

Brief Communication

A two-in-one breeding strategy boosts rapid utilization of wild species and elite cultivars

Yue Xie^{1,2,3}, Tinghao Zhang^{1,2,3}, Xiaozhen Huang^{1,2} and Cao Xu^{1,2,3,*} ¹State Key Laboratory of Plant Genomics, National Center for Plant Gene Research (Beijing), Institute of Genetics and Developmental Biology, The Innovative Academy of Seed Design, Chinese Academy of Sciences, Beijing, China²CAS-JIC Centre of Excellence for Plant and Microbial Science, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China³University of Chinese Academy of Sciences, Beijing, China

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*Correspondence (Tel +86-10-64806508; fax +86-10-64806503; email caoxu@genetics.ac.cn)

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Global climate change challenges modern agriculture and food security. Intensive selection in crop breeding considerably narrows genetic diversity for climate adaption (Atherton and Rudich, 1986; Lin *et al.*, 2014). For instance, modern cultivars approximately account for only about 5% of the total genetic variability of tomato resources (Atherton and Rudich, 1986). These challenges raise an urgent need to develop novel strategies to exploit wild species, a largely untapped source of desirable stress-resistant traits, to accelerate breeding of climate-smart crops. Genome editing has shown its power as a rapid and precise breeding technique, but it is still challengeable to create complex and polygenic traits underpinned by multiple quantitative loci, such as stress resistance traits (Gao, 2021). In particular, many desirable traits are difficult to create by gene editing due to its inefficient knock-in and knock-up property in plants. Introgression of stress-tolerance traits from wild relatives into elite cultivars via genetic crosses can achieve such success. However, the introgression progress is often slow and labour-consuming due to multiple hurdles presented by genetic barriers, large growth habit variations of wild species and labour cost of manual emasculature in elite cultivars. For example, while tomato seed catalogues are presently dominated by F₁ hybrids, seed production in tomato is expensive and laborious because it requires manual emasculature of the seed parent line one-by-one and also its pollination (Atherton and Rudich, 1986).

Here, we show a 'two-in-one' strategy to accelerate breeding by combining male-sterility production by CRISPR in elite cultivars with *de novo* domestication of wild species. Crossing *de novo* domesticated wild species into male-sterile elites allows faster introgression of stress-tolerance trait and selection of new germplasms because male-sterility reduces labour and time cost for crosses and *de novo* domestication save time from rounds of backcrosses caused by huge differences of growth habits of wild species. New varieties can be produced by backcrosses using desirable F₂ progenies as male and male-sterile elite as female (Figure 1a).

First, we created male-sterility in a classical inbred line, Ailsa Craig (AC), which has been grown over 100 years and had served as a popular part of a traditional English breakfast due to its juicy and exceptional flavour (Atherton and Rudich, 1986). Unfortunately, it is disease-susceptible and sensitive to abiotic stresses. To produce specific male-sterility without penalty of plant growth and yield, we screened the genes specifically involved in early stages of pollen development by analysing the public organ and tissue specific transcriptome data (Liu *et al.*, 2018). We selected the *SILAP3* gene that specifically expressed in microspore, a tomato ortholog of *Arabidopsis* *LESS ADHERENT POLLEN 3* (*LAP3*) encoding a strictosidine synthase essential for pollen exine formation (Figure 1b,c; Dobritsa *et al.*, 2009; Du *et al.*, 2020). We used CRISPR/Cas9 technique to target *SILAP3* and identified two independent null alleles with identical phenotypes (Figure 1d). The *CR-silap3* mutant showed completely male-sterile without setting any fruit and seed at all tested growth conditions, including greenhouse (Figure 1e-h), open field and vertical farm. *silap3* plants produced flowers indistinguishable from AC, including normal morphology of stamen and stigma, but dissection of the stamen showed aborted pollens that result in the male sterility (Figure 1f,g). Notably, *silap3* plants exhibited normal vegetative growth, flowering transition, shoot and inflorescence architecture (Figure 1h,i). We manually pollinated wild-type pollens to *silap3* and found fruit-setting was quite well with higher setting rate than self-pollinated AC plants. The fruit shape was normal with slightly increased yield (Figure 1j,k), indicating the rapid generation of specific sporophytic male-sterility in tomato.

Second, we selected wild species *Solanum pimpinellifolium* (*S. pimp*) as the introgression donor, an ancestor of modern tomato with excellent stress tolerance (Lin *et al.*, 2014). A rapid domestication of major yield traits in *S. pimp*, including day-length sensitivity, shoot architecture, flower and fruit production, can quickly eliminate flowering asynchrony and trait discrepancy of wild parental lines that often require rounds of backcross in conventional breeding. We used our CRISPR/Cas9 multiplex-editing system to simultaneously edit *SELF-PRUNING 5G* (*SP5G*, Solyc05g053850) and *SELF PRUNING* (*SP*, Solyc06g074350) to create day-neutral *S. pimp* plants with synchronized flowering and compact shoot architecture (Li *et al.*, 2018). The Cas9-free T₁ progenies with *SP5G* and *SP* mutations flowered after producing about eight leaves in contrast to 13–15 leaves in *S. pimp*, comparable to AC plants. Simultaneous mutation of *SP* and *SP5G* also converted the indeterminate vine architecture of *S. pimp* into determinate growth with early termination of sympodial cycling,

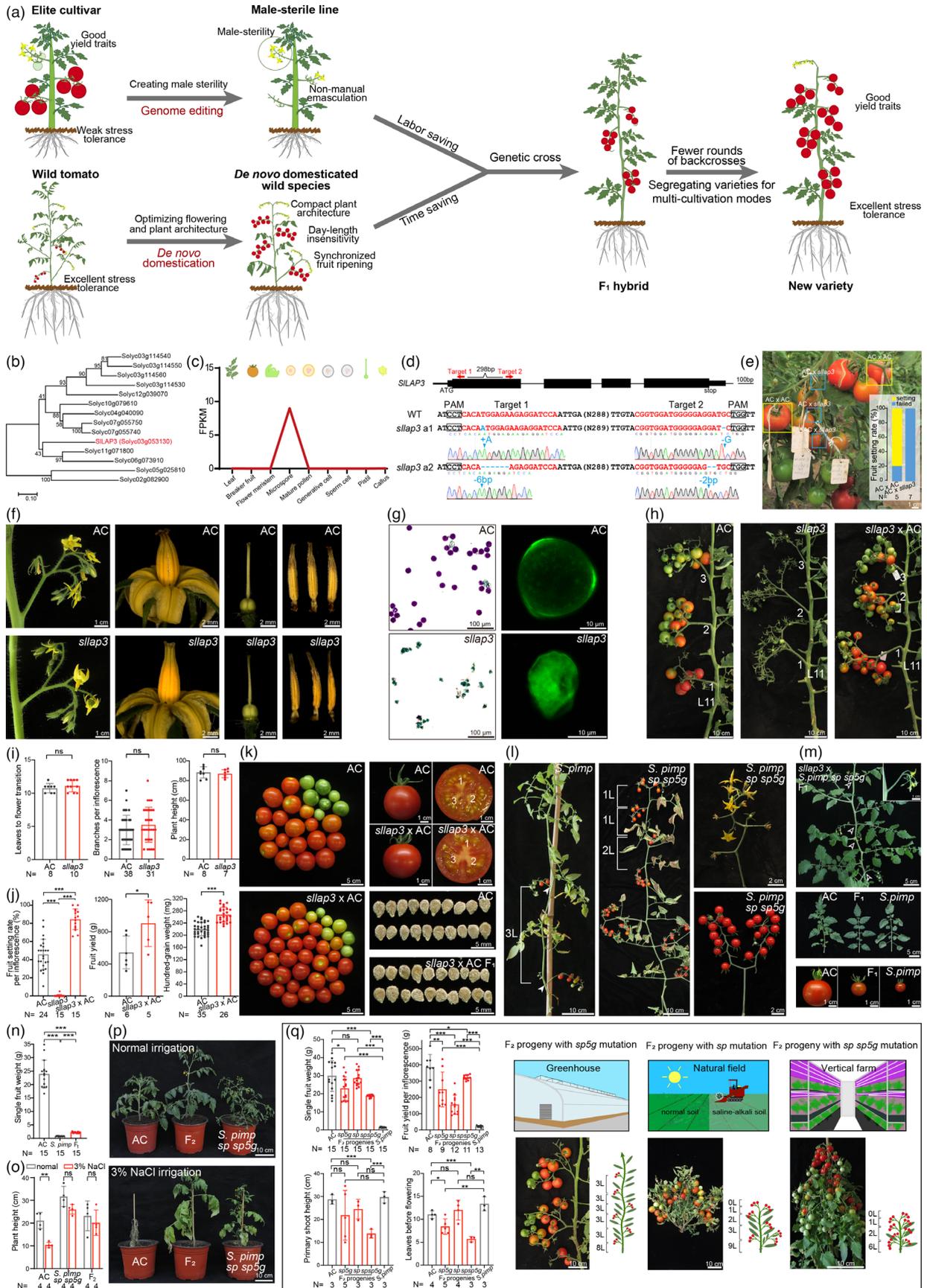


Figure 1 A two-in-one breeding strategy promotes rapid utilization of wild species and elite varieties. (a) Schematics illustrating the breeding strategy. (b) Phylogeny of tomato strictosidine synthase family proteins. (c) Expression atlas of *SILAP3*. (d) The CRISPR construct for *SILAP3* and verified null mutation alleles. (e) Reciprocal crosses between AC and *sllap3* confirming the male sterility. (f) Images showing reproductive organs of AC and *sllap3* plants. (g) Alexander staining (left panel) and confocal imaging (right panel) showing pollen defects of *sllap3* plants. (h–k) Images and quantitative data showing shoot architecture, fruits and seeds from self-pollinated AC, *sllap3* and manual-pollinated *sllap3* by AC pollens. (l) Representative images showing wild and *de novo* domesticated *S. pimp* with *sp sp5g* mutations. (m, n) Phenotypes and statistics of F₁ progeny from cross between *sllap3* and *S. pimp sp sp5g*. (o–p) Phenotypic comparison and quantification between parental plants and F₂ progenies upon salt solution irrigation. (q) Representative plants, statistics and schematics showing F₂ progenies segregated to fit three different cultivation modes. All data are shown in means ± s.d. (two-tailed *t*-test). The *P* values are **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns, non-significant difference.

thus, resulting in compact tomato plants with intensive and almost synchronously ripening fruits (Figure 1l).

Third, we crossed *de novo* domesticated *S. pimp* lines with male-sterile AC plants. The F₁ progenies exhibited typical intermediate phenotypes (Figure 1m,n). We then screened desirable F₂ individuals that carry stress-tolerant traits from *S. pimp* and yield traits from AC. As the *S. pimp* accession we *de novo* domesticated is salt-tolerant, we first examined if this trait had been introgressed. We irrigated F₂ progenies that phenotypically resemble AC plants with 3% NaCl solution, the salt-stress treatment optimized for screening *S. pimp* accessions (Li *et al.*, 2018). After 1-week irrigation, in contrast to the withering to death of AC plants, selected F₂ progenies and *S. pimp sp sp5g* parental plants kept growing and flowering, albeit some bottom leaves showed early senescence (Figure 1o,p), suggesting the stress-tolerant traits had been successfully introduced into AC only after two generations. Strikingly, three types of plant architecture, day-neutral (*sp5g*), determinate (*sp*) and double-determinate (*sp sp5g*) segregated in F₂ population, which fit three currently widely used cultivation modes, including protected cultivation, open fields and vertical farming (Figure 1q).

Together, we devised a ‘two-in-one’ breeding strategy that boosts rapid utilization of wild relatives and elite germplasms for stress-tolerant and multi-scenario cultivation breeding. Advances of CRISPR technology and *de novo* domestication will promote application of ‘two-in-one’ breeding strategy in different major and orphan crops.

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Conflict of interest

The authors declare no competing interest.

Authors' contributions

C. X. designed the research; Y. X, T. Z. and X. H. performed the experiments; C. X. wrote the manuscript. All authors read and approved the final manuscript.

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