadenza‘
931 plants sprayed and rubbed, respectively, with viral particles (VPs) containing PVX-GFP, SMV-
932 GFP, and FoMV-GFP constructs. VPs were previously isolated from *N. benthamiana* plants
933 infected with PVX-GFP, SMV-GFP, and FoMV-GFP vectors using agroinfiltration. Detailed
934 description on the spraying and rubbing procedures is given in **Methods** (dpr: days post
935 rubbing). Photos of GFP-expressing transfected leaves and corresponding untreated controls are
936 shown side-by-side in **Supplementary Fig. 2**.

937 **Fig. 2: Induction and repression of flowering with viral vectors in several plant species.**
938 (**a**,**b**) *Arabidopsis thaliana* wild-type Col-0 and a mutant containing the null allele *ft-10* are
939 induced to flower in response to the infection with TMV carrying the *Arabidopsis FT* gene (FT).
940 Plants were sprayed with agrobacteria carrying viral vectors. For each plant, days to flowering
941 were counted from the day of spray until the day of bolting as days post spray (dps). (**c**) TMV
942 vector-driven expression of the FT protein in *N. benthamiana* leaves detected by SDS-PAGE
943 with Coomassie staining and immunoblotting with FT-specific antibodies. (**d**,**e**) Induced
944 flowering in wheat ‘Cadenza' by FoMV-based overexpression of Hd3a (*O. sativa*) using viral
945 particle (VP) delivery. VPs were previously isolated from *N. benthamiana* plants infected with
946 FoMV constructs by agroinfiltration. (**f,g**) Five heterologous FT family genes from *Brassica*
947 *napus* were expressed in tobacco 'Maryland Mammoth' using PVX vectors. Only two FT family
948 members induced flowering, in both plant species. “∞” indicates no flowering by the end of
949 experiment. (**h,i**) Tomato and pepper were induced to flowering by PVX-based vectors

expressing FT. (**j**) Vernalization-dependent *Arabidopsis thaliana* ecotypes were induced to flowering in non-vernalizing condition using TMV vectors expressing FT. TMV-FT vectors had a weak or no effect (depending on the experiment) on the ecotype Lov-5 only. (**k**) Silencing of *FLC* gene by TRV vectors resulted in robust induction of flowering in *Arabidopsis thaliana*. Also ecotypes with stricter vernalization requirements including Lov-5 were induced to flower with this alternative approach. (**l,m**) Tobacco was induced to flowering by PVX-FT vector delivered as viral particles. VPs were previously isolated from *N. benthamiana* plants infected with PVX-FT using agroinfiltration. In the graphs mean values with standard deviation are indicated; statistical significance was evaluated by one-way ANOVA test using GraphPad Prism v. 8.0.2. * = $p<0.05$; **= $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$. n=number of plants for each column. (**j**) n=3-6; (**k**) n=10-12, and n= 7-11; (**m**) n= 5-6.

Fig. 3: Modification of plant stature in several crop species via modulation of gibberellin metabolic pathway by viral vectors. (**a**,**b**) Plant height reduction in tomato caused by PVX-based overexpression of gibberellin pathway genes. Tomato 'Tamina' plants were sprayed with agrobacteria harboring PVX vectors with genes for GA2ox1 (*O. sativa*), DREB1A (*A. thaliana*), DREB1B (*A. thaliana*), DREB-LP1 (*C. annuum*), and GA2ox8 (*G. max*). Controls consisted of untreated plants and plants treated with PVX-GFP. Plant height was measured at 28 dps. (**c**,**d**) PVX-mediated GA2ox8 overexpression caused height reduction in tomato 'Balcony Red', pepper 'ECW', and tobacco 'Samsun' plants. Viral vectors were delivered to plants using spraying with agrobacteria. (**e**,**f**) Height reduction in pea 'Dinga' plants sprayed with *Agrobacterium* harboring ClYVVT27I-GA2ox8 vector. **(g**,**h)** Stem shortening and elongation in tomato 'Tamina' due to PVX-mediated overexpression of GA2ox8 (*G. max*) or GA20ox1 (*A. thaliana*) genes, respectively. Viral vectors were delivered to plants using spraying with agrobacteria. Plant height

was measured at 35 dps. (**i**,**j**). Height reduction in wheat ‘Cadenza' by FoMV-based overexpression of GA2ox6 (*O. sativa*) using viral particle delivery. VPs were previously isolated from *N. benthamiana* plants infected with FoMV constructs by agroinfiltration. (**k**,**l**) Stem shortening and reduction in tomato by modulating gibberellin metabolism using viral particle delivery. VPs were previously isolated from *N. benthamiana* plants infected with PVX constructs by agroinfiltration. (**m**,**n**) Spraying of tomato 'Tamina' with agrobacteria carrying PVX-GA2ox1 vectors in the field resulted in stem shortening. In the graphs mean values with standard deviation are indicated; statistical significance was evaluated by one-way ANOVA test using GraphPad Prism v. 8.0.2. **= $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$. n = number of plants for each column. (**n**) n = number of plants for each column, consisting of 68-88 plants.

Fig. 4: Transient reprogramming of other agronomic traits in tomato with PVX vectors delivered by *Agrobacterium* using spraying. (**a**,**b**) PVX-mediated NCED3 complementation increases drought stress tolerance in ABA deficient tomato mutant *notabilis.* Upon drought, only the tomato mutant plants ('Ailsa Craig' *notabilis*) which were transfected with NCED3 (*A. thaliana)* showed a tolerant phenotype. The mutant plants not subjected to drought were used to determine the content of abscisic acid (ABA) and its derivative abscisic acid-glucose ester *(*ABA-Glc) using HPLC. (**c**,**d**) PVX-mediated overexpression of *DREB1A* (*A. thaliana*) and *DREB-LP1 (C. annuum*) augments drought stress tolerance in wild-type tomato 'Tamina' plants. Increased relative water content (RWC) found after the drought correlated with tolerant phenotype. (**e**,**f**) PVX-driven overexpression of *SP3D* (*S. lycopersicum*) gene converted *single flower truss* (*sft*) mutant phenotype to wild-type inflorescence. Number of flowers and fruits per truss were counted on untreated and sprayed tomato plants 'Ailsa Craig' MSU100 *sft* at 44 and 90 dps, respectively. (**g**,**h**) Overexpression of *ANT1* (*S. lycopersicum*) gene induces anthocyanin

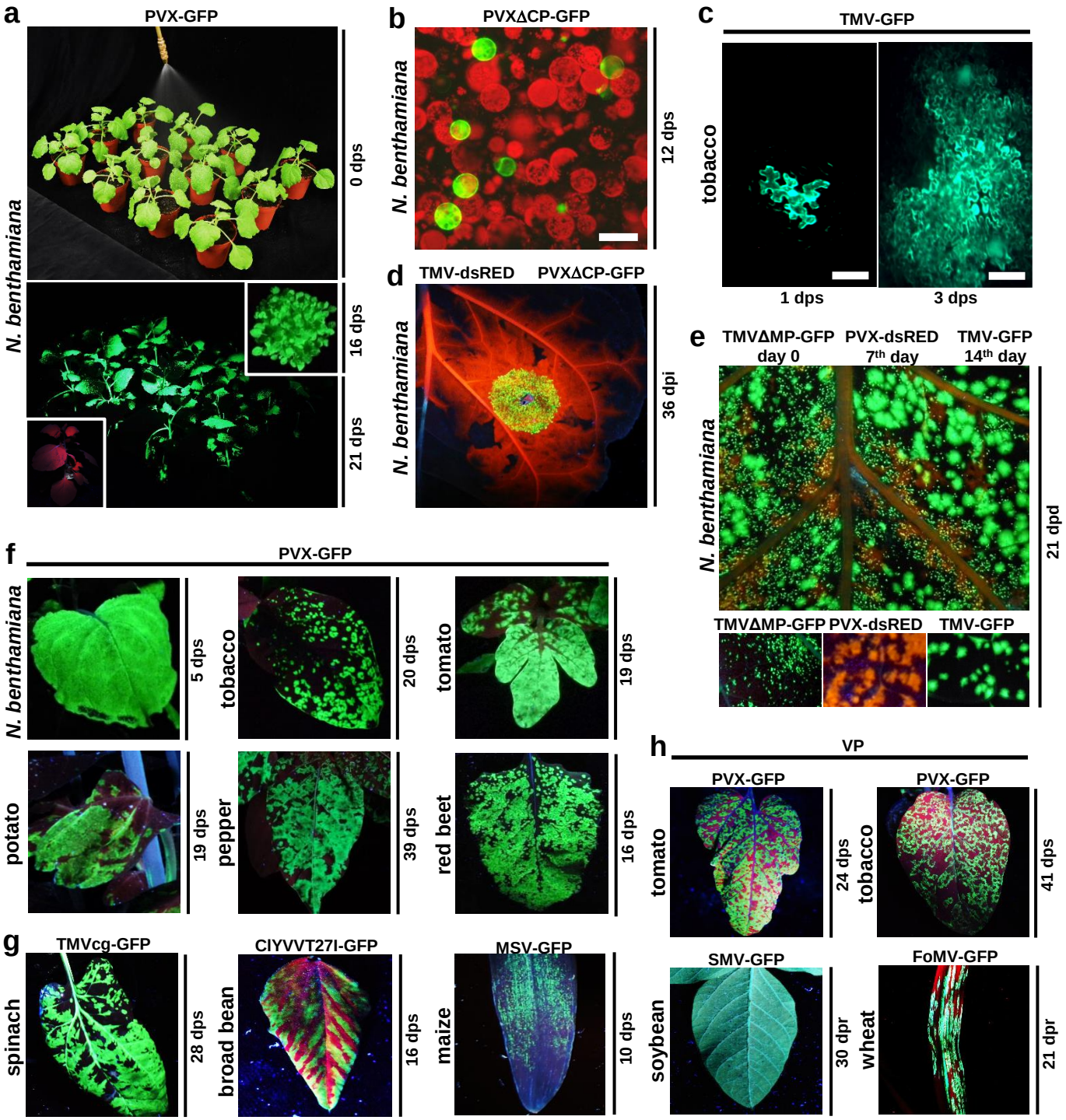
production in tomato fruits. Visual changes in the fruit color of tomato 'Balcony Red' reflect the difference in anthocyanin concentrations measured in extracts. (**i**,**j**) Delay in fruit ripening of tomato 'Balcony Red' plants by silencing of the *DML*2 gene using PVX vector with a fragment of DML2 coding sequence in antisense orientation. Y axis shows the duration of fruit ripening calculated as a time post anthesis till complete fruit maturation. In the graphs, mean values with standard deviation are indicated; statistical significance was evaluated by one-way ANOVA test using GraphPad Prism v. 8.0.2. ** = $p<0.01$; *** = $p<0.001$; **** = $p<0.0001$. n = number of plants for each column (**b**,**d,f**,**h**). (**d**) n = 5-10. For (**f**), all the flowers of each plant were considered and counted (range 24-26). For (**h**), all the fruits of each plant were pooled and measured. For (**j**), n = number of flowers/fruits, which was in the range 17-135 (taken from all plants of each treatment. Number of plants was 7 for each column, except for untreated and PVX-GFP, where the number of plants was 2.

Fig. 5: Enhanced virus spread, amplification and recombinant protein accumulation using modified PVX vectors. (**a**) Stabilizing PVX constructs using codon-optimized inserts. Tomato 'Tamina' plants were inoculated using syringe with agrobacteria carrying PVX constructs containing wild-type or codon-optimized genes of TAGL1, OVATE, and ANT1 from *S. lycopersicum*. Stability of the inserts in systemic leaves at 27 days post infiltration (dpi) was determined by RT-PCR with PVX specific primers using generated cDNA (sl) and plasmid control (Pl) as templates. (**b**) Enhancement of reporter gene expression in *N. benthamiana* by CPMMV nucleic acid-binding protein (NABP) gene insertion in PVX backbone. Plants were syringe inoculated with agrobacteria carrying constructs PVX-GFP and PVX-GFP_{NABP}. (**c**) Enhancement of reporter gene expression in tobacco 'Samsun'. Plants were syringe inoculated with agrobacteria carrying the constructs PVX-GFP and PVX-GFP_{NABP}. GFP fluorescence is

shown for several systemic leaves. (**d**) SDS-PAGE analysis of plant samples showing the enhancement of GFP expression in presence of NABP in the viral vector backbone. (**e**) GFP expression enhancement in tomato 'Tamina'. Plants were transfected with *Agrobacterium* harboring different GFP-expressing PVX constructs using spraying, resulting in a further increase with CVB-CRP in the backbone. (**f**) dsRED expression enhancement in tomato 'Tamina‘: dsRED fluorescence is clearly visible only when CVB-CRP is added to the PVX backbone. Plants were transfected by agroinfiltration using syringe. (**g**) SDS-PAGE analysis of samples obtained from PVX-dsRED and PVX-dsRED$_{CVB-CRP}$ treated plants showing the enhancement of dsRED accumulation. (**h**,**i**) Enhanced dwarfism effect by NABP insertion in PVX backbone. Tobacco 'Samsun' plants were transfected with different PVX constructs by agroinfiltration using syringe. (**j**,**k**) Enhanced flowering repression effect by NABP insertion in PVX backbone. Tomato 'Balcony Red' plants were transfected with different PVX constructs by syringe agronfiltration. Genes are: *FT1* (from tobacco), and *SP5G* (from tomato). In all these experiments except for (**e**), we used syringe inoculation of first true leaf (*Nicotiana benthamiana*) or both cotyledon and first true leaf together (tobacco and tomato) instead of spraying the whole plant so as to be able to track virus systemic movement and assess viral RNA stability and subtle quantitative differences in viral vector performance. In the graphs, mean values with standard deviation are indicated; statistical significance was evaluated by one-way ANOVA test using GraphPad Prism v. 8.0.2. n = number of plants for each column.

Fig. 6: Fate of *Agrobacterium* and viral vectors in transfected plants and soil (greenhouse and open field). (**a**) PVX vectors loose foreign gene inserts upon systemic movement in tomato. Plants were sprayed with agrobacteria harboring PVX constructs with *GA2ox1* (*O. sativa*), *DREB1A* (*A. thaliana*), *GA2ox8* (*G. max*), and *GA20ox1* (*A. thaliana*) genes. Vector stability was

determined in systemic leaves (sl) by RT-PCR using PVX specific oligos. PCR fragments generated using corresponding plasmid DNA as a template (Pl) were used as a positive control. (**b**,**c**) Reducing the GC-content destabilizes GFP gene inside the PVX vector. Tomato plants were agroinfiltrated by syringe with PVX-GFP constructs containing GFP sequences with original and reduced GC-content. Stability of the inserts in systemic leaves (sl) at 25 dpi and in fruits at 110 dpi (fr) was determined by RT-PCR as described above. In this group of experiments, we used syringe inoculation of both cotyledon and first true leaf together instead of spraying the whole plant so as to be able to track the virus systemic movement and assess viral RNA stability. (**d**) Absence of PVX in progeny of agrosprayed plants. The presence of PVX and cystatin housekeeping control gene was determined by RT-PCR using cDNAs generated from tomato 'Balcony Red' as templates and specific primers. Seedlings germinated from seeds collected on plants sprayed with the PVX constructs (pr) did not show the presence of PVX, while control leaves of sprayed plants (sp) generated a clear band. (**e**,**g**) Transient presence of genetically modified agrobacteria in tobacco leaves and surrounding soil analyzed in a field experiment. (**e**) Tobacco 'Maryland Mammoth' plants were sprayed with auxotrophic *Agrobacterium* strain NMX021 harboring TMV-GFP vector and monitored for GFP fluorescence. (**f**) Time course after spraying, in which samples of leaves were collected and analyzed for the presence of *Agrobacteria* by counting cfu per g leaf material (wps: weeks post spraying, mps: months post spraying). (**g**) Time course after spraying, in which samples of soil with a constant distance to the plants were collected and analyzed for the presence of *Agrobacteria* by counting colony forming units (cfu) per g of soil. In the graphs, mean values with standard deviation are indicated. (**f**) n = number of plants for each column. (**g**) n = number of soil samples collected for each column.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16 dps
21 dps
b
PVXΔCP-GFP
N. benthamiana
12 dps
c
TMV-GFP
tobacco
1 dps
3 dps
d
TMV-dsRED
PVXΔCP-GFP
N. benthamiana
36 dpi
e
TMVΔMP-GFP day 0
PVX-dsRED 7th day
TMV-GFP 14th day
N. benthamiana
21 dpd
TMVΔMP-GFP
PVX-dsRED
TMV-GFP
f
PVX-GFP
N. benthamiana
5 dps
tobacco
20 dps
tomato
19 dps
potato
19 dps
pepper
39 dps
red beet
16 dps
g
TMVcg-GFP
spinach
28 dps
ClYVVT27I-GFP
broad bean
16 dps
MSV-GFP
maize
10 dps
h
VP
PVX-GFP
tomato
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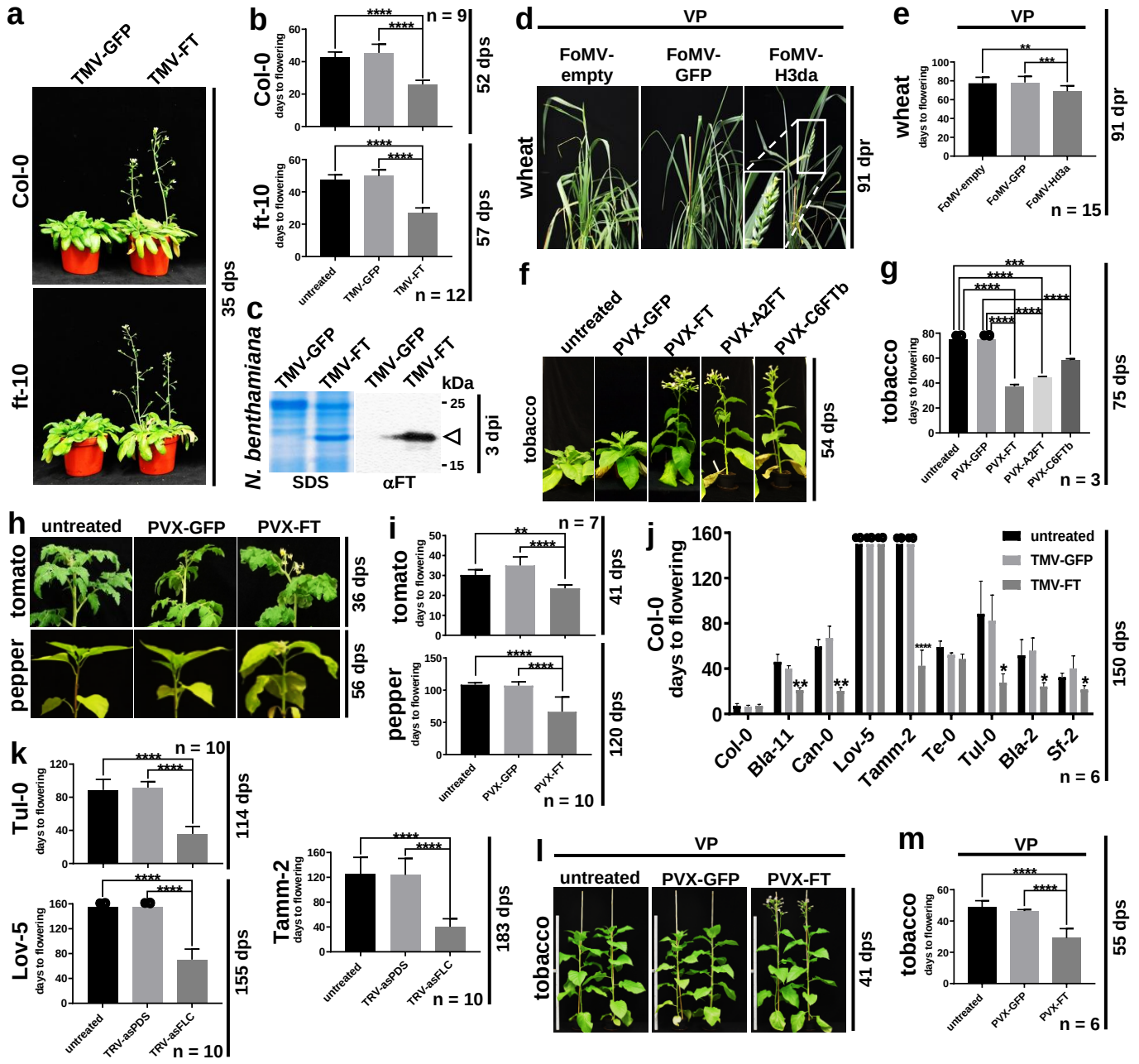
PVX-GFP
tobacco
41 dps
SMV-GFP
soybean
30 dpr
FoMV-GFP
wheat
21 dpr

Figure 1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35 dps
b
Col-0
ft-10
days to flowering
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52 dps
57 dps
untreated
TMV-GFP
TMV-FT
n = 12
c
N. benthamiana
TMV-GFP
TMV-FT
TMV-GFP
TMV-FT
kDa
25
15
3 dpi
SDS
αFT
d
VP
FoMV-empty
FoMV-GFP
FoMV-H3da
wheat
91 dpr
e
VP
wheat
days to flowering
FoMV-empty
FoMV-GFP
FoMV-Hd3a
n = 15
91 dpr
f
untreated
PVX-GFP
PVX-FT
PVX-A2FT
PVX-C6FTb
tobacco
54 dps
g
tobacco
days to flowering
untreated
PVX-GFP
PVX-FT
PVX-A2FT
PVX-C6FTb
n = 3
75 dps
h
untreated
PVX-GFP
PVX-FT
tomato
pepper
36 dps
56 dps
i
tomato
pepper
days to flowering
n = 7
41 dps
120 dps
untreated
PVX-GFP
PVX-FT
n = 10
j
Col-0
days to flowering
untreated
TMV-GFP
TMV-FT
Col-0
Bla-11
Can-0
Lov-5
Tamm-2
Te-0
Tul-0
Bla-2
Sf-2
n = 6
150 dps
k
Tul-0
Lov-5
Tamm-2
days to flowering
n = 10
114 dps
155 dps
183 dps
untreated
TRV-asPDS
TRV-asFLC
n = 10
l
VP
untreated
PVX-GFP
PVX-FT
tobacco
41 dps
m
VP
tobacco
days to flowering
untreated
PVX-GFP
PVX-FT
n = 6
55 dps

Figure 2

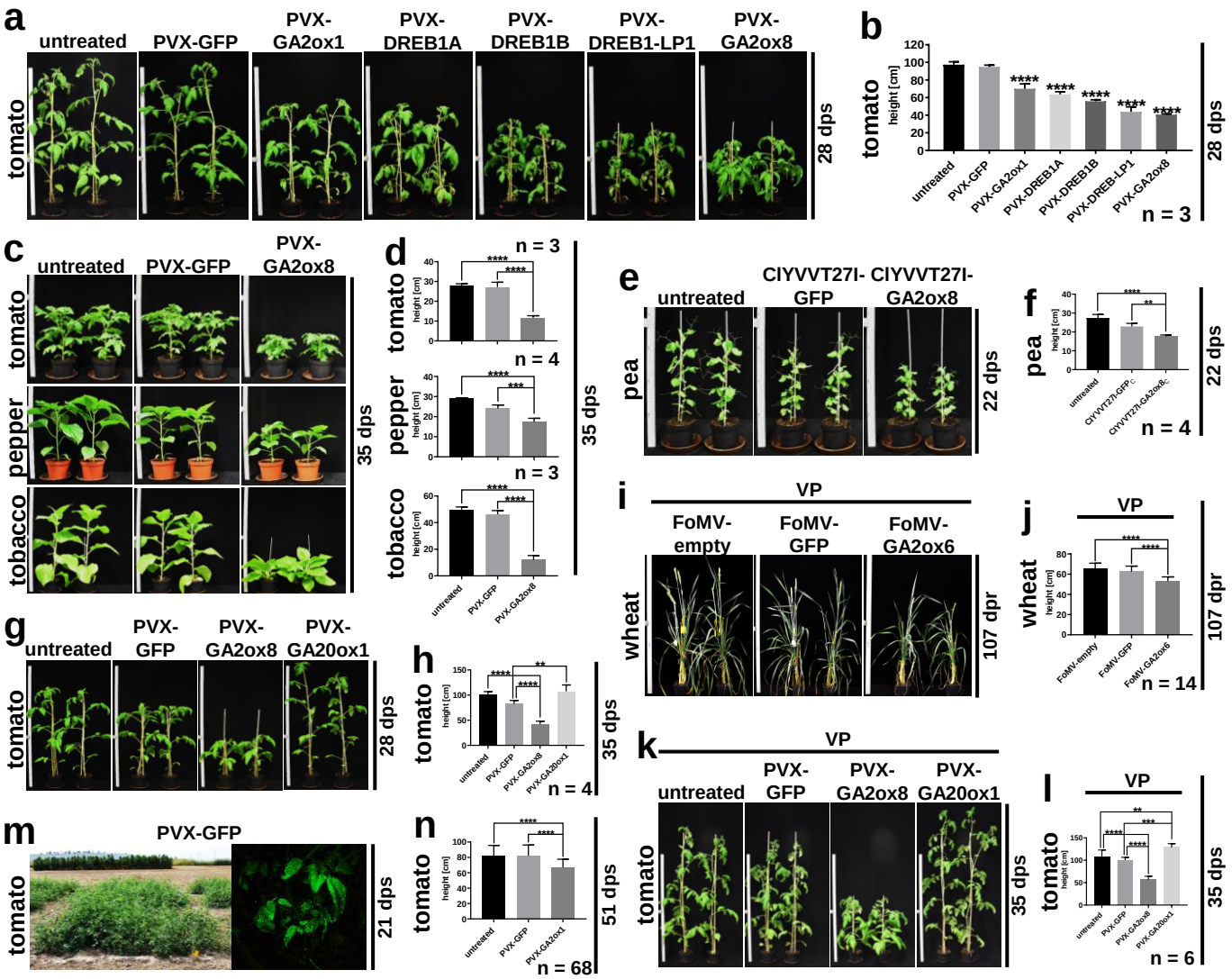
a
untreated
PVX-GFP
PVX-GA2ox1
PVX-DREB1A
PVX-DREB1B
PVX-DREB1-LP1
PVX-GA2ox8
tomato
28 dps
b
tomato
height [cm]
untreated
PVX-GFP
PVX-GA2ox1
PVX-DREB1A
PVX-DREB1B
PVX-DREB-LP1
PVX-GA2ox8
n = 3
28 dps
c
untreated
PVX-GFP
PVX-GA2ox8
tomato
pepper
tobacco
35 dps
d
n = 3
n = 4
n = 3
tomato
pepper
tobacco
height [cm]
35 dps
e
ClYVVT27I-GFP
ClYVVT27I-GA2ox8
untreated
pea
22 dps
f
pea
height [cm]
n = 4
22 dps
g
untreated
PVX-GFP
PVX-GA2ox8
PVX-GA20ox1
tomato
28 dps
h
tomato
height [cm]
n = 4
35 dps
i
VP
FoMV-empty
FoMV-GFP
FoMV-GA2ox6
wheat
107 dpr
j
VP
wheat
height [cm]
n = 14
107 dpr
k
VP
untreated
PVX-GFP
PVX-GA2ox8
PVX-GA20ox1
tomato
35 dps
l
VP
tomato
height [cm]
n = 6
35 dps
m
PVX-GFP
tomato
21 dps
n
tomato
height [cm]
n = 68
51 dps

Figure 3

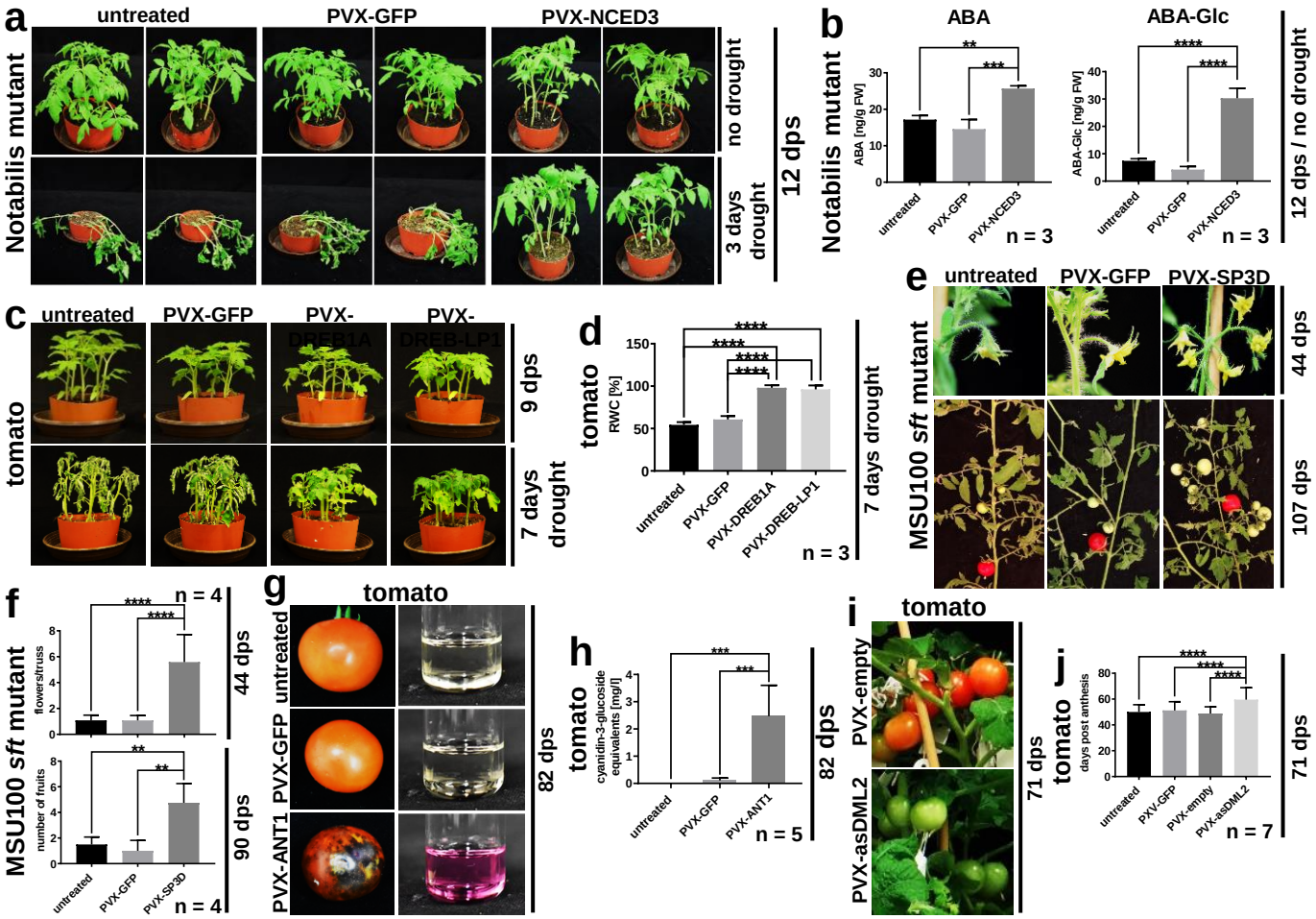
a
untreated
PVX-GFP
PVX-NCED3
Notabilis mutant
no drought
3 days drought
12 dps
b
ABA
ABA-Glc
Notabilis mutant
ABA [ng/g FW]
ABA-Glc [ng/g FW]
untreated
PVX-GFP
PVX-NCED3
n = 3
12 dps / no drought
c
untreated
PVX-GFP
PVX-DREB1A
PVX-DREB-LP1
tomato
9 dps
7 days drought
d
tomato
RWC [%]
untreated
PVX-GFP
PVX-DREB1A
PVX-DREB-LP1
n = 3
7 days drought
e
untreated
PVX-GFP
PVX-SP3D
MSU100 sft mutant
44 dps
107 dps
f
MSU100 sft mutant
flowers/truss
number of fruits
untreated
PVX-GFP
PVX-SP3D
n = 4
44 dps
90 dps
g
tomato
untreated
PVX-GFP
PVX-ANT1
82 dps
h
tomato
cyanidin-3-glucoside equivalents [mg/l]
untreated
PVX-GFP
PVX-ANT1
n = 5
82 dps
i
tomato
PVX-empty
PVX-asDML2
71 dps
j
tomato
days post anthesis
untreated
PXV-GFP
PVX-empty
PVX-asDML2
n = 7
71 dps

Figure 4

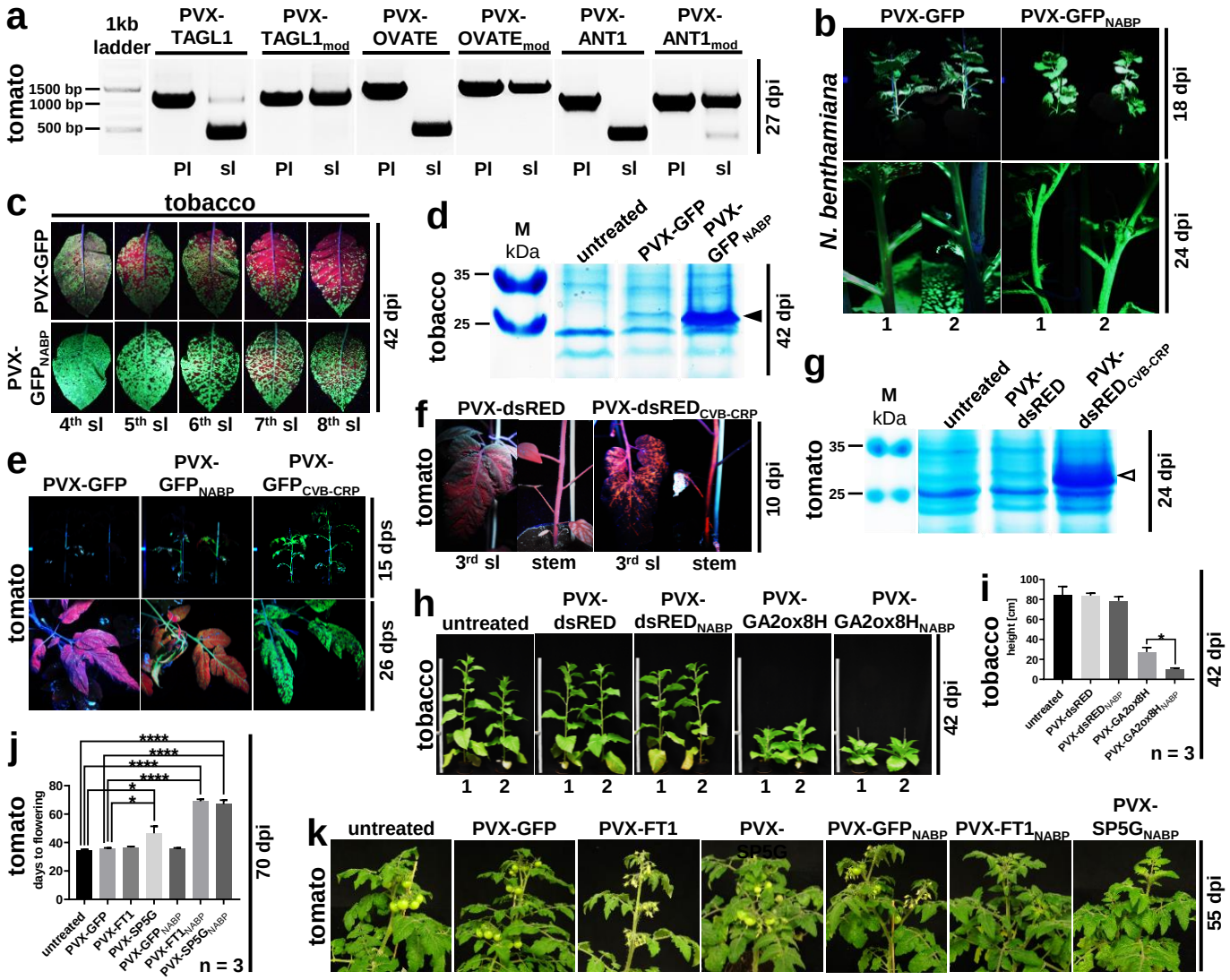
a
1kb ladder
PVX-TAGL1
PVX-TAGL1mod
PVX-OVATE
PVX-OVATEmod
PVX-ANT1
PVX-ANT1mod
tomato
1500 bp
1000 bp
500 bp
PI sl PI sl PI sl PI sl PI sl PI sl
27 dpi
b
PVX-GFP
PVX-GFPNABP
N. benthamiana
18 dpi
24 dpi
1 2 1 2
c
tobacco
PVX-GFP
PVX-GFPNABP
42 dpi
4th sl 5th sl 6th sl 7th sl 8th sl
d
M kDa
untreated
PVX-GFP
PVX-GFPNABP
tobacco
35
25
42 dpi
e
PVX-GFP
PVX-GFPNABP
PVX-GFPCVB-CRP
tomato
15 dps
26 dps
f
PVX-dsRED
PVX-dsREDCVB-CRP
tomato
10 dpi
3rd sl stem 3rd sl stem
g
M kDa
untreated
PVX-dsRED
PVX-dsREDCVB-CRP
tomato
35
25
24 dpi
h
untreated
PVX-dsRED
PVX-dsREDNABP
PVX-GA2ox8H
PVX-GA2ox8HNABP
tobacco
42 dpi
1 2 1 2 1 2 1 2 1 2
i
tobacco
height [cm]
100
80
60
40
20
0
untreated
PVX-dsRED
PVX-dsREDNABP
PVX-GA2ox8H
PVX-GA2ox8HNABP
*
*
n = 3
42 dpi
j
tomato
days to flowering
80
60
40
20
0
****
****
****
****
*
*
untreated
PVX-GFP
PVX-FT1
PVX-SP5G
PVX-GFPNABP
PVX-FT1NABP
PVX-SP5GNABP
n = 3
70 dpi
k
untreated
PVX-GFP
PVX-FT1
PVX-SP5G
PVX-GFPNABP
PVX-FT1NABP
PVX-SP5GNABP
tomato
55 dpi

Figure 5

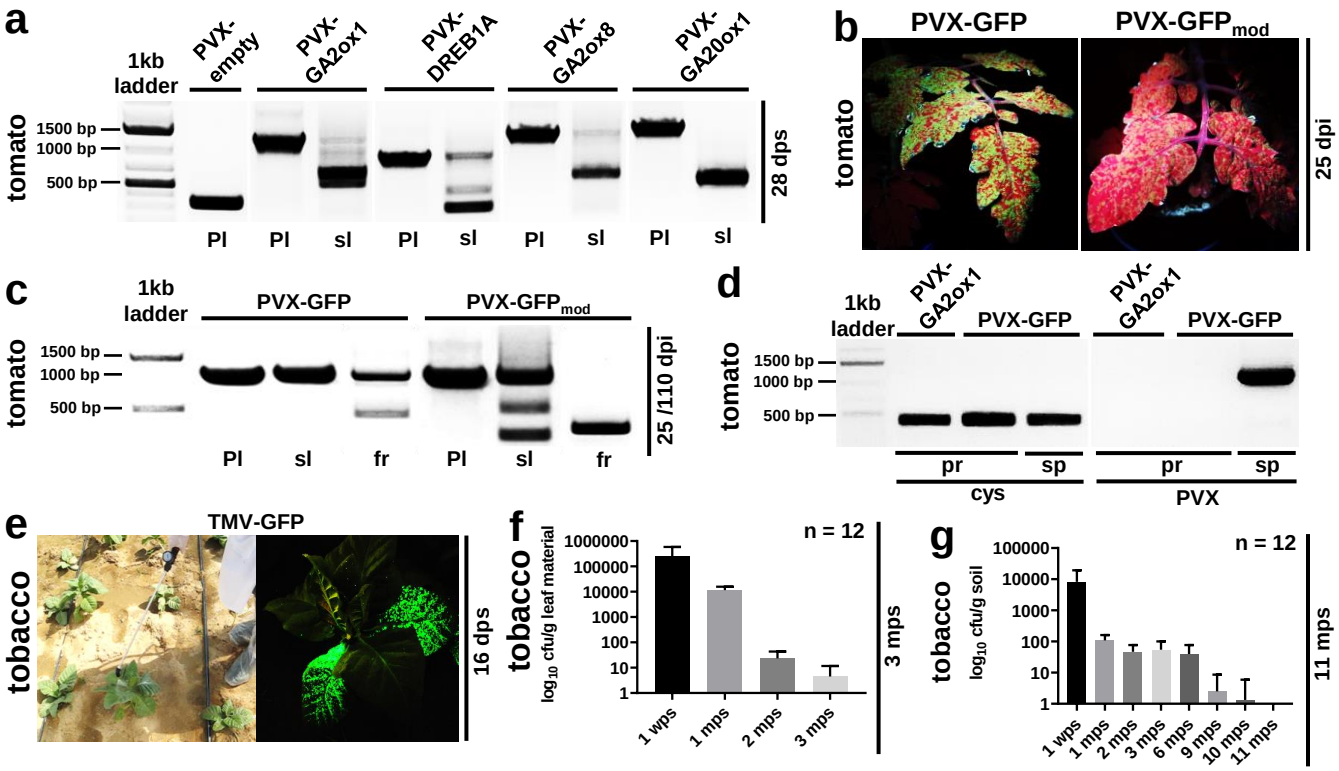
a
1kb ladder
PVX-empty
PVX-GA2ox1
PVX-DREB1A
PVX-GA2ox8
PVX-GA20ox1
tomato
1500 bp
1000 bp
500 bp
Pl
Pl
sl
Pl
sl
Pl
sl
Pl
sl
28 dps
b
PVX-GFP
PVX-GFPmod
tomato
25 dpi
c
1kb ladder
PVX-GFP
PVX-GFPmod
tomato
1500 bp
1000 bp
500 bp
Pl
sl
fr
Pl
sl
fr
25 /110 dpi
d
1kb ladder
PVX-GA2ox1
PVX-GFP
PVX-GA2ox1
PVX-GFP
tomato
1500 bp
1000 bp
500 bp
pr
sp
pr
sp
cys
PVX
e
TMV-GFP
tobacco
16 dps
f
tobacco
log10 cfu/g leaf material
n = 12
1000000
100000
10000
1000
100
10
1
1 wps
1 mps
2 mps
3 mps
3 mps
g
tobacco
log10 cfu/g soil
n = 12
100000
10000
1000
100
10
1
1 wps
1 mps
2 mps
3 mps
6 mps
9 mps
10 mps
11 mps
11 mps

Figure 6