Feature Review

Are we ready for back-to-nature crop breeding?

Michael G. Palmgren[1], Anna Kristina Edenbrandt[2], Suzanne Elizabeth Vedel[2], Martin Marchman Andersen[3], Xavier Landes[3], Jeppe Thulin Østerberg[1], Janus Falhof[1], Lene Irene Olsen[1], Søren Brøgger Christensen[4], Peter Sandøe[2,5], Christian Gamborg[2], Klemens Kappel[3], Bo Jellesmark Thorsen[2], and Peter Pagh[6]

[1] Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
[2] Department of Food and Resource Economics, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark
[3] Department of Media, Cognition, and Communication, University of Copenhagen, Karen Blixens Vej 4, DK-2300 Copenhagen S, Denmark
[4] Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark
[5] Department of Large Animal Sciences, University of Copenhagen, DK-1870 Frederiksberg C, Denmark
[6] Centre for Public Regulation and Administration, Faculty of Law, University of Copenhagen, Studiestræde 6, DK-1455 Copenhagen K, Denmark

Sustainable agriculture in response to increasing demands for food depends on development of high-yielding crops with high nutritional value that require minimal intervention during growth. To date, the focus has been on changing plants by introducing genes that impart new properties, which the plants and their ancestors never possessed. By contrast, we suggest another potentially beneficial and perhaps less controversial strategy that modern plant biotechnology may adopt. This approach, which broadens earlier approaches to reverse breeding, aims to furnish crops with lost properties that their ancestors once possessed in order to tolerate adverse environmental conditions. What molecular techniques are available for implementing such rewilding? Are the strategies legally, socially, economically, and ethically feasible? These are the questions addressed in this review.

Reverse breeding

Agriculture has only been practiced for about 10 000 years. In this relatively short historical period, humans have developed crops that feed more than seven billion people on this planet [1]. However, it is uncertain whether current agricultural practices will be able to feed the world in 2050, when the human population is predicted to reach more than nine billion [2–4]. Can this goal be reached without applying modern plant biotechnology techniques? Much food can be saved by reducing waste, promoting less meat-intensive diets, and using resources more efficiently; however, increased food production seems to be a necessity.

Agricultural land should not be expanded at the expense of the remaining natural ecosystems on earth. Thus, we are faced with the challenge of getting the most out of existing agricultural systems, a concept known as 'sustainable intensification' [5]. Food production is further threatened if substantial areas are used to grow crops for biofuels [6]. In contrast to industrial agricultural methods, new agricultural systems have been proposed that allow for the sustainable production of food with a minimal input of resources [7]. These include cover crops, long crop rotations, tillage, increased biodiversity, and crop and animal integration. Although the efficacy of these systems is debated, they all advocate ecosystem approaches to crop management with the aim to reduce the need for pesticides and fertilizers.

To date, the process of domestication has focused on securing specific traits that occurred at random, either spontaneously in nature or as a result of radiation treatment or exposure to mutagenic chemicals. Important traits that have been selected for are easy harvest, high yield, and low toxicity. By contrast, mutations that compromise the hereditary basis of crop survival during environmental stresses, both biotic (such as pests, pathogens, herbivores, and diseases) and abiotic (such as drought, flooding, nutrient deficiencies, and salinity) are rarely selected against. As a result, many of these survival traits may have been weakened or completely lost.

Reverse breeding as defined here implies simply back-to-nature breeding, or the reversal of the unintended results of breeding. The term 'reverse breeding' was originally introduced to describe a technique in plant cell culture where homozygous lines are produced from heterozygous parent lines [8,9]. Here, the term 'reverse breeding' includes the earlier proposed usage but goes beyond the original definition by widening the methods used to produce homozygous lines. Much remains to be learnt about

---

Corresponding author: Palmgren, M.G. (palmgren@plen.ku.dk).
Keywords: rewilding; reverse breeding; sustainable agriculture.

1360-1385/

http://dx.doi.org/10.1016/j.tplants.2014.11.003

the mutations in today's crop varieties that compromised or disabled valuable original traits. Detecting mutations in crop plants not found in their wild relatives will be a formidable task involving high-throughput sequencing techniques. Nevertheless, the task is becoming increasingly feasible due to rapid technological advancements and reductions in cost. Once the genes that have been mutated unintentionally have been identified, the next step would be to reestablish wild type properties. Rewilding would allow crop plants not only to better utilize available resources in the environment and have higher nutritional value, but also to better resist diseases, pests, and weeds.

In this review, we outline an important agricultural strategy for fortifying the crops we produce today so that they can better thrive under adverse conditions. To reach this goal, we must reestablish in crop plants specific original traits that are important for plant survival under adverse conditions, while at the same time preserving other traits obtained through breeding related to food quality and yield (Figure 1). Any proposed strategy for crop modification should be evaluated based on its legal, social, economic, and ethical feasibilities (Figure 2).

The rise of agriculture and the origins of breeding

Grasses such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rice (*Oryza sativum*), and maize (*Zea mays*) were among the first plants to be domesticated. Contrary to popular belief, domestication of grasses did not occur because of the invention of agricultural practices, but because mutant versions of grasses, with properties that made large-scale collection of grains possible, were noticed and utilized by humans.

Wheat provides an example of how the disappearance of a trait that is required for survival in the wild proved to be essential for its domestication as a crop. The first domestication of einkorn wheat (*Triticum boeoticum*), a wild relative of wheat, is believed to have taken place in southeast Turkey in around 7500 BC [10,11]. In wild wheat, the rachis (i.e., the structure to which grains are attached in the spike) becomes brittle during grain maturation, and easily shatters into spikelets that fall to the ground or blow away. Furthermore, once spikelets are collected, the grain is tightly held by the husk (glumes) surrounding it and is difficult to release. These combined features made the large-scale collection of early grains cumbersome or even impossible [1,12].

In the first domesticated einkorn wheat, *Triticum monococcum*, the rachis was hardened, and the seed was only loosely held at the base of the glumes. These properties allowed for easy harvest of wheat in the field and subsequent threshing. About 60 years ago, this trait was found to be the result of a mutation in a single gene, which was designated '*Q*' [13], and the responsible gene was identified in 2006 [14]. The *Q* gene encodes an AP2 transcription factor that regulates the expression of several other genes, which in turn influences a number of features related to inflorescence structure and flowering, including rachis fragility. Compared to the corresponding gene in wild wheat, termed '*q*', *Q* carries a dominant mutation that results in a single amino acid change in the encoded

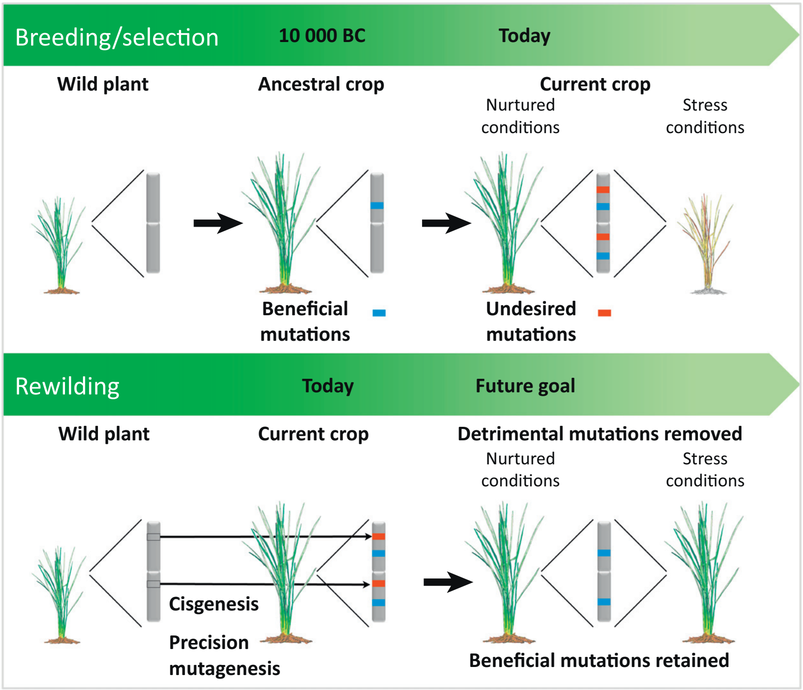

Figure 1. Rewilding maintains beneficial mutations while eliminating undesired mutations. Ancestral crops were based on wild plants carrying mutations that proved to be beneficial for agriculture, such as mutations that made plants easier to harvest and/or resulted in higher yield. During extensive periods of breeding and inbreeding, undesired mutations accumulate and may remain unnoticed because they only affect traits that are important for the plant when growing under adverse conditions, such as nutrient and water deficiency, salinity, and the presence of pests ranging from microorganisms to herbivores. Enabling the plant to overcome these deficiencies by cisgenesis or precision mutagenesis may result in crop plants in which detrimental mutations are removed but beneficial mutations retained.

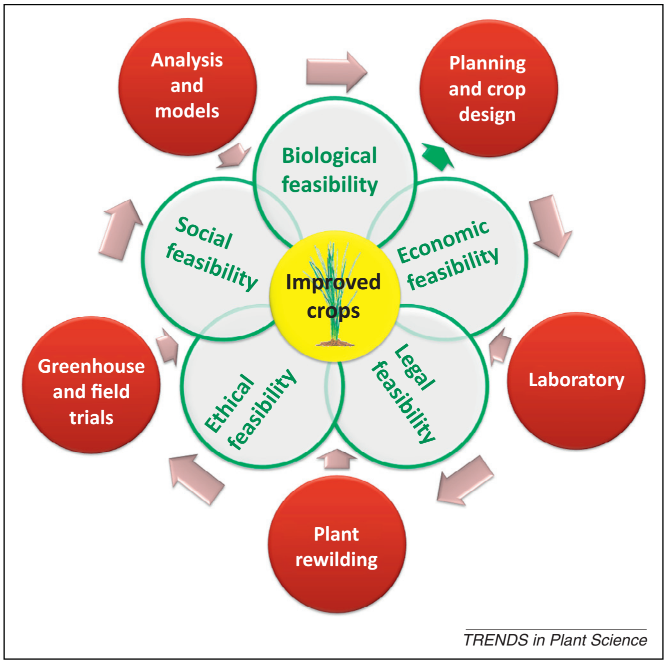

Figure 2. A proposed scheme for developing new crops by reverse genetics techniques. Throughout the breeding process, strategies for crop improvement are evaluated based on their legal, social, economic, and ethical feasibilities.

protein. This mutation might by chance have occurred only once in the wild, but the fortuitous prehistoric isolation and sowing of plants with a tough rachis and an easier threshing trait became the basis for wheat domestication. In parallel, other mutant grasses with suppressed seed shattering and belonging to species such as sorghum (*Sorghum vulgare*), rice, and maize, were isolated in other parts of the world, which allowed for the domestication of these plants [15].

Traits lost by breeding

Inspired by the work of Gregor Mendel, conventional modern breeding originated from efforts to combine desirable traits found in various plants. Thus, a plant with a desired trait would be cross-pollinated with pollen from another plant lacking this trait, but harboring another desirable trait. The subsequent generations often displayed a combination of the traits. In the 1950s and 1960s, mutations began to be introduced into plant seeds by radiation or chemical treatments to increase the degree of genetic variation used as a basis for selection in breeding programs [16].

The domestication of maize provides an example of how breeding efforts may select for specific traits, but unintentionally cause others to disappear. The maize gene *acyl-CoA:diacylglycerol acyltransferase* (abbreviated *DGAT*) is important for producing oil with the healthy monounsaturated fatty acid oleic acid. However, a mutation occurred during maize domestication that resulted in a small deletion of three bases in the *DGAT* gene and, consequently, a significant loss in the activity of the encoded mutant protein [17]. Introducing the natural gene without this mutation into a modern variety of maize has resulted in plants that are able to produce oil containing oleic acid [17].

In the example above, the trait resulted from a mutation in a single gene which resulted in allelic diversity. However, many if not most traits depend on the simultaneous presence of mutations in multiple genes [18,19]. When several different genes influence a single desirable trait, breeding for this trait by traditional means is complicated and was almost impossible until recently [20]. Thus, many traits were lost during domestication, because they simply could not be selected for.

How to bring the lost traits back again

Plant genomes can be modified by several techniques. In this review, we will only consider techniques that do not introduce foreign gene material into plants, or that do so only to a minimal extent, where foreign is defined as belonging to an organism with which the plant cannot naturally reproduce. This is not because we consider 'foreign' DNA as a problem as such. No study has ever shown that so-called transgenes in plants have caused any problems related to public health or the environment. Nevertheless, plants expressing transgenes have been the subject of intense public debate and resistance from interest groups, and are currently subject to stringent public regulatory procedures in many countries, especially in Europe, which complicate their approval process.

Genes lost during domestication are traditionally recovered by introgression breeding in which all the genes of a modern cultivar and a wild relative are at first combined in a cross (Box 1, i). However, this technique is time consuming as it involves backcrossing for several generations and, especially when more than one gene has to be recovered, the process can become very demanding if not impossible. A decade ago, no methods were available for gene-specific reverse breeding; however, several have recently emerged. A number of excellent reviews describe the molecular mechanisms underlying these new methods [21–26]. Here we will only broadly describe the principles of the methods and, where possible, will use rice as an example, as rice was developed as a model crop for plant biotechnological efforts.

In principle, there are two ways in which reverse breeding can be carried out using plant biotechnological methods (Box 1, ii and iii). First, full-length 'modern' genes can be replaced with 'ancient' ones. Second, individual mutations that occurred in 'ancient' genes during the process of domestication can be identified in 'modern' genes and reverted.

Legal feasibility concerning new techniques in plant biotechnology

Following the first reports on the expression of genes introduced into plants by biotechnological methods [27,28], expectations were high that this strategy would soon be used to benefit mankind. Although some concerns were raised, the approach was positively received, as reflected in the first global legal regime addressing biotechnology, the Biodiversity Convention (CBD) from 1992, which in Article 16(1) recognizes that access and transfer of biotechnology 'are essential elements for the attainment of the objectives of this Convention' – meaning that biotechnology could help assure that biodiversity is not lost. The

Box 1. Techniques available for rewilding that do not involve transgenes

(i) Introgression breeding. This technique has been used for decades for the introduction of alleles from wild germplasm into modern cultivars [77–79], frequently to introduce resistances to diseases [80] or to abiotic stress [81]. For introgression breeding, crosses are performed between a modern genotype and a wild relative. Subsequently the undesired alleles from the wild genotype are removed from the progeny by repeated backcrosses with the modern genotype, yet keeping the desired wild allele(s). Nowadays this is often supported by using molecular DNA markers. However, introgression breeding can be hampered seriously if more than one allelic variant has to be introduced and if plants are not self-compatible, have long generation times or reduction in fitness due to deleterious genes being introduced along with the beneficial gene during backcrossing (linkage drag).

(ii) Specific insertion of lost genes (cisgenesis). Cisgenesis is the introduction of genes into plants that originated from the same plant or from cross-compatible relatives [82]. The genes contain their own regulatory elements and introns. A variant of this concept, intragenesis, allows for regulatory elements to be mixed between genes, as long as they originate from the same plant or from a cross-compatible relative [82]. Selection markers that originate from crop plants such as rice are now available [83,84] and, if desirable, the selection marker can be removed after selection by Cre-loxP site-specific recombination [85,86] or other marker gene elimination methods [87,88].

(iii) Precision mutagenesis. Sequence-specific nucleases engineered to modify target DNA sequences have already demonstrated great promise for introducing site-specific mutations in plants [89,90]. Several variants are described in the literature, such as those based on zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and, most recently, RNA-guided engineered nucleases (RGENs) derived from the bacterial clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated (Cas) system. Five papers were published in 2013 in which CRISPR–Cas technology was used to mutagenize rice [23,91–94] and, based on its efficiency, it can be predicted that this technique will be widely used in future.

concern of risk is reflected in the CBD Article 8(g), which calls for measures ‘to regulate, manage or control the risks associated with the use and release of living modified organisms (LMOs) resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health.’

Two decades later, the number of plants that have been modified by biotechnological methods and have reached the market is limited for several reasons, with the main stumbling block that plants genetically modified (GM) in the laboratory remain a highly controversial issue [29].

Today, the legislation, definitions, and regulatory approaches for crops derived from biotechnology vary considerably between different countries [30]. In 2013, Food Standards of Australia and New Zealand (FSANZ) concluded that food produced using a method of precision mutagenesis would be similar to food produced using traditional mutagenic techniques and should therefore not be regarded as GM food [31]. The European Commission Joint Research Centre (JRC) had reached a similar conclusion [32]. However, it cannot be expected that the European Food Safety Authority (EFSA) will reach the same conclusion, because, in the EU, the regulation of crops resulting from biotechnology is still based on the premise that there is a fundamental difference between conventional and biotechnologically derived crops based on the methods that are employed to generate them [33] (Box 2). Because the current legislation does focus on the underlying technology used rather than the resulting trait, rewilded plants produced by the methods discussed above are likely to be classified as genetically modified organisms (GMOs) (Box 3).

Social, economic, and moral feasibility

Even when reverse breeding is technically feasible and legally permissible, it may still not be implemented in practice, should the resulting plants be considered as GMOs and therefore be subject to regulatory practices that seriously restrict their use. Will it be possible to convince citizens, decision makers, and farmers that reverse breeding provides a path for agriculture that is compatible with conventional breeding? The public’s choice of food is governed by many factors, which include social, economic, and moral concerns.

Social feasibility – more than a question of naturalness

Studies have been conducted on the public acceptance of the insertion of complete genes via cisgenesis, whereas no studies have analyzed the public acceptance of crops modified by precision mutagenesis. Hence, we focus on the public acceptance of cisgenesis.

Cisgenesis was first introduced in a chapter on the ethical assessment of genetic modification [34], and launched with the aspiration that it would be a new tool

Box 2. What is a GMO?

Paradoxically, it might not be an easy task for molecular techniques to reestablish a natural trait without the resulting organism having to be legally classified as a GMO [25,33]. The Cartagena Protocol on Biosafety to the Convention on Biological Diversity is an international treaty governing the transfer of LMOs resulting from modern biotechnology from one country to another by establishing a Prior Informative Consent regime. The protocol was adopted in 2000 as a supplementary agreement to the Convention on Biological Diversity and endorsed in 2003. Notably, it defines in Article 3(g) that a LMO is ‘any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology’ (Article 3). Further, ‘modern biotechnology’ is in Article 3(i) defined as ‘the application of a) *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b) fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection.’

Thus the methods used, rather than the end result, define whether an organism is genetically modified. As all methods discussed above employ modern biotechnological tools, the resulting plants are likely to be classified as GMOs according to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. GMOs are similarly defined in the GMO directive of the EU.

The legal feasibility of cisgenesis and precision mutagenesis within the EU is influenced by the regulatory burden and controversies associated with the approval process for GMOs and by the effect of the modification on biodiversity. The same goes for the GMO Directive of the EU. However, the legal feasibility of cisgenesis and precision mutagenesis can be seen as not only a regulatory burden to prevent environmental degradation and damage to public health but also as a contribution to ensure biodiversity as reflected in the Nagoya Protocol on benefit sharing under the CBD.

Box 3. Are rewilded plants to be considered as GMOs?

It can be questioned whether the methods used in reverse breeding will provide a means of sidestepping controversies related to the complex and time-consuming approval process. The GMO Directive Article 2(2) defines a 'genetically modified organism' as an 'organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.' According to this legal definition, GMOs are not restricted to organisms harboring transgenes and requiring laboratory methods for the insertion of complete cisgenes. Further, the introduction of specific mutations in cisgenes would under the legal definition result in the production of GMOs. However, a derogation in Article 3(1) that exempts GMOs from the legal requirements of the GMO Directive seriously questions this definition since it excludes:

'Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below, are:

(i) mutagenesis;
(ii) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.'

This derogation indicates that if GMOs are generated by the use of mutagenesis or cell fusion, the GMOs are exempt from the GMO Directive provided the method does not involve the use of recombinant nucleic acid molecules or (other) GMOs. However, the scope of this derogation seems to be restricted to 'techniques', indicating that the GMOs generated using this technique do not escape the GMO Directive. Although no formal decision has been taken by the EU Commission, the case law of the European Union Court of Justice regarding the GMO Directive strongly indicates that the derogation in Article 3(1) must be interpreted narrow as only referring to 'techniques'.

In conclusion, the techniques used to introduce complete genes or specific mutations make it impossible for the resulting plants to escape the legal framework of the GMO Directive, not even if the genes or mutations originate from wild plants. Nevertheless, they might affect the criteria for approval, since techniques such as cisgenesis and precision mutagenesis should not only be seen as a threat to biodiversity, but also as tools that can be used to improve biodiversity and sustainable development as stated in Article 16(1) of the CBD.

for improving crops intended to be '. . .safer and ethically more acceptable and therefore [to] require a less stringent assessment' [35]. Hence, the hope was that the resulting crops would be subject to a more lenient regulatory regime than the one presently used for transgenic crops [36–38].

The controversies surrounding transgenic modification of crops have led to widespread public rejection and limited the commercialization of such technologies [39]. To assess how the public views new technologies in the field of food production, explorative focus groups, individual interview studies, and (representative) national and international surveys have been combined [40]. Factors used to explain the attitudes of citizens include perceived risk [41], perceived benefit, trust [42], consumer autonomy and labelling [43,44], level of knowledge [45], technology's and science's role in agriculture [46], perceived naturalness [47], as well as other moral concerns [48,49].

Worldwide, plant-related biotechnology applications are considered more acceptable than animal-related applications. In Europe, GM plants are linked to higher levels of perceived risk than in North America (and the reverse with regard to perceived benefit) [43]. When it comes to the perception of cisgenic as distinguished from transgenic crops a Swiss study found that cisgenic apples would be considered more acceptable than transgenic ones in the context of fire blight [50]. Also, data from the 2010 Eurobarometer in the 27 EU member states show that cisgenic production of apples gets higher support (55%) than transgenic apples (33%). Moreover, 72% of respondents in the 27 EU member states agreed or tended to agree that transgenic crops are 'fundamentally unnatural', whereas the corresponding figure for cisgenic crops was 52% [48]. However, a recent paper concluded from a representative study in EU27 that most of the respondents consider both options unnatural and that 'cisgenics is very unlikely to be equated with [conventional] breeding' [51]. Moreover, not all people distinguish between cisgenic and transgenic options. Across Europe, only 28% of respondents differentiate between the two options to a degree implying differential praise [51].

Despite considerable variation across Europe (and between Europe and the US), some general tendencies in the public view of various forms of GM crops emerge: (i) skepticism towards GM food is a lasting phenomenon, and hence not just a matter of getting used to something new; (ii) perceived risk plays a considerable role; (iii) perceived utility is a key factor, with emphasis being placed on whether the modifications will result in crops that address problems perceived as being significant, and not on whether biotech companies and/or farmers make a profit; (iv) consumer autonomy is important – the public want to be allowed to choose whether or not to buy the modified crops; (v) naturalness matters – the closer the technology to conventional plant breeding and the less human intervention the better; and finally (vi) other moral considerations such as establishing who benefits from the technology and who bears the risks are also important.

Although cisgenic crops address point (v) above, it is unclear whether they would actually be perceived as being more natural than transgenic crops. Lack of consensus regarding the definition of 'natural' is part of the reason [52]. Moreover, one may question whether a higher degree of perceived naturalness would be sufficient to foster a positive attitude towards cisgenesis. A frequent expert assumption is that the public would consider cisgenics to be more natural and therefore more acceptable than transgenics; however, as pointed out [51], such estimates of laymen's views are not necessarily correct. In fact, it may be as problematic to assume the perceived naturalness of cisgenic crops as it was to assume consumer acceptance of GMOs in the 1990s. Furthermore, even if cisgenic crops were to be regarded as being more natural than transgenic crops, they may still be deemed unacceptable because they are not natural enough [53].

In summary, cisgenesis is largely viewed as being more natural than transgenesis, and the example of cisgenic production in the 2010 Eurobarometer study received higher support than the transgenic option [48]. However, across Europe, 72% of respondents do not tell cisgenic and transgenic options apart to a degree indicating differential treatment [51]. Nevertheless, other public concerns relating to transgenesis are not addressed by the concept of cisgenesis; notably, utility (e.g., addressing public good aspects), consumer autonomy (e.g., the issue of labeling), and other moral concerns (e.g., who bears the risks, and where).

Economic feasibility and consumers' willingness to pay for avoiding GM food

Numerous economic studies have focused on consumers' assessments of various breeding and biotechnological plant improvement techniques, most notably transgenesis [54], and a smaller body of studies on producers' concerns. Two fundamental questions studied by economists are: (i) what aspects of biotechnology give rise to the skepticism and hence lack of willingness to buy biotech-derived products? And (ii) what factors enhance or reduce this skepticism?

A core axiom in economics and consumer choice theory is that consumers select products based on which products give them the highest utility. The utility of a food product depends on aspects such as its price, taste, nutritional value, convenience, environmental impact, and perhaps the technology used to produce it, such as conventional, biotech-derived, and organic technology. Because consumers have diverse preferences, the same product may give different levels of utility to different individuals. As preferences and utility levels are not observable, economists infer them from the study of consumers' choices, either in real market situations or in experiments, and from that derive the likelihood of consumers buying products with specific traits and their willingness to pay (WTP) for these traits.

There is consistent evidence that consumers expect to pay less for biotech-derived food products than for identical products obtained by conventional breeding. Furthermore, this WTP gap is significantly higher in the EU than in the US [55], and this is commonly attributed to differences in culture, in the levels of trust in regulatory institutions, and in regulations. Specifically, mandatory labeling in the EU may be interpreted as an indication that biotech-derived food is unsafe, while the voluntary labeling in the US may have the opposite interpretation. The WTP gap between biotech-derived and non-biotech-derived food is, however, significant in both regions [56].

Will preferences be altered if reverse breeding is implemented? Consumer acceptance of biotech-derived foods is commonly argued to improve when enhancing the qualitative characteristics of the products (e.g., taste or nutritional value) and providing consumers with information. The first possibility is supported in empirical studies; increased consumer benefits do increase consumer acceptance of biotech-derived foods, although a WTP gap remains between biotech- and non-biotech-derived foods [57,58].

The second possibility is based on the assumption that more information leads to higher levels of knowledge, and that this is associated with higher acceptance of biotech-derived foods. On this issue, the results are less clear; individuals that consider themselves well informed about biotechnology have a higher acceptance of GM crops, but when the actual knowledge is measured (through tests), there is no significant relationship between knowledge and acceptance [59]. There is ample experimental evidence that information can affect the WTP gap between biotech- and non-biotech-derived foods. Whether information increases or decreases this gap depends on who communicates the information (firms, scientists, or non-governmental organizations), what the message is (positive, neutral, or negative) [60,61], and the individual's prior beliefs about biotech-derived foods [62].

Another aspect of consumer acceptance of biotech-derived food concerns attitudes towards the technology used and the perceived naturalness of the food. Several empirical studies reveal higher consumer acceptance towards cisgenesis than transgenesis, although conventional breeding remains the preferred technology [56,63], which in turn is likely to affect the WTP gap.

Biotech-derived crops have been designed to benefit farmers by reducing risks and costs and increasing productivity [64]. This approach has been somewhat successful [65]. Due to the limited number of GM crops in Europe, existing studies on European farmers' perceptions and attitudes are based on landowners' stated perceptions. These economic studies on farmers' willingness to adopt biotechnological approaches reveal that the expected profitability is indeed an important driver for adoption [54,66–68], but so are issues such as their neighbors' perceived viewpoints, potential demand by the public, and dependence on seed suppliers [66,67,69]. Providing that acceptable benefits exist, between a third and a half of the farmers in five European countries were willing to adopt herbicide-tolerant maize and oilseed rape transgenic crops [66].

In conclusion, there is ample evidence that consumers generally prefer food not to have biotech-derived traits and would pay less for it if it did. The literature on restricted versions of GM plants is sparser, but the evidence suggests that consumers find it more acceptable than transgenic crops [56,63]. The even more restricted precision mutagenesis could therefore be expected to be at least as acceptable as cisgenesis, although these approaches may not be considered equal to conventional breeding methods. Thus, reverse breeding may have several advantages over transgenesis; yet, a number of concerns remain. Farmers tend to focus on profitability, and the success of new technology will thus depend on expectations about consumers' demands.

Ethical theory and public skepticism about GM plants

As discussed above, public skepticism regarding genetic modification of plants is still emphatic, and would probably include reverse breeding. Public skepticism may limit producers and research institutions, and may sustain rather restrictive legislative regimes, such as those adopted by the EU. This section will address the ethical significance of public skepticism. We will first consider what general ethical theories imply about biotech-derived crops in general (including those derived from reverse breeding), and about the fact that a considerable proportion of citizens are skeptical. Second, we will consider what theories about political legitimacy imply regarding public skepticism, and the current fairly restrictive governance policies.

We will assume that public skepticism is nurtured by the view that biotech-derived plants (i) are intrinsically unnatural or created by an unnatural process, (ii) warrant concerns about adverse risks and health effects, and (iii) warrant other distributive concerns (e.g., about exploitation of farmers, sustainment of traditional human life forms, and profits reaped by large companies, or other forms of social injustice).

Ethical theories are philosophical theories about what is morally right. Traditionally, the dominant ethical theories include various forms of kantianism (i.e., act according to rules that everyone can accept as universal rules), consequentialism (act to promote the best consequences), contractarianism (act according to what we would hypothetically agree about), and combinations of these elements. Although these theories vary greatly, they also have important traits in common, as we discuss in the next three paragraphs, and we will refer to them as the KCC theories.

The KCC theories do not support the idea that a product can be less morally valuable merely by being unnatural or that manipulating parts of nature can be morally wrong merely because it is unnatural, or that such manipulations violate a presumed prerogative of a creator to form nature. Therefore, none of the KCC theories support the distinctions between traditional breeding and modern plant biotechnologies that public skepticism seems to assume. Nothing in the KCC theories supports distinguishing between two biologically identical products merely on the grounds that their causal history or genesis is different, as the legislative system in the EU tends to do.

KCC theories do address ethical concerns about how the development, production, and consumption of biotech-derived plants affect health and safety, as well as the distribution of power, property, and freedom. However, as KCC theories are normally understood, risks and adverse effects would have to be estimated using the best available scientific evidence of their likelihood. Non-specialists' conflicting assessments of the risks would be considered irrelevant. Moreover, increasing the food supply to a starving population through the successful implementation of biotech-derived crops would be considered a highly relevant factor in favor of promoting the development of biotech-derived crops.

However, what about the fact that part of the population is skeptical about biotech-derived crops? Many versions of KCC theories assign some weight to what they consider mistaken moral and factual views. One of the most accommodating theories in the context of public decision-making is probably preference utilitarianism, according to which the best policy is the one that leads to greatest overall preference satisfaction. Therefore, the preferences of worried citizens would count in favor of a restrictive policy. Of course, the preferences of the non-skeptical part of the population would count as well, as would those of future generations, and this could vastly outweigh the discomforts of current skeptics. This would count in favor of policies that promote the development of biotech-derived crops, despite public disagreement.

Theories of political legitimacy and skepticism towards biotech-derived crops

An influential view in political philosophy asserts that we should focus on political legitimacy, rather than on trying to find the truth about controversial moral questions [70] (for a glossary of relevant terms in political philosophy, see Box 4). A legitimate policy must in some way reflect or respect the views of the citizens affected by it. Thus, what does political legitimacy imply when institutions have to deal with public skepticism for the regulation of biotech-derived plants?

According to classical liberalism, the state must secure individual rights and freedoms, and provide equal opportunities to all citizens. The state should refuse to pass legislation on moral grounds other than these. Citizens might well have other moral views, say about naturalness, but these should be treated as the private views of individuals and should not influence legislation. This idea is captured in what is known as The Harm Principle [71], according to which the only valid ground for imposing a restrictive legislation is to prevent harm to non-consenting others. This would imply that biotech-derived plants should be treated the same as traditional products.

A similar ideal is the Neutrality Principle, according to which the justification of government policies should not be based on values that in some sense belong to citizens' private worldviews or choice of lifestyles [72]. The Neutrality Principle would exclude justifications of policies based

Box 4. Glossary of terms in ethics and political philosophy

Classic liberalism: a modest form of liberalism holding that the only legitimate aim of the state is to secure fundamental individual rights and freedoms. In some versions, however, this includes the aim of equal opportunities to all citizens.

Consequentialism: a class of ethical theories holding that the morally right act (or policy) is the one out of the available alternatives that maximizes (actual or expected) consequences.

Contractarianism: a class of ethical theories holding that the morally right act must conform with some sort of contract that individuals have consented to, or would consent to under certain hypothetical circumstances.

Ethical theory: philosophical theories about moral rightness and wrongness.

Kantianism: ethical theories derived from German philosopher Immanuel Kant's work holding that one should act in accordance with principles that one can rationally want to be universal laws, or in such a way that humans (rational beings) are treated as ends in themselves.

Liberal principle of legitimacy: a principle detailing a specific view about when a policy is legitimate; legitimacy requires some sort of qualified consent by its citizens. The controversy concerns, among other things, what type of consent is required.

Neo-republicanism: a political theory (or set of political theories) holding that liberty consists in the absence of arbitrary domination, which means for instance that democratic decisions must be open for critic.

Neutrality principle: a principle stating that the state should not appeal to any specific conception of the good such as religious outlooks or specific moral views when justifying policies.

Political philosophy: the domain of philosophy concerned with what the just or right political order is, that is, the proper distribution of resources, power, and freedom, ideally speaking. Includes theories of legitimacy.

Preference utilitarianism: a form of consequentialism holding that what should be maximized is the satisfaction of preferences.

The harm principle: a principle holding that the only purpose for which power can be rightfully exercised over any member of a civilized community is to prevent harm to others. If some behavior is not harmful to anyone, but merely thought to be morally objectionable, the state should not prohibit it.

Theories of political legitimacy: theories about when it is morally appropriate for the state to impose restrictive legislation on its citizens. Fair democratic voting, for example, is thought to produce legitimate political outcomes. Proceduralist views of legitimacy hold that policies acquire legitimacy by being adopted through certain procedures, for example, by voting or deliberation.

on the view that unnatural products or unnatural interventions are morally problematic.

A related influential view is the Liberal Principle of Legitimacy, according to which a policy is legitimate only if no one affected can reasonably object to it [73]. Thus, according to this principle, it is not legitimate to impose restrictive policies on citizens who can reasonably reject those policies. It is, however, a matter of controversy who is considered to be affected by a policy, for example, a policy that regulates biotech-derived crops, and also whether reasonable objections may be based on views about naturalness that are not widely shared or difficult to rationalize in traditional philosophical and scientific discussions.

The influential political philosopher John Rawls has proposed a very restrictive view about what counts as admissible reasons for or against public policies [73]. Essentially, Rawls argues that citizens should be bound by the idea of reciprocity. A citizen should appeal only to reasons that he/she sincerely believes other reasonable citizens would endorse as a part of public reason. Skepticism based on private views about naturalness would presumably not qualify, and legislators should therefore not invoke them in favor of (or against) public policies. Many have criticized Rawls' restrictive notion of public reason for being far too restrictive. Others have proposed a highly permissive alternative, according to which any objection would count as valid, if only it is comprehensible to others [74]. However, this view also argues that citizens are entitled to object to policies that restrict their freedom, but not with similar force to policies that do not. It is not clear if skeptical citizens could plausibly argue that a permissive policy regarding biotech-derived plants restricts their freedom.

Finally, proceduralist views about political legitimacy hold that legitimate policies are those that are adopted by the right type of decision procedure, such as public deliberation (rational exchange of arguments and reasons) followed by some form of democratic voting procedure [75] or contestation, such as in neo-republican tradition [76]. Skeptics of biotech-derived plants would, accordingly, be entitled to participate in deliberations and influence decision-making, but so would non-skeptics. The current rather restrictive policies regarding biotech-derived plants would be fully legitimate if adopted according to democratic procedures of deliberation and contestation. However, reversing these polices could be perfectly legitimate if correct procedures were followed, even if the level of resistance were unchanged, or even increased.

Concluding remarks

We hypothesize here that during the process of domestication, crop plants have lost natural traits that confer selective advantages, such as efficient water and nutrient utilization and resistance to microorganisms and pests. The purpose of reverse breeding is to regenerate lost abilities of crop plants and thereby enable plants to compete in interplant agricultural ecosystems under adverse environmental conditions. Once identified, the mutated genes that have unintentionally been selected for during the process of domestication can be reverted to their wild type forms using novel molecular breeding techniques that do not involve the transfer of genes between unrelated organisms. Developing crop plants through the process of rewilding, with continuous input regarding legal, social, economic, and ethical feasibility (Figure 2), may provide a more socially acceptable route to reaping the benefits of plant biotechnology. However, when reverse breeding strategies employ modern biotechnology tools, the resulting plants are classified as GMOs according to the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. This remains a major legal bottleneck for the implementation of reverse breeding.

Acknowledgments

We thank Preben Bach Holm for critical reading of the manuscript. This work was supported by the University of Copenhagen Excellency Program KU2016.

References

1 Doebley, J.F. *et al.* (2006) The molecular genetics of crop domestication. *Cell* 127, 1309–1321
2 Godfray, H.C. *et al.* (2010) Food security: the challenge of feeding 9 billion people. *Science* 327, 812–818
3 Foley, J.A. *et al.* (2011) Solutions for a cultivated planet. *Nature* 478, 337–342
4 Ray, D.K. *et al.* (2013) Yield trends are insufficient to double global crop production by 2050. *PLoS ONE* 8, e66428
5 Mueller, N.D. *et al.* (2012) Closing yield gaps through nutrient and water management. *Nature* 490, 254–257
6 Fargione, J. *et al.* (2008) Land clearing and the biofuel carbon debt. *Science* 319, 1235–1238
7 Francis, C.A. and Porter, P. (2011) Ecology in sustainable agriculture practices and systems. *Crit. Rev. Plant Sci.* 30, 64–73
8 Dirks, R. *et al.* (2009) Reverse breeding: a novel breeding approach based on engineered meiosis. *Plant Biotechnol. J.* 7, 837–845
9 Wijnker, E. *et al.* (2012) Reverse breeding in *Arabidopsis thaliana* generates homozygous parental lines from a heterozygous plant. *Nat. Genet.* 44, 467–470
10 Heun, M. *et al.* (1997) Site of einkorn wheat domestication identified by DNA fingerprinting. *Science* 278, 1312–1314
11 Zaharieva, M. and Monneveux, P. (2014) Cultivated einkorn wheat (*Triticum monococcum* L. subsp. *monococcum*): the long life of a founder crop of agriculture. *Genet. Resour. Crop Evol.* 61, 677–706
12 Zhang, Z. *et al.* (2011) Duplication and partitioning in evolution and function of homoeologous Q loci governing domestication characters in polyploid wheat. *Proc. Natl. Acad. Sci. U.S.A.* 108, 18737–18742
13 MacKey, J. (1954) Neutron and X-ray experiments in wheat and revision of the speltoid problem. *Hereditas* 40, 65–180
14 Simons, K.J. *et al.* (2006) Molecular characterization of the major wheat domestication gene Q. *Genetics* 172, 547–555
15 Lin, Z. *et al.* (2012) Parallel domestication of the *Shattering1* genes in cereals. *Nat. Genet.* 44, 720–724
16 van de Wouw, M. *et al.* (2010) Genetic diversity trends in twentieth century crop cultivars: a meta analysis. *Theor. Appl. Genet.* 120, 1241–1252
17 Zheng, P. *et al.* (2008) A phenylalanine in DGAT is a key determinant of oil content and composition in maize. *Nat. Genet.* 40, 367–372
18 Murren, C.J. (2012) The integrated phenotype. *Integr. Comp. Biol.* 52, 64–76
19 Falke, K.C. *et al.* (2013) The spectrum of mutations controlling complex traits and the genetics of fitness in plants. *Curr. Opin. Genet. Dev.* 23, 665–671
20 Tester, M. and Langridge, P. (2010) Breeding technologies to increase crop production in a changing world. *Science* 327, 818–822
21 Gaj, T. *et al.* (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.* 31, 397–405
22 Belhaj, K. *et al.* (2013) Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. *Plant Methods* 9, 39
23 Shan, Q. *et al.* (2013) Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.* 31, 686–688

24 Chen, K. and Gao, C. (2014) Targeted genome modification technologies and their applications in crop improvements. *Plant Cell Rep.* 33, 575–583
25 Fichtner, F. *et al.* (2014) Precision genetic modifications: a new era in molecular biology and crop improvement. *Planta* 239, 921–939
26 Hartung, F. and Schiemann, J. (2014) Precise plant breeding using new genome editing techniques: opportunities, safety and regulation in the EU. *Plant J.* 78, 742–752
27 Herrera-Estrella, L. *et al.* (1983) Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. *Nature* 303, 209–213
28 Murai, N. *et al.* (1983) Phaseolin gene from bean is expressed after transfer to sunflower via tumor-inducing plasmid vectors. *Science* 222, 476–482
29 Halford, N.G. (2012) *Genetically Modified Crops*, Imperial College Press
30 Lusser, M. and Davies, H.W. (2013) Comparative regulatory approaches for groups of new plant breeding techniques. *N. Biotechnol.* 30, 437–446
31 FSANZ (2013) *New Plant Breeding Techniques*, Food Standards Australia New Zealand
32 Lusser, M. *et al.* (2011) *New Plant Breeding Techniques. State-of-the-Art and Prospects for Commercial Development*, Joint Research Centre (JRC), European Commission
33 Ammann, K. (2014) Genomic misconception: a fresh look at the biosafety of transgenic and conventional crops. A plea for a process agnostic regulation. *N. Biotechnol.* 31, 1–17
34 Jochemsen, H. and Schouten, H.J. (2000) Ethische beoordeling van genetische modificatie. In *Toetsen en Begrenzen. Een Etische end Politieke Beoordeling van de Modern Biotechnologie* (Jochemsen, H., ed.), pp. 88–95, Buijten and Schipperheijn
35 Jochemsen, H. (2008) An ethical assessment of cisgenesis in breeding late blight resistant potato. *Potato Res.* 51, 59–73
36 Schouten, H.J. *et al.* (2006) Cisgenic plants are similar to traditionally bred plants. *EMBO Rep.* 7, 750–753
37 Espinoza, C. *et al.* (2013) Cisgenesis and intragenesis: new tools for improving crops. *Biol. Res.* 46, 323–331
38 Holme, I.B. *et al.* (2013) Intragenesis and cisgenesis as alternatives to transgenic crop development. *Plant Biotechnol. J.* 11, 395–407
39 Dale, P.J. (2004) Public-good plant breeding: what should be done next? *J. Commer. Biotechnol.* 10, 199–208
40 Dunwell, J.M. (2014) Genetically modified (GM) crops: European and transatlantic divisions. *Mol. Plant Pathol.* 15, 119–121
41 Houdebine, L.M. (2007) Transgénique ou cisgénique: quel niveau de risqué? *Cah. Agric.* 16, 63–64
42 Gupta, N. *et al.* (2012) Socio-psychological determinants of public acceptance of technologies: a review. *Public Underst. Sci.* 21, 782–795
43 Frewer, L. *et al.* (2013) Public perceptions of agri-food applications of genetic modification – a systematic review and meta-analysis. *Trends Food Sci. Technol.* 30, 142–145
44 Sleenhoff, S. and Osseweijer, P. (2013) Consumer choice: linking consumer intentions to actual purchase of GM labeled food products. *GM Crops Food* 4, 166–171
45 Mielby, H. *et al.* (2013) The role of scientific knowledge in shaping public attitudes to GM technologies. *Public Underst. Sci.* 22, 155–168
46 de Krom, M.P.M.M. *et al.* (2013) Understanding relations between science, politics, and the public: the case of a GM field trial controversy in Belgium. *Sociol. Rural.* 54, 21–39
47 Lassen, J. *et al.* (2002) Ethics and genetic engineering – lessons to be learned from GM foods. *Bioprocess. Biosyst. Eng.* 24, 263–271
48 Gaskell, G. *et al.* (2010) *Europeans and Biotechnology in 2010: Winds of Change?* Report for the European Commission's Directorate-General for Research
49 Myskja, B.K. (2006) The moral difference between intragenic and transgenic modifications of plants. *J. Agric. Environ. Ethics* 15, 267–278
50 Haller, T. (2009) Apples compared to apples: attitudes towards cisgenic and transgenic breeds. *Agrarwirtschaft und Agrarsoziologi* 2, 3–34
51 Kronberger, N. *et al.* (2014) How natural is "more natural"? The role method, type of transfer and familiarity for public perceptions of cisgenic and transgenic modification. *Sci. Commun.* 36, 106–130
52 Siipi, H. (2008) Dimensions of naturalness. *Ethics Environ.* 13, 71–103
53 Mielby, H. *et al.* (2013) Multiple aspects of unnaturalness: are cisgenic crops perceived as being more natural and more acceptable than transgenic crops? *Agric. Hum. Values* 30, 471–480
54 Qaim, M. (2009) The economics of genetically modified crops. *Annu. Rev. Resour. Econ.* 1, 665–693
55 Dannenberg, A. (2009) The dispersion and development of consumer preferences for genetically modified food – a meta-analysis. *Ecol. Econ.* 68, 2182–2192
56 Lusk, J.L. and Rozan, A. (2006) Consumer acceptance of ingenic foods. *Biotechnol. J.* 1, 1433–1434
57 Onyango, B.M. and Nayga, R., Jr (2004) Consumer acceptance of nutritionally enhanced genetically modified food: relevance of gene transfer technology. *J. Agric. Res. Econ.* 29, 567–583
58 Lusk, J.L. *et al.* (2005) A meta-analysis of genetically modified food valuation studies. *J. Agric. Res. Econ.* 1, 28–44
59 House, L. *et al.* (2004) Objective and subjective knowledge: impacts on consumer demand for genetically modified foods in the United States and the European Union. *AgBioForum* 7, 113–123
60 Huffman, W.E. (2003) Consumers' acceptance of (and resistance to) genetically modified foods in high income countries: effects of labels and information in an uncertain environment. *Am. J. Agric. Econ.* 85, 1112–1118
61 Rousu, M. *et al.* (2007) Effects and value of verifiable information in a controversial market: evidence from lab auctions of genetically modified food. *Econ. Inquiry* 45, 409–432
62 Huffman, W.E. *et al.* (2006) The effects of prior beliefs and learning on consumers' acceptance of genetically modified foods. *J. Econ. Behav. Organ.* 6, 193–206
63 Colson, G.J. *et al.* (2011) Improving the nutrient content of food with genetic modification: evidence from experimental auctions on consumer acceptance. *J. Agric. Resour. Econ.* 36, 343–364
64 Nature Editorial (2013) Fields of gold. *Nature* 497, 5–6
65 Nature News Feature (2013) GM crops. A story in numbers. *Nature* 497, 22–23
66 Areal, F.J. *et al.* (2011) Attitudes of European farmers towards GM crop adoption. *Plant Biotechnol. J.* 9, 945–957
67 Breustedt, G. *et al.* (2008) Forecasting the adoption of GM oilseed rape: evidence from a discrete choice experiment in Germany. *J. Agric. Econ.* 59, 237–256
68 Lawson, L.G. *et al.* (2009) Perceptions of genetically modified crops among Danish farmers. *Acta Agric. Scand. C Food Econ.* 6, 99–118
69 Hall, C. (2008) Identifying farmer attitudes towards genetically modified (GM) crops in Scotland: are they pro- or anti-GM? *Geoforum* 39, 204–212
70 Peter, F. (2008) *Democratic Legitimacy*, Routledge
71 Mill, J.S. (1859) *On Liberty*, Longman, (Roberts & Green)
72 Larmore, C. (1987) *Patterns of Moral Complexity*, Cambridge University Press
73 Rawls, J. (1993) *Political Liberalism*, Columbia University Press
74 Gaus, G. and Vallier, K. (2009) The roles of religious conviction in a publicly justified polity. *J. Philos. Soc. Crit.* 35, 51–76
75 Waldron, J. (1999) *Law and Disagreement*, Oxford University Press
76 Pettit, P. (1997) *Republicanism: A Theory of Freedom and Government*, Oxford University Press
77 Brar, D.S. and Khush, G.S. (1997) Alien introgression in rice. *Plant Mol. Biol.* 35, 35–47
78 Chahel, G.S. and Gosal, S.S. (2002) *Principles and Procedures of Plant Breeding: Biotechnological and Conventional Approaches*, Alpha Science International
79 Warschefsky, E. *et al.* (2014) Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. *Am. J. Bot.* 101, 1791–1800
80 Knott, D.R. (1989) In *The Wheat Rusts – Breeding for Resistance, Monographs on Theoretical and Applied Genetics* (Vol. 12), pp. 162–181, Springer-Verlag
81 Atwell, B.J. *et al.* (2014) Could abiotic stress tolerance in wild relatives of rice be used to improve *Oryza sativa*? *Plant Sci.* 215/216, 48–58
82 Schouten, H.J. and Jacobsen, E. (2008) Cisgenesis and intragenesis, sisters in innovative plant breeding. *Trends Plant Sci.* 13, 260–261
83 Endo, M. *et al.* (2007) Molecular breeding of a novel herbicide-tolerant rice by gene targeting. *Plant J.* 52, 157–166

84 Saika, H. *et al.* (2011) Application of gene targeting to designed mutation breeding of high-tryptophan rice. *Plant Physiol.* 156, 1269–1277

85 Cuellar, W. *et al.* (2006) Self-excision of the antibiotic resistance gene nptII using a heat inducible Cre-loxP system from transgenic potato. *Plant Mol. Biol.* 62, 71–82

86 Dang, T.T. *et al.* (2013) Gene editing a constitutively active OsRac1 by homologous recombination-based gene targeting induces immune responses in rice. *Plant Cell Physiol.* 54, 2058–2070

87 Thole, V. *et al.* (2007) The pCLEAN dual binary vector system for *Agrobacterium*-mediated plant transformation. *Plant Physiol.* 145, 1211–1219

88 Holme, I.B. *et al.* (2012) Cisgenic barley with improved phytase activity. *Plant Biotechnol. J.* 10, 237–247

89 Lozano-Juste, J. and Cutler, S.R. (2014) Plant genome engineering in full bloom. *Trends Plant Sci.* 19, 284–287

90 de Lange, O. *et al.* (2014) From dead leaf, to new life: TAL effectors as tools for synthetic biology. *Plant J.* 78, 753–771

91 Feng, Z. *et al.* (2013) Efficient genome editing in plants using a CRISPR/Cas system. *Cell Res.* 23, 1229–1232

92 Jiang, W. *et al.* (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Res.* 41, e188

93 Miao, J. *et al.* (2013) Targeted mutagenesis in rice using CRISPR-Cas system. *Cell Res.* 23, 1233–1236

94 Xie, K. and Yang, Y. (2013) RNA-guided genome editing in plants using a CRISPR-Cas system. *Mol. Plant* 6, 1975–1983