

Gene editing to provide traits of value in pasture and forage plants

John CARADUS¹, Marissa ROLDAN², Kim RICHARDSON², Nick ROBERTS², Richard JOHNSON², Christine VOISEY² and Linda JOHNSON²

¹ Grasslanz Technology Ltd, PB 11008, Palmerston North New Zealand

² BSI - AgResearch Group, PB 11008, Palmerston North New Zealand

Corresponding author: john.caradus@grasslanz.com

Abstract

Plant breeding has and will continue to be required to deliver cultivars to assist with overcoming current and future challenges facing pastoral agriculture whether they be economic, environmental or societal. The role of gene editing in achieving success through delivery of crops and forages of benefit is discussed. Research into the use of gene editing of forages, which are important to New Zealand's primary sector, has been a low priority compared with gene editing in row crops and food plants. Records of forage species that have been gene edited, the traits targeted and their likely impact in forages are described. Gene editing of New Zealand's major pasture species and their microbial symbionts is possible and could provide trait improvements of value to pastoral agriculture. However, to effectively deliver gene edited forages and pasture species, costs of development and regulation need to be proportionate to the value of the resulting pasture or forage crop. Concerns about coexistence also need to be effectively managed, benefits need to be quantified, and risks understood to allay societal concerns. The regulatory processes need to be trusted and deliver evidence-based risk assessments and understanding of how genes contribute to complex traits in forage species needs clarification.

Keywords: forage, gene editing, New Breeding Techniques, pasture, traits

Introduction

Modern plant breeding has a short history of about 100 years (Stenseth et al. 2022) but during that time thousands of varieties or cultivars in major food crops and forage plants have been produced leading to improvements in yield and quality of resulting agricultural products (Hallauer 2011). The role of plant breeders continues with new challenges facing primary production systems related to environmental and societal demands (Caradus 2023a). New methods of editing genes with increased precision for managing beneficial plant traits have been used over the past decade (Brummer and Wang 2020) and with the likely changes to New Zealand's gene use regulations

(MBIE 2024) may provide opportunities for delivery of improved plant cultivars of economic value. Gene editing has been identified as an opportunity "for NZ to maintain its current global competitiveness" through the implementation of innovative solutions (Fritsche et al. 2018). That review identified opportunities using genome editing technologies not just for pasture and forage plants but also for woody species, horticulture and vegetable crops.

The understanding that new plant cultivars will be needed to assist with overcoming potential intractable current and future challenges facing pastoral agriculture whether they be economic, environmental or societal, has led to questioning as to whether gene editing may be a way to effectively achieve this. So, is this realistic? The prediction is that the answer to this is 'yes' assuming the regulatory regime is workable. The focus here will be to discuss the potential for using gene editing of pasture and forage plants that are grazed by sheep or cattle to provide solutions to these current and future challenges facing pastoral agriculture.

Understanding genetic modification and gene editing terminology

Genetic modification has been defined as the manipulation of an organism's genes by introducing, eliminating or rearranging specific genes using the methods of molecular biology (USDA 2022). A primary advantage of this process is that it allows the introduction of new traits and variation otherwise not available in the unmodified genome (EU Directive 2001). The advantages of gene editing using CRISPR (Clustered regularly interspaced palindromic repeats), Zinc Finger Nucleases, or TALENs (Transcription activator-like effector nucleases) over genetic modification (Christou 1996) include modification of one or a few target sites with improved precision (Lee et al. 2019), targeted loss of gene function with no foreign DNA introduced (Veillet et al. 2019), and a faster process time (Wolter et al. 2019). Both methods can use *Agrobacterium* and/or biolistics to facilitate gene insertion (Basso et al. 2020), but for gene editing there is more control over the location of the insertion site, and the selectable marker and integrated *Agrobacterium* T-DNA can be

Table 1 Plant traits that could contribute to current and future challenges facing pastoral agriculture.

Plant trait	Target			
	Productivity / production ceiling	Climate change adaptation	Reducing environmental impacts	Biosecurity breaches
Yield	✓	✓	✓	
Disease resistance	✓			✓
Insect pest resistance	✓			✓
Drought tolerance	✓	✓		
Improved nutrient uptake	✓		✓	
Improved winter hardiness	✓			
Salinity tolerance		✓		
Improved digestibility	✓		✓	
Improved metabolizable energy levels	✓		✓	
N-fixation in non-legumes	✓		✓	
Reduced methane and urinary N	✓		✓	
Reproductive efficiency	✓			
Improved animal welfare	✓			
Reduced levels of unwanted metabolites	✓			
Modification of microbes associated with forages	✓	✓	✓	✓

removed through recombination during plant crossing (Petersen et al. 2021). Removing the selectable marker and integrated *Agrobacterium* T-DNA is not unique to gene editing processes and can be also achieved for genetically modified organisms (Breyer et al. 2014). Genetic modification has been extensively tested in forage and pasture species (Caradus 2023b; Bryan et al. 2025), but only two have been commercialised, Roundup Ready® lucerne to aid weed management (Putnam et al. 2013) and HarvExtra® lucerne to provide low lignin feed to improve digestibility (Barros et al. 2019). Reasons for this include lack of investment and small market size, regulatory hurdles, consumer concerns, real or perceived co-existence challenges and life cycle effects due to most pasture plants being perennial and outcrossing.

Challenges facing pastoral agriculture

Current and future challenges facing pastoral agriculture for which there might be a plant breeding solution include – breaking through the current productivity/production ceiling (Mackay et al 2023; Chapman et al 2024), climate change adaptation (Lundquist et al. 2011), reducing environmental impacts, and biosecurity breaches which could threaten New Zealand's bioeconomy (Caradus 2024). The main environmental impacts from agriculture that could be addressed through plant breeding include reducing greenhouse gas emissions (Mfe 2024), and improved nutrient use efficiency and capture which could reduce nutrient loss to waterways (Caradus 2024).

Traits for improved adaptation

A number of plant traits can be identified that through genetic manipulation could improve productivity/production, adaptation to and mitigation of climate change, and biosecurity breaches (Table 1). However, identifying the plant traits of potential value is just one step in the process. The often more intractable action is to identify the specific genes required to enhance or alter the plant traits of interest and identify the expression system required to regulate them appropriately. This would then be followed by understanding the role of gene editing in delivering these traits that will assist in solving these challenges.

Defining gene editing

“DNA serves as a stable information storage medium and every protein which is needed by the cell is produced from this blueprint via an RNA intermediate code” (Witzany 2011). Editing of genes can occur (1) naturally through effects of viruses and viral-derived elements that edit the genome in host organisms (Villarreal 2005), (2) through random mutagenesis using chemical or radiation (and has been successfully used for decades to ‘create’ new traits resulting in over 3000 cultivars being commercialised (Hull et al. 2021)) although very few of these are in pasture or forages plants, and (3) through targeted gene modification using a variety of processes further described below. The simplest and most used form of gene editing in plants reduce or knock out gene function (Subedi et al. 2022). Many assume this is what defines gene editing

is, but gene editing uses machinery that targets specific DNA sequences which can result in not just loss of gene function but also the targeted insertion of new DNA for improved trait expression.

Natural DNA changes that resemble gene editing occur spontaneously in genomes through random mutagenesis. Farmers have been exploiting the natural and non-directed natural variation to improve varieties for over 10,000 years (Songstad et al. 2017). More recently, induced mutagenesis resulting from radiation-based or chemical-based methods have resulted in many plant cultivars of value, but again this is a non-targeted process requiring large numbers of plants to be screened for the desired trait. Similarly, natural sequence variation within genes can be exploited for benefit. Cultivars resulting from these uncontrolled mutagenesis processes are unregulated globally and socially accepted. Targeting Induced Local Lesions in Genomes (TILLING) has been used to create novel mutant alleles for both functional genomics and improvement of crops through combining traditional chemical mutagenesis with high-throughput genome-wide screening for point mutations in desired genes (Chen et al. 2014). A recent example of using TILLING, is the discovery of a mutant for cyclic nucleotide-gated channel (CNGC) 15 that when silenced increased flavonoids that enhance arbuscular mycorrhiza, root nodule symbiosis and resulting nutrient acquisition (Cook et al. 2025). This outcome could also have been achieved using targeted gene editing. Interestingly, the TILLING method is accepted by society and yet produces many off target ‘unknown’ changes (Siddique et al. 2023) while gene editing is directed to one specific often single target site with little/no other genome changes (Doudna and Charpentier 2014).

Targeted gene editing technologies have been developed recently which target the modification of DNA at one or more specific sites within a genome using a variety of methods including Oligonucleotide-Directed Mutagenesis (ODM), site directed zinc finger nucleases (ZFNs) and transcription activator-like (TAL) effector nucleases (TALENs) and then most recently clustered regularly interspaced palindromic repeats (CRISPR) and their associated Cas (CRISPR-associated protein 9) nucleases (Doudna and Charpentier 2014; Hsu et al. 2014; Sauer et al. 2015; RSNZ 2016; Zhang et al. 2018; Kumar et al. 2020). CRISPR/Cas9 has been described as “a straightforward, reliable, and potent tool for targeted gene mutagenesis, knockouts, knock-ins, and transcriptional regulation” (Hussain et al., 2018) and as being easier to use with much higher editing efficiency than other gene editing options (Ma and Liu 2016). Two components are required for successful targeted CRISPR/Cas9 gene editing - a guide RNA (gRNA) that matches a short DNA sequence within the desired target

gene and Cas9, an endonuclease that creates a double-stranded DNA break (Jinek et al., 2012). Cas9-based genome engineering tools are based on components from the microbial antiphage defence system (Hille et al. 2018) and have been publicised as “one of the most powerful and versatile platforms for engineering biology” (Hsu et al. 2014). Successful gene edits have led to improved plant yield, quality, disease resistance and herbicide resistance, breeding and accelerated domestication (Zhu et al. 2020). Currently cultivars resulting from these targeted gene editing methods are regulated to some degree in many jurisdictions (Caradus 2023c).

CRISPR/Cas (Bezie et al. 2021) and other nucleases deliver three site directed nuclease (SDN) outcomes - gene disruption (SDN-1) where the nuclease cuts the DNA and it is imperfectly repaired by natural cellular repair mechanisms; gene correction or modification (SDN-2) where a donor DNA template is added that directs a precise within-gene edit at the cut site, or DNA insertion (SDN-3) where the donor template inserts large DNA inserts at the cut site, commonly whole genes (Doudna and Charpentier 2014; FAO 2022). In addition to the commonly used CRISPR/Cas9 system new emerging CRISPR/Cas systems include spCas9-NG, Cas6, Cas9, Cpf1, Cas 12, Cas13, Cas14 and base and prime editing which use modified enzymes to make specific base changes in DNA without a double-stranded DNA break (Koonin et al. 2017; Hille et al. 2018; Manghwar et al. 2019).

It should be noted that the intellectual property landscape related to genome editing technologies such as CRISPR/Cas can be complex with U.S. Patent and Trademark Office having granted hundreds of patents pertaining to the use of CRISPR in some form (Sherkow 2018).

Can gene editing deliver traits for improved adaptation of forage and pasture plants?

Research into gene editing of forages has been a low priority compared with gene editing in higher value and more extensive row crops and food plants (Bao et al. 2022; Fu et al. 2024). In 2016, there was no published literature reporting targeted genome editing in any forage species (Badenhorst et al. 2016). In 2019, reviews listed only model plant species such as *Nicotiana benthamiana* and *Arabidopsis thaliana*, along with crops such as rice (*Oryza sativa*), maize (*Zea mays*) and cotton (*Gossypium hirsutum*), and vegetables such as potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*) (Eş et al. 2019). At that time important applications of gene editing were to improve plant virus and diseases resistances, improve tolerance to drought and herbicide, enhance crop production and nutritive value, and manipulate bioactive compounds,

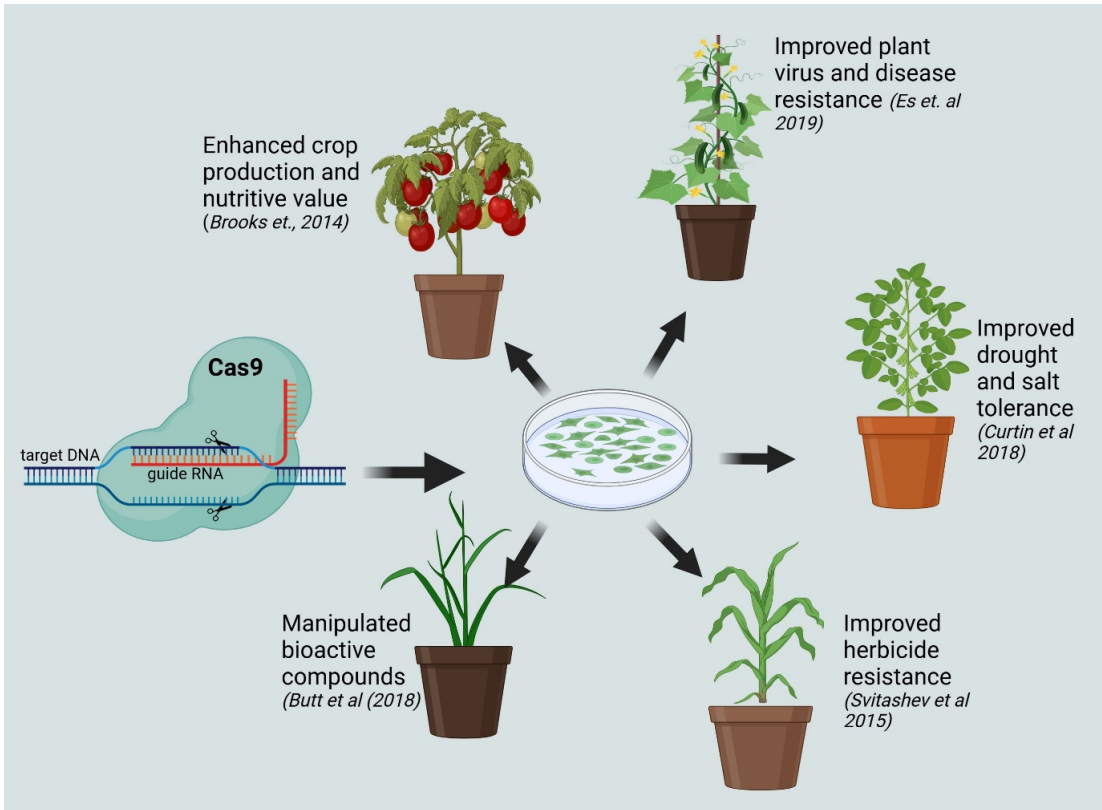


Figure 1 Some applications of gene-editing to enhance important plant traits. Created in BioRender. Roldan, M. (2025) <https://BioRender.com/i28k140>

such as alkaloids, hormone and flavonoids (Figure 1). For example, gene deactivation or knockout was used early in the application of CRISPR/Cas9 to deliver high amylopectin starch content in maize (DuPont Pioneer, Johnston Iowa, described in Waltz (2018)), drought and salt tolerance in soybean (Curtin et al. 2018; Waltz 2018; Zhong et al. 2022), delayed flowering in *Setaria viridis* (Walz 2018; Yang et al. 2021), and increased omega-3 oil content of *Camelina sativa* (Ozseyhan et al., 2018y, Waltz 2018). Improved resistance to Northern Leaf Blight in maize resulted from an NLB18-sensitive allele replaced with resistant allele via homology directed repair (HDR) mechanism. (Dinkar et al. 2024). Even in 2023 it was noted that while “editing strategy was immediately applied in model and crop plants, almost ten years ago not much has been achieved in the forage grasses landscape” (Sustek-Sanchez et al. 2023).

A recent re-assessment of the literature indicates that gene editing using CRISPR/Cas in grasses and legumes has been achieved in the forage legumes *Medicago truncatula*, lucerne, *Lotus japonicus*, and red clover, and grasses *Brachypodium distachyon*, tall fescue (*Festuca arundinacea*), *Panicum virgatum*, perennial

ryegrass (*Lolium perenne*) and *Leymus chinensis* (Liu et al. 2018; Khoshhal Sarmast 2019; Hus et al. 2020; Bilal et al. 2025). Some model grass or legume species were used because of the ease of transformation. This includes for legumes *Medicago truncatula*, and *Lotus japonicus*, and for grasses *Brachypodium distachyon*. CRISPR/Cas9 has also been successfully applied to *Epichloë* endophytes of perennial ryegrass, which are filamentous fungi to manipulate a secondary metabolite pathway to reduce animal toxicity while retaining insect resistance (Miller et al. 2022b). So, gene editing technology has only recently been applied to forages. Records of forage species that have been gene edited, the traits targeted and their likely impact in forages are summarised in Table 2. In addition to those listed in Table 2, a highly efficient system for production of bi-allelic mutants of perennial ryegrass (Kumar et al. 2022) and tall fescue (*Festuca arundinacea*) (Zhang et al. 2021) that should also be applicable in other related grass species using CRISPR/Cas9 has been documented. Similarly for legumes CRISPR/Cas9 based transgene-free genome editing protocol have been developed (Chen et al. 2020; Wolabu et al. 2020;

Table 2 Opportunities for developing traits of value using gene editing directly in pasture species or in crops as an exemplar.

Trait	Species	Phenotype	Type of gene edit	Gene	Reference	
Yield and shoot morphology	Lucerne	No distinct phenotype, used as proof of concept to demonstrate CRISPR/Cas in lucerne	Knockout of gene function – SDN-1	<i>MsSPL9</i> (Squamosa Promoter binding protein-like 9)	Gao et al. 2018;	
	Lucerne	Hormone control resulting in dwarf plants; multiple lateral branches, a high leaf/stem ratio, and high crude protein	Knockout of gene function – SDN-1	<i>MsGA3ox1</i> (Gibberellic Acid 3-oxidase)	Zheng et al. 2022	
	Lucerne	Under nitrogen deficiency, both mutant lines E26a and E26b displayed stunted growth, yellow/chlorotic leaves and aberrant nodules	Knockout of gene function – SDN-1	<i>MsNOD26a</i> and <i>MsNOD26b</i>	Frare et al. 2022	
	<i>Brachypodium distachyon</i>	Variation in tillering, leaf patterning, inflorescence, and flower development	Knockout of gene function – SDN-1	<i>NBCL</i> (Nootbop-Co-ch-Like) paralogs <i>BdUniculme4 (CUL4)</i> and <i>BdLaxatum-A (LAXA)</i>	Liu et al. 2021	
	<i>Panicum virigatum</i> (Switchgrass)	Produced significantly more tillers and higher fresh weight biomass than the wild-type plants	Knockout of gene function – SDN-1	Teosinte branched 1 (<i>tb1</i>)/Branched 1 (<i>BRC1</i>)	Liu et al. 2020	
	<i>Leymus chinensis</i>	Significantly increased tiller number and biomass	Knockout of gene function – SDN-1	Teosinte Branched1 (<i>TB1</i>)	Lin et al. 2023	
<i>Hordeum vulgare</i>	<i>Hordeum vulgare</i>	Increase in plant height, tiller number, grain protein content, and yield	Knockout of gene function – SDN-1	Abnormal cytokinin response1 repressor 1 (<i>HvARE1</i>) gene	Karunaratne et al. 2022	
	<i>Hordeum vulgare</i>	Increased coleoptile length and level of seed dormancy	Knockout of gene function – SDN-1	Gibberellin (GA) 3-oxidase1 (<i>GA3ox1</i>)	Cheng et al. 2023	
	Root traits	<i>Hordeum vulgare</i>	Greater root length, increased surface area, and greater numbers of root hairs,	Knockout of gene function – SDN-1	<i>HvCKX1</i> and <i>HvCKX3</i> genes <i>CKX</i> genes encode the cytokinin oxidase/dehydrogenase enzyme	Gasparis et al. 2019
	Disease resistance	Wheat	Powdery mildew resistance - (<i>Blumeria graminis f. sp. tritici</i>)	Gene insertion -SDN-3	<i>TaMLO</i>	Wang et al. 2014
		Wheat	Powdery mildew resistance (<i>Blumeria graminis f. sp. tritici</i>)	Knockout of gene function – SDN-1	Mildew Resistance Locus <i>TaEDR1</i> Enhanced disease resistance1 (<i>EDR1</i>)	Zhang et al. 2017
Rice		Bacterial blight resistance <i>Xanthomonas oryzae pv. oryzae</i>	Knockout of gene function – SDN-1	<i>OsSWEET13</i> sucrose transporter gene	Zhou et al. 2015	
Insect pest resistance	Rice	Tryptamine converted to serotonin resulting in reduced growth in plant hoppers and stem borers	Knockout of gene function – SDN-1	<i>CYP71A1</i> gene encoding tryptamine 5-hydroxylase	Lu et al. 2018	
Drought and heat tolerance	Lucerne	Improved ability to withstand water-deficit; reduced leaf size, early flowering, decreases in internode length, plant height, shoot and root biomass, and root length.	Small insertions/deletions (indels) no template used – SDN-1	<i>MsSPL8</i>	Singer et al. 2022	
	Maize	Drought stress tolerance, increased grain yield	Novel allelic variation through insertion of a native maize promoter GOS2 – SDN-3	<i>ARGO58</i>	Shi et al. 2017	
	<i>Brachypodium distachyon</i>	Earlier flowering and hypersensitivity to water stress	Knockout of gene function – SDN-1	Regulator of Flowering and Stress (<i>RFS</i>)	Ying et al. 2022	
Tall fescue	Decline in thermotolerance	Knockout of gene function – SDN-1	Fa-heat shock protein 17.8 Class II (<i>FaHSP17.8-CII</i>)	Bi et al. 2021		
Improved nutrient uptake	Lucerne	Hyper-accumulate phosphate	Knockout of gene function – SDN-1	Phosphate2 (<i>PHO2-B</i> and <i>PHO2-C</i>)	Miller et al. 2022a	
Improved winter hardiness/cold tolerance	Rice	Enhanced chilling tolerance at the seedling stage	Knockout of gene function – SDN-1	<i>OsVPE2</i> Plant vacuolar processing enzymes (<i>VPEs</i>)	Deng et al. 2024	
	Rice	Significantly enhanced tolerance to the low temperature	Knockout of gene function – SDN-1	<i>OsNAC050</i>	Wang et al. 2023	
Salinity tolerance	Rice	Enhanced tolerance to salinity	Gene knockout -SDN-1	<i>OsRR22</i>	Zhang et al. 2019; Sheng et al. 2023	

Improved digestibility	Switchgrass (<i>Panicum virgatum</i>)	Lignin reduction and improved sugar (glucose/xylose) production	Knockout of gene function – SDN-1	4-Coumarate: coenzyme A ligase (<i>Pv4CL1</i>).	Park et al. 2017
	Lucerne	Reduced lignin content, altered lignin composition, and improved nutritional value	Knockout of gene function – SDN1	Coumarate 3-Hydroxylase (<i>MsC3H</i>)	Wolabu et al. 2024
	Lucerne	Leaves after detachment retained greenish appearance	Gene knockout of 4 alleles – SDN-1	<i>Medicago sativa</i> stay-green (<i>MsSGR</i>) gene	Wolabu et al. 2020
Improved metabolizable energy levels	<i>Camelina sativa</i>	Improved seed oil composition; with significant decrease in less desirable PUFA	Knockout of gene function – SDN-1	Fatty Acid Desaturase (<i>FAD2</i>)	Jiang et al. 2017
N-fixation in legumes and non-legumes	<i>Lotus japonicus</i>	Overproduced superoxide radicals and hydrogen peroxide. Disruption of Lb genes resulted in early nodule senescence	Knockout of gene function – SDN-1	Leghaemoglobin genes (<i>Lbs</i>)	Wang et al. 2016, 2019
	<i>Lotus japonicus</i>	Over-expressing LjCZF1 exhibited increased numbers of infection threads and nodules	Knockout of gene function – SDN-1	Cytokinin receptor <i>LHK1</i> (<i>Lotus</i> Histidine Kinase 1) and <i>LjCZF1</i> encoding a zinc finger (C3HC4-type RING finger)	Cai et al. 2018
	<i>Lotus japonicus</i>	Knockout mutants show significantly decreased nodule numbers	Knockout of gene function – SDN-1	<i>MIR2111-5</i>	Okuma et al. 2020
	<i>Lotus japonicus</i>	No abnormal symbiotic phenotype compared with the wild-type plants, suggesting that Lj β CA1 or LjaCA1/2 are not essential for the nitrogen fixation	Knockout of gene function – SDN-1	<i>LjβCA1</i> or both <i>LjaCA2</i> and its homolog, <i>LjaCA1</i>	Wang et al. 2021
	<i>Lotus japonicus</i>	Significant reduction in infection thread formation in root hairs leading to nodule formation	Knockout of gene function – SDN-1	Cyclops response element (<i>CYC-RE</i>) which binds to Nodule Inception (<i>NIM</i>) transcription factor	Akamatsu et al. 2022
	<i>Medicago truncatula</i>	Control of lateral root number and nodulation	Knockout of gene function – SDN-1	<i>MtCEP1, 2, and 12 - CEP</i> (C-terminally Encoded Peptide) gene family	Zhu et al. 2021
	<i>Medicago truncatula</i>	Aberrant nodules, chlorosis and impaired grow under nitrogen-limiting conditions	Knockout of gene function – SDN-1	<i>Medtr8g087710, Medtr8g087720 -</i>	Frare et al. 2022
	<i>Medicago truncatula</i>	Differing combinations of NPD gene inactivation had progressively smaller nodules, earlier onset of nodule senescence, or ineffective nodules	Knockout of gene function – SDN-1	Nodule-specific PLAT domain genes (<i>NPDs</i>)	Trujillo et al. 2019
	<i>Medicago truncatula</i>	Knockouts to elicit the role of certain NCRs in symbiotic nitrogen fixation	Knockout of gene function – SDN-1	Nodule specific cysteine-rich (<i>NCR</i>)	Güngör et al. 2023
Potential to reduced methane and urinary N	<i>Camelina sativa</i>	Increased seed oil content and fatty acid compositional changes in oilseed species	Knockout of gene function – SDN-1	<i>FAD2</i>	Jiang et al. 2017; Subedi et al. 2020
Reproductive efficiency	Foxtail millet (<i>Setaria italica</i>)	Reduced seed shattering	Rendered nonfunctional by a retrotransposon insertion but no template used – SDN-1	Less Shattering1 (<i>SvLes1</i>)	Mamidi et al. 2020
	Perennial ryegrass and Italian ryegrass (<i>Lolium multiflorum</i>)	Male sterility	Knockout of gene function – SDN-1	<i>DMC1</i> (Disrupted Meiotic cDNA1)	Zhang et al. 2020
	Lucerne	Male sterility	Knockout of gene function – SDN-1	<i>MtNP1</i> – (No Pollen 1)	Ye et al. 2022
	Lucerne	Delayed flowering time had normal inflorescences during the flowering phase but no visible pollen grains.; but also, higher forage biomass	Knockout of gene function – SDN-1	Flowering locus Ta1 (<i>MsFTa1</i>)	Wolabu et al. 2023
Improved animal welfare	<i>Epichloë</i> endophyte in perennial ryegrass	Manipulation of secondary metabolite expression with aim of reducing ryegrass staggers	Knockout of gene function – SDN1	<i>idtM, idtD, idtO, idtA, idtF</i> and <i>idtA/F</i> (indole diterpene pathway genes)	Miller et al. 2022b
Reduced levels of unwanted metabolites	Red clover (<i>Trifolium pratense</i>)	Reduced levels of isoflavones - formononetin, biochanin A and genistein	Knockout of gene function – SDN-1	Isoflavone synthase (<i>IFS1</i>)	Dinkins et al. 2021

Bottero et al. 2021). Gene editing using CRISPR/Cas9 has also been demonstrated in apomictic, tetraploid grasses (*Paspalum notatum*) (May et al. 2023). Several of the SDN-1 gene knockouts described in Table 2 appear to have been undertaken to demonstrate gene function rather than to deliver an improved phenotype. Some gene edits, in this case knockouts, have been of genes that are highly conserved among monocots, are highly expressed, and involved in various biological processes, such as MicroRNA528 (miR528) in *Leymus chinensis* (Li et al. 2023). This gene has a role in flowering time, pollen development, lodging resistance, antiviral defence, and abiotic stress responses.

While gene editing is being heralded as “a groundbreaking technology with many advantages, including ease of manipulation, high efficiency, and wide applications that can improve the yields, nutrition value, disease resistance, and other properties of forage crops” there is still the challenge of efficient delivery methods allowing precise gene knock-in/replacement in target plants (Ul Haq et al. 2022). There is no doubt that as the effort to use gene editing in pasture and forage crops increases these challenges will be overcome as they have in the more researched horticulture, vegetable and cereal crops (Zhang et al. 2018).

Discussion and implications

Gene editing of New Zealand’s major pasture species is possible and has been demonstrated. Reflecting on the plant trait improvements that would be of value to pastoral agriculture (Table 1), many appear amenable to gene editing (Table 2). However, the traits of importance for many forage species are controlled by many/several genes across many/several loci. The effects of each gene and loci can be additive such that editing a single gene is only likely to be as influential as the contribution of that gene at those loci and the loci within the trait. The examples given in Table 2 are the ‘low hanging fruit’ which represent editing of single gene controlling Mendelian traits. However, a significant upside of gene editing in forage species is to improve understanding of how (and which) genes affect the expression of complex traits.

The summary provided here is reliant on very preliminary studies, with gene editing of forage and pasture species only being used effectively in the last 7 years and with the main focus being on higher value row crops and horticulture. The potential use in pasture and forage species is relatively untapped. Despite the low level of investment to date into this area of research, gene editing of forages holds considerable promise for achieving significant improvements in forage and their microbial symbionts, such as grass endophytes, used in pastoral agriculture.

To effectively deliver gene edited forages and pasture species, consideration of the following is required:

1. Costs of development and regulation need to be proportionate to the value of the resulting pasture or forage crop.
2. Concerns about co-existence of gene edited and non-gene edited plants of the same species need to be effectively managed to prevent cross-contamination.
3. Benefits need to be quantified, and risks understood to allay societal concerns.
4. Regulatory processes need to be trusted and deliver evidence-based risk assessments.
5. Poor understanding of how single genes contribute to complex traits in forage species and the subsequent impact of knocking them out. It is possible that single gene changes may not realise the gains observed in some row crops when undertaken in pasture and forages plants.
6. With many pasture and forage species being outcrossing the time periods for breeding and development of cultivars with homozygous genes of interest while still maintaining heterozygosity can be long and take many years.

Despite these considerations, gene editing has the potential to provide a means to an end in solving some of the productivity and environmental challenges facing managed grasslands systems. As to whether New Zealand will embrace this opportunity is reliant on legislative changes proposed in the new Gene Technology Bill (New Zealand Parliament 2024) which is currently being debated and resulting regulations may provide increased freedom to operate with technologies delivered using some gene edited processes.

Disclosure statement

JRC is employed by Grasslanz Technology Ltd which has an R&D investment portfolio that includes both genetic modification and gene editing of forages and microbes to provide mitigating solutions to current environmental and animal welfare issues facing both New Zealand and other pastoral economies.

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