



# Plant molecular pharming in Africa for sustainable health and economic development

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## Abstract

Plant genetic engineering goes back to over 40 years ago with the first genetically modified tobacco expressing the nopaline synthase gene. Since then, genetic engineering has emerged as an important technology as various sectors have included it in their strategic plans with potential impact on healthcare, agriculture and industry. One important aspect is the engineering of plants as bioreactors for production of vaccines, monoclonal antibodies, pesticides, orphan drugs, dyes and many other recombinant novel products. This process is referred to as plant molecular pharming. Plant molecular pharming has numerous benefits over conventional methods such as the use of animal cell lines and prokaryotic systems to produce recombinant products. These benefits include cost effectiveness, rapid and high mass production, easy scalability, accessibility and sustainability. Some African countries such as South Africa have adopted plant molecular pharming and have increased human and infrastructural capacity development, collaborations, and public sensitisation coupled with enabling policies and regulatory frameworks. However, in most of Africa, the sector faces numerous challenges including the lack of funding and expertise, low public acceptance, and poor to inadequate policies and regulatory frameworks. Despite the caveats surrounding the technology, it remains a significantly important tool for addressing global health challenges and fostering biotechnology innovations. Therefore, this review explores plant molecular pharming methods and associated products, advantages, strategies taken to improve the technology, regulatory and social- economic issues surrounding the technology in Africa.

**Keywords** Genetic engineering · Molecular pharming · Virus like particles · Monoclonal antibodies

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## Introduction

The beginning of plant genome engineering can be traced back to the early 1980's when nopaline synthase, a bacterial gene, was successfully transferred and expressed in tobacco plants (Bevan et al. 1983). Hoekema et al. (1983) developed a binary vector system by transferring plasmids that have a vir- and T-DNA region independently into *Agrobacterium tumefaciens* facilitating integration of the T-DNA into plant cells. In addition, *A. tumefaciens* was used to integrate infectious cauliflower mosaic virus DNA into plants via T-DNA transfer providing an alternative system for transmission plant viruses (Grimsley et al. 1986). These pioneering technologies led to a new era in plant biotechnology. Some of the most significant milestones in plant genetic engineering include the commercialization of genetically modified crops such as transgenic tobacco with resistance to cucumber mosaic virus in China (Clive and Krattiger 1996), Bt stands

for *Bacillus thuringiensis* (Bt) Bt cotton with resistance to cotton bollworm (*Helicoverpa armigera*) (Zhang and Tang 2000).

The field of plant genetic engineering has continued to evolve and expand its applications. Today, scientists engineer crops not only for disease and pest resistance, nutritional content and environmental adaptability but also as bioreactors for vaccines, antibodies, metabolites and growth factors. This transformative approach that harnesses the genetic potential of plants to produce valuable recombinant pharmaceuticals, therapeutic proteins and bioactive molecules is referred to as plant molecular pharming (PMP) (Goven and Morris 2012).

Recent advances in synthetic biology, systems biology, genomics, metabolomics, bioinformatics, plant physiology, plant genetic and protein engineering have paved the way for PMP. The concept of utilising plants as bioreactors was first proposed in the early 1990's with the landmark production of hepatitis B surface antigen in transgenic tobacco plants (Hiatt et al. 1989). In 2012, Protalix Biotherapeutics made a significant breakthrough by developing the first Food and Drug Authority (FDA) approved PMP product taliglucerase alfa, a genetically modified form of human glucocerebrosidase, marking a pivotal moment in the treatment of the lysosomal storage disorder Gaucher's disease (Stoger et al. 2014; Tekoah et al. 2015).

Plant molecular pharming PMP offers numerous advantages over conventional methods in the synthesis of biologically inspired molecules such as cost effectiveness, rapid production, diversity of production systems, accessibility, safety and sustainability (Abiri et al. 2016; Rybicki et al. 2012). Africa has a rich biodiversity which can be utilised in the generation of various products. In pursuit of low cost technologies to produce sustainable and eco-friendly products, some countries in Africa such as South Africa have adopted PMP in their strategic plans (Mir-Artigues et al. 2019). However, the development of PMP in Africa faces a lot of challenges such as lack of capital, infrastructure, poor regulatory frameworks and lack of skilled personnel (Murad et al. 2020). Therefore, this review explores the development of PMP, its products, solutions to problems affecting the technology, regulatory and social-economic issues surrounding the technology in Africa.

## Plant molecular pharming

Plant molecular pharming is a robust technique that employs plants as bioreactors to synthesize various industrial proteins like enzymes, therapeutic proteins such vaccines and monoclonal antibodies. This has been achieved due to tremendous and novel developments in genetic engineering

that allow introduction of transgenes in plants to express various products (Li et al. 2011). For example, Sainsbury et al. (2009) developed a multiplex vector system using the pEAQ plasmid that facilitates rapid and high production of multiple proteins in plants. In addition, integration of synthetic 5' and 3' untranslated regions (UTRs) in expression vectors (pHRE and pHREAC) resulted in high level of protein expression in *Nicotiana benthamiana* (Peyret et al. 2019). A number of plant species have been used to express various recombinant proteins with *N. benthamiana* being the most widely used expression host (Eidenberger et al. 2023). The development of PMP products follows a number of processes as shown in Fig. 1.

Depending on the intended transgenesis outcome, single or multiple genes can be introduced to a suitable expression system. Various approaches to stacking of genes of interest (GOIs) in a plant exist such as (i) cloning all the genes in a single vector that is used to transform the plant of interest, (ii) cloning GOIs in different vectors and crossing the multiple genetically modified lines to achieve the required characteristics (Krichevsky et al. 2012). Cloning of GOIs in different vectors has the major drawback of requiring different plasmids which might have different vector backbones and selection markers which limits not only the type and number of constructs but also integration and expression of the GOIs. In addition, the last approach requires a very long time and the transgenes can be integrated at a different and distant loci thus leading to genetic instability (Krichevsky et al. 2012).

Expression of GOIs, whether stable or transient, is influenced by various factors such as amount of product required, time frame, regulatory requirements and the type of product produced. Stable expression necessitates production of recombinant proteins in plant cells for a long period of time and in large amounts which can be achieved through integration of GOIs of into plant cells using *Agrobacterium*-mediated transformation and biolistic bombardment (Dubey et al. 2018). However, transient expression is the temporary, small-scale and fast production of proteins in host cells and can also be achieved by integration of the GOIs into plant cells using methods like *Agrobacterium*-mediated infiltration, virus-based infection, or electroporation (Stoger et al. 2014). The GOI is then expressed in plant cells but it is not incorporated into the plant's genome. This approach is highly versatile, as it entails protein production across broad range of tissues and plant species. Table 1 summarizes the key differences between stable and transient expression.

## Advantages and potential applications of PMP

Plant molecular pharming offers tremendous opportunities and inventiveness in the global pharmaceutical industry and

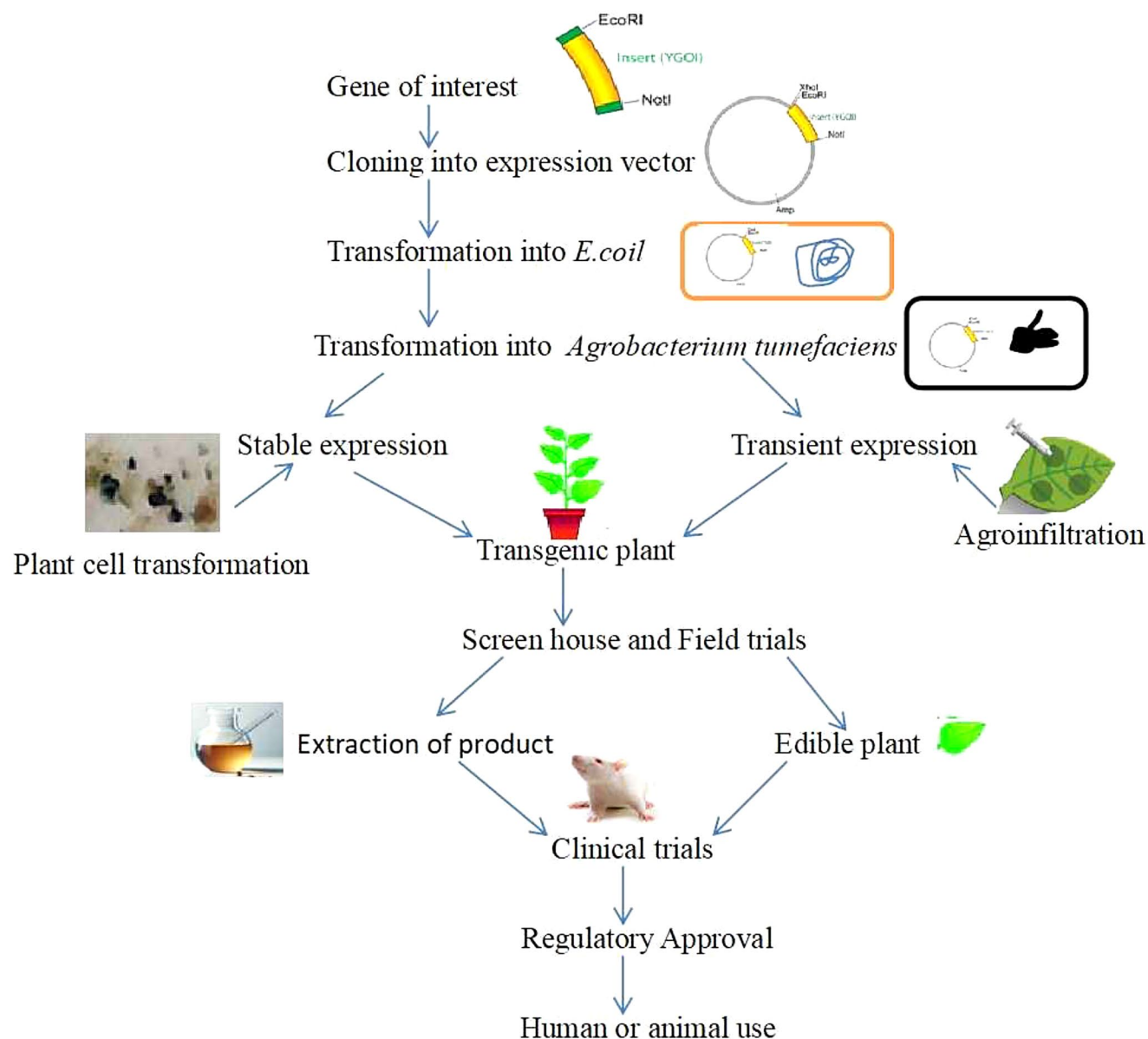


**Fig. 1** The process of acquiring plant molecular pharming (PMP) products

**Table 1** Differences between stable and transient expression

| No. Factor                 | Stable expression | Transient expression |
|----------------------------|-------------------|----------------------|
| 1. Level of production     | Low               | High                 |
| 2. Time Frame              | Long              | Short                |
| 3. Regulatory requirements | More stringent    | Less stringent       |
| 4. Cost                    | High upfront      | Low upfront          |

has numerous advantages over conventional approaches used in drug production. For example, production of monoclonal antibodies in plants was found to be cost effective and safe compared to Chinese hamster ovary cells (Mir-Artigues et al. 2019). Plants do not harbour human viruses

and so they do not produce human pathogen related toxins which make them ideal for parenteral and oral administration with simple purification steps (Parvathy 2020). Plants can be grown in green houses which makes them easy to scale at a low cost whereas cell-based systems require bioreactors which are not only expensive but also limited in capacity (Huebbbers and Buyel 2021).

Plant molecular pharming can be used to develop products rapidly in case of an emergency such as pandemics by using transient expression which can produce required quantities of proteins in a relatively short time (Tusé et al. 2020). Interestingly, plants have been used to make products that are difficult to produce in other systems such secretory IgA antibodies (Stoger et al. 2014). Ma et al. (1995)

demonstrated the production of recombinant secretory IgA antibodies from a single cell type which overcomes the requirement of two cell types in mammalian expression systems. Plant based systems are easy to maintain as they require few raw materials such as light, fertilizers, and water whereas cell based systems require expensive media and equipment servicing (Mir-Artigues et al. 2019). Plant molecular pharming products have comparable efficacy to those produced from conventional systems. A plant derived subunit anthrax vaccine (PA83-FhCMB) induced immunogenicity with no adverse effects in healthy adults during a phase 1 trial (Paolino et al. 2022).

Several plant species have found applications in the production of vaccines e.g., influenza vaccine in *N. benthamiana*, hepatitis B vaccine in tomato (*Solanum lycopersicum* L.) (Li et al. 2011), rotavirus vaccine in potato (*Solanum tuberosum* L.) (Wu et al. 2003) and foot and mouth disease vaccine in *N. benthamiana* (Li et al. 2006). Plants have also been used to produce a number of antibodies, for example, monoclonal antibody (mAb) E559 in tobacco and maize (*Zea mays* L.), for treatment against rabies (Zungu et al. 2008), anti-HIV-1 mAb 2G12 in barley (Hensel et al. 2015) and mAbs (E559 and 62-71-3) in *N. benthamiana* (Tsekoa et al. 2016). Additionally, plant-based systems have been utilised in production of human growth factors (Magnusdottir et al. 2013), cytokines (Kolotilin 2022) and diagnostic reagents (He et al. 2012).

### Plant based monoclonal antibodies

Monoclonal antibodies (mAbs) can be produced cost effectively in plants compared to conventional systems like animal cell lines (Dhama et al. 2020). Their production involves introducing genes encoding the antibody of interest into the plant which then produces and assembles the antibodies (Eidenberger et al. 2023). This process necessitates the expression of a minimum of two types of polypeptides and their accurate assembly into a multimeric structure and complex-type glycan modifications (Chen 2022).

Scientists have managed to synthesize a considerable number of mAbs in plants such as tobacco. For example, an anti-Chikungunya virus (CHIKV) mAb has been successfully expressed in wild-type and glycoengineered *N. benthamiana* plants with enhanced efficacy in a mouse model (Hurtado et al. 2020) and another study demonstrated the production of SARS-COV-2 mAbs B38 and H4 in *N. benthamiana* (Shanmugaraj et al. 2020). In addition, geminiviral vectors enabled production of heteromultimeric protein (humanized 2A10G6 mAb) in *N. benthamiana* with stable binding capacity and neutralization efficiency (Diamos et al. 2020).

### Plant-based vaccines

Vaccines stimulate the immune system by training it to recognize and remember a pathogen. This involves introducing a harmless part of the pathogen or a weakened/inactivated form (Liu et al. 2024). Vaccines have been produced conventionally through cell cultures and eggs which are costly. However, alternative systems such as plants have continued to gain attention and traction in the production of human and veterinary vaccines (Tregoning 2020). For example, the Newcastle virus vaccine for chicken was the first approved plant-based vaccine for commercial use by the United States Department of Agriculture (Vermij and Waltz 2006).

Vaccines consist of broadly three types that is: whole organism vaccines, subunit vaccines and nucleic acid vaccines (Moyle 2015). Whole organism vaccines include live attenuated vaccines and inactivated vaccines while subunit vaccines include polysaccharide vaccines, toxoid vaccines, conjugated vaccines and nucleic acid vaccines include mRNA vaccines and DNA vaccines (Ghattas et al. 2021). Subunit vaccines have been significantly produced in plants and they consist of specific isolated components of a pathogen such as proteins or peptides which prevents exposure of the immune system to unnecessary elements minimizing potential side effects (Cid and Bolívar 2021). For example, HIV antigens Pr55Gag, truncated Gag (p17/p24) and p24 capsid subunit were successfully expressed in *Nicotiana* spp. using stable and transient expression via *Agrobacterium tumefaciens*, and recombinant tobamovirus vectors (Meyers et al. 2008).

Recent developments in plant-based vaccine technology such as the use of virus-based nanoparticles and recombinant immune complexes (RICs) may have a huge impact on the delivery and production of new vaccines (Stander et al. 2022). Virus-based nanoparticles serve as carriers of foreign antigens enabling the production of vaccines by eliciting an immune response without causing disease (Zanganeh et al. 2023). An important subset of VNPs are virus-like particles (VLPs) which are competent upon vaccine candidates and do lack status the viral genome conferring them non-infectious and safer (Venkataraman et al. 2021). Interestingly, VLPs can be made from plant viruses such as cowpea mosaic virus and tobacco mosaic virus and they can act as strong vaccines and adjuvants (Venkataraman et al. 2021). Virus-based nanoparticles have been produced in various plants for instance, bluetongue virus (BTV) structural proteins VP3 and VP7 were transiently expressed and used to produce large quantities of green fluorescent protein-encapsidating core-like particles from *N. benthamiana* leaves (Brillault et al. 2017).

Recombinant immune complexes (RICs) are engineered complexes which consist of specific antigens and antibodies.

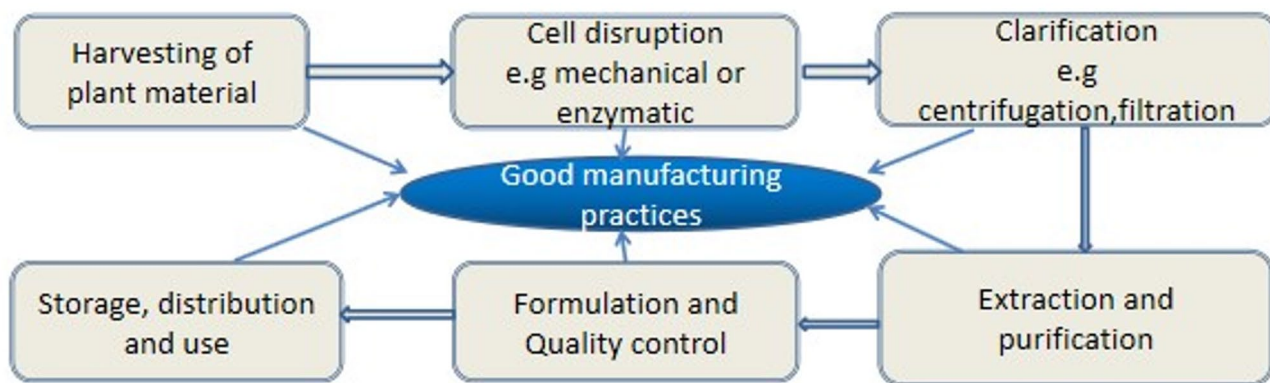


**Fig. 2** Procedure for downstream processing of plant molecular pharming (PMP) products

There is a growing interest in employing immune complexes as preventive vaccines driven by the progress in therapeutic antibody applications and improved comprehension of immunoregulatory responses (Stander et al. 2022). Recently, RICs have been developed through modifying a mAb where its corresponding antigen is added to the heavy chain C-terminus (Stander et al. 2022). For example, RICs have been produced in transgenic tobacco plants by fusing early secreted Ag85B and latency-associated Acr antigen of *Mycobacterium tuberculosis* as an individual polypeptide to the mAb heavy chain against Acr and the RICs formed through the antibody's binding to Acr facilitating self-polymerisation of the complexes (Pepponi et al. 2014).

### Improving heterologous protein production in plants

Various aspects of the plant expression system must be optimised in order to enhance the yield, quality, and stability of the target protein (Watts et al. 2021). Factors that affect production of heterologous proteins in plants include selection of the expression system, promoter selection, codon usage and optimisation, intracellular localisation, transcriptional and post-transcriptional regulation, protein stability and solubility, post translational modifications, protein degradation, choice of vector system and host plant selection. A number of algorithms for synthetic gene design and codon optimisations such as Codon Optimizer, JCat, OPTIMIZER have been developed (Webster et al. 2017). Codon optimisation involves adapting the DNA sequence of the gene to match the preferred codon usage of the host plant and thus improving translation rates.

Promoters with strong activity and tissue specificity are often preferred to achieve stable and high levels of expression in the target tissues (Streitfield 2007). A recent study utilized orthogonal regulatory systems from *Saccharomyces spp.* and developed transcriptional regulators that were validated in *N. benthamiana* and *Arabidopsis thaliana* (Belcher

et al. 2020). Plants may perform post-translational modifications differently than the donor organism. A recent study utilized N-glycosylation machinery of *N. benthamiana* to engineer the glycosylation patterns of helminth glycoproteins omega-1 and kappa-5 (Wilbers et al. 2017). Transient expression of a silencing construct targeting CysP6 in tobacco leaves increased interleukin-10 (IL-10) levels compared to the control and it was suggested that CysP6 was localized to the endoplasmic reticulum demonstrating the role of the ER in the degradation of IL-10 (Duwadi et al. 2015).

### Downstream processing

Downstream processing is an important step in PMP and it involves the extraction, purification, and characterisation of recombinant proteins produced in plants. Since there are a lot of molecular pharming platforms, downstream processes for plants need to accommodate the distinct characteristics of each host system especially in the initial stages (Tschofen et al. 2016). The major goal of downstream processing is to isolate the recombinant product from the plant material with the required quality. The yield, quality and cost of the plant-derived pharmaceuticals can be hugely impacted by the effectiveness and efficiency of downstream processing (Buyel et al. 2015). Downstream processing is done under aseptic conditions and adhering to Good Manufacturing Practices (GMP) throughout the process is essential (Harris 2008). Figure 2 shows the steps involved in downstream processing.

### Advancements in plant genome engineering for high-throughput Recombinant protein production

Traditionally, PMP has relied on classical genetic engineering which involves random integration of transgenes in plant genomes using methods such as biolistic bombardment

or *agrobacterium*-mediated transformation. During stable transformation, integration of T-DNA sites in plants may lead to gene silencing due to accumulation of small interfering RNAs and DNA methylation at insertion sites (Jupe et al. 2019). In addition, chromatin modifications around T-DNA insertion sites may influence transgene expression (Jupe et al. 2019). The random integration of transgenes leads to positional effects that may negatively influence gene expression. Mini et al. (2018) showed that insertions occurred in exons or untranslated regions which may affect the stability and expression of the transgenes. New genome engineering techniques such as genome editing could overcome these limitations by offering precise and specific modifications.

Genome editing involves use of site-specific endonucleases to make targeted modifications in host organisms. Genome editing involves use of zinc finger nucleases (ZFNs), Transcriptional Activator Like Effector Nucleases (TALENs) and CRISPR/Cas9 systems (Belhaj et al. 2013). Of these genome editing tools, CRISPR/Cas9 has emerged as the best alternative for plant genetic engineering. The CRISPR/Cas9 genome editing system basically consists of a Cas9 endonuclease and a single guide RNA (sgRNA) that guides Cas9 endonuclease to make a double stranded break on the target gene (Doudna and Charpentier 2014). Cellular repair mechanisms such as non-homologous endjoining (NHEJ) or homology-directed repair (HDR) facilitate gene knockouts or insertions respectively (Zaboikin et al. 2017).

CRISPR/Cas9 genome editing systems are widely used for crop improvement against biotic and abiotic stress however they have been less adopted for PMP. Interestingly, CRISPR/Cas9 genome editing has the potential to increase recombinant protein production in plants, improve post-translational modifications, protein stability and efficacy. A multiplex CRISPR/Cas9 genome editing has been used to knockout rice N-glycan genes  $\alpha$ 1, 3-fucosyltransferase and  $\beta$ 1, 2-xylosyltransferase in order to improve the efficiency of biopharmaceutical production from the crop (Jung et al. 2021). The immunogenicity of hepatitis B virus (HBV) vaccine produced from CRISPR/Cas9 edited *N. benthamiana* plants lacking  $\alpha$ 1, 3-fucosyltransferase and  $\beta$ 1, 2-xylosyltransferase genes increased in mice as compared to its wild-type (Pantazica et al. 2023). In addition, the targeted knockout of cysteine protease genes (NbVPE-1a, NbVPE-1b and NbCysP6 genes) using the CRISPR/Cas9 system resulted in a decrease degradation of mAb CAP256-VRC26.25 in *N. benthamiana* (Singh et al. 2022). RNA-dependent RNA polymerase 6 (RDR6) *N. benthamiana* mutants produced using the CRISPR/Cas9 technology produced higher levels of recombinant green fluorescent protein as compared to the wild-type plants (Matsuo and Atsumi 2019).

## Developing PMP platforms in Africa

Development of PMP platforms in Africa is essential to improving access to affordable, safe and effective healthcare products. This is the reason why there is an ever-growing adoption by African scientists. Notably, development of PMP technologies for vaccines and treatment of non-communicable and infectious diseases are at different stages in Africa. As a result, the effective products are increasingly being scaled up for commercialisation which will increasingly create a positive impact in Africa. This technology will have a huge impact on the way drugs, vaccines and nutraceuticals are designed and manufactured in Africa (Maima and Munyendo 2018) and so far, only South Africa has successfully developed PMP technology in Africa. There are a number of challenges to developing PMP in Africa which include insufficient funding, lack of skilled personnel, infrastructure and poor regulatory frameworks (Murad et al. 2020; Tsekoa et al. 2020).

Some strategies have been adopted to address these challenges due to a mounting interest in PMP products. For example, the Council for Science and Industrial Research in South Africa which produced RabiVir, in plants started training a number of graduate students and postdocs in PMP (Ma et al. 2013). Additionally, some key steps that can be taken to develop PMP in Africa include the following:

- Increased investment in research, development and acquisition of new PMP technologies, and training of a critical mass of technical personnel to facilitate the production of high-quality plant-based pharmaceuticals.
- Clear and concise regulatory processes developed in consultation with technical personnel and in line with global standards. This can fast track introduction of PMP products in the market more effectively.
- Collaborations through building strong and healthy public-private partnerships which can mobilise resources and expertise from governments, industry and academia.
- Technology transfer to facilitate appropriate training of personnel, joint research projects sharing of intellectual property rights or granting access to patented products and voluntary transfer of machinery.

## South Africa's biopharming industry

South Africa has a large community for plant genome engineers in addition to increased government support and collaborations between institutes making biopharming of great national importance (Ma et al. 2013). Notably, South Africa's biopharming industry is spearheaded by University of Cape town (UCT), Council for Scientific and Industrial

Research, Stellenbosch University and University of Pretoria (Rybicki et al. 2012). Scientists at UCT developed and assembled Bluetongue virus VLPs in *N. benthamiana* using the cowpea mosaic virus-based HyperTrans and associated pEAQ plant transient expression vector system which demonstrated a strong immune response in sheep (Thuenemann et al. 2013).

Furthermore, a matrix protein 2 ectodomain (M2e) was human and plant codon optimized and fused with N-terminal proline-rich domain (Zera<sup>®</sup>) of the  $\gamma$ -zein protein of maize and the recombinant product Zera<sup>®</sup>M2e was expressed in *N. benthamiana* and then administered to mice which elicited M2e-specific antibodies and thus demonstrated the production of an inexpensive universal influenza vaccine (Mbewana et al. 2015). In addition, rotavirus VLPs with good immunogenic properties were expressed in *N. benthamiana* demonstrating production of a cheap subunit vaccine (Pêra et al. 2015).

### Economic and social impacts

Plant molecular pharming will have a huge impact on the economic and social development of numerous countries. Economically, PMP is expected to generate significant cost savings, making essential medicines more affordable and accessible particularly in low and middle income countries where healthcare resources are often limited (Murad et al. 2020). Moreover, PMP could create new industries and employment in areas such as feed additives, biocatalysts, and diagnostics reagent manufacturing (Tschofen et al. 2016). These industries can boost local innovation and promote rural industrialisation.

In pursuit of equitable sharing of benefits, PMP has a far-reaching impact to the social development of stakeholders involved. By increasing access to affordable and effective medicines, PMP has the potential to improve health outcomes worldwide, reducing the burden of disease and mortality (Ma et al. 2013). For example in early 2014 during the Ebola outbreak in Liberia, two American citizens were treated with ZMapp, an experimental drug whose antibody components were produced in *N. benthamiana* (Zhang et al. 2014). In addition, PMP could contribute to global food security by producing valuable nutrients and supplements, addressing malnutrition and hunger.

### Regulatory and ethical considerations

Appropriate regulatory and ethical considerations are crucial aspects of PMP ensuring the responsible and safe use of this technology (Goven and Morris 2012; Menary et al. 2020). These considerations aim to safeguard the environment, human health, and the integrity of ecosystems as well

as to address ethical concerns surrounding the technology. Some of the regulatory considerations include biosafety regulations, environmental risk assessment, regulatory approvals, intellectual property rights, product quality and safety standards and pharmaceutical approval (Goven and Morris 2012).

All stakeholders involved in PMP including farmers, researchers, and consumers should have access to transparent information about the technology (Menary et al. 2020). The risks and benefits related to ecological impacts, biodiversity conservation, and use of resources must also be taken into consideration (Tsatsakis et al. 2017). The handling of animals used in research and the potential effects on human health should be taken into consideration (Carvalho et al. 2018) and efforts should be made to ensure equitable sharing of benefits that arise from PMP by various stakeholders.

### Conclusion

With a Africa's rich source of plant biodiversity, plant molecular pharming (PMP) has the ability to revolutionise the continent's entire pharmaceutical and medical industries. The use of plants as bioreactors for the production of valuable products such as vaccines, and monoclonal antibodies presents an innovative yet sustainable and cost-effective approach to address the continent's unique challenges. However, there is need to increase investments in research infrastructure, training of critical human resource, and public awareness campaigns to foster understanding and acceptance of plant molecular pharming technology. In addition, collaborations will play a crucial role in facilitating knowledge exchange, technology transfer, and the development of a robust regulatory framework that balances innovation with safety considerations. Adopting novel techniques such as gene editing could expand the scope of developing PMP in Africa.

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**Data availability** There are no datasets that were generated or analyzed since this is a review article.

### Declarations

**Competing interests** The authors have no competing financial interests or personal relationships that might influence the findings reported in this manuscript.

**Ethics approval and consent to participate** This is a review article and thus no ethical approval is required.

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