Adapting forages to climate changes and lower environmental footprint

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Abstract

New Zealand (NZ) agriculture is likely to enter the NZ greenhouse emissions budget and see additional costs and restrictions. However, farmers do not have options to adapt to new environmental requirements with NZ crops and pastures currently bred and managed with reliable water, fertilisers, and pesticides. This requires breeding and testing across a large sample of genetic resources and strategic climatic conditions. The Margot Forde Forage Germplasm Centre has thousands of forage accessions ready to be utilised for pre-breeding efforts under environmental stresses. No genetic tool or breeding method can be successful if germplasm choice is poor. Once germplasm is identified for desired environmental traits, breeding techniques for genetically complex traits (e.g. drought and heat tolerance, nitrogen use efficiency) can be applied to develop cultivars with desirable attributes. The use of alternative recurrent selection methods from other species may now be possible in forage breeding programmes, purging deleterious alleles from populations faster than traditionally achieved. The exploitation of heterosis with reciprocal recurrent selection programmes can provide the next generation of successful forage semi-hybrid cultivars. New developments in agronomy should complement breeding research to best utilise environmental interactions with new genetics. This discussion paper encourages forage breeding programmes, forage value indices, and government funded science to tackle critical needs to develop forage cultivars with enhanced performance under low input systems. This will ultimately provide an option currently unavailable to farmers to adapt to climate changes and reduce the environmental impacts on their farms.

Additional keywords: genetic resources, germplasm, environmental traits, maize, pre-breeding, recurrent selection, ryegrass, seed production

Background, Lessons from the Past, Visions for the Future

Exactly what New Zealand’s (NZ) climate will look like in 20 or 30 years is difficult to predict. The Ministry for the Environment (2018) produced a detailed report on expected regional climatic changes over the next century. Numerous changes are expected including higher temperatures, potential transpiration deficit, drought and flooding. Estimates of future precipitation and river flows vary between seasons and locations (Collins *et al.*, 2018), decreases in summer precipitation and river flows will likely result in an increase in irrigation demand and reduction in availability. It is only a matter of time before challenges and opportunities to adapt crops and forages to climate changes will be embraced. By 2040 national mean temperature is expected to increase by 0.7 to 1.0°C, the number of days exceeding 25°C is expected to increase by between 40 and 100% and precipitation change predictions are variable and differ by season and region (Ministry for the Environment, 2018). Higher temperatures through dryer, hotter parts of the year will result in a higher Potential Evapotranspiration Deficit (PED). The Ministry for the Environment (2018) predicts a 60 to 80 mm increase in average PED for most of the country, with Taranaki and the South Island west and south coasts seeing less or no increase, and the North Island east coast seeing a greater increase. The increase in PED roughly equates to an increase in water demand, manifested either as an increase in irrigation use or as water stress in plants in areas where growth is or becomes water limited. At the same time, the Ministry for the Environment (2018) predicts an increase in the frequency and severity of droughts, meaning more frequent water limited seasons in areas outside of Taranaki and the west and south coasts of the South Island.

Statistics New Zealand (2019c) found a 94% increase in irrigated agricultural land between 2002 and 2017, bringing the total to 747,000 ha or 3% of NZ land area. Canterbury accounts for the majority of land using irrigation at 64%, with Otago the second largest at 13%. Dairy production accounts for the majority of irrigated land uses at 59%. Brown (2016) found a major shift toward more efficient irrigation systems between 2002 and 2015, estimating 55-60% of the irrigated area had an application efficiency of at least 80%. Irrigation schemes from ground and surface water have been developed over the years in regions such as Canterbury. Water storage has become more common, taking water from rivers while the river flow is high and storing it in dams for dry periods. An increase in demand will put further pressure on dams, aquifers and infrastructure, not only for storage volume but also for the capacity of equipment such as pump stations and bores. Infrastructure solutions such as water storage dams may help mitigate the effects of droughts, but if irrigation is unavailable or unable to replace the increase in soil moisture deficit production may decline. Developing new drought and heat tolerant cultivars is a solution to adaptation to climate changes such as increasing soil moisture deficits as are agronomic practices specifically improved to combat these changes.

The Ministry for the Environment (2019) claims agriculture is responsible for 48% of the total NZ greenhouse gas emissions and the current interim NZ Climate Change Committee is considering the inclusion of agriculture in the emission budgets. The usage of Nitrogen (N) fertiliser in NZ increased by 627% between 1990 and 2015 (Statistics New Zealand, 2019b). Ministry for the Environment and Ministry for Primary Industries (2018) state the amount of nitrogen leached from agriculture increased by 29 per cent between 1990 and 2012. Statistics New Zealand (2019a) also states nitrate-nitrogen leached from livestock increased from 189,000 tonnes nationwide in 1990 to 199,000 tonnes in 2017, a 6% increase.

There is a strong need in NZ for applied research that will address future demands of crops and forages with tolerance to climate changes (Carena, 2011) and to low inputs (Carena, 2017). Breeding for adaptation to climate changes and under low input environments is a challenge not yet addressed (Hallauer *et al.*, 2010).

Sustainable breeding is a viable scientific alternative to maintain enough food supply under the environmental challenges facing our planet (Carena, 2017). It aims for efficient and wise use of resources, genetic diversity, maximisation of conservation, and minimisation of waste.

Facing future climate changes and environmental regulations

Lowering fertiliser and water demands for farmers has not been a high priority for public and private scientists but would be desirable, increasing sustainability and lowering production risks and costs. Current and future climatic and environmental challenges can be solved with the addition of useful germplasm diversity (Carena, 2011; Carena, 2013a) and the utilisation of target stressful environments with low water and N inputs for faster genetic improvement (Carena, 2013b; Carena, 2013c), integrating germplasm adaptation and improvement with cultivar development (Carena *et al.*, 2010; Hallauer *et al.*, 2010).

Breeding and agronomic inventions to facilitate the collection of data on genetically complex traits that are difficult to measure and largely influenced by the environment without plot destruction can be cost-efficient. Sharma and Carena (2016a) developed BRACE, a non-destructive high throughput breeding methodology for short-season drought tolerance involving visible brace root systems in maize. Phenomic tools targeted at genetically complex traits such as BRACE may be adaptable to other forage species. Yang *et al.* (2010) provided AUDDC, a high throughput methodology to develop fast drying maize cultivars without the need to destroy plot samples for laboratory measurements. This method, through a $200 wood moisture meter, has identified inbred lines and hybrids in industry and public breeding programs significantly reducing the use of fossil fuels to dry grain at farmer elevators. Millions of dollars have already been saved in industry and on farms with these cost-effective 'phenomics' tools. Similarly automated tools for assessing dry matter yield in ryegrass are being developed and validated for use in breeding programs (Ghamkhar *et al.*, 2018). For a general discussion on field based phenomics in plant genetics research see White *et al.* (2012).

Genetically broad-based populations can be incorporated and improved under short-season environments through intra- and inter-population recurrent selection programs (Carena & Hallauer, 2001a, b; Laude & Carena, 2014) as sources of not only new inbred lines and hybrid cultivars but also new improved populations and population hybrids. As a cross-pollinated crop, maize (*Zea mays* L.) breeding techniques are readily applicable to forage species like perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). However, utilisation of these techniques has not typically been the case. Historical events might help understand this.

Integrating pre-breeding efforts with cultivar development: A case study from maize

Maize is an economically important crop for feed, fibre, fuel and food. The return on investment for research and development in maize genetic improvement just in the public sector has been impressive even before biotechnology was available (3 billion vs. 260 billion U.S. dollars) (Hallauer *et al.*, 2010). Genetic gains for maize yield have been over 2.0% per year when compared with dry matter ryegrass yield improvement at 0.25-0.73% per year (McDonagh *et al.*, 2016), or white clover at just 0.16% per decade (Hoyos-Villegas *et al.*, 2019). In short-season environments, about of 75% of the maize yield improvement has been attributed to genetic gain and the rest to improved agronomic practices (Tollenaar & Lee, 2006).

After domestication, maize was widely grown by Native Americans in the United States (USA). As cultivation of maize became more extensive, selected open-pollinated strains were developed in a similar way as ryegrass and clover cultivars are being developed today. By the end of the 1800s, U.S. farmers had 800 to 1,000 unique landrace options to grow (Sturtevant, 1899), some of them showing high yields when properly cultivated (Leaming, 1883). Maize was introduced in NZ in the late 1800's (Rhodes & Eagles, 1984) and became an important part of Maori agriculture with open-pollinated varieties (Hardacre *et al.*, 1991).

Native Americans were the first 'corn breeders' of open-pollinated varieties in the 1600s in the USA (Carena, 2017). In the early 1900s, public scientists created the inbred-hybrid concept for maize (East, 1908; Shull, 1908). The private sector exploited this research idea even though it was considered impractical and public research on inbreeding and hybridisation was abandoned. Inbred lines became the gold mine of confidential secrets for hybrid providers who led a new competitive market worth billions of dollars. However, the emphasis on the inbred-hybrid system relegated population genetic improvement efforts to just a few public maize breeding programs integrating pre-breeding with cultivar development.

Pre-breeding includes the introduction, adaptation, evaluation, and improvement of germplasm resources for use in breeding programs (Hallauer & Carena, 2009). It includes the mid- to long-term systematic goals needed to increase the genetic diversity of cultivars that is often ignored. The EarlyGEM (Carena *et al.*, 2009; Sharma & Carena, 2016b), EarlyQPM and EarlyQPMF programs (Dong *et al.*, 2012), stratified mass selection (Carena *et al.*, 2008; Hallauer & Carena, 2009), EarlyTROP (Carena, 2020), and recurrent selection programs (Carena & Wicks III, 2006; Hallauer & Carena, 2014), are some of the pre-breeding efforts that have integrated the creation and improvement of genetically broad-based synthetic varieties with the development of genetically diverse inbred lines and hybrids in USA short seasons. The key factors in developing useful and unique cultivars are the adaptation of exotic germplasm (Carena, 2017), and the maximisation of the rate of genetic improvement (Carena *et al.*, 1998; Hyrkas & Carena, 2005; Hallauer & Carena, 2013). In addition, genetic divergence and reciprocal recurrent selection programs have been the source of the population-hybrid maize concept (Carena, 2005), the same way semi-hybrids operate in pastures with the advantage of exploiting heterosis. Maize populations and population hybrids have been sold commercially to farmers as alternative low-cost seed, generating royalties and fees to the public sector. Even though the odds of developing cultivars from recurrent selection programs is small it only requires one to make an impact (Hallauer & Carena, 2009). For example, B73 was developed from a public recurrent selection program on Iowa Stiff Stalk Synthetic variety, which has been used either directly in proprietary hybrids or modified by pedigree selection to develop improved strains, making billions of dollars for farmers and industry before modern intellectual property restrictions. Public maize breeding programmes developing inbred lines have continued to generate royalties and fees with exclusive and non-exclusive licenses with Foundation Seed Companies. However, intellectual property restrictions have often prevented farmers to grow the best possible hybrid combinations (Carena, 2013a).

The importance of genetic diversity

Current NZ forage and crop cultivars represent a narrow sample of their total species genetic diversity. Within the most important NZ forage species, perennial ryegrass cultivars have mainly been bred from two locally adapted ecotype sources, supplemented more recently by germplasm introductions from Northwest Spain (Stewart, 2006). Ryegrass has an environmentally diverse natural range, from Scandinavia to the Mediterranean (Blackmore *et al.*, 2015). The Margot Forde Forage Germplasm Centre (MFFGC) has genetic resources from locations spanning this range with the desirable traits suited to different climates. Molecular markers could be used to confirm diversity at the DNA level (Pembleton *et al.*, 2018), but ultimately identification of accessions based on collection location environment and evaluation for the desired traits under NZ stressful environments is more desirable to link DNA level diversity with genetically complex traits such as drought and heat tolerance. Interspecific hybrid species such as festuloliums are a further potential source of genetic diversity.

New genetic materials are needed to prepare NZ farms to be profitable and sustainable under climate changes. A systematic applied approach to improve crops resilience to climate changes is possible and has been successful in maize (Hallauer *et al.*, 2010). Tropical germplasm carrying unique genes have been successfully integrated with short season varieties (Carena, 2020). The EarlyGEM maize program (Carena, 2002), is a public-private pre-breeding initiative to incorporate unique alleles to commercial cultivars that could be useful to pastures in NZ (Figure 1). EarlyGEM was a programme designed to increase the genetic diversity of northern US hybrids through the incorporation of exotic useful germplasm and development of early maturing high quality inbred lines for utilization in the northern U.S. Corn Belt (Carena, 2011). It was developed in the USA and has successfully been replicated in China to increase the genetic diversity of maize commercial cultivars (Zhang *pers. comm.*, 2019).

The narrow genetic diversity currently present in crop and forage species makes them vulnerable to pest and disease epidemics on farms with potential negative economic consequence to NZ production and exports. The development of a new generation of forage and crop cultivars with resilient to climate changes will require exploitation of unique genes residing in germplasm collections (such as the MFFGC), integrating long-term pre-breeding efforts with a new approach for genetically broad-based cultivar development.

Maize production in NZ has not yet met domestic requirements and has the potential to do so considering the availability of short-season germplasm and the potential to breed under NZ conditions. Results have shown that germplasm carrying unique genes can break environmental margins for maize production (Carena, 2011). Breeding maize under NZ drought (northern Otago) and cold (Southland) environments has proven successful not only for the NZ dairy industry but also for U.S. short-season maize breeding efforts for new cultivar releases carrying drought and cold tolerance (Carena *et al.*, 2009; Carena, 2017). Side by side irrigated and dryland trials under target environments have identified more stable cultivars. Similar approaches are underway to improve drought tolerance in wheat without sacrificing yield in optimal years (Lewis & Christiansen, 1981; Christopher *et al.*, 2015) and could be adapted to other important forage and crop species.

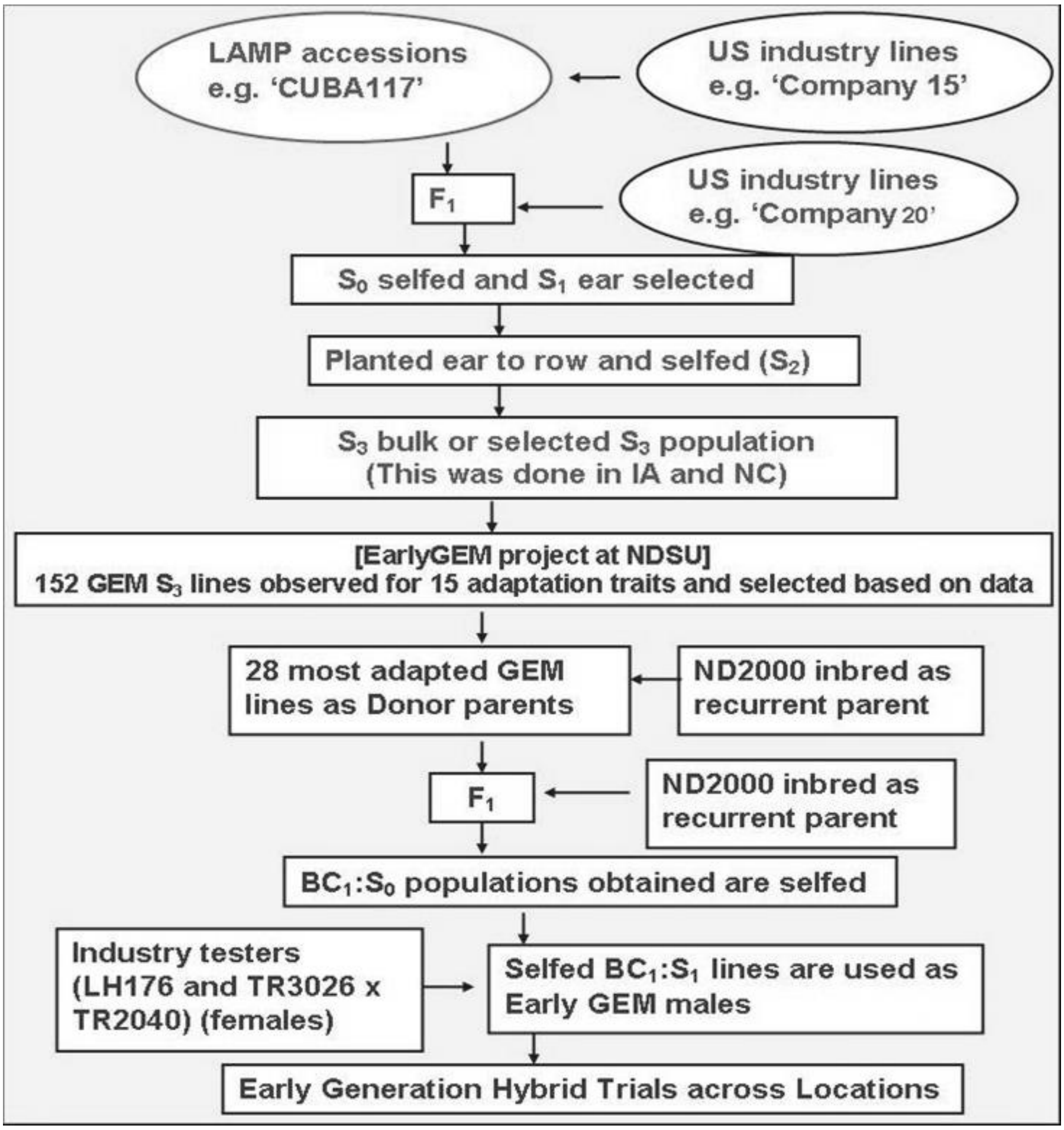

Figure 1: Integrating EarlyGEM pre-breeding efforts to increase genetic diversity with cultivar development for adaptation to climate changes (adapted from Carena & Sharma, 2016). Abbreviations are as follows: LAMP = Latin American Maize Project; GEM = Germplasm Enhancement Maize; IA = Iowa; NDSU = North Dakota State University; NC= North Carolina; F_x = Filial cross generation x; S_x = self of generation x; BC_x = backcross of generation x.

Alternative breeding systems for pastures

Cross-pollinated pasture breeding techniques have been limited to intra-population mass selection efforts for individuals and half-sib family progenies, with limited use of full-sib families. Purging deleterious alleles can most effectively be achieved when employing one to several generations of self-pollination. Utilising just one or two self-pollinations for developing S_1-S_2 progenies followed by multi-location evaluation of progenies and recombination of top progenies cyclically has been a successful breeding methodology for intra-population recurrent selection efforts in maize with fast genetic gains (Hallauer & Carena, 2014). The presence of albino plants in commercial ryegrass cultivars indicates masking of undesirable alleles is common in commercial populations. Moreover, challenges arise for self-incompatible species such as ryegrass and white clover. Some options to break self-incompatibility in ryegrass have been investigated. Wilkins and Thorogood (1992) found breakdown of self-incompatibility above 34°C during pollen shed. Yamada (2001) proposed the use of interspecific hybrids with *Lolium temulentum* and backcrossing to reduce self-incompatibility in ryegrass.

Anther cultures for the creation of double haploids has been extensively used in maize for developing inbred lines in one generation and they have been used in ryegrass previously (Olesen *et al.*, 1988; Madsen *et al.*, 1993). More recently that work has been expanded (Begheyn *et al.*, 2016; Begheyn, 2017) although not deployed in a commercial scale. Additionally Kindiger (2016) identified a tall fescue mutant which when crossed with annual ryegrass (*Lolium multiflorum* L) spontaneously created haploid annual ryegrass or tall fescue genotypes. Both studies observed that ryegrass tends to spontaneously create double haploids when single haploids are produced artificially.

Herridge *et al.* (2019) provided an in-depth review on the prospects of an F1 hybrid ryegrass, suggesting the use of CRISPR to create male sterile and maintainer lines or environment-sensitive genic male sterile lines. CRISPR is a modern gene editing technology which can be used to inject foreign DNA into a genome, the insertion could be a sequence from within the same species, or from an entirely different species. However, current regulations will not allow this in NZ, and in the future even if de-regulated, may be unfavourable to higher value markets. The usefulness of F1 hybrid commercial production is highly dependent on cost and specific combining ability and heterosis (hybrid vigour) levels for pair crosses. Instead, investing in reciprocal recurrent selection programmes (Figure 2) utilising half-sib or full-sib progenies is an excellent option to improve both populations and semi-hybrids in pastures without the need for self-pollination. Reciprocal recurrent selection programmes improve both general and specific combining ability simultaneously, once pair crosses with the highest heterosis are identified through mating designs (e.g. diallels) (Comstock *et al.*, 1949; Hallauer & Eberhart, 1970).

Forage production challenges

Temperature dependence is species specific, white clover has a wide optimal window, between 16 and 30°C (Brock *et al.*, 1989) while ryegrass has a much narrower window, 18 to 21°C. Canterbury produces most of the ryegrass seed in NZ and is already near the upper end of the temperature window for growth. A widening of the window to the upper end is needed to ensure resilience under an increase in temperature. Thus, breeding efforts with increased genetic diversity for higher heat tolerance are needed (Hampton *et al.*, 2016).

Increased restrictions and regulations associated with the application of N, either through emissions trading schemes or through N leaching restrictions or charges be mitigated with farmer access to new low N use cultivars supplemented with best agronomic practices. Decreasing the need for supplementary N will reduce application costs for farmers under current conditions and reduce environmental impacts. Breeding under low N conditions will assist agronomists in their extension trials with various N levels to provide farmers the information (and cultivars) they need to reduce supplementary N use without reducing production.

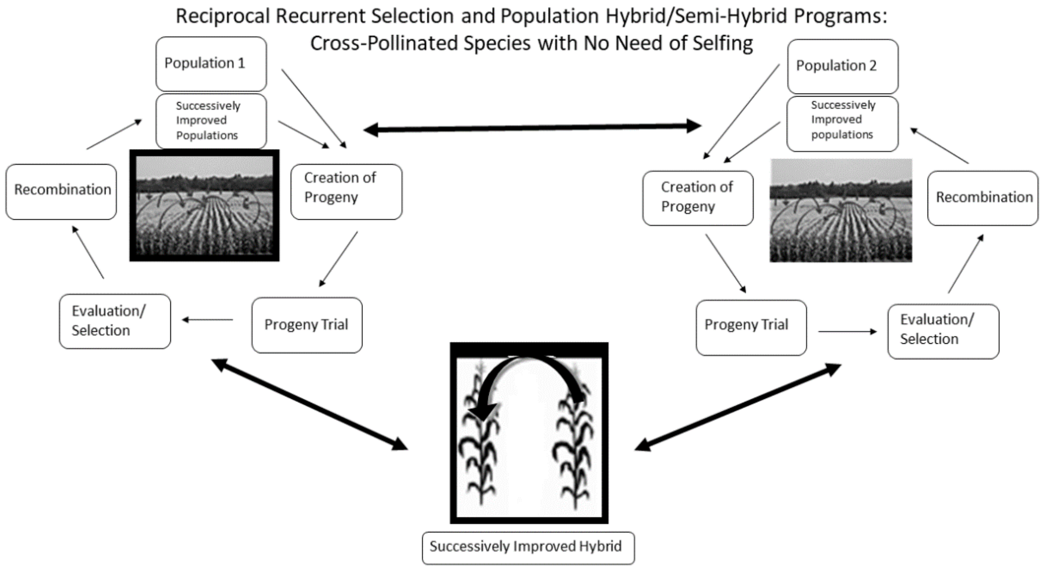

Figure 2: Reciprocal recurrent selection to maximise genetic improvement of populations and their crosses for population hybrid and semi-hybrid programmes. Mating designs can initially identify population hybrids with largest high-parent heterosis.

Drought tolerance is becoming increasingly important for forage species around the world. Norton *et al.* (2016) suggested increasing the depth and density of ryegrass rooting systems and breaking the trade-off between summer dormancy and yield through both breeding and agronomic practices. Cattivelli *et al.* (2008) suggested that high yielding genotypes are not stable over environments, often displaying high levels of sensitivity to water stress. Ultimately this dependence, if possible, needs to be broken with enhanced utilisation of genetic diversity along with screening and evaluation under strategic environments to create cultivars which perform well in less predictable environments. What metrics are needed to identify, measure and break any relationship between yield and water stress sensitivity in New Zealand's forage species requires further investigation. There is high variability in ryegrass drought tolerance traits from differing geographical areas (Jonavičienė *et al.*, 2014), indicating breeding for drought tolerance by selecting accessions or crosses between accessions and elite cultivars from known drought prone areas should be feasible. It is important that breeding is undertaken in conjunction with advances in agronomic practices.

Agronomic research needs to work with the genetic changes to investigate the best way to exploit or enact them.

Practical implications for NZ farmers

The agribusiness sector needs to provide farmers with options and solutions for them to be able to comply with environmental regulations and targets. Seed company breeding nurseries have been developing pastures and crops assuming water and nitrogen availability continues as is the status quo and government funded science has not tackled the critical need to develop forage cultivars with enhanced performance under low input systems associated with less environmental impact. Multiple solutions can be addressed initially such as transitioning breeding to environmental conditions where stress can increase genotypic differences. Stakeholders could develop better selection indices for developing adequate cultivars for our farmers. Commercial cultivars are currently genetically vulnerable to epidemics that can cost millions of dollars to NZ farms, especially under climate changes. Prevention efforts increasing genetic diversity are essential to avoid major national economic losses.

In 2018, the AgResearch Ltd. Forage Genetics team developed a proposal for a national pre-breeding programme for adaptation of forages to climate changes and lower environmental footprint (FENZ or Forage Enhancement NZ). This programme was based on the EarlyGEM maize program to increase the genetic diversity of U.S. short-season hybrids (Figure 1) (Carena, 2002). This is a public and private initiative followed by over 30 stakeholders that can serve as an example to pasture species breeding in NZ. The outcomes of the EarlyGEM programme were not only novel synthetic varieties but also novel inbred lines for genetically diverse hybrids. In forages, the overall goal of the FENZ programme was to develop novel germplasm for commercial use by industry and farmers to increase sustainable production and profitability of NZ farms under climate changes. This could influence a transformational change in the current Forage Value Index (FVI), an economic measure used by farmers and industry to decide which ryegrass-based pastures to use. Better measures to identify top cultivars are available. A heritability index including environmental traits would be desirable to minimise rank changes caused by the current economic index. Coordinated initiatives like this one should broaden the germplasm base to NZ breeding programmes and track utilisation of unique germplasm from the MFFGC. This could be a long-term program to increase the genetic diversity of commercial varieties with the incorporation of elite exotic germplasm. Genetically diverse products will successfully improve the sustainability and stability of NZ forage production and profitability. Once developed significant genetic gains can be achieved through several intra- and inter-population recurrent selection programs, like the earlyGEM programme, focusing on genetically complex traits to more rapidly develop the next generation of NZ’s sustainable commercially important forage species adapted to future climates.

A solid foundation needs to be built for the development of more robust future forage cultivars. Genetic diversity studies need to be complemented with the identification of accessions carrying genetically complex traits expressing heat and drought resistance and high nitrogen use efficiently. The extensive multi-location evaluation of accessions and/or accession x cultivar crosses under strategic stressful environments will provide needed data for this purpose. These genetic resources will be the sources of the next generation of sustainable cultivars needed by farmers.

Intra- and inter-population recurrent selection programmes on closed but genetically broad-based populations will systematically increase the frequency of favourable genes while maintaining genetic diversity with adequate recombination protocols between selection cycles. The utilisation of these alternative breeding methodologies will increase pasture genetic gains. If self-pollination and heterosis are included genetic gains may be higher and faster but population specific as genetic effects are population specific.

Further investigation on the effect of temperature on seed production and vigour in NZ forage species as well as identifying phenotypic markers for selection of nitrogen use efficacy and drought tolerance will be beneficial. These are the resources needed for breeders and agronomists to jointly develop the next generation of NZ forage cultivars to be more robust to future climates on farms, reducing seasonal risk and increasing profitability.

Well selected genetically diverse products will successfully improve the sustainability and stability of NZ forage and crop production profitability. Increasing biodiversity on farms will address long-term sustainability and influence a transformational change in the way farmers and industry decide what cultivars to use, reducing inputs and increasing profitability.

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