**CRISPR/Cas9 genome editing of rubber producing dandelion *Taraxacum kok-saghyz* using
Agrobacterium rhizogenes without selection**

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Abbreviations: TK, *Taraxacum kok-saghyz*; 1-FFT, fructan:fructan 1-fructosyltransferase;
ZFNs, zinc finger nucleases; TALENs, transcription activator-like effector nucleases; CRISPR,
clustered regularly interspaced short palindromic repeats; Cas9, CRISPR associated protein 9;
QTLs, quantitative trait loci; PEG, polyethylene glycol; 1/2 MS, half-strength Murashige and
Skoog medium; sgRNA, single guide RNA; CTAB, cetyl trimethyl ammonium bromide; PCR,
polymerase chain reaction; InDels, insertions and deletions; PAM, protospacer adjacent motif.

Abstract

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9) is a highly accessible genome editing tool. Here we demonstrate its potential for use in *Taraxacum* species. *Taraxacum kok-saghyz* (TK, Kazak Dandelion) is notable for its ability to produce high molecular weight rubber in its roots and promise as an alternative source of natural rubber. In order to accelerate the domestication of TK, we have established a simple strategy to deploy CRISPR/Cas9 in this species. A critical gene encoding *fructan:fructan 1-fructosyltransferase (1-FFT)*, implicated in inulin biosynthesis was selected as the target, as inulin is an expected antagonist of rubber production. TK plantlets were inoculated with *Agrobacterium rhizogenes* harboring a plasmid encoding a Cas9 and sgRNA (single guide RNA) targeting TK *1-FFT*. We were able to rapidly induce hairy roots harboring knockout alleles without the selection of stable, herbicide or antibiotic resistant transformants. Mutagenesis was affirmed by observing a loss of restriction sites within *1-FFT*, followed by sequencing. Of 11 hairy root samples tested, 10 showed the presence of genome editing, with mutation rates as high as 88.9%, suggesting a high efficiency mutagenesis induced by CRISPR/Cas9 via *A. rhizogenes*-mediated transformation. Whole TK plants were regenerated from hairy roots harboring knockout alleles. The regenerated plants were confirmed to contain knockout alleles with mutation rates as high as 80.0%. TK plants with edited genomes were obtained within 10 weeks. By omitting a selection step, it was possible to generate edited TK plants lacking stably transformed CRISPR elements, which may potentially reduce off-target mutagenesis. Application of high efficiency CRISPR/Cas9 genome editing will facilitate the domestication and commercialization of TK as a rubber producing crop, and may accelerate basic research on the regulation of rubber biosynthesis.

- 44 *Keywords:* genome editing, CRISPR/Cas9, alternative natural rubber, *Taraxacum kok-saghyz*,
45 hairy roots, *Agrobacterium rhizogenes*

1. Introduction

The targeted modification of native genomic DNA in organisms and cells, termed genome editing, is a reverse genetics tool that allows basic and applied research. Previously developed genome editing approaches, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), rely on protein-DNA interactions to determine specificity (Sander and Joung, 2014). Due to challenges in producing large, custom proteins and limited multiplexing capacity, the application of these approaches in plants has been limited (Bortesi and Fischer, 2015). However, a new technology, termed CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has accelerated this field due to its simplicity and accessibility. Unlike ZFNs and TALENs, CRISPR relies on RNA-DNA complementary base pairing to determine specificity, which allows for quick construct design and simultaneous editing of multiple genes (Gao et al., 2015; Lowder et al., 2015; Xie et al., 2015). CRISPR/Cas9 (CRISPR associated protein 9), which is derived from the type II CRISPR prokaryotic immune system, has been adapted to conduct gene knockouts and regulatory changes, as well as transgene insertions (Jinek et al., 2012; Bortesi and Fischer, 2015). Genome editing through CRISPR/Cas9 provides a platform for research focusing on gene function, regulatory elements and genetic mechanisms underlying quantitative trait loci (QTLs). With the ability to introduce multiple targeted edits into plant genomes, CRISPR can also facilitate metabolic engineering, which usually requires gene manipulation of one or multiple pathways.

CRISPR/Cas9-induced genome editing has been demonstrated in multiple plant species, including model species, major food crops and specialty crops (Li et al., 2013; Gao et al., 2015; Nekrasov et al., 2013; Shan et al., 2013; Jiang et al., 2013; Ron et al., 2014; Wang et al., 2015;

Jia and Wang, 2014). However, the application of CRISPR in industrial crops has not been reported. Kazak dandelion (*Taraxacum kok-saghyz*, TK) is a rubber producing dandelion species being developed as an alternative source of natural rubber and as a model system for rubber biosynthesis studies. Virtually all commercial natural rubber comes from the Brazilian rubber tree (*Hevea brasiliensis* Muell. Arg.), which has a narrow growing range, little genetic diversity and requires manual tapping to extract latex (Mooibroek and Cornish, 2000). TK does not suffer from these shortcomings; however, it is largely undomesticated, competes poorly with weeds and has highly variable rubber production. To accelerate the domestication of TK as a rubber producing crop, CRISPR may provide an avenue for editing the TK genome to increase rubber yield and introduce desirable agronomic traits. Moreover, the application of CRISPR to TK will facilitate the investigation of rubber biosynthesis mechanisms, which are not yet fully understood (Cornish and Xie, 2012).

The introduction of CRISPR/Cas9 elements has been carried out mainly by PEG (polyethylene glycol)-protoplast transfection and *Agrobacterium tumefaciens*-mediated transformation, while particle bombardment has been reported for rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Bortesi and Fischer, 2015). Although *Agrobacterium rhizogenes*-mediated transformation has been widely used in plant transformation, its use in genome editing has been limited. Ron et al. introduced CRISPR/Cas9 into tomato (*Solanum* spp.) hairy roots (Ron et al., 2014). Soybean (*Glycine max*) genome editing was also achieved by CRISPR/Cas9-mediated mutagenesis introduced by *A. rhizogenes* infection (Sun et al., 2015; Jacobs et al., 2015; Cai et al., 2015). However, since both tomato and soybean lack the ability to regenerate plants from hairy roots, other transformation methods were used to obtain whole plants with edited genomes. Recently, a high efficiency *A. rhizogenes*-mediated transformation system was developed for TK,

showing the regenerative capacity of TK hairy roots without the addition of phytohormones (Zhang et al., 2015), which may provide a fast approach to introduce CRISPR/Cas9 elements.

In TK, natural rubber is stored in roots as an end product. However, a greater amount of carbon is typically stored as inulin, which is metabolized to survive winter and rapidly produce shoots after overwintering. It has been shown that cold induction decreases inulin and increases rubber in large TK root systems (Cornish et al., 2013), suggesting antagonism between these major carbon sinks. In this new study, we have demonstrated CRISPR/Cas9 editing, by mutagenizing *fructan:fructan 1-fructosyltransferase (1-FFT)*, one of the most important enzymes involved in inulin biosynthesis (Van Laere and Van Den Ende, 2002). Future characterization of such mutants may demonstrate increased rubber production and a reduction in weedy character (e.g. decreasing winter survivorship).

In the research described here, we demonstrate that CRISPR/Cas9-induced targeted genome editing can rapidly be achieved in TK through *A. rhizogenes*-mediated transformation. Whole plants with edited alleles can be regenerated from hairy roots. This research will expedite research in TK and potentially other dandelion species.

2. Materials and methods

2.1. Plant material

Plants from TK USDA accession KAZ08-017 (W6 35172) were used for CRISPR/Cas9-induced mutagenesis. Sterile plant cultures were obtained according to Zhang et al., (2015). Plantlets used for transformation were obtained by culturing root fragments on solid, half

strength Murashige and Skoog (1/2 MS) medium for three weeks (Murashige and Skoog, 1962; Gamborg et al., 1968; Zhang et al., 2015).

2.2. Selection of target sequence within *I-FFT*

The CRISPR/Cas9 target sequence, as well as the primers used for validation were selected using RNA-seq data from six discrete TK genotypes (Iaffaldano, unpublished). The second exon of *I-FFT* was selected, as it was present in all predicted isoforms, while the first exon was only present in about 90% of predicted *I-FFT* isoforms. Furthermore, the second exon was present in all Blast results against *I-FFT*, while some published *I-FFT* genes lacked the first exon. For these two reasons we elected to pursue mutagenesis of the second exon of *I-FFT*. Potential CRISPR sites within this exon were identified using CRISPRdirect (Naito et al., 2015). Potential sites were then manually entered into NEBcutter V2.0 (Vincze et al., 2003) to identify which target sites would result in the loss of a restriction site. Sequence variations within the second exon of *I-FFT* were identified by loading all *I-FFT* reads into Integrative Genomics Viewer (Robinson et al., 2011; Thorvaldsdóttir et al., 2013). Validation primers as well as the CRISPR site were designed in regions where no variants were flagged among the six TK genotypes sequenced. These primers were then used to affirm the target region of *I-FFT* by Sanger sequencing.

2.3. Construction of expression vectors

A guide RNA (sgRNA) targeting the gene to be knocked out, and a nuclease Cas9, are required for CRISPR/Cas9 mutagenesis: Plasmid pICH86966::AtU6p::sgRNA_PDS which expresses sgRNA was a gift from Sophien Kamoun, (Addgene plasmid # 46966; Nekrasov et al., 2013). The sgRNA was modified to specifically target the second predicted exon of TK *I-FFT* (Supplemental Figure 1) using Q5® Site-Directed Mutagenesis Kit according to the

manufacturer's instructions (New England Biolabs Inc., Ipswich, MA, USA). Primers FFT_Ins
 _forward: 5'-CGCACCAATTGTTTTAGAGCTAGAAATAGCAAG-3' and FFT_Ins _reverse:
 5'-TACGGGTTGTAATCGCTATGTCGACTCTATC-3' were used to amplify plasmid
 pICH86966::AtU6p::sgRNA_PDS to replace the original sgRNA targeting sequence by
 ACAACCCGTACGCACCAAATT (The primers were designed using NEBaseChanger™;
 restriction enzyme *Mlu*CI cutting sites overlapping with Cas9 cutting sites are underlined). The
 sgRNA expression cassette was then amplified using primers sgRNA_cl_forward: 5'-
 ACTAGATCGACGCTGCAAGA-3' and sgRNA_cl_reverse: 5'-
 TTGTACAAGAAAGCTGGGTCT-3' and digested using *Eco*RI and *Xba*I. The Cas9 expression
 vector pFGC-pcoCas9 (a gift from Jen Sheen, Addgene plasmid # 52256) was linearized with the
 same enzymes mentioned above. The sgRNA expression cassette and pFGC-pcoCas9 were
 combined using T₄ ligase (New England Biolabs Inc., Ipswich, MA, USA). The final construct,
 pFGC-pcoCas9_FFT, was introduced into *A. rhizogenes* K599 wild type by electroporation, and
 further used for plant transformation.

2.4. *A. rhizogenese-mediated transformation of TK*

A. rhizogenes cell suspensions were prepared according to Zhang et al., (2015). Plantlets
 at the four leaf stage with roots removed were used as explants for transformation. The wounded
 surface of the plantlets was dipped into cell suspensions of *A. rhizogenes* harboring CRISPR
 elements for 10 seconds. Plantlets were then placed on 1/2 MS medium with 200μM
 acetosyringone. After a 3-day co-culture period, using protocols described previously (Zhang et
 al., 2015), plantlets were transferred to 1/2 MS medium with 400mg L⁻¹ Timentin. Similar plant
 materials were inoculated by *A. rhizogenes* K599 wild type as a control.

2.5. Detection of genome editing

DNA was extracted from hairy roots using the CTAB (cetyl trimethyl ammonium bromide) method previously described (Kabelka et al., 2002). Mutagenesis was detected using extracted DNA by the restriction enzyme site loss method (Parry et al., 1990). Genomic regions (160 bp) flanking the 20 bp targeted sequence within the second exon of *1-FFT* were amplified by FFT_v_forward: 5'-ATTGTTCCACATGGGTTGGT-3' and FFT_v_reverse: 5'-GCCTTCGATGTCGTACCATT-3' (Supplemental Figure 1). To clean the polymerase chain reaction (PCR) products before digestion, 1/10 volume sodium acetate and 2.5 volume pure ethanol were used to precipitate DNA. PCR products were washed with 70% ethanol, air dried, then dissolved in double distilled water. The restriction enzyme *MluCI* was used to detect the loss of the cut site targeted by CRISPR/Cas9. PCR products from wild type plants are expected to be digested completely by *MluCI*, resulting in two gel bands, with sizes of 54bp and 106bp. Since the predicted Cas9 cutting site was designed to overlap with the *MluCI* cutting site, CRISPR/Cas9-mediated mutagenesis is likely to disrupt *MluCI* digestion, resulting in a digestion resistant band. Mutation rates were calculated by dividing the intensity of the digestion resistant band by the intensity of the uncut band using an equal amount of initial PCR product. Band quantification was conducted using KODAK molecular imager software (version 4.5, Carestream Health Inc., Rochester, NY, USA). Digestion resistant PCR products from mutagenized samples were cloned into a plasmid vector using the TOPO® TA Cloning® Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) and analyzed by Sanger sequencing.

2.6. Regeneration of mutagenized plants

Hairy roots confirmed to contain mutagenized alleles were removed and placed on 1/2 MS medium with 400mg L⁻¹ Timentin (Zhang et al., 2015). After 6 to 8 weeks regeneration, plantlets were obtained and DNA was extracted from leaf tissue using the method described in

Section 2.4. Genome edits were detected in regenerated plants using the restriction enzyme site loss method and Sanger sequencing described in Section 2.4. Control plants were regenerated from hairy roots induced by wild type *A. rhizogenes* K599.

3. Results

3.1. *Agrobacterium rhizogenes*-mediated transformation of TK

Hairy roots were produced in three weeks on media with 400 mg L⁻¹ Timentin. Plantlets were inoculated by wild type *A. rhizogenese* or *A. rhizogenese* harboring CRISPR/Cas9 elements. Both treatments resulted in plagiotropic and high density root growth, typical of hairy root transformation (Figure 1A, HR and HR+CRISPR). These phenotypes were not seen in plantlets without *Agrobacterium* inoculation (Figure 1A, WT).

3.2. CRISPR/Cas9 genome editing in TK hairy roots

Among hairy root populations tested, 10 of 11 contained digestion-resistant PCR products of *I-FFT*. Mutagenesis was confirmed by sequencing. Digestion resistant bands of four samples treated with CRISPR/Cas9 are shown in Figure 1B and Supplemental Figure 2A. Mutation rates are listed under gel lanes with digestion resistant bands. Mutation rates ranging from 39.4% to 88.9% were observed. Sequencing of targeted regions in samples exhibiting digestion resistant PCR products revealed introduced InDels (Insertions and deletions) and single nucleotide substitutions. Alignments showing types of mutations introduced within four CRISPR/Cas9 treated root populations are shown in Figure 1C and Supplemental Figure 2B. Single nucleotide insertions and substitutions, as well as deletions up to 22 nucleotides were

observed in sequenced samples. InDels observed in all four samples caused frame shifts and introduced stop codons immediately or shortly after mutations. Since the targeting sequence was specifically chosen at the beginning of the second exon of *I-FFT*, the introduction of pre-mature stop codons are likely to result in highly truncated proteins that are not functional. The frameshift mutations here resulted in the predicted translation of 17 to 39 amino acids of the second exon of *I-FFT*, which has an expected wild type length of 286 amino acids. The amino acid number where a frameshift is expected to occur (when compared to the wild type protein sequence), and the predicted length of the mutagenized amino acid chain, are listed in Figure 1C and Supplemental Figure 2B.

3.3 CRISPR/Cas9 genome editing in regenerated TK plants

Whole plants were regenerated from TK hairy roots in as little as six weeks (Figure 2A). Multiple plants were obtained from each hairy root population. No obvious morphological differences were observed between regenerated plants produced from mutagenized hairy roots and control plants regenerated from hairy root produced by inoculation with wild type *A. rhizogenes*. Restriction enzyme digestion analysis showed that all plants tested in this study exhibited digestion resistant PCR products. The mutation rates obtained in regenerated plants varied from 15.2% to 80.0% (Figure 2B and Supplemental Figure 3A). Among all the mutations, InDels, including single nucleotide insertion and deletions as big as 12 nucleotides, were detected, causing amino acid deletions, frame shifts and the introduction of premature stop codons (Figure 2C and Supplemental Figure 3B). One or more mutagenesis types were revealed by sequencing, indicating that plants were likely regenerated from a group of mutagenized cells (Benson, 2002). However, some mutagenesis events were enriched by regeneration. For example,

only one type of mutagenesis event was detected in regenerated plant 401 (Supplemental Figure 3B).

4. Discussion

This research has demonstrated editing of the TK genome using a CRISPR/Cas9 approach without selection (Figure 3). Novel alleles can be quickly generated using hairy root phenotypes induced by *A. rhizogenes* to enrich for mutagenized tissue, instead of selection with herbicides or antibiotics. PCR detection of integrated Cas9 and sgRNA showed that genome editing could be achieved without stable transformation of Cas9 and sgRNA (data not shown).

The mutation rates obtained in previous reports are highly variable and depend on the methods used for transformation, as well as the plant species. Most of the previous CRISPR studies have been summarized by Bortesi and Fischer, (2015). Therefore, we only compare our results with studies using *A. rhizogenes*-mediated transformation. Mutation rates in soybean hairy roots ranged from 0.6% to 95.0%, with most below 50.0% (Cai et al., 2015). Sun et al. (2015) calculated the mutation efficiencies by dividing the number of genome-edited hairy soybean roots by the total number of hairy roots tested, and reported mutation efficiencies ranging from 3.2% to 20.2%. Jacobs et al. (2015) calculated Indel induction efficiencies, by dividing reads with edited Indels by the total number of reads, and concluded that seven of nine constructs had average Indel frequencies greater than 70% (Jacobs et al., 2015). In this study, we calculated the mutation rate by dividing the intensity of the digestion resistant band by the

intensity of the uncut band using an equal amount of initial PCR product (see Section 2.5). We observed mutation rates of 39.4% to 88.9% in TK, in line with the soybean reports above.

The method described here has the potential to minimize off-target effects, by limiting the exposure of a genome to CRISPR elements. The band pattern in the restriction enzyme site loss analysis showed that most of the mutations are heterogeneous (Figure 1B and 2B, Supplemental Figure 2A and 3A). Mutation rates being less than 100% might be due to two cases: 1) All of the cells were not mutagenized, or 2) one copy (allele) within certain cell lines may have been mutagenized, while one copy may remain wild-type. It is more than likely to be an amalgam of these two scenarios. Both of these phenomena have been observed by the mutagenesis of green florescent protein (GFP) followed by confocal microscopy (Jacobs et al., 2015). The efficiency of mutagenesis might be increased by including a selection step. Furthermore, the efficiency of mutagenesis may be further increased by using newer CRISPR elements with guide RNAs driven by stronger promoters (Xie et al., 2015).

Taking advantage of the strong regenerative ability exhibited by TK hairy roots, whole plants with edited genomes were obtained within a short period of time. Unlike earlier application of *A. rhizogenes*-mediated hairy root induction to only restrictive root-related or model system research, this novel approach allows a large amount of plants harboring different types of mutations to be obtained quickly and used for a wide range of research. This is the first report of *A. rhizogenes* being used to develop knockout alleles in entire plants. The *I-FFT* knockout plants produced in this research were not morphologically discrete from hairy root plants produced by inoculation with wild type *A. rhizogenes*. This may be because a minority of unmutagenized *I-FFT* alleles are able to complement the knockout, or because plants are in a low stress environment where inulin reserves are not required. We have not yet evaluated the

metabolic impact of knocking out *I-FFT*, as measuring the phenotypic impact of our knockouts would require multiple generations, both to develop controlled populations with homozygous knockouts and to remove the *A. rhizogenes* genes underlying hairy root phenotypes. This latter point is particularly important for evaluating metabolic traits, as the hairy root phenotype greatly alters root phenotype and metabolism (Cai et al., 1995; Kim et al., 2002; Srivastava and Srivastava, 2007). Furthermore, hairy root events may vary in what *A. rhizogenes* genes were inserted, the copy number of each and positional effects, which may further modulate expression. As TK is self-incompatible, the generation of homozygous knockouts may require multiple generations or a large pool of mutation events in order to generate double knockouts lacking hairy root elements. To screen easily scorable traits that are not complicated by hairy root phenotypes, additional breeding steps may not be required.

By using simple cloning strategies, such as those described here, genes of interest in *Taraxacum* genomes may be rapidly knocked out and changes in phenotype can be observed. This approach may lead to the accelerated domestication of rubber producing dandelions such as *T. kok-saghyz* and *T. brevicorniculatum*, by minimizing metabolites that compete with rubber for energy and eliminating traits such as seed dispersal. These methods of genome editing in *Taraxacum* may also allow for genes potentially implicated in important traits, such as rubber biosynthesis and apomixis, to be rapidly screened for functionality.

Overall, we have demonstrated a simple, rapid method to deploy CRISPR/Cas9 in *Taraxacum* species. Enriching for mutagenized tissue by subculturing hairy roots permits genome edited plantlets to be rapidly generated. Plantlets regenerated from *Taraxacum* hairy roots have been shown to be reproductively viable (Zhang et al., 2015). This will allow new

alleles to be crossed into controlled populations and *A. rhizogenes* T-DNA insertions (hairy root phenotypes) to be removed.

5. Conclusions

The rapid introduction of CRISPR/Cas9-induced genome editing into TK hairy roots has been achieved using *A. rhizogenes*-mediated transformation. TK whole plants with edited genomes were regenerated from hairy roots within 6 weeks. This simple approach relies on hairy root induction to select for tissue exposed to CRISPR elements instead of using antibiotics or herbicides to select cells stably transformed with CRISPR constructs. This method provides a fast and simple workflow to apply CRISPR to TK and may potentially be expanded to other species with a strong capacity to regenerate from roots.

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Legends for Figures

Figure 1

CRISPR/Cas9 genome editing in *Taraxacum kok-saghyz* hairy roots. (A) Phenotypes of a *Taraxacum kok-saghyz* (TK) wild type plant (WT); hairy root induction of TK transformed by wild type *Agrobacterium rhizogenes* K599 (HR) and *A. rhizogenes* harboring a sgRNA and Cas9 expression cassette (HR+CRISPR). Size bars indicate 1 cm. (B) Detection of *I-FFT* (*fructan:fructan 1-fructosyltransferase*) targeted genome editing by the restriction enzyme site loss method. L, NEB 100bp ladder; C, control plants transformed by wild type *A. rhizogenes* K599; 1 and 2, hairy root populations from two plants transformed using TK *I-FFT* targeted sgRNA and Cas9; -, undigested PCR products; +, PCR products digested by *MluCI*. Mutation rates are listed under gel lanes showing digestion resistant bands. (C) Alignment of reads with CRISPR/Cas9-induced mutagenesis in TK *I-FFT*. The wild type sequence is shown at the top. The targeted sequence is shown in red, while mutations are shown in blue. PAM denotes the

Protospacer Adjacent Motif. The sample names are shown on the left (hairy root population number-mutation type). The sizes of the InDels or substitutions introduced are shown in purple. The amino acid number where a frameshift is expected to occur (FS, when compared to the wild type protein sequence) and the length of the amino acid chain expected to be truncated (AA) by mutagenesis are shown on the right. Mutation types without numbers did not change amino acid sequences.

Figure 2

CRISPR/Cas9 genome editing in regenerated *Taraxacum kok-saghyz* plants. (A) Phenotypes of a one-month old and two-month old *Taraxacum kok-saghyz* (TK) plants regenerated from hairy roots harboring CRISPR/Cas9-mediated mutagenesis. Size bars indicate 1 cm. (B) Detection of *1-FFT* (*fructan:fructan 1-fructosyltransferase*) targeted genome editing by the restriction enzyme site loss method. L, NEB 100bp ladder; C, control plants regenerated from hairy roots induced by wild type *Agrobacterium rhizogenes* K599; 101 and 102, individual regenerated plants from hairy root population 1 in Figure 1; 201 and 202, individual regenerated plants from hairy root population 2 in Figure 1; -, undigested PCR products; +, PCR products digested by *Mlu*CI. Mutation rates are listed under gel lanes showing digestion resistant bands. (C) Alignment of reads with CRISPR/Cas9-induced mutagenesis in TK *1-FFT*. The wild type sequence is shown at the top. The targeted sequence is shown in red, while mutations are shown in blue. PAM denotes the Protospacer Adjacent Motif. The sample names are shown on the left (regenerated plant number-mutation type). The sizes of the InDels or substitutions introduced are shown in purple. The amino acid number where a frameshift is expected to occur (FS, when compared to the wild type protein sequence) and the length of the amino acid chain expected to be truncated (AA) by mutagenesis are shown on the right. Mutation types without numbers did

446 not change amino acid sequences. Gray numbers indicate the position of mutagenized amino
447 acids, where frameshifts do not occur.

448 Figure 3

449 Pipeline of CRISPR/Cas9 genome editing in *Taraxacum kok-saghyz* using *Agrobacterium*
450 *rhizogene*-mediated transformation. Media used for each step are shown in blue. Minimum time
451 needed for each step are shown in red.

Figure 1

Figure 2

Figure 3