

kept intact to keep the remaining vascular-free tissue together. Microdissection of the vascular bundles including the starch-containing bundle sheath did not disturb positive gravitropic upward bending of horizontally tilted plants. In experiments where the root cap was removed, roots did not show gravitropic curvature any more. Nevertheless, roots, which were also incubated in latrunculin (inhibits polymerization of actin), showed distinct upward bending. These experiments are clear indications that sedimentation of amyloplasts is not necessary for gravitropism of plants. According to Popper, Edelmann [12] did not try to confirm the hypothesis of starch-amyloplast, but to disprove it. The result of the investigation, no amyloplasts but still gravitropism, clearly refutes the starch-amyloplast hypothesis. Only if an ulterior mechanism or parameters explain the observed curvature can the hypothesis be maintained. In the coleoptile gravitropism experiment, the intact remaining tissue at the tip might still have contained amyloplasts (as we have observed when we reproduced the experiment). As the growth hormone auxin is mainly released from the shoot's tip, gravity-induced rearrangement of auxin carriers in the tip may be responsible for an auxin asymmetry and subsequent bending of the organ. To exclude such an effect, we would suggest searching for amyloplasts in the tip of the coleoptile. In addition, we would suggest performing experiments in which the tip is completely removed and the shoot is kept together mechanically (optionally auxin can be supplied to allow cell elongation). To ensure that the observed bending of roots is independent of sedimentation of starch, we suggest performing experiments with isolated roots (ideally supplied with auxin and nutrients via the cut surface), because auxin asymmetry in the roots is likely supplied from the coleoptile by basipetal auxin transport. Auxin concentration in the lower flank of a tilted coleoptile (experiments

concerning root gravitropism were performed with intact coleoptiles) is higher than in the upper flank, a gradient that may also spread into the root system and induce the observed bending. Alternatively, the experiment can be performed with decapitated coleoptiles. This would prove that root bending is connected to an intact coleoptile tip. If Edelmann's results can be verified or they can be not falsified, science needs to reject the starch-amyloplast hypothesis. This opens the way for elucidation of the enigma of graviperception in plants, but some questions remain: What is the role of the pronounced amyloplast-bearing cells? What are possible alternative mechanisms? An alternative model is the protoplast pressure hypothesis, according to which the whole cytoplasm of a cell exerts force on the physical lower membrane inducing a signal transduction chain.

And finally, one has to admit that simple questions do not necessarily have simple answers.

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Spotlight

A CRISPR Way for Fast-Forward Crop Domestication

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Precision crop breeding, using genome editing tools such as clustered regularly interspaced short palindromic repeats (CRISPR) systems to improve useful traits in crop plants, holds great potential for the future of agriculture. Using CRISPR-Cas9, recent studies have engineered domestication traits in wild-relative species of tomato crop for higher nutritive value and better adaptation to diverse stresses.

Conventional Breeding Techniques

Technological advances in agricultural, especially plant breeding techniques, have led to an increase in agricultural productivity. Conventional plant breeding techniques take advantage of naturally available resources to combine desirable



traits for crop improvement, but are often accompanied by stress susceptibility and loss of genetic diversity. These issues make it difficult to resolve the challenges of global food security. Although introgression of genes for desirable traits through conventional breeding has been successfully exploited over millennia and has resulted in increased agricultural production, new plant breeding techniques offer cheaper and rapid development of improved crop varieties [1].

Revolution in Precision Breeding

Advances in genomics and genome editing have revolutionized the field of precision breeding. The availability of high-throughput sequencing techniques and computational analysis has added valuable information in genomic data. This information based on genomic data can be exploited to identify desirable genes/traits that can be incorporated in wild relatives of crop plants. Combining this with the genome editing technologies such as CRISPR and the associated Cas9 nuclease (CRISPR-Cas9), it is now possible to edit the plant genome with extreme precision and accuracy. CRISPR-Cas9 allows the manipulation of DNA in many ways; for example, by simply incorporating random mutation (insertion or deletion) through nonhomologous end joining to disrupt genes, by generating targeted point mutations in genes using precise base editors, and by whole-gene insertion employing the cell's homology-directed repair pathway [1]. Moreover, multiple loci can be edited at the same time using multiplex approaches, enabling researchers to incorporate multiple traits at once [2]. Several recent studies have reported the potential of CRISPR-Cas9 technology to improve the yield or quality of major cereal crops including rice, maize, and wheat [3]. Grain length and width increase in wheat, resistance to powdery mildew in wheat, resistance to bacterial blight in rice, and threefold increase in fruit size and tenfold increase in fruit number in tomato are

among the major milestones that have been achieved via CRISPR-Cas9 [1,3,4].

De Novo Domestication and Crop Improvement

The process of domestication is the result of a selection procedure that has led to increased adaptation/acclimatization of plant and animals for agriculture. Domestication of plants by human activities has drastically altered the evolution of the ecological niche. The process of domestication, followed by plant breeding, has altered crop architecture at both the phenotypic and the genomic level. This alteration has resulted in the gradual transformation of species that were once wild into elite, high-yield cultivars [5]. However, the focus of most plant breeding programs has been major cereal crops like wheat, rice, and maize. There are other locally important crops, like chickpea, sorghum, cassava, groundnut, and sweet potato, often known as 'orphan crops', that have the potential to address food security issues [6]. Now, with the availability of powerful tools like CRISPR-Cas9, it is possible to engineer desirable traits in traditional orphan crops in a very short time. This will have an economic impact that can address food security issues (Figure 1).

The studies by Zsögön *et al.* and Li *et al.* [7,8] provide evidence that genome editing of wild tomato not only enhanced fruit size and yield but also increased the nutritional value of the fruit. They targeted a set of six genes [*SELF-PRUNING (SP)*, *OVATE (O)*, *FRUIT WEIGHT 2.2 (FW2.2)*, and *LYCOPENE BETA CYCLASE*] using a multiplexed CRISPR-Cas9 approach to create a novel variety with desirable traits. In parallel, another study by Lemmon *et al.* has shown 'de novo' domestication of an orphan Solanaceae crop, 'ground cherry' (*Physalis pruinosa*). The concept of *de novo* domestication is to identify and introgress the genes/mutations that led to the

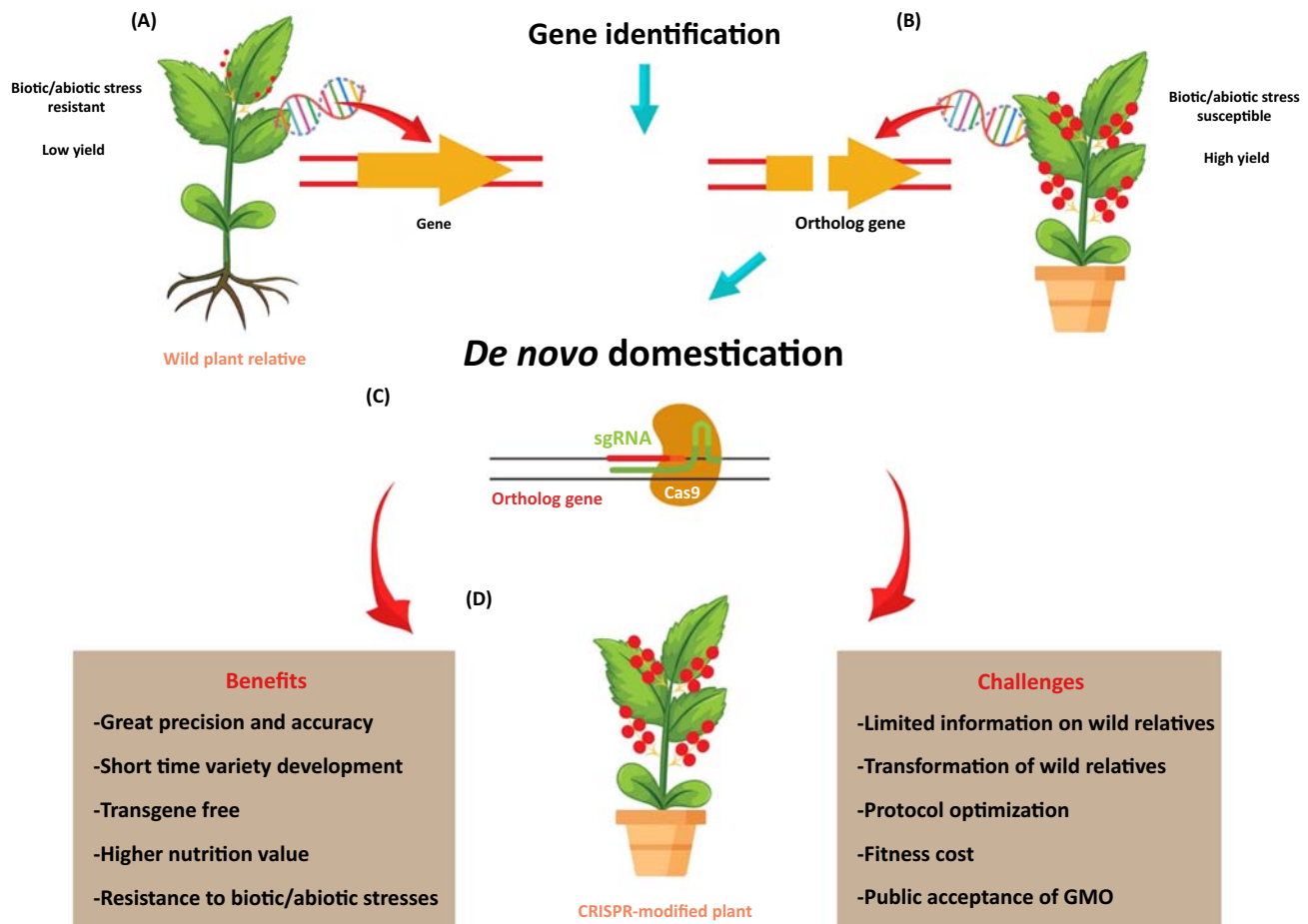
success of the domestication and adaptation of the crop varieties. Lemmon *et al.* showed that CRISPR-mediated editing of genes for improved plant architecture, flower production, and fruit size holds the potential to accelerate domestication by improving these major productivity traits [9]. In short, these studies have demonstrated the successful *de novo* domestication of wild species via CRISPR-mediated precision breeding.

Orphan Crops' Domestication: The Best Is Yet to Come

Orphan crops have been used for centuries by local communities because of their better nutritional attributes and adaptation to environmental conditions. However, they are unable to compete with major crops, mainly because of their lower yield or limited scope and moderate resilience to diseases and pests [7–9]. However, given the limited interest and funding, research to improve the quality and yield of orphan crops remains in its infancy [6]. While conventional breeding for quality enhancement of orphan crops is challenging, genetic modification through guided nucleases is an ideal platform [7,8,10]. This robust domestication is proposed to cope not only with the changing climate scenario but also the growing food security issues.

An important advantage of CRISPR-mediated plant breeding is the availability of protocols to produce potentially 'transgene-free' crop varieties. This provides researchers with an opportunity to create genetically modified crop varieties identical to conventionally bred crop varieties. Although the acceptance of CRISPR crops is controversial, and (rapidly changing) GMO regulations vary from country to country, these crops still hold great potential for rapid variety development and immediate impact on farmers' lives.

Besides CRISPR-mediated *de novo* domestication, other promising



Trends in Plant Science

Figure 1. CRISPR-Mediated De Novo Domestication. (A) Although wild relatives (e.g., ground cherry) are not as high yielding as their cultivated-relative species (e.g., tomato), they demonstrate certain highly valuable traits such as biotic and abiotic stress tolerance. Genes for such traits can be (and have been) identified and used in downstream precision breeding programs. (B) By contrast, cultivated crop varieties, despite having high yield and useful agronomic traits, can be severely affected by certain biotic and abiotic stresses, especially plant pathogens. One way of improving this is to utilize resistance-gene orthologs from wild relatives. Vice versa, domestication genes from the cultivated varieties can be transferred to the wild relatives to increase their yield and productivity. The latter is referred to here as *de novo* domestication. (C) Domestication traits can be engineered in wild relatives using a guided CRISPR-Cas9 system. (D) The benefits and challenges of CRISPR crops are highlighted.

technologies, such as speed breeding [11], can further decrease the time and cost of variety development. It has been observed that multiple domestication traits follow a Mendelian inheritance pattern and are often accompanied by gain or loss of gene functions. Knowing the proper genetic loci and required mutation, precision breeding can be leveraged to incorporate these useful agronomic traits from wild plant species into staple and orphan food crops [6].

However, several challenges, like the transformation of wild species/orphan crops, might delay the delivery of product to farmers' fields and a research focus on the optimization of transformation protocols is needed.

Given the fact that most of the orphan crops (e.g., cassava, banana, quinoa) are not only important for food and feed but also hold great economic potential for industrial applications, effective

management and improved orphan crop varieties will not only fulfil the caloric content and phytonutrient requirements of the growing population but will also contribute towards poverty reduction and global food security.

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Forum

Greenhouse Gas Fluxes From Tree Stems

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Tree stems exchange carbon dioxide, methane, and nitrous oxide with the atmosphere. The biophysical mechanisms controlling these fluxes are not fully understood, and consequently are not included in process-based models. We highlight advances and opportunities that will allow quantification of

the role of these plant structures in the local-to-global balance of greenhouse gases (GHGs).

Terrestrial ecosystems play a key role in the global balance of GHGs and, with an estimated 3 trillion trees across the world [1], woody plants represent an important biophysical link between soils and the atmosphere. Consequently, unraveling soil–plant–atmosphere interactions is crucial for understanding the role of terrestrial ecosystems in the biogeochemical cycles of the Earth system. In this Forum article we discuss current knowledge on GHG emissions from tree stems and propose general priorities for research.

Most studies on woody plants have focused on biophysical processes in leaves and fine roots because these are considered to be the most active structures for mass and energy exchange. Arguably, the role of tree stems has mainly been considered as structural support or as conduits for transport of mass (e.g., water and carbohydrates), but their specific role in soil–plant–atmosphere interactions has been less well studied or incorporated into process-based models. Recent discoveries are challenging this traditional view because these structures could represent important surfaces for plant–atmosphere interactions [2,3].

Plant respiration is a key process that releases energy stored in the chemical bonds of carbohydrates produced during photosynthesis. Byproducts of this catabolic reaction include carbon dioxide (CO₂) and water, which can be exchanged with the atmosphere across different plant structures including leaves, roots, and tree stems. Stem respiration (i.e., stem CO₂ efflux) has been studied for >40 years [4]. Most of the CO₂ within a tree stem originates from cell respiration within the tree stem or roots, but stem

CO₂ efflux rates are dependent on internal CO₂ axial/radial transport and diffusion rates [4]. We postulate that the thermodynamic principles developed for transport and diffusion of CO₂ in tree stems [4] may be applicable to fluxes of other GHGs in process-based models [5].

Since the 1970s it has been known that trees can store methane (CH₄) inside stems at high concentrations [6], but it was recently discovered that tree stems can emit CH₄ [2,5]. Most studies regarding stem CH₄ emissions have been carried out on forested wetlands or floodplains, where soils are usually net producers of CH₄ [2]. In these ecosystems it is hypothesized that CH₄ can be transferred from soils to the atmosphere via tree stems, with important implications for local-to-regional CH₄ budgets [7]. In upland forests, tree stems also emit CH₄ but the mechanisms of CH₄ production, oxidation, and transport are a matter of debate [5]. There is evidence that CH₄ can be produced in the soil and transported to stems [8], or is produced internally within the stem (by anaerobic bacteria) and emitted radially [9]. In upland forests, the magnitudes of CH₄ efflux from stems could counterbalance the uptake from soils (usually considered as net CH₄ sinks), and may influence whether a forest acts as a net CH₄ sink or source [9].

The largest knowledge gap is in our understanding of nitrous oxide (N₂O) fluxes from tree stems. Recent studies report contrasting results that N₂O can either be emitted or absorbed by tree stem surfaces [10]. It is possible that these fluxes could be associated with cryptogamic covers living on the surface of the bark [11], but it is unclear whether N₂O production or consumption might be associated in any way with microbial or fungal activity within the tree stem or with any plant biophysical process (e.g., photosynthesis, transpiration).