



Update of Regulatory Options of New Breeding Techniques and Biosafety Approaches among Selected Countries: A Review

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ABSTRACT

A new set of breeding techniques, referred to as New Breeding Techniques developed in the last two decades have potential for enhancing improved productivity in crop and animal breeding globally. These include site directed nucleases based genomic editing procedures-CRISPR and Cas associated proteins, Zinc Finger Nucleases, Meganucleases/Homing Endonucleases and Transcription- Activator Like-Effector Nucleases for genome editing and other technologies including- Oligonucleotide-Directed Mutagenesis, Cisgenesis and intragenesis, RNA-Dependent

DNA methylation; Transgrafting, Agroinfiltration, Reverse breeding. There are ongoing global debates on whether the processes of and products emerging from these technologies should be regulated as genetically modified organisms or approved as conventional products. Decisions on whether to regulate as GMOs are based both on understanding of the molecular basis of their development and if the GMO intermediate step was used. For example- cisgenesis, can be developed using *Agrobacterium tumefaciens* methods of transformation, a process used by GMO but if the selection is properly conducted the intermediate GMO elements will be eliminated and the final product will be identical to the conventionally developed crops. Others like Site Directed Nuclease 3 are regulated as GMOs in countries such as United State of America, Canada, European Union, Argentina, Australia. Progress in genome editing research, testing of genome edited bacterial blight resistant rice, development of Guidelines for regulating new breeding techniques or genome editing in Africa is also covered with special reference to South Africa, Kenya and Nigeria. Science- and evidence-based approach to regulation of new breeding techniques among regulators and policy makers should be strongly supported.

Keywords: *New breeding techniques; regulation; USA; Canada; Australia; EU; Argentina; Africa.*

1. INTRODUCTION

In the last two decades eight new breeding techniques (NBTs) have been developed with potential for biotechnology-based crop improvement but whose regulatory approaches are at various stages of development in several countries. These include site directed nucleases (SDN) based genome methods that include CRISPR and Cas associated proteins such as CAS9, Zinc Finger Nucleases (ZFN), Meganucleases also called Homing Endonucleases and Transcription- Activator Like-Effector Nucleases (TALENs), Oligonucleotide-Directed Mutagenesis (ODM) for genome editing and other technologies including Cisgenesis and intragenesis, RNA-Dependent DNA methylation (RdDM), Transgrafting, Agro-infiltration, and Reverse breeding. This paper is a review of the molecular basis of these approaches, the updated current global status of their biosafety regulation, the opportunities and regulatory challenges in developing regulations in forthcoming countries and gives current initiatives in Africa for regulation of NBTs.

2. CATEGORIES OF NEW BREEDING TECHNIQUES

Previous reviews of regulation of NBTs focused on the broad spectrum diverse methods [1,2,3] with exception of recent publication by Eckerstorfer et al. [4] while more recent review papers have concentrated on regulation of genome editing technology which comprises of SDN applications and ODM [5,6,3,7,8,9], perhaps because the later have more immediate economic applications than the whole spectrum of NBTs. Other reviews have approached regulatory perspective from EU verses the rest of

the world [4], while others looked at it from process-verses-products based approaches [3]. However, most National regulations developed or under development globally cover beyond just genome editing to include more NBTs. The following section briefly reviews the broad spectrum of NBTs inclusive of new improved CRISPR-based genome editing methods.

2.1 Cisgenesis and Intragenesis

In cisgenesis and intragenesis technologies, only genetic components from the gene pool of the same species or sexually compatible species are transferred from the donor and to recipient organism [10,11]. In cisgenesis, the transferred cisgene(s) comprises of an identical natural gene copy with respect to structural and regulatory genetic elements with no transgenes or foreign genetic elements [10]. Intragenesis, the intragene(s) transferred consist of rearranged/recombined structural and regulatory genetic elements derived from genes of same donor species or sexual compatible species [10]. Both methods use similar gene delivery methods used in transgenesis such as agrobacterium-mediated transformation or biolistic transformation [10]. These differences in molecular basis between cisgenesis and intragenesis, methods of transformation influence the different regulatory approaches among countries as outlined later.

2.2 Oligonucleotide-Directed Mutagenesis

Introduction of short to medium sized oligonucleotides complementary to genome sequence except for one or a few bases into a genome induces mutations, a phenomenon termed oligonucleotide- directed mutagenesis

(ODM) [12,13,2,14]. The introduced oligonucleotides associate with complementary genomic sequences and cause sequence mismatches which during subsequent DNA replication cause mutations such as point mutations, reversions, deletions or insertions at the mismatched positions of the target genome sequences [15,14,10]. This technique has been applied successfully for gene editing in various organisms such as bacteria, yeast, mammals and plants [16,17,18,19]. Methods used to introduce ODM into target cells include polyethylene glycol-mediated DNA transfer, electroporation, biolistic or natural cellular DNA uptake [10,20]. Although the genetic change induced by ODM closely emulates conventional mutation breeding methods such as point mutation, there is diversity in regulatory approaches adopted by countries as discussed later in this paper.

2.3 RNA-Directed DNA Methylation

DNA methylation is process in which methyl groups are added to either cytosine or adenine or both DNA bases and is catalyzed by DNA methyltransferases. In certain methylation processes, small interfering RNAs (siRNAs) direct DNA methyltransferases to sites for de novo methylation. These sites usually have complementary sequences to siRNA or are loci from which the siRNAs were transcribed [21,22,23,24]. This pathway of DNA methylation is termed RNA-directed DNA methylation (RdDM) and was first reported by Wassenaar et al. [21].

DNA methylation can change the activity of a DNA segment without changing the DNA sequence. Such changes could include repression of transcription of gene, silencing transposons, or repression of endogenous genes or transgenes [25]. This phenomenon where heritable changes are imparted in an organism gene expression without concomitant changes in DNA sequence is termed epigenesis and is self-perpetuating but reversible [26]. Some epigenetic changes can be inherited and sometimes are persistent in the target organism for a few generations [27]. Studies using genetic analysis and biochemical methods have elucidated many components involved in the establishment and maintenance of RdDM [25]. Application of RNA-Directed DNA methylation technology therefore draws a lot of regulatory discussions and questions among countries as will be outlined later [27].

2.4 Agroinfiltration

Agroinfiltration is a plant biotechnology technique applied to induce transient expression of genes in whole plants or detached leaves or cell culture [28,29,30,31,32] and can also be used to deliver transgenes into plant for large-scale manufacture of recombinant proteins [33]. Agrobacterium-mediated gene delivery is the preferred method in agroinfiltration [34,35,36] and it is introduced into the plant leaf by direct injection using syringe agroinfiltration [36] or vacuum infiltration. The introduced Agrobacterium interacts with a range of plant cells and through a type IV secretion system injects single stranded DNA (ssDNA) into the plant cell. The ssDNA is transported into the nucleus, replicated to make dsDNA and integrated into the host chromosome [37]. Agroinfiltration has several advantages over traditional plant transformation because it is fast, convenient and produces high and consistent quantities of recombinant protein.

2.5 Reverse Breeding

Reverse breeding (RB) is a plant breeding technique designed to produce complementary homozygous breeding lines from heterozygous plants to be used in subsequent breeding of improved crops [38]. With traditional breeding of heterozygous crops, it is not possible to fix heterozygous genotypes by attaining homozygosity. The RB procedure involve reduction in genetic recombination in heterozygote by eliminating meiotic chromosomal crossover. This is followed by *in vitro* culture of the resultant male or female spores obtained from non-recombinant parental chromosomes to produce homozygous doubled haploid plants (DHs). Elite heterozygous plants are produced from these DHs through random hybridization and subsequent selection for the best complementary parents [38].

2.6 Transgrafting on GMO Rootstock and Vice Versa

Transgrafting uses similar methods as in traditional horticultural grafting practices where a scion is grafted onto rootstock usually of same species or a different related species [10]. However, in transgrafting three scenarios are possible: i) conventional scions grafted onto GM rootstocks; ii) GM scions grafted onto conventional rootstocks or iii) GM scions grafted onto GM rootstocks. The primary goal of transgrafting is to improve the quality

characteristics of the rootstocks using recombinant DNA technology. However, in certain cases the conventional scions are grafted onto GM rootstocks to improve their performance while precluding transgenic traits from harvested products [10]. Transgrafting attracts regulatory concerns because of the influence of GM scions on GM rootstocks and vice versa. For example, proteins and metabolites can be translocated across the graft union from rootstock to the scion and vice versa [39].

2.7 Nuclease- Directed Site Mutagenesis

Nuclease-directed mutagenesis are methods of introducing mutations in plants and other organisms based on enzymes categorized as Site specific-nucleases (SSN). Four classes of synthetic site-specific nucleases have been developed for genetic modification of many organisms including crops [40,41,42]. These nucleases combine two functions, a domain that recognizes a specific DNA sequence called DNA recognition domain and a second domain called DNA breaking domain that introduces double strand breaks at specific sites of genomic DNA. The breaks subsequently trigger either one of the two naturally occurring DNA repair mechanisms found in plants namely - non-homologous end joining (NHEJ) or homologous recombination (HR). Because of the differences in the source of enzymes and differences in enzymatic characteristics of enzyme domains four classes of SSN have been identified: Zinc-finger nucleases (ZFN) [43,44,45,46]; Transcription activator effector-like nucleases (TALENs) [47,48,49,46,50], Meganucleases / Homing endonucleases (HEs) [51,52,53,54,55] and CRISPR/Cas-nucleases [56,57,58,59,60,61,62, 63,64,65].

2.7.1 Classification of site-specific nuclease applications

The broad classification of Site-specific nuclease (SSN): SSN-1, SSN-2 and SSN-3 [66] correspond to the classification into Site-Directed Nucleases (SDNs)- Site- Directed Nuclease-1 (SDN-1), SDN-2, and (SDN-3) as outlined below and these influence global regulation approaches to genome editing.

SDN-1. The SDN-1 mechanism is achieved when the SDN is designed to target a specific DNA sequence without addition of a DNA repair template. In addition, the SDN-1 process can be designed to make one double strand DNA break

(DSB) or two double strand neighboring DNA breaks. Single strand DNA break produced targeted site-specific random mutations at the genomic site(s) that have the nuclease recognition sequence. Because of precision in selection of DSB while the host cell DNA repair mechanism is random, the final products could have base deletions, additions or substitutions. In somatic plant cells the single DSB in the genomic DNA are mainly repaired by the non-homologous end joining mechanism [66,42]. In cases where SDN-1 is designed to make DSB, deletions are generated between the two target sites [67].

SDN-2. SDN-2 mechanism is achieved when SSN is introduced into genome in the presence of a short template DNA. The template DNA also called donor template is designed to match the targeted DSB DNA sequence site except for one or a few bases. SSN will make double DNA strand break at the targeted DNA sequence and the donor template used to repair the break resulting in substitution of the original host DNA sequence with the template sequence that contains the desired mutations [68].

SDN-3. SDN-3 is like SDN-2 process above except that the DNA template added will also contain genes of interest which can be transgenes and during the repair process these genes will be incorporated into the host genome. Both SDN-2 and SDN-3 use the homologous recombination (HR) mechanism to repair the double DNA break. One advantage of SDN-3 approach is that gene of interest is integrated precisely at specific target location compared to conventional GMO technology which produce random DNA integration [10].

Gene Delivery Mechanisms. The methods used for gene delivery of expression vectors containing SSN and donor DNA into recipient plant cells include electroporation, agrobacterium-mediated transfer and shot-gun DNA delivery method. The final product SSN can be modified to either produce temporary integration by segregating out the SSN-transgene [69,1]. In permanent SSN integration leads to stable integration of the SSN-encoding gene and its subsequent expression in host genome [10].

Despite the application of TALENs, Zinc Finger Nucleases, Meganuclease in genome editing CRISPR/CAS systems have emerged as the most widely used [70,71]. In addition, five new CRISPR/Cas based systems have been

engineered with improved efficacy and reduced off targets, attributes that would reduce regulatory concerns.

2.7.2 Recent developments in CRISPR-based genome editing approaches

Five recent developments in CRISPR-based genome editing approaches include use of Cas12a, base editors, prime-editing, DNA-free editing and CRISPR/cpf1 [72]. Two categories of Base editors include cytosine-base editors (CBEs) and adenine-base editors (ABEs) [72]. CBEs were first reported in 2016 [73] with improved efficacious versions [74,75]. Similarly, ABEs were reported in 2017 [76] with improved versions [77,74,78]. Dual base editors combining both CBEs and ABEs have been developed [79,80]. ABEs and CBEs are favorable from regulatory perspective because of improved efficacy and reduced off-target effects compared to CRIPR/CAS9 based systems.

DNA free genome editing, using biolistic delivery system to introduced CRISPR/Cas9 ribonucleoprotein in Arabidopsis, lettuce, wheat among others has been reported [81,82,83,84,85]. The method results in transient expression of CRIPR/CAS9, precludes integration of CRISPR/Cas9 genomic DNA, eliminating off-target effects [81]. CRISPR/Cas12a System belonging to Class 1 (types I, III and IV) systems uses multiple Cas proteins in CRISPR ribonucleoprotein effector nucleases [86,87]. It is an RNA guided endonuclease with improved precision and reduced off-target effects [86].

Prime editing uses a modified Cas9 protein and a guide RNA called pegRNA. The Cas9 protein makes single strand nick of the double helix while the pegRNA contains an RNA template used to synthesis new DNA sequence at target site by transcriptase enzyme attached to Cas9 [88,89]. This method has application in human health such as in providing remedies for disease such as sickle cell anemia.

CRISPR-cpf1 is a class 2 type IV CRISPR/Cas system and Cpf1 cleaves DNA via a staggered DNA double-stranded break [90]. Despite several advantages over Cas9 its adoption is curtailed because of target-dependent insufficient indel efficiencies [91], a constraint alleviated by new engineered CRISPR RNA (crRNA) with highly efficient genome editing by Cpf1 [91].

Globally, several countries and regions have adopted variable regulatory options to regulate the preceding outlined NBTs while others are in the process of developing regulations and or Guidelines. The following section covers North America: USA and Canada, Latin America: Argentina, European Union, Australia, Australia, Food Standard of Australia and New Zealand and Africa.

3. REGULATION OF NEW BREEDING TECHNIQUES IN NORTH AMERICA-USA

Regulation of Biotechnology in the USA is product-triggered and not process-triggered. The regulatory responsibility is implemented by three separate agencies: USDA- Animal and Plant Health Inspection Services (APHIS), US Environmental Protection Agency (EPA) and US Food and Drug Administration (FDA) [92]. In 2015, the USA endeavored to modernize the Biosafety Regulatory systems and enhance public confidence in the system. Four key aspects of focus were: to improve its transparency, enhance predictability, improve coordination (among FDA, USDA and EPA) and enhance its efficiency [93,94]. Clarity in regulation NBTs in USA emanated first from statement by USDA Secretary in 2018 [95] and later in 2020 from revised USDA Regulations [96]. Both pronouncements are in concurrence, but more clarity is in the later than the former.

3.1 Recently Published USDA-for New Regulations Biotechnology Innovation

In the recently published USDA regulations which covers NBTs the following will not be regulated [96]: 1) a genetic modifications resulting from the a cell repairing a targeted DNA break where no external repair template is added; or 2) the target genetic modification that results in a single base pair substitution; or 3) a genetic modification that introduces a gene into plant that comes from the same gene pool (*gene pool refers to all genes in an inter breeding populations*) as the modified plant, or 4) makes changes in a targeted sequence to correspond to a known allele of such a gene (*allele refers to alternative form(s) of a gene*) or to a known structural variation present in the gene pool. In addition, the Administrator was empowered to exempt plants having additional modifications, if similar modification

could be achieved through use of conventional breeding techniques [96].

Cisgenesis. From the recently published USDA-APHIS regulations, cisgenesis would be exempt from regulation under category under 3 above [95,96]. This is exemplified by exemption cisgenic salinity tolerant developed by NexGen using biolistic transformation. The event contains gene duplication and recombination processes that gave extra copies of the native salt tolerance gene DREBJA [97]. In contrast the cisgenic Scab resistant apple cultivar Gala developed by Wageningen UR by Schouten in 2012 applied for release in USA would be regulated under "AIR" because of use of plant pest *Agrobacterium tumefaciens* as transformation vector [98]. Intragenesis on the other hand would be on case-by-case but may be considered under 4 above [96].

Cibus Canola example of non-Regulation of ODM. In USA products from products from ODM would not be regulated under USDA exemption of regulating plants derived from processes similar to that could be developed through traditional breeding, provided that genes were not derive from a plant pest, nor is the vector for transformation or the final product is plant pest [95]. Cibus Canola with increased tolerance to imidazolinone and sulfonylurea herbicide, developed by ODM single nucleotide mutation in the *BnAHAS1C* and *BnAHAS3A* genes was exempted on this basis [92,96,99,100].

Exemption of SDN-1 from Regulation. Exemption of SDN application falls under the "category 1 above" provided the donor of the genes or DNA sequences inserted, or vector used for transformation should not be a plant pest [95,96]. An example of SDN, exemption is USDA-APHIS Decision on non-regulatory status SDN1 in waxy maize- SDN1/NHJE genome editing via CRISPR/Cas9 that is also a null segregant in which the waxy gene (*Wx1*) was deleted [101].

Non-regulatory Status of SDN-2 Maize resistant to Northern Blight in USA. The Northern Blight of resistant maize developed through CRISPR CAS 9 is not a regulated product pursuant to 7 CFR part 340" [102]. It developed by deleting the northern blight susceptible allele (NLB18) in genotype target and replacing with resistant allele (NLB18) from disease resistant maize genotype through double DNA strand breaks [10].

Exemption of Rice Resistant to Bacterial Blight. Genome edited (*SWEET11*, *SWEET13* *SWEET14*) rice resistant to bacterial blight developed by the University of Missouri [10] used *Agrobacterium tumefaciens* to introduce CRISPR-Cas gene editing reagents into rice cells. No DNA repair template was provided, and conventional breeding was used to select progeny that contained the intended edits without the introduced exogenous DNA [103]. Recently, USDA-APHIS stated that this genome edited rice line is not regulated pursuant to 7 CFR part 340, because the rice is not a pest and no plant pest sequences were inserted in it [104].

The regulatory approach adopted in the USA in regulating GM and NBTs is mainly classified as products-based, however if the source of genes or genetic components are derived from a crop pest or used plant pest it depicts some features of process-based approach. It is also notable that in US herbicide tolerant ODM developed by Cibus Canola was exempt from GM Regulation while in Canada it was classified as "Novel" and regulated as outlined below.

3.2 Regulation of Novel Foods Including New Breeding Techniques in North America, Canada

Health Canada and the Canadian Food Inspection Agency (CFIA) regulate all foods sold in Canada, including "novel foods" [105]. "Novel food" means: a) substance, including a microorganism, with no history of safe use as a food; b) manufactured food, prepared, preserved or packaged by a process that: i) has not been previously applied to that food, and ii) causes the food to undergo a major change; and c) a food derived from a plant, animal or microorganism that has been genetically modified such that: i) the plant, animal or microorganism exhibits characteristics not previously observed in that plant, animal or microorganism, ii) the plant, animal or microorganism no longer exhibits characteristics previously observed in that plant, animal or microorganism, or iii) one or more characteristics of the plant, animal or microorganism no longer fall within the anticipated range for that plant, animal or microorganism [105,106].

With regards to new breeding techniques, in Canada all foods classified as "novel" are regulated this would include cisgenesis or intragenesis, ODM, transgrafting, site directed mutagenesis (SDNs) and RdDM. Cibus Canola

Event 5517 developed through ODM present an example of application of "Novelty". In Canada unlike in USA, Cibus Canola Event 5517 produced through ODM was regulated. In Canada this ODM produced foods was classified "novel" according to the preceding regulations safety food assessment of novel foods and novel food ingredients in *Food and Drug Regulations* (Division 28) and regulated [105].

In the following sections regulation of NBTs in Latin America is represented by Argentina which is one of the global pioneers in developing regulations for NBTs and is often used as model for forthcoming regulatory systems for NBTs in Africa.

4. REGULATION OF NEW BREEDING TECHNIQUES IN LATIN AMERICA- ARGENTINA

The National Advisory Commission on Agricultural Biotechnology of Argentina (CONOBIA) regulates GMOs in Argentina. Argentina uses the Cartagena Protocol on Biosafety definition of Living Modified Organisms (LMOs) which defines "LMO" as any living organism that possesses a novel combination of genetic material obtained using modern biotechnology [107]. Argentina uses this definition as "new combination of genetic material" to help classify what their legislation regulates under GM Act. In 2015, Argentina was among the first countries globally to discuss the regulation of NBTs and the team covered cisgenesis, intragenesis, SDNs, RNA-RdDM, agroinfiltration, ODMs, transgrafting [107]. Argentina made the following outlined decisions on regulation of NBTs.

Cisgenesis and intragenesis. Cisgenesis, intragenesis are considered as generated using artificially made genetic constructs which may have a structural or regulatory gene functions. Therefore, products from cisgenesis and intragenesis are regarded as "new combination of genetic material" and regulated as GMO [107]. This is in accord with report by Schuttelaar et al. [1] where Community Environment Working Group (CEWG) considered cisgenesis to be GM due to presence of complete genes or parts of genes.

SDNs. *SDN-1* and *SDN-2*, were considered as modifications in plant genome through small deletions in the already existing native plant

genome sequence that do not lead to "a new combination of genetic material". However, where a transgenesis intermediate with SDN gene is obtained, there should be evidence of its removal in the final product for exemption from GMO regulations [107,108]. In a recent consideration because of practical applications, SDN-1 is exempt for GMO regulation [108] while SDN-2, will be on case-by-case basis. However, it will be regulated as GMO if open reading frames (ORFs) are modified or if new DNA sequences are introduced into coding regions. In contrast, for SDN-3 artificial genetic constructs are introduced into the host genome and it is a "a new combination of genetic material" and regulated as GMO [1,108].

ODM, RdDM, Reverse Breeding Transgrafting and Agroinfiltration. In ODM, the nature and extent of modification on the plant genome is variable and could led to GM products or non-GMO, but because of its practical application ODMs don't qualify as GMOs [109,110]. Similarly, RdDMs are exempted from GM regulations and are not "a new combination of genetic material" and in addition because of instability and ability to revert they may have little commercial application [107]. Products obtained through *Reverse breeding* technique would not be regulated are GM. However, if the transgene intermediate was used in their development, it should be removed and evidence provided [107]. For transgrafting, commercially released whole plants will be regulated as GM and assessed for food and environmental safety irrespective whether GM part is the rootstocks or scions or both [107].

The regulation of NBTs in Argentina is both process- and product-based, founded on sound science and also considers practical application of technologies. In the following, the European Union approach to regulation of mutagenesis is covered and the approach shows a distinct departure from the preceding North America and Argentina approaches.

5. REGULATION OF NEW BREEDING TECHNIQUES IN EUROPEAN UNION

The European Union GMO Legal framework consist of five main legislation pieces and among them is Directive 2001/18/EC on the deliberate release and placing on the market of GMOs [111]. Others include Regulation (EC) No. 1829/2003 [112], Directive 2009/41/EC, Directive

(EU) 2015/412 [113]. Regulation (EC) 1830/2003 [114].

The EU High Level Group of Scientific Advisory categorized NBTs into genome editing techniques- comprising of site-directed nucleases and ODM and also covered cisgenesis and intragenesis, epigenetic modification- RdDM, reverse breeding and agroinfiltration; transgrafting [115,116]. In November 2018, the European Court of Justice ruled that products developed from new mutagenesis i.e. genome editing fall under the scope and obligations of the GMO directive in the EU and should be regulated as GMOs [117]. It has been suggested that static definition of GMOs attributed to this broad categorization of genome editing under “mutagenesis” under EC GM Act [117]. EU law defines GMOs as 'genetically modified organism means 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' – Directive 2001/18/EC, Article 2(2). This definition refers to both characteristics of the techniques used and organism obtained [117]. One key challenge in adopting the EU approach in regulation of genome edited crops will be inconsistency when you consider two organisms with equivalent genetic change such as base indel, one developed through conventional mutation breeding and another through genome editing will be subject to two contrasting legislations.

The following section covers recent regulations of NBTs in Australia that cover broad spectrum of NBTs beyond genome editing and progress in the FSANZ on the same.

6. REGULATION OF NEW BREEDING TECHNIQUES IN AUSTRALIA

In Australia, gene technology is regulated by the Commonwealth Gene Technology Act 2000 and Gene Technology Regulation 2001, in conjunction with corresponding State and Territory legislation. Seven Amendments have been made on the Act and Regulations [118]. However, the amended Gene Technology Amendment (2019 Measures No. 1) is pertinent to the regulation of NBTs. NBTs classified as GMOs include “Organisms modified by ODM; “Organisms modified by repair of single-strand or double-strand breaks of genomic DNA induced by a site-directed nuclease, if a nucleic acid

template was added to guide homology-directed repair”; An organism modified by repair of single-strand or double-strand breaks of genomic DNA induced by a site-directed nuclease, if a nucleic acid template was not added to guide homology-directed repair; An organism that was modified by gene technology but in which the modification, and any traits that occurred because of gene technology, are no longer present [119].

Cisgenesis and ODM. In Australia, there will be case-by-case assessment of products obtained cisgenesis to determine if adventitious foreign genetic material such as T-DNA or border sequences are present and if absent then they will be exempt from GM regulations. On the other hand, ODM produced products will be regulated as GM as specified under the amended Gene Technology Amendment (2019 Measures No. 1) [118,120].

SDNs. Based on OTGR technical review report description, SDN-1 fall under the category of organisms that are produced from repair of targeted double-strand break where no template is added [121]. According to the revised guidelines organisms these should not be regulated as GMOs [119]. This is in accord with recent report that SDN-1, null segregants are not regulated under GM Act while SDN-2 are regulated [122]. In Australia SDN-3 will be regulated under GM Act. From recent report, SDN-3 involves template-guided repair of a targeted double-strand break using a long template to insert new sequences [121] and falls within the description of regulated NBTs under the amended regulations [119] and in concurrence with recent report by Thygesen [122].

Agroinfiltration, Reverse Breeding and Transgrafting. In Australia, agroinfiltration is regulated as GM used for research purposes and under research conditions. However, commercial products produced from plants selected through agro-infiltration will not be regulated under the GM Act, provide the applicant gives proof of no *Agrobacterium tumefaciens* or foreign genetic material in the progeny and this will be handled on case-by-case basis [121]. In contrast, transgrafted plants will be regulated as GMOs (OGTR, 2016). Reverse Breeding and null segregants will not be regulated under GM Act [121]. Similarly, in a recent report, it was stated that null segregants including reverse breeding don't fall under GMO Regulations [122].

7. REGULATION NEW BREEDING TECHNIQUES BY FOOD STANDARDS OF AUSTRALIA AND NEW ZEALAND

In June 2017, Food Standards Australia New Zealand (FSANZ) reviewed Australia New Zealand Food Standards Code (the Code) to consider regulation of food derived from NBTs covering cisgenesis, intragenesis, ODM, SDNs, transgrafting and RNA-directed DNA methylation (RdDNAm) [123]. The following section outlines selected example remarks on SDNs and progress to date in regulation NBTs under FSANZ.

SDNs. FSANZ covered two mechanisms of cell's own enzyme machinery used to repair the break in the DNA – non-homologous end joining (NHEJ) or homology directed repair (HDR) [124]. FSANZ recognized that under HDR a DNA template which has a DNA sequence that complements the DNA sequence at the break site is used and this can be supplied externally and can be designed to introduce precise modifications to the DNA sequence during the repair process ranging from indels to insertions of new pieces of DNA such as whole genes. Use of SDNs to introduce new genes is a form of transgenesis, the only difference is that the DNA is inserted at a precise location, rather than randomly. This is description equivalent to SDN3 [124]. However, the final FSANZ regulatory determination is still under consideration for all the NBTs outlined above.

The following section covers initiatives contributing to developing regulation/guidelines for regulating new breeding techniques or genome editing in Africa covering South Africa and forthcoming Africa Union Member states.

8. TECHNICAL REPORT ON REGULATION OF NEW BREEDING TECHNIQUES IN SOUTH AFRICA

The Academy of Science of South Africa (ASSAf) conducted a technical review to provide evidence-based scientific advice to South African policymakers on regulation NBTs [125]. NBTs considered were ZFNs, Meganucleases, TALENs and CRISPR/Cas system (Cas9) and ODM products; cisgenesis, transgenesis, transgrafting; null-segregants including reverse breeding, accelerated breeding, RdDM and other technologies including gene drives and synthetic biology [125]. The ASSAf technical analysis and

proposed potential regulatory recommendations are outlined in the following section.

Cisgenesis, Intragenesis, ODM, RdDM and Transgrafting. Cisgenesis involves targeted insertion of homologous, functional gene sequences, including genes and/or regulatory sequences (cisgenes) and therefore it was recommended not to be regulated as GMO. Intragenesis involves “targeted insertion of reorganized, homologous, functional gene sequences, including genes and/or regulatory sequences (intragenes) and recommended to be regulated under the GMO Act. ODM mutation involve small, targeted inserts or deletions and recommended not to be regulated as GMs [125]. RdDM enables release of varieties with inherit epigenetic modification, but does not induce transgene and was recommended exemption from GMO Act. In case of GM rootstock and non-GMO scion or vice versa: the GMO part and its products should be regulated as GM while the non-GMO part and its products should not be regulated as GM [125].

SDNs. According to ASSAf [125] SDN-1 involves “small, targeted and untargeted inserts or deletions based on non-homologous end joining (NHEJ)” resulting from ZFNs, MNs, TALENs and CRISPR/Cas and should be exempt from GM Act. SDN-2 uses homologous donor DNA template, that is a copy of the target gene sequence with only a small modification, that is used in HDR repair process thus inducing SDNs and should not be regulated under GMO Act. SDN-3 applications unlike the homologous donor DNA includes (a) complete gene sequence(s) e.g. cis-, intra- and/or transgene sequences and therefore should be regulated. In addition, “targeted and untargeted insertion of heterologous, functional gene sequences, including genes and/or regulatory sequences that are transgenesis whether by “conventional GM technology” or SDNs should be regulated under the GMO Act.

Agroinfiltration, Reverse Breeding and Null Segregants. ASSAf [125] discussed global experiences in regulating agro-infiltration but provided regulatory recommendations. Reverse Breeding could be generated through a GM intermediate which if segregated out give null segregants, that should be GMO Act.

Unlike South Africa, which commission a study on regulation of NBTs other Africa countries have received technical support from Africa Union

Development Agency-NEPAD among other agencies as Program for Biosafety Systems of IFPRI in genome editing.

9. AFRICA UNION DEVELOPMENT AGENCY-NEPAD MANDATE AND BIOSAFETY IN AFRICA

Africa Union's support for the safe application of biotechnology for socio-economic development of Africa countries started in 2007 based on Panel of Expert Report on "Freedom to Innovate". The report covers safe application of biotechnologies to improve agriculture, health, industry, economic competitiveness, sustainable environment including biodiversity for socio-economic transformation of Africa [126]. Pertinent to ensuring biosafety the African Biosafety Network of Expertise a program in AUDA-NEPAD was established in 2010 to support building of functional biosafety regulatory systems and today it has impacted on 23 AU Member States but with future mandate to reach 55 countries [127]. It is notable that regulation of genome editing or the broader NBT in Africa is building on the regulatory systems developed based on regulation of GMOs.

9.1 Genome Editing Research in Africa and towards Revised Regulations and or Guidelines

Today, Africa has ongoing genome editing research. The Kenya National Biosafety Authority (KNBA) approved development of banana resistant to viruses and aphid vectors by the International Institute of Tropical Agriculture (IITA); yams resistant to yam mosaic virus, anthracnose and with enhanced vitamin A (IITA), vaccines to control African Swine Fever under International Livestock Research Institute (ILRI); enhancement of nutritional and agronomic traits of grass pea (ILRI) and goats resistant to trypanosomiasis (ILRI) [128]. In addition, there is application for testing of genome edited rice.

9.2 Evaluation of Genome Edited Rice Resistant to Bacterial Blight

In 2020, an application for testing of genome edited rice resistant to bacterial blight (BB) developed using CRISPR-Cas9 induce mutations in all three *SWEET* gene promoters [129] was submitted in West Africa. The BB is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), secretes one or more of six known

transcription-activator-like effectors (TALEs) that bind specific promoter sequences and induce, at minimum, one of the three host sucrose transporter genes *SWEET11*, *SWEET13* and *SWEET14*, the expression of which is required for disease susceptibility [129]. It is notable that this is the same genome edited rice covered under USDA "AIR" in the preceding sections.

9.3 Pioneers in Development of Genome Editing Guidelines- Kenya and Nigeria

In late 2019, both Kenya and Nigeria embarked on developing Guidelines to clarify the regulation of genome edited organisms and their products. In Kenya, it was found appropriate that genome editing be regulated under Biosafety Act 2009, and Regulations 2001 and no revision was made on this regulatory framework however Draft Guidelines have been developed to clarify their regulations [130].

9.4 Kenya's Genome Editing Guidelines

The Guideline outlines genome editing methods including ODM and SDNs applications using meganuclease, ZFNs, TALENS, CRISPR/Cas system; covers regulation of genome editing in plants, animals and microorganisms, and uses the Cartagena Protocol on Biosafety definition of LMOS to define GMOs. Genome edited organisms/products not to be regulated will include all modifications done using genes from sexually compatible species and also where gene regulatory elements are from the same species; all deletions/knockouts provided the regulatory elements are from the same species; processed products whose inserted foreign DNA sequences cannot be detected; natural processes such as conventional breeding, mutations, polyploidy. GM regulation will apply to - all insertions containing foreign genes, regulatory elements from a non-sexually compatible species where foreign DNA sequence are detectable, uses of markers- selectable and reporter genes present in the product in subsequent generations; cases where developmental phase starts with a GMO, KNBA will regulate up to the stage where GMO intermediate is removed [130]. This Guideline mainly focuses on genome edited organisms, but also description covers cisgenesis, they recognize genome edited products with transgene intermediate which if segregated out should be exempt from GM regulations.

9.5 Nigeria Draft Genome Editing Guidelines

In 2019, the Nigeria National Biosafety Management Authority (NBMA) inceptioned a stakeholder capacity strengthening in the science and regulations of genome editing to identify key steps needed to develop policy guidelines. Three distinct potential products from genome editing were identified- products equivalent to conventional breed crops, products that have GMO intermediate which is eventually segregated out and products which are transgenic in nature. NBMA has embarked on development of Guidelines on regulation genome edited technology.

10. GENERAL OBSERVATIONS AND RECOMMENDATIONS

Regulation of NBTs and or genome editing among pioneer countries globally takes diverse approaches and hopefully the key primary goal is to apply new technologies for socio-economic development of the societies while ensuring safety to humans, animals and environment. Because these diverse approaches will shape the future regulatory approaches adopted by emerging countries in Africa, South America and Asia, it is important that solid science and understanding of the technologies will be a key consideration in developing new regulatory systems with aim to ensure predictability, consistence, efficiency and coordination in regulatory decision.

The published USDA-AHPIS regulation on New breeding techniques gives a comprehensive coverage of new breeding techniques. Argentina and Australia provide good science-based approach to regulating new breeding techniques. The EU legal Court ruling based regulatory approach to “mutagenesis” may challenging to emulate by many countries and may lead to inconsistency and lack of predictability in regulatory decision.

Many Africa Union Members have inceptioned capacity strengthening and developing Guidelines in regulation of genome editing technology and have expressed interest in adopting a science-based approach in developing Guidelines on regulation of genome editing.

11. CONCLUSIONS

The application of broad New Breeding Techniques or the narrow genome editing

technology is expanding rapidly and commercial products have already reached market and many more are in pipeline which if harnessed safety could positively impact on socio-economic development of societies globally. The global advancement in development of regulatory approaches to NBTs is commendable but slightly lagging behind progress in technology and this disparity needs to be alleviated. The globally adopted regulatory approaches to NBTs or genome editing though diverse could be synchronized by using sound science-based approaches as a common denominator in decisions pertinent to safety of NBTs. Developing countries such as Africa are gradually aligning their regulatory frameworks to address NBTs/genome editing and preliminary appraisal is that within each country's sovereignty many prefer to use science-based approach in developing regulatory frameworks to ensure predictability, consistence, efficiency in making regulatory decisions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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