Pasture biotechnology — not as you know it

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ABSTRACT

The breeding of improved varieties of agriculturally important species requires constant effort and innovation. The ways we in New Zealand use emerging technologies to maintain our low-cost, low-input, sustainable, pastoral agricultural systems are extremely important. When it comes to developing elite varieties and strains of ryegrass we have been, until very recently, disrupting much to achieve little: we rely on chance to find the right combinations of traits in our breeding lineages, whether animal germplasm or plants cultivars. The use of Cisgenics®, where heritable material is moved only within a species by either traditional or modern biotechnological means offers a better way for ryegrass breeding in line with a key principle of science and economics.

Keywords: Cisgenics; plant biotechnology; protoplast fusion; breeding; marker assisted breeding.

There is a well-known principle that we should never use more when we can make do with less. This 'principle of economy' asks us to find the way forward in the most prudent way, causing the least disruption to achieve a goal. While it is true that you can't make an omelette without breaking eggs, it is always valid to ask whether we can do as well or even better with less breakage. This is not philosophy: it is relevant to the breeding of improved varieties of agriculturally important species such as ryegrass and also important in the ways we in New Zealand will use emerging technologies to maintain our low-cost, low-input, sustainable, pastoral agricultural systems. When it comes to developing elite varieties, strains and cultivars of ryegrass we have been, until very recently, disrupting much to achieve little. To make even small changes to, for example, the breeding worth of dairy cattle germplasm or to the flowering date of a perennial pasture grass cultivar, we import traits or enhancements from distant relatives of the organism in which we are interested. This is a considerable undertaking and many eggs are broken.

Each breeding lineage is a well-organized and competent assemblage of genes and proteins working together to make cells and organs. Genes not only contribute to the prosperity of ryegrass but also provide a context (or an 'environment') for all the other genes present; as elsewhere, a good team is one that works well alongside one another to achieve the goal (Dawkins, 1989). To import a trait like late flowering into a pasture grass cultivar we typically take at least two lineages, one of established agricultural worth ('elite line') and others bearing the importable trait but otherwise poor ('non-elite line') and we scramble them together quite randomly. These lineages may have been separated by geography or by thousands of years spent adapting to different conditions, each team of genes working until now to create a successful organism that is a specialist for its environment. The elite gene team may also be the result of decades, even millenia in the case of maize (Eyre-Walker *et al*., 1998), of focussed human effort to breed for agricultural value. In traditional biotechnology, namely plant breeding, we force these gene teams together. The resulting unruly crowd, after a round of firings, downsizings and redundancies, must then perform the task of creating a successful organism. It is our hope that we will chance upon the right combination, in which a new, mixed gene team works together sufficiently well that we have something like our agronomically useful elite line but with some substitutes that bring the non-elite delayed flowering trait to the mix. We must make many attempts because in this case fortune favours only the persistent. If we find a successful mix, we call it a new cultivar or improved germplasm, we thank our lucky stars, we hope that the new team will continue to behave under all other conditions, and we christen it *Tolosa* or *Brown Swiss* or *Aries HD* or *HyGreen*. Could we do better? Could we do this in a way that uses the principle of prudence? Can we make this omelette without breaking quite so many eggs? We already do.

The above is an almost ridiculously simplified description of the work of a professional breeder. There is science and artistry in breeding and the skills in working out the details of a breeding scheme—and in recognizing a good result when it comes to hand—are not to be underestimated. To transport a trait or behaviour like drought tolerance via improved root architecture from a non-elite to an elite line requires much more than the process outlined above, and there is much more to breeding that making a cross and picking a name. For example, plant breeders make extensive use of backcrossing, where the progeny containing unruly crowds of genes are rebuilt to be as much like the agronomically successful gene team as possible via repeated interbreeding with the elite parent line. The result is a new cultivar at least as good as the old in all respects, and better in one new way. Ideally, *only* those genes that are necessary to confer the imported trait will remain as intruders into the well-organized and

competent assemblage that made up the elite cultivar. In practice, this endpoint is never achieved: it would take many decades of focussed breeding effort to eliminate all traces of the non-elite parent DNA with little certainty of success. In many cases it would be impossible to detect the difference between elite and non-elite DNA and so know when the job is complete. The lack of precision in the breeding method forces us to break many eggs in this process, but our omelette is at least a little better for it.

We can use technology to assist the elimination of non-contributory non-elite DNA. Molecular breeding can help breeders identify which DNA is from which parent. Using markers as known points of difference between the elite and non-elite gene teams, a breeder with access to the right tools can confirm the elimination of unwanted non-elite DNA and retain any required for the imported trait. This can both speed up and optimize the backcrossing process to produce an agronomically adequate variety more quickly but there is little incentive even with these tools to eliminate all non-elite DNA. Because time and money are scarce, compromises are sought where attrition of elite traits is minimized (and also offset by the advantage of the newly imported trait). To get better omelette today we must tolerate some imperfections.

Another traditional biotechnology, protoplast fusion, can be used to introduce new traits in traditional crops. Nearly a century old, this technology is re-gaining popularity in the climate of public concern about transgenic crop improvements. In our metaphor, it is akin to selecting from many duck and hen eggs to get a better combination of tastes and textures. Protoplasts are plant cells in which the strong, protective cell wall has been removed. These relatively naked cells can be fused into one cell where the genomes from both parent cells recombine, shuffling the gene teams. The effect is similar to breeding but greater gains are possible because more gene variety can be brought to the mix from more distant relatives. Undomesticated varieties of many crops (including ryegrass) that have not been subjected to human directed breeding pressures possess heritable capabilities, such as tolerance to environmental extremes, that have been lost in elite lines; in almost all cases these are the 'non-elite' lines used in breeding. In cases where the differences between lines have grown large, straightforward breeding may be impossible or very inefficient; protoplast fusion is one way of crossing this divide, bringing the teams together and searching for those new combinations that perform well or better.

Contemporary commercial uses of protoplast fusion in plant breeding are seen in tobacco (Zheng *et al.*, 2003), potato (Austin *et al.*, 1986), several brassicas (e.g. Hu *et al.*, 2002), and citrus fruits (Pereira de Carvalho Costa *et al.*, 2003), and have brought improved pest and disease resistances and flavour enhancements. Probably the most notorious example is the German “Tomoffel”, a fusion product of tomato and “Kartoffel” (potato) that unfortunately had few commercially attractive features. Some sources claim that nectarine is a protoplast fusion of plum cells and cells from a spontaneous peach mutant (we have not been able to substantiate this claim). It is not only genomes that are mixed, combined and sorted in this method: new combinations of organelles can result in improved cultivar performance that cannot occur in ordinary breeding due to the mechanics of pollen formation. It is as though the best eggs can be selected regardless of whether they are from ducks or chickens or even, given patience, some technological improvements, clearly identifiable benefits and most importantly consumer permission, ostriches.

With the onset of the step change in our abilities to manipulate genomes precisely via transgenic technology, protoplast fusion became less prominent but more recently interest has revived. To our knowledge it has never been achieved its potential in the hands of any pasture ryegrass seed companies. However there is some research effort: for example the National Grassland Research Institute in Japan once reported using the technology to produce a hybrid pasture plant from Italian ryegrass and tall fescue (Takamizo *et al.*, 1991). The resulting plants had attributes that were intermediate of both parents.

It is not always necessary to screen wild lineages for capabilities that have been lost from agronomically important breeds. Sometimes we can rely on good fortune: they may arised spontaneously in our elite lines because ryegrass genomes constantly change in small, random ways. Again, fortune favours the most patient and the lucky. The impatient or the unlucky need not despair; they can increase the rate of change in a variety of ways. One is called somaclonal variation. Individual plant cells grown in tissue culture medium accumulate genomic changes which come to light on regeneration of full sized plants as altered genes or modified epigenetic control mechanisms are activated. ViaLactia has a perennial ryegrass variety based on *Bronsyn* formed using exactly this method which does not flower. The mechanism is currently unknown and the plants remain flowerless despite vernalisation and hormonal treatments. Flowering delay is a desirable trait in pasture ryegrasses but with complete ablation of flowering this cultivar has no foreseeable future in New Zealand: viable seed cannot be produced to propagate the line. This is a most interesting egg but we have only one and cannot make more for our omelette.

All of the above recipes for generating and capturing agriculturally useful variety for crop improvement are valid replacements for the transgenic methods for the introduction of traits into plants such as ryegrass. Every one is inefficient and highly unpredictable because combining many thousands of genes from two lineages, perhaps after random scrambling through somaclonal variation or some other procedure, can only provide surprises and is never without the risk of wasted time or effort. Furthermore, all the above processes must play the numbers game, making many attempts before finding a combination that is tolerable. In all cases, techniques such as marker assisted selection must be used to re-introduce as much 'elite' behaviour as possible in the time available. All else aside, these problems are

TABLE 1: Comparison of impacts, considerations and concerns over a range of traditional and modern biotechnologies for plant improvement.

Concerns	**Transgenics**	**Cisgenics®**	**Plant Breeding**	**Protoplast fusion**
Introduction of foreign DNA	Yes	No	In some cases yes (e.g. Matrix ryegrass)	Yes
Escape of genes to other (organic) ryegrass plants	Possible	Possible but other ryegrass plants already contain that gene.	Possible but other ryegrass plants already contain that gene.	Possible
Escape of genes to other plant species	Limited to close relations	Limited to close relations	Limited to close relations	Limited to close relations
Generation of new allergens	Possible	No new allergens from introduced gene but possible	Possible	Possible
Subject to ERMA regulatory approval	Yes	Yes	No	No, as long as parents are derived from NZ pasture plants

undoubtedly circumvented when transgenic approaches are used instead.

By working within a single lineage and only introducing single or small numbers of non-elite genes, transgenesis is clearly more precise by an order of magnitude or more. The multiple gene teams, the backcrossing and the rounds of firing and downsizing, the reliance on luck and the laws of large numbers – all are absent in modern biotechnologies based on transformation. Such target-oriented control has the potential to revolutionise any biological industry when not subject to the unprecedented scrutiny, debate and emotion we see today.

Transgenic approaches have their own problems, and public concerns are often centred on (but certainly not limited to) those listed in table 1. We suggest that one perceived benefit of non-transgenic approaches is that, except in the case of protoplast fusion, only genes that are found within the species are moved around or recombined. In the case of protoplast fusion, only genes that are working in successful gene teams are allowed in the mix. The conclusion that may be drawn is this: the best kind of transgenesis would be the kind where *only* ryegrass DNA is (re-)introduced into the ryegrass genome. We call this approach Cisgenics®, where genes are only moved within existing gene teams or between closely related ones. The term applies equally to the use of only rice genes in rice, loblolly genes in loblolly, and so on. It can even apply to the use of undomesticated varieties of a plant or animal as non-elite parents in breeding. It therefore encompasses both traditional and modern approaches but not all biotechnologies. We are concerned only with the source of the heritable material that ends up in the final, agriculturally useful variety and believe that Cisgenics® is a useful concept. Cisgenic® approaches lack some of the shortcomings of transgenics and also of other biotechnologies such as breeding (see table 1).

ViaLactia is actively researching the utility of Cisgenics® under controlled laboratory conditions as an option for the New Zealand dairy farmer if this technology becomes acceptable in the future. We believe that the cisgenic approach, where genes, promoters, regulatory elements, all units of heritability that can confer useful traits and characteristics to a plant are sourced only from within the gene team of the plant of interest is the best way forward. There will be situations when the Cisgenic® approach is inadequate or inappropriate; in these cases we consider that the other approaches discussed in this article are required, including transgenesis. For example, the most widely planted transgenic crops in the world today contain DNA sourced from soil bacteria – this is appropriate only because resistance to the herbicide glyphosate cannot today be provided via Cisgenic® means, whether marker assisted breeding, some types of protoplast fusion, transformation with only endogenous DNA, or some other approach. While the goal of herbicide resistance has been here achieved using the best available means, Cisgenics® imposes a further duty to make improvements as new tools and breakthroughs occur. Other desired traits, such as flowering control in pasture grasses, will be achievable by Cisgenic® means in the short term. In the medium term many agricultural crop improvements will certainly be Cisgenic® and we believe this is entirely appropriate. Breaking eggs is easy; plant biotechnology will be breaking many more in the decades to come as we face the challenges of the twenty-first century; all we ask is that we be principled about it.

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