**New Plant Breeding Techniques and their regulatory implications: An opportunity to advance metabolomics approaches**

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**Abstract**

Over the previous decades, biotechnological innovations have led to improved agricultural productivity, more nutritious foods and lower chemical usage. Both in western societies and Low Medium Income Countries (LMICs). However, the projected increases in the global population, means the production of nutritious food stuffs must increase dramatically. Building on existing genetic modification technologies a series of New Plant Breeding Technologies (NPBT) has recently emerged. These approaches include, Agro-infiltration, grafting, cis and intragenesis and gene editing technologies. How these new techniques should be regulated has fostered considerable debate. Concerns have also been raised, to ensure over-regulation does not arise, creating administrative and economic burden. In this article the existing landscape of genetically modified crops is reviewed and the potential of several New Plant Breeding Techniques (NPBT) described. Metabolomics is an omic technology that has developed in a concurrent manner with biotechnological advances in plant breeding. There is potentially further opportunities to advance our metabolomic technologies to characterise the outputs of New Plant Breeding Technologies, in a manner that is beneficial both from an academic, biosafety and industrial perspective.

**Keywords**

Metabolomics, New Plant Breeding Techniques, Sequence Specific Nuclease

**Abbreviations**

LMICs-Low Medium Income Countries, NPBT-New Plant Breeding Technologies, MAS-Marker-Assisted Selection,GM**-**Genetically Modified, EFSA-European Food Standard Agency, LC-Liquid Chromatography**,** MS-Mass Spectrometry, NMR-Nuclear Magnetic Resonance, DiMS-Direct infusion Mass Spectrometry, GC-Gas Chromatography, FID-Flame ionisation detector, UHPLC-, HPLC-High Performance Liquid Chromatography, PDA-Photodiodearray, FLD-Fluorescence detector, MRM-Multiple Reaction Monitoring, SECURE-Ecological, Consistent, Uniform, Responsible, Efficient, GMO-Genetically Modified Organism, ODM-oligonucleotide directed mutagenesis, SSN-sequence specific nuclease, RdDM-RNA-dependant DNA methylation, DSBs-double stranded breaks, ZFNs-Zinc-finger nucleases (ZFN), TALENS-effector nucleases, CRISPR/Cas-Clustered Regularly Interspaced Short Palindromic Repeat associated protein, NHEJ-Non-Homologues End Joining, HDR-Homologous Directed Repair.

**Introduction**

Food and nutritional security represent a major global challenge. In order to feed a global population of 9.2 billion by 2050, crop/food production must increase by 70% (FAO, 2009). These increases must also occur with less arable land availability, less water, energy input, fertilisers and chemicals and under changed climates (Baulcombe et al., 2009). Additionally, the dwindling of fossil fuel reserves has prompted the need to switch our reliance on petro-chemical derived products to renewable sources, typically using Plant-based initial feedstocks. This is the proposed approach necessary to underpin the bioeconomy (Bugge et al., 2016).

For 1000s of years humankind has used innovative approaches to improve plant-based crops for both food and sources of valuable commodities, including phytomedicines. For example, in the “pre-green” revolution era phenotype selection involving preference, experience and germplasm collections were the predominant approaches used (Doebley et al., 2006). Then, in the 1940s to 60s the “green revolution” delivered a dramatic increase in our capability to produce plant-based foods and products. The technological inputs were vital to this step-change in production, with new varieties and hybrids introduced (Khush, 2001). These new varieties were often outputs from mutagenesis (physical and chemical) programmes. The new germplasm was integrated with novel chemical inputs in the form of fertilisers and pest management formulations.

Subsequently, in the 1990s and beyond, biotechnology input became prevalent with transgenesis and marker-assisted selection (MAS) used as processes to generate new plant varieties. By 1994 the first GM food, Zeneca’s Flavr Savr Genetically Modified (GM) tomato, was marketed in the USA (Barclay, 1994; www, genewatch.org) and then in 1996 Monsanto’s herbicide tolerant GM Soy was commercialised in the USA (www.genewatch.org). The global hectarage used to produce GM crops has continued to grow, and 2014 represented the 19th consecutive year of growth following their commercialisation (James, 2014). However, although GM crops have been successfully implemented in North America, in Europe the situation is the opposite where very few GM crops have been grown. This is mainly due to consumer perception (Bonny, 2003) and the burden of costs associated with regulatory approval (Wesseler et al., 2019). To date, GM crops represent one of the most tested technologies for consumer and environmental safety, which has led to a very comprehensive panel of testing within Europe. The generation of European Food Standard Agency (EFSA) dossiers require the detailed acquisition of data on the GM variety in comparison to their genetic comparators (EC No 1829/2003).

Over the last decade, biotechnological innovations have led to the establishment of New Plant Breeding Techniques (Lusser et al., 2011). These include (i) genome editing via different techniques such as oligonucleotide directed mutagenesis (ODM) and sequence specific nuclease (SSN); (ii) variants of conventional transformation methods including cisgenesis and intragenesis, grafting, and agro-infiltration; and (iii) negative segregants obtained through manipulation of epigenetic landscape (via RNA-dependant DNA methylation, RdDM). These technological advances offer great potential to meet the global challenges associated with food and nutritional security, posed by population growth, consumer demand and climate change. However, the technologies are also creating new challenges for regulators when applying the traditional GMO definition and regulatory framework.

In this article our aim is to describe some of the New Plant Breeding Techniques (NPBT) available, where they are presently located with respect to the regulation of genetic modified materials and the techniques and advances required from an academic perspective to improve the characterisation of these new varieties, with a particular focus on metabolomics. The need for evaluation in a risk assessment framework (Kawall et al., 2020; Paoletti et al., 2008; Zimny et al., 2019) and consideration of the economic burden (Ali-Zaidi et al., 2019; Wesseler et al., 2019) in developing these new varieties and implementing their potential is acknowledged but beyond the present expertise and scope of this article. However, it is hoped that some of the technological advances described could help promote the use of modern analytics and actually reduce economic and regulatory burden in the long-term.

**New Plant Breeding Techniques (NPBT) presently available**

*Cis and intragenesis*. A common feature of NPBT is the increased similarity to their non-GM comparator. For example, traditional transgenesis occurs when genes from any organism are inserted into the plant genome, whereas cisgenesis and intragenesis refer to the introduction of genes from a sexually compatible species. The difference between cis and intragenesis is the utilisation of elements of genetic elements from various cross-compatible species for intragenesis, whereas cisgenesis uses a more conservative approach with the presence of unchanged introns and regulatory elements within the target gene (Holme et al., 2013). The high similarity homology between the transferred gene and its endogenous counterpart could cause routine detection/traceability problems. This is because cis/intragenic lines must be free from foreign sequences (*i.e*. selectable markers or reporter genes). The molecular markers used are typically modified endogenous or over expressed gene products linked to herbicide resistance (Liu et al., 2013). It would be interesting to know the long-term effects on the transcriptome and metabolome caused by these selection treatments (Miki et al., 2009).

*Agro-infiltration/infection*. The transient expression of gene products in plants via agro-infiltration has been successful in producing high-value pharmaceuticals, but is also a routine research tool to show gene function. The main production host used for this technique is *Nicotiana benthamiana* or more precisely a specific laboratory line of *N.* *benthamiana* (Bally et al., 2018), that is engineered accordingly. This line contains a loss-of-function mutation in a RNA–dependent RNA polymerase gene (*Rdr*1) (Wylie et al., 2015). That results a higher susceptibility to viruses or *Agrobacterium*, a plant-pathogen capable of mediating genetic transformation of plant cells. *Agrobacterium tumefaciens,* harbouring Ti plasmids, is the vehicle used to transfer the gene of interest into the plant cell. Ideally the *Agrobacterium* is infiltrated in the leaf material and, subsequently, production of the recombinant protein or metabolite of interest occurs three to five days after. Unlike traditional stable plant transformation, multiple *Agrobacterium* strains harbouring different genes of interest can be used simultaneously. Recently, agro-infection has been developed that uses viral vectors for heterologous expression of proteins (Majer et al., 2017). This approach generates systemic viral infection and logistically improves vector delivery and replication inside host cells. The speed of the approach makes the platform ideal for protein products used in the pharma industry, such as influenza (H1N1 and H5N1) vaccines (Landry et al., 2014) and ZMapp, which is an anti-Ebola antibody cocktail (Chen and Davis, 2016). Moreover, it has been suggested that this rapid transient approach could be used to manufacture vaccines and therapeutic antibodies to address the COVID-19 pandemic (Capell et al., 2020). Presently, high-value, low volume products are favoured by this approach which economically promotes pharma product targets. Scale-up into full industrial factories has been achieved (<https://www.pharmaceutical-technology.com/projects/fraunhofernewplant>) for agro-infiltration. In order to optimise production further, the EU Horizon 2020, Newcotiana consortium is developing new *N.* *benthamiana* chassis with reduced protease activities and altered glycosylation properties to improve protein yield and reduce allergenicity. In comparison to recombinant protein, the production of valuable small molecules by agro-infiltration has not received as much attention. This is somewhat surprising because the transient approach and destructive nature of the down-stream processing means, unlike the stable transformation system, toxic molecules or high level production can be achieved using plant-based transient expression, without adverse or lethal pleiotropic effects occurring. To date, a wide array of chemically diverse compounds have been generated using agro-infiltration. These compounds include carotenoids (Nogueira et al., 2019), taxol intermediates (Li et al., 2019), anthocyanins (Fresquet-Corrales et al., 2017), artemisinin precursors (van Herpen et al., 2010) and betalain (Polturk et al., 2016). The process has also been used to achieve gram-scale quantities of bioactive triterpene analogues (Reed et al., 2017). Despite the popular use of agro-infiltration very few studies exist that have characterised the transcriptional and/or metabolome changes arising during the *agrobacterium* infiltration process as a means of directing further chassis development for optimised production of specific chemicals as well as protein yield and quality.

*Grafting*. Rootstocks have been used in agriculture and horticulture for many centuries. Typically the shoot of one plant (known as the scion) can be attached to an elite rootstock. Depending on the rootstock’s properties, traits such as vigour, disease resistance and increased yield can be conferred to the chimeric plant (Goldschmidt, 2014). When the rootstock is GM and the scion not transgenic, the resulting vegetative tissues are classified as non-GM. However, the rootstock can deliver small RNAs to the vegetative tissues. Therefore, GM rootstocks designed to target genes by RNA interference can be used to silence genes of interest in the vegetative tissues and potentially confer a key trait of interest (Zhao and Song, 2014). The potential of grafting has further been exploited, through the discovery of horizontal gene transfer from nuclear and plastid DNA transfer across graft junctions; this transfer can occur between different plant species (Stegemann and Bock, 2009; Stegemann et al*.*, 2012). Using this approach interspecies grafting between tobacco species *Nicotiana tabacum* and *Nictotiana glauca* has led to the generation of a new species (Fuentes et al., 2014). Recently, studies have also bypassed the limitation of genetically close-related species and acheived interfamily grafting. This was revealed to be facilitated by the involvement of extracellular-localised glucanases in cell wall reconstruction near the graft interface. *N.benthamiana* lines were engineered to work as interscion and successfully established grafts with a wide range of species from distinct evolutionary families (Notaguchi et al., 2020). These new grafting technologies open the potential to combine new pathways and create promising novel plant metabolomes.

*Gene editing*. Of all the NPBT, gene editing offers the greatest potential to revolutionise the way we produce food and feedstocks for energy and other commodities. Gene editing in plants has been widely achieved via engineered Sequence Specific Nucleases (SSNs) to provide double stranded breaks (DSBs) at sequence specific points in the DNA. These include Zinc-finger nucleases (ZFN), TAL effector nucleases (TALENS) and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated (Cas) protein SSNs, (Gaj et al., 2013). In common approaches, when SSNs enzymatically produce DSBs in the DNA, repair can occur via Non-Homologues End Joining (NHEJ) or Homologous Directed Repair (HDR), (Shrivastav et al., 2008). SSN-1 is the term used to describe small insertions or deletions derived from NHEJ, which can lead to loss or gain of function mutations in the gene targeted. SSN-2 is used to describe an edited DNA sequence at the site of the DSB in the presence of a homologous donor DNA template, usually via HDR. SSN-3 can result in total allele replacements, targeted gene insertions or their stacking at the DSB site as a result of HDR and suitable donor DNA templates. The SSN technology of choice presently is RNA-guided CRISPR/Cas, which is highly efficient and has ease of use, and has proven to be extremely versatile.

More recently, the Cas9 enzymes have been engineered with extra catalytic domains to carry out base editing and the newest function prime editing (Anzalone et al., 2020; Chen et al., 2019). Both techniques enable gene targeting without requiring donor DNA templates, DSBs or the low efficiency HDR repair. Base deamination can occur resulting in precise simple nucleotide substitutions, whereas prime editors rely on reverse-transcribed DNA that is further incorporated into a targeted site hence broadening editing possibilities beyond substitutions.

Effectively the tools are available to confer precise alterations in a gene that could mimic alleles responsible for conferring important traits of interest in a background specific manner without genetic crossing. Alternatively, the replacement of genes can arise or the stacking of genes improving multiple traits can be achieved. Another option is to use CRISPR/Cas in a multiplex mode where numerous genes of interest can be altered simultaneously. In all cases, following the introduction of the mutation in the primary generation, the option exists to segregate away the Cas gene, further crossing schemes can segregate away the Cas and guide RNA genes. In this way progeny containing potentially no foreign or intragenic DNA can be achieved.

Typically the CRISPR/Cas machinery used to alter target genes/sequences is delivered into plant cells by *Agrobacterium*-mediated transformation or biolistic transformation. The option also exists to introduce the CRISPR/Cas ribonucleotide protein directly into the plant via protoplasts. Indeed, plant regeneration through tissue culture remains a fundamental difficulty in the application of genome editing across a wide range of crops. Promising studies have reported effective gene editing by co-delivering CRISPR reagents and developmental regulators to plant meristems in somatic tissues (Maher et al., 2020).

Applications derived from CRISPR mediated gene editing have been deployed in different perspectives in plant breeding. For instance, multiplexed CRISPR/Cas has been used to perform *de novo* domestication (Zsogon et al., 2017 and 2018), whereby several genes linked to domestication were altered in *Solanum pimpinellifolium,* a wild tomato relative. This approach by-passed years of domestication to generate a new tomato variety that resembled those presently cultivated, yet permits the recovering of resilience lost during domestication. How the transcriptomes and metabolomes of these plants have changed compared to their parental or ancestral counterparts and similar domesticated varieties would be very informative.

**Regulatory considerations for New Plant Breeding Techniques**

The European Commission defines GMOs (with the exception of human beings) as an organism, in which genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Mutagenesis using chemical or ionising radiation is excluded from the definition or regulatory requirements because of its history of use (Lusser et al., 2011). In the US the regulatory process mainly focuses on the product, while in Europe the process involved in generating the GM plant is the focus. This can result in EFSA requirements that detail the molecular characterisation of the GM variety and the process used to generate the event, protein characterisation and equivalence, and compositional analysis. The latter mainly focussing on key constituents recommended by the OECD (OECD, 2012). These data must be determined typically under multiple environmental conditions and statistically analysed. In addition, food and feed safety assessments are required, along with toxicology and nutritional assessments (EC No 1829/2003; EFSA, 2013). The safety dossiers need to include detection, identification and quantification methodologies, and the reference material should also be made available. It is required that these analytical methods are specific to unambiguously identify the GMO in question. NPBT approaches can be used to generate varieties without the presence of any distinguishing foreign DNA. This approach differs from conventional mutagenesis, whereby the random mutation of multiple genes simultaneously occurs or the random insertion of new genes, as occurs with conventional GMOs. In 2018, the European Court of Justice (ECJ) ruled that organisms obtained by new mutagenesis techniques, *i.e.* genome editing are not exempted from the GMO legislation, in contrast to conventional mutagenesis techniques *"that have conventionally been used in a number of applications and have a long safety record"* (CJEU C-528/16; CJEU, 2018). So this has posed the question; how can gene-edited crops/foods, which contain no foreign/distinguishable DNA and potentially natural alleles be effectively detected, both in authorised and non-authorised materials present in the food chain? To address this challenge, new emerging scientific technologies/frameworks will need to be considered, perhaps rethinking the way we evaluate GM derived materials or even change to our current GMO policies. In the US gene edited crops have not been subjected to the same regulation required for traditional GMOs. Predominantly, because the end-products of the process do not contain foreign genetic information or material from plant pests (Camacho et al., 2014). However, the regulations have been revised in 2020 as part of the Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient (SECURE) rule. In this case some gene editing outputs come under the traditional GMO regulations but those varieties with minor and limited modifications are not necessarily applicable. The developers can also self-determine whether they qualify for exemptions (www.geneticliteracyproject.org/2020/06/11/viewpoint-usda-relaxed-its-gmo-gene-edited-crop-rules).

Significant investments have previously been made in the development of “omic” technologies to evaluate and characterise GM plants. Although these approaches generated important informative datasets about the varieties in question and helped foster generic technological advances within the scientific community, the methodologies *per se* were not incorporated into the risk assessments or dossiers, predominantly on the basis of the cost burden associated (Fedorova and Herman 2020). This was particularly pertinent for Low Medium Income countries (LMICs) where the technology could be most useful. Since the initial application of “omic” technologies (Hoekenga, 2008; Noteborn et al., 2000) as a means of evaluating GM crops, the technologies have advanced further. This is particularly true of genomics, where rapid, accurate genome sequencing is now available, adding genotyping and transcriptomics by RNA-Seq to the evaluation techniques. Proteomics is now more robust with improved coverage and ease of quantification. The analysis of plant metabolomes, which are the end-products of the genome, has made notable advances but not to the same extent as other “omic” technologies. The metabolome also represents a chemical composition and will thus still be representative in processed food or feeds, where DNA and protein integrity is compromised. Therefore, potentially the metabolome may not yield non-specific identifying constituents of gene-editing but could provide valuable comparative analysis to existing natural variation, and outputs from NPBT (see Figure 1 for the potential contributions NPBT to the approval process in Europe). In addition, after initial infrastructure acquisition, metabolomics can be cost-effective compared to other “omic” technologies

**Upgrading the metabolomics platforms for better characterisation of NPBT**

The metabolomic characterisation of transgenic plants and mutants has not only played an important role in advancing our understanding of plant metabolism but also advancing metabolomic technologies in their own right. For example, the assessment of substantial equivalence between genetically modified potatoes and conventional cultivars (Catchpole et al., 2005) and the use of NMR to profile transgenic peas (Charlton et al., 2004) were initial examples that brought together robust experimental design, analytical coverage of metabolites and molecular features coupled with discriminative statistical analysis. The studies performed by Roessner et al., 2001a utilised metabolite profiling as a forensic tool to identify why a certain metabolic engineering strategy had not been successful. In addition to these examples, metabolomics has been routinely used to identify changes in plant metabolism associated with abiotic and biotic stresses (Lugan et al., 2010; Roessner et al., 2001b). It has also been used in plant breeding to identify naturally occurring variation (e.g. quantitative trait loci - QTLs) among core collections or mapping populations (Schauer et al., 2006). Reports also exist on the use of metabolomics to identify herbicide mode of action by comparison of herbicides with known and unknown targets (Trenkamp et al., 2009) or to address health-safety aspects (toxicological studies and traceability). Considering the vast efforts in combinatory chemistry and the absence of a novel herbicide mode of action for thirty years, it is surprising that metabolomic approaches to herbicide development have not been adopted in a more universal manner. The use of metabolomics to analyse genome-edited plants were no foreign-DNA is present is also been suggested for a more product orientated evaluation (Fraser et al., 2020). This approach has however, been strongly counter balanced, in view the approach being risk disproportionate (Fedorova and Herman 2020)

Given this prior track-record, there is no reason why metabolomics will not play an important role in characterising outputs from NPBT both academically, as well as from an industrial research and development and safety perspective. One of the major issues is how the significant advances in metabolomics will be made to ensure it is comparable or has the robustness to complement other “omic” technologies. The ultimate goal would be a metabolomic technology that in one analysis enables you to pinpoint the biochemical step or regulatory process altered or how a derived food product differs from its traditional counterparts. Presently and for the foreseeable future this is unlikely to be achieved, due to the intrinsic chemically diverse and dynamic range of the metabolome. However, improvements to the present *status quo* can and should be made. These advances will typically be associated with the biological questions being addressed. For example, if you were characterising a plant with a known alteration in a biosynthetic enzyme the approach would be very different to elucidating the effects of an uncharacterised transcription factor or the surveillance of food products. In the case of a biosynthetic pathway enzyme or regulator responsible for a specific pathway, it is likely that the pathway in question would first be analysed to ascertain end-products and intermediates. Ascertaining this question would then lead to addressing the question how modifying the pathway of interest has altered associated metabolism. A clear example is the characterisation of transgenic plants producing elevated anthocyanins and phenylpropanoids (Tohge et al., 2015; Zhang et al., 2015). In this case the products of the target pathway were comprehensively analysed and then GC/MS metabolite profiling used to ascertain associated changes in primary/intermediary metabolism; showing that a transcription factor regulating anthocyanin production, actually modulated precursor supply. Other examples include the manipulation of carotenoid biosynthesis leading to changes in intermediary metabolism, plastid type and/or phytohormones (Diretto et al., 2019; Fraser et al., 2007).

A common feature of these examples, that is applicable to NPBT outputs, is the comprehensive nature of the analysis performed on the targeted pathway. The precursors are analysed in a quantitative manner, and extractions are optimised. In addition, the intermediates of the targeted pathway are analysed as well as the end-products and their derivatives. For the future it will be important to identify sources of authentic standards or well characterised mutant extracts. In fact, gene–edited mutants across a pathway could act as sources of authentic standards or extracts for identification purposes. One of the earliest and impactful examples of metabolomics/metabolite profiling was described using GC-MS to analyse mutant and transgenic *Arabidopsis* (Fiehn et al., 2000). Since this report, GC-MS has become the “gold standard” in assessing metabolite changes in plants. GC-MS routinely requires derivatisation to reduce polarity and facilitate chromatographic separation. The ability to create customised searchable libraries, typically using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) is an advantage of this approach (Halket et al., 2005). The outputs from these analyses typically result in the relative quantification of 100 to 150 compounds depending on the plant species and tissue used. The classes of compounds amenable to this approach are sugars, polyols, organic acids, amino acids, fatty acids and some terpenoids. There are still a large number of unknowns present in this type of analysis, often matched as “known unknowns” (Goodacre et al., 2004). One approach to increasing the coverage of compounds identified by GC-MS is running more authentic standards or the use of GC separations coupled to high resolution accurate mass spectrometry.

The alternative scenario could be a phenotype or food product (including processed products) where the precise effects of the modification are unknown. For example, outputs from *de novo* domestication, or outputs generated by trans-grafting techniques. In this instance, it would appear that metabolome coverage is key. Untargeted analysis by NMR, direct infusion MS or LC-MS can provide informative chemical fingerprints in this case (Dunn et al., 2011). These technologies typically use extraction protocols that are a compromise to obtain the widest representation of chemical classes possible. LC-High Resolution Mass Spectrometry is becoming the most popular technique to use for this fingerprinting purpose. Molecular features with accurate mass are typically used as variables as opposed to know metabolites, although high resolution MS has the accuracy to determine exact chemical composition but not structure.

The application of multivariate statistical analyses, such as Principle Component Analysis (PCA) and discriminant analysis (DA) like Partial Least Squares (PLS-DA or OPLS-DA) and other packages (Random Forest, K-means clustering, etc.) can typically be used to identify the key differentiators between varieties and comparators (Ching et al., 2019). These molecular features or putative metabolites can then be used to direct more metabolite profiling or even targeted analysis. This hierarchical or multi-layered approach has been used in the determination of metabolite changes in cassava associated with conferring white fly tolerance (Perez-Fons et al., 2019). There are numerous metabolomics work flows that could be used to provide varying degrees of metabolomics characterisation depending on the biological question to be addressed. The potential workflows that could be implemented to characterise outputs from NPBT and the biological questions they raise have been illustrated in Figure 2. Furthermore, the definitions of the different types of metabolome analysis have been provided in Table 1, as this is an area were descriptors are used in an interchangeable manner that can cause confusion.

It would appear from the literature that, in comparison to the initial reports related to the characterisation of GM plants, there has been a move from the use of a single analytical platform to multiple platform workflows as outlined in Figure 2. The major factor linking advances remains the same, that being the annotation of metabolites and coverage. One way metabolite coverage has been increased, is through the use of sequential extraction with different solvent mixtures of contrasting chemistries. The extractions targeted to different platforms covering complementary classes of compounds (Salem et al., 2020). Other reports describe the collection and implementation of large-scale libraries of metabolites, the WEIZMASS library being a recent example (Shahaf et al., 2016). In addition to metabolite libraries (Kopka et al., 2004), crop databases have also been developed that can effectively act as chemical core collections (Price et al., 2020). The pan-genome approaches (Zhao et al., 2018) could also be extrapolated to metabolomes and in this way it could be possible to identify biochemical variation resulting from NPBT.

**Conclusions and perspectives**

NPBTs offer a new package of innovations with the potential to address global food and nutritional security challenges. Scientifically they also provide an excellent opportunity to upgrade and advance our analytical technologies to characterise these NPBT outputs. This is especially true of metabolomics which, due to the intrinsic nature of the cells’ biochemistry, has not reached the breadth of coverage that has been achieved with genomic technologies. In the long-term, delivering such informative data from both an academic and industrial perspective can only benefit all parties. It is clear, and should be ~~forcefully~~ stated, that a multidisciplinary approach with co-operation from all parties is required when dealing with NPBTs so that the economic, legislative and administrative burden does not restrict the potential and benefits of the technology (Ali-Zaidi et al., 2019; Fedorova and Herman, 2020).

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence this paper.

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**Tables**

**Table 1**. Summary of the terms used to describe the different metabolomics/metabolite profiling approaches used and the analytical techniques employed (modified and updated from Goodacre et al 2004).

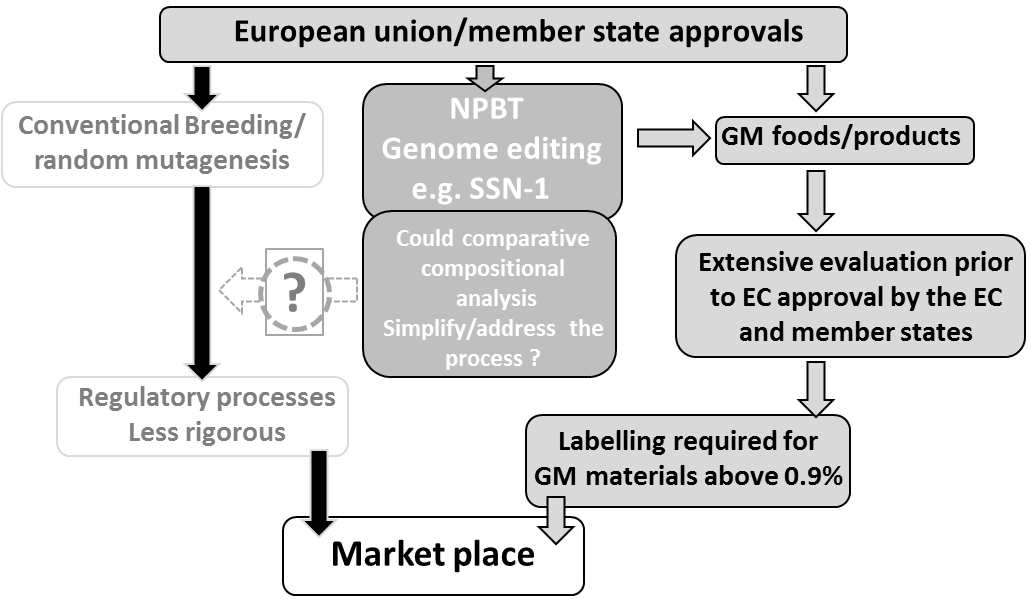
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| --- | --- | --- |
| **Terms** | **Description** | **Predominant platforms used** |
| Untargeted Metabolomics | Typically a comparative approach that captures all ions across a certain mass range in a global manner within a sample. The extraction method being a compromise and not optimised for a specific class of metabolites. | LC-MS & NMR |
| Metabolite profiling | The determination of multiple metabolites (ca. 100) across different biosynthetic classes. Searchable libraries are typically used to provide unambiguous identification, although “known unknowns” can be included. | GC-MS focussing on primary or intermediary metabolism.  LC-MS Specialised (secondary) metabolism. |
| Lipidomics | Large-scale analysis of cellular lipids providing a representation of the lipidome. | DiMS, LC-MS & GC-MS |
| Targeted analysis | Quantitative determination of a selected metabolite and its derivatives. Often includes a complete pathway with end-products and accumulating intermediate pools. The extraction optimised for specific compounds. | GC-MS, GC-FID, LC-MS & UHPLC/HPLC-PDA or FL. MRM used to capture specific metabolites in a quantitative manner.  GC-MS in a selective ion monitoring  mode has also been used. |

**Figure Legends**.

**Figure 1**. A diagrammatic representation of the existing European approvals process for GMPs and NPBT (genome editing) is provided. Presently, NPBT are considered GMPs but could rapid comparative metabolite analysis be employed to alter their classification, reducing costs and extensive evaluation process?

**Figure 2.** Potential metabolomics schemes for the characterisation of genotypes generated by New Plant Breeding Techniques. Modules in green (route 1) and blue (route 2) illustrated approaches to characterising a new metabolome without prior knowledge of the genome changes. The approaches in route 1 and 2 will maximise the coverage of the metabolome. The approach to characterising a genotype of known genotype is shown in pale red. The location of data integration modules are shown in yellow.

**Figure 1.**

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**Figure 2**

