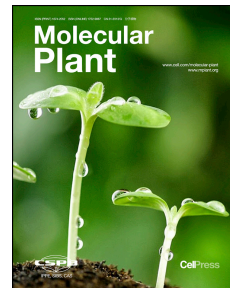


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SPOTLIGHT: Targeting key genes to tailor old and new crops for a greener agriculture

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Growing crops closer to the consumer is one of many approaches proposed to lessen the environmental footprint associated with food production (Banerjee and Adenauer, 2014). This is complicated by an increasingly urban population, the lack of available land in cities and the consequent need for rapid crop cycling. Given these constraints to date only lettuce and related leafy greens are currently cultivated at scale in urban farms (Touliatos et al., 2016). There is, however, considerable recent interest in the urban agriculture of fruits and berries since controlled automated vertical farming systems offer the twin advantages of optimizing land use and the ease in which they can be designed to be more environmentally friendly and sustainable than traditional agriculture. A considerable challenge is however that commercial crop varieties were historically, as well as still being contemporarily, bred to be high yielding under typical field and greenhouse parameters. Moreover, given the perishable nature of fruits and berries a reduced distance from farm to fork renders the shelf life trait (and its associated detrimental effects on fruit quality), less imperative. Thus the possibility to improve fruit and berry quality traits is a particularly attractive aspect of urban agriculture. For these species the environmental benefit is additionally perhaps even greater than for other crops since they are more often transported by air. A recent study uses multi-site CRISPR-Cas9 genome editing to restructure vine line tomato plants into compact early-yielding plant suitable for urban agriculture, whilst demonstrating that a single gene editing strategy can produce a compact stature in the related plant groundcherry (Kwon et al., 2019).

Before discussing this elegant study in detail we feel it is important to provide a broader context to the work since this will enable us to offer a wider-reaching perspective of the power of the approach. Of the tens of thousands of edible plants only a few hundred are cultivated throughout the world with just 15 contributing to the vast majority of calories consumed (Fernie and Yan, 2019). The domestication of cultivated crops has historically largely been studied in the model grasses of the Poaceae family. However, recent studies based on quantitative trait locus mapping, genome-wide association mapping and whole genome re-sequencing have expanded our knowledge to cover a far greater range of species (Meyer and Purugganan, 2013). These studies have

led to a great acceleration in the identification of domestication genes with a couple of dozen being reported a mere 13 years ago to over a hundred at the turn of this decade. As we recently argued the identification of these genes alongside the opportunities afforded by genome editing or introgression breeding offer the change of re-domesticating or *de novo* domesticating new crops that may be better suited to regional climatic conditions and/or agronomical practices (Fernie and Yan, 2019). Specifically, the grand challenges that face future agriculture including enhanced sustainability, tolerance to extremes of climate and the need to bio-fortify our crops could be met by these approaches. The use of CRISPR for *de novo* domestication has been demonstrated in a trio of papers (Lemmon et al., 2018; Li et al., 2018; Zsogon et al., 2018), including one describing work by the Lippman group in which they developed the genomic resources and transformation capabilities for the Solanaceous crop groundcherry (*Physalis pruinosa*) (Lemmon et al., 2018). One of the other papers included increasing vitamin C content as one of the target traits (Zsogon et al., 2018), with fruit quality being something that will undoubtedly be important in the future development of urban tomato farming. By contrast, the prior work of Lemmon and coworkers used CRISPR-Cas9 to mutate orthologous of tomato domestication and improvement genes and focused on conferring improved plant architecture, flower production and fruit size. As such they demonstrated the ability to transfer knowledge from model crops to orphan crops opening up the opportunity of massively expanding our crop species portfolio. We previously listed a vast number of other orphan crops for which this strategy appears to be highly suitable as alternatives to our current staples and which may well prove better suited to current and future agronomical practices and environmental conditions (Fernie and Yan, 2019). It is important to note that whilst an attractive approach the CRISPR technology is by no means the only route by which such changes can be achieved with mutagenesis and classical introgression breeding also being capable of similar such success.

Returning to the issue of urban agriculture a major current obstacle for tomato growth is its indeterminate growth. Previous work had demonstrated that mutating *SELF PRUNING* (*SP*) and its paralog (*SP5G*) results in early yielding compact varieties which are rapidly cycling and productive when grown at high density in both greenhouses and fields. However, even smaller plants would be required to make urban agriculture of the species commercially viable. Genetic

analysis of ethyl methanesulfonate dwarf mutants revealed them to be allelic to the tomato homolog of *ERECTA* (*SIER*), with the short internode phenotype conferred by mutations in *SIER* identified this as an interesting candidate manipulation (Kwon et al., 2019). In order to test this further, all combinations of double and triple *sp*, *sp5g* and *sler* mutants were generated. In greenhouse and field conditions *sp sler* produced more condensed shoots but yielded similarly to *sp*. The *sp sp5g sler* triple mutant was still early flowering and produced the same number of flowers, however, a smaller fruit size led to a slight decrease in yield compared to the *sp, sp5g* double determinant fruit, however, the harvest index of double and triple determinant plants was invariant (Kwon et al., 2019). Trials under restricted space revealed even more promising results that both under field and vertical farming under high density fruit yield is no longer compromised. Intriguingly, targeting the *Physalis pruinosa* ortholog of *ERECTA*, PpER, by CRISPR resulted in a highly dwarves phenotype reminiscent of the triple determinant tomato. As for tomato the fruit size was compromised by this modification, however, as the authors speculate this could perhaps be overcome by targeting regulators of fruit size such as the homolog of the *CLAVATA1* gene. Intriguingly, targeting *SP* function in kiwifruit and cucumber also result in compact growth habit and accelerated flowering respectively (Varkonyi-Gasic et al., 2019; Wen et al., 2019). However, as Kwon *et al.* mention this approach is not likely to be universally applicable for example pepper would more likely benefit from changes in *ER*. Beyond the example of plant stature, the enabling of this approach by next-generation sequencing and the range of species for which genome-editing techniques are applicable renders several other improvements to sustainability tractable. Indeed, as we previously stated (Fernie and Yan, 2019) we would anticipate that higher disease resistance, higher nutrient use efficiency, better climate tolerance and ultimately better nutritional balance and flavor could be achieved both in current and re- or *de novo* domesticated crops. Several of these improvements would also enhance the sustainability of our crops in a manner similarly to that showed in the elegant work of Kwon *et al.* Indeed it is likely that in the future multiplexing of such strategies will allow even greater improvements.

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Fig 1. Schematic diagram of urban tomato creation via CRISPR-Cas technique: the sympodial meristems switch *SP* (SELF-PRUNING) gene mutation change the indeterminate growth to determinate growth (**Upper**); the flowering repressor *SP5G* (SELF-PRUNING 5G) gene mutation promote earlier flowering in long-day (**Medium**), and a comparison of *sp sp5g* double-mutant performed more compact and flowering; the stem length regulator gene, *S/ER* (Sl: tomato is denoted with ‘Sl’ prefix; ER: Arabidopsis *ERECTA*) mutation shorter the internode (**Below**) and making the plant more compact on the background of *sp sp5g*. Taken together, the rapid cycling and compact urban tomato plant was generated by mutating three genes.

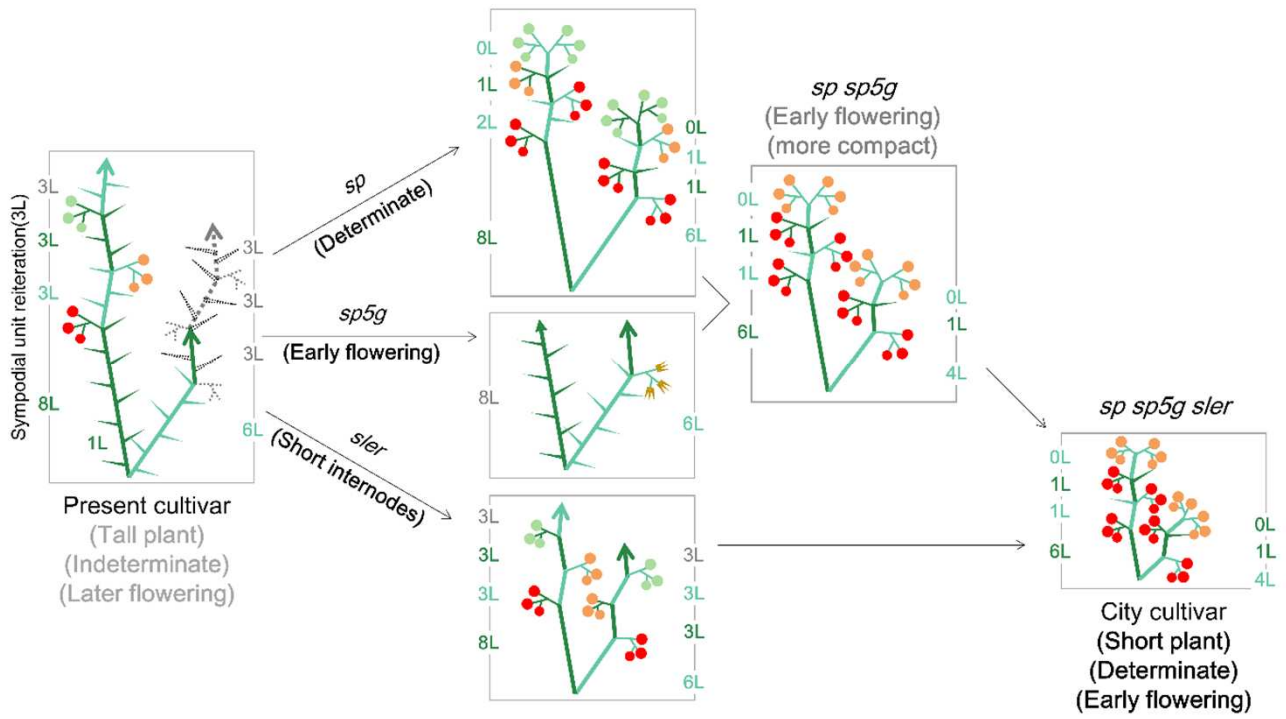


Figure 1