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Brief Communication

Gene-editing of the strigolactone receptor *BnD14* confers promising shoot architectural changes in *Brassica napus* (canola)

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Plant architecture, especially in important crop plants, has been under various human selection regimes over agricultural history. In plants, shoot form is determined by the complex interplay of hormones which integrate various environmental cues such as light and nutrient availability to influence growth and architecture (Tarancón *et al*., 2017). Shoot architecture is a highly complex polygenic trait known to play a fundamental role in crop yield. One key example is the shoot architecture in modern maize in comparison to its ancestor teosinte, where a naturally occurring transposon insertion in the *TB1* gene results in increased apical dominance causing a single flowering stalk. Selection for this mutation over time has led to the current maize variety from its high-tillering ancestor (Studer *et al*., 2011).

One group of hormones that is required for suppressing shoot branching and regulating axillary meristem activity are the Strigolactones (SLs), a class of carotenoid-derived terpenoid lactones (Umehara *et al*., 2008). SLs also regulate various developmental processes including internode elongation, leaf shape, secondary stem thickening, as well as root architecture (Waters *et al*., 2017). SL deficient mutants exhibit increased branching phenotypes caused by a combination of enhanced auxin flux and decreased expression of the *TB1*-homolog *BRANCHED1* in axillary buds (Bennett *et al*., 2006; Waters *et al*., 2017). Given that yield increase in canola *(Brassica napus)* is a major industry priority, alteration of SL signalling could lead to a highly branched morphotype similar to the dwarfed plants of the green revolution with favourable shoot architecture for addition of more inputs.

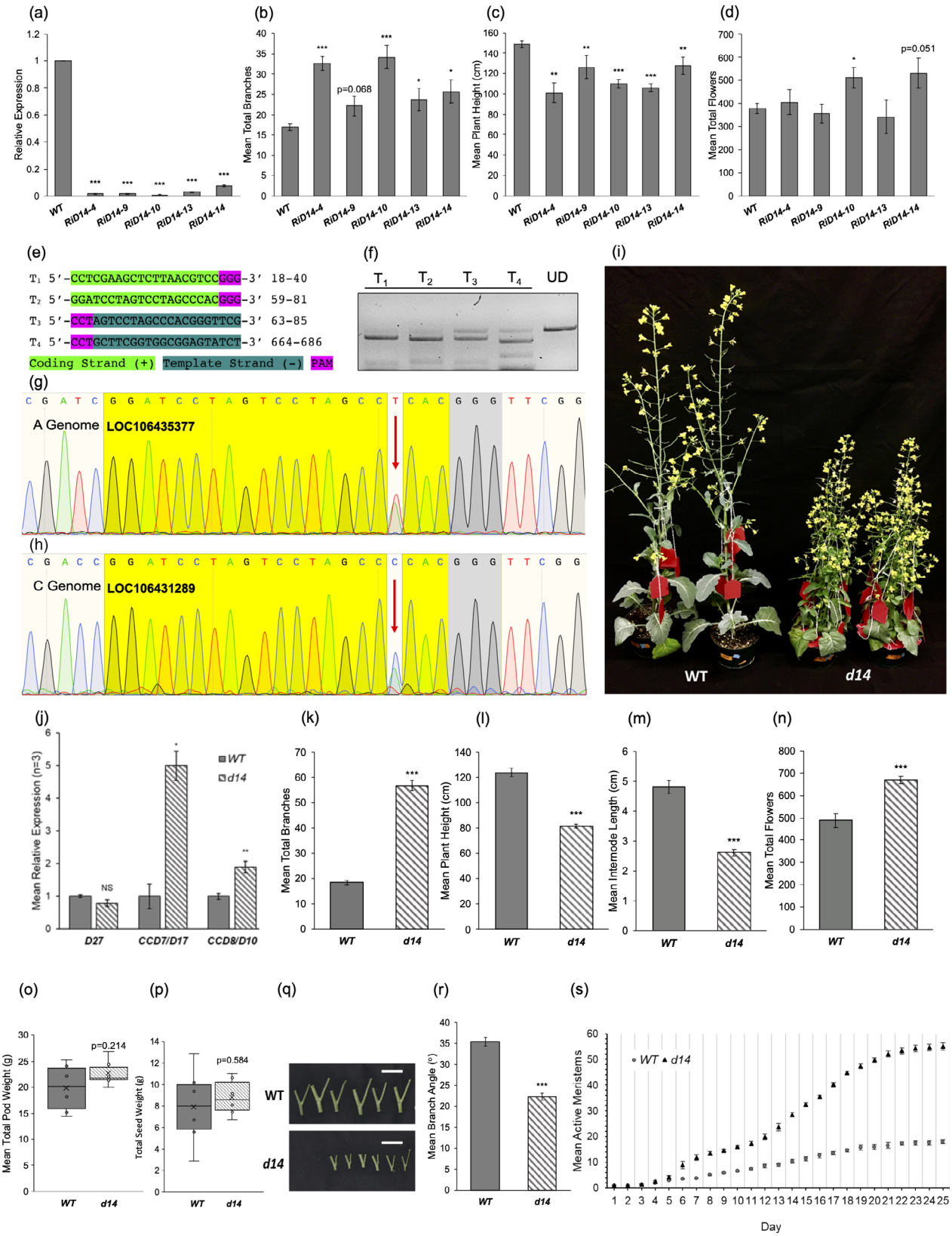
In order to examine if suppression of *D14* receptor could lead to these desired changes, canola (Westar) were transformed with an RNAi suppression construct driven by the *35SCaMV* promoter which targets the SL receptor *BnD14*. We analysed five independent lines (*RiD14*; T1 generation) which showed a drastic reduction in *BnD14* transcript levels (Figure 1a). Most of T_2 generation from these plants exhibited an increased branching phenotype relative to WT plants, (Figure 1b) concomitant with a significant reduction of plant height in all the lines, similar to the classical SL mutants (Figure 1c). Only one line (line 10) exhibited a significant increase in mean total flowers (35%) per plant relative to WT (Figure 1d). The incomplete suppression of *D14* in these RNAi lines may have resulted in the observed partial phenotypes but provided valuable evidence that creating *D14* knockout lines would be a viable option.

To achieve this, we generated CRISPR/Cas9-mediated knock-out lines of the genes encoding the SL receptor *BnD14*. We designed a custom-made multiplex construct with the coding sequence of *S. pyogenes* Cas9 under a *35SCaMV* constitutive promoter, followed by various single guide RNAs (sgRNAs) (Cong *et al*., 2013) under the control of different U3 and U6 promoters to target four conserved regions of the *BnD14* homeologs (A genome:LOC106435377) (C genome: LOC106431289) (Figure 1e). *In vitro* digestion of amplified *BnD14* sequence with recombinant Cas9 enzyme and *in vitro* transcribed sgRNA demonstrated the expected activity of Cas9/target sgRNAs (Figure 1f). We transformed Westar canola lines with this construct and isolated two dwarfed lines in T1 primary transformants. Sequencing of the *D14* genomic DNA from A and C genomes (tetraploid) of these lines, revealed a single biallelic insertion in all four chromosomal copies of *BnD14* (Figure 1g, h).

All T_1 CRISPR/Cas9-edited lines exhibited a similar branched phenotype (Figure 1i) and sequencing showed consistent edits at the specified target sites, and therefore, T2 and T3 seeds from a single line, designated *d14*, were chosen for further downstream analyses. When SL biosynthetic genes were examined in the roots of *d14* plants, characteristic feedback up-regulation of *CCD7* and *CCD8* transcripts were observed in the absence of strigolactone perception (Figure 1j).

The *d14* plants showed a prolific branching phenotype with an approximately 200% increase in mean total branches per plant relative to the WT as well as a dwarfed phenotype with a 34% reduction in mean plant height and reduced internode length (Figure 1k-m). Most importantly, the *d14* plants exhibited a significant increase of 37% total flowers per plant relative to WT plants (Figure 1n). These observations clearly indicate that lack of *BnD14* function leads to promising alterations in yield-relevant traits.

When we compared yield characteristics from mature *d14* and WT plants, a slight, although insignificant, increase of 12.5% ($P = 0.214$) in total pod weight per plant was found in the *d14* line compared to the WT (Figure 1o). Total seed weight per plant showed a slight, however insignificant, increase of 10.4% ($P = 0.584$) in *d14* compared to the WT (Figure 1p). These results show that lesions in SL signalling do not result in detrimental effects on yield in canola.



One key morphology that was altered in the *d14* lines is the branch angles or the gravitropic setpoint angle. Typically, lower branch angles are preferred for their tighter architecture and options for greater planting densities. As observed from previous studies (Liang *et al.*, 2016), *d14* plants showed a significant reduction in mean gravitropic setpoint angle of 37% relative to WT plants (Figure 1q, r). This trait has been associated with

Figure 1 (a) Quantitative RT-PCR of SL receptor gene *BnD14* in leaf tissue from 6-week old T_1 plants. Relative values are normalized to *BnACTIN2* (± SEM, $n = 4$). Comparison of mean number of (b) total branches per plant (c) height (d) total flowers per plant (±SEM, $n = 6$). (e) Selected CRISPR target sequences highlighted in light green (coding strand) or dark green (template strand), PAM sites in pink. CRISPR targets numbered based on proximity to start codon, position provided in nt distance from start codon. (f) *In vitro* digestion of *BnD14* using recombinant Cas9 endonuclease (UD = undigested control). (g, h) DNA sequencing chromatograms of edited *BnD14* A and C genome sequence. Yellow: 20bp Target 2 site, red arrows: insertions in A and C copies, grey: PAM sites. (h) Phenotypic comparison of *WT* (left) and *d14* (right) plants. (j) Quantitative RT-PCR of SL biosynthetic genes (*D27, CCD7/D17* and *CCD8/D10*) in seedling root tissue from 7-day old T_3 plants. Relative values are normalized to *BnACTIN2* (± SEM, $n = 3$). (k-n) Mean quantitative analyses of the T_2 CRISPR-Cas9 *d14* mutant line (± SEM, $n = 6$). Comparison of mean, number of total branches per plant (k), plant height (l), internode length (m). total flowers per plant (n). (o) Total pod weight per WT and *d14* plant. (p) Total seed weight per plant (± SEM, $n = 6$). (q) Visual comparison of *d14* branch angle relative to WT. Bars = 1cm. (r) Comparison of mean branch angle between *d14* and WT plants (±SEM, $n = 6$). (s) Emergence of active meristems over a 25-day period beginning with the onset of flowering. (±SEM, $n \geq 5$). All statistical significance identified using Student's T-test comparing transgenic lines to WT (unpaired test assuming equal variance, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

increased planting density in rice crops (Ferrero-Serrano *et al*., 2019) and may be of value in canola as well.

When meristem activity was compared in the WT and *d14* lines over a 25-day period beginning with the onset of flowering, the *d14* plants exhibited a significant increase in rate of lateral meristem activation over this 25-day period relative to the WT plants (Figure 1s). This indicates that the increase in total flowers per plant was a result of an elevated axillary meristem activity rather than a prolonged flowering period. Given that canola is grown in many temperate regions with short seasons, augmented meristem activity is always a favoured trait over a prolonged flowering period.

Collectively, we have been able to generate a new morphotype of canola that is quite similar to the dwarfed plants of the green revolution which are best suited for increased resource inputs that could significantly boost yield. The dwarf stature and the lack of increased yield characteristics in SL mutants using model systems such as Arabidopsis and petunia (Simons *et al*., 2007) may have deterred the exploitation of SL pathway for promoting yield in crop plants. Our observations with the *d14* deficient canola lines clearly indicate the potential for tweaking the SL pathway for crop improvement strategies in canola. Recently, it has been shown that specific SL partial loss-of-function alleles were also artificially selected for, along with GA mutant alleles, in the generation of elite dwarfed rice varieties during the green revolution (Wang *et al*., 2020). Incorporation of this trait into elite breeding lines could lead to primary producers having access to a new generation of canola lines with a tighter architecture, increased flowering and a lodging-tolerant stature amenable for responding to more inputs. As our population grows exponentially, it is imperative that we enhance the resource use efficiency of our existing crop lands to improve yield.

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Author contributions

M.S., N.H. and R.D. conducted the experiments. M.S., N.H. and M.A.S. designed the experiments and wrote the manuscript.

Conflict of interests

The authors declare no competing financial interests.

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