Chapter 18
Methods of Strain Improvement for Crop Improvement

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Abstract Biofertilizers are suitable substitutes to chemical-based fertilizers and pesticides, which cause serious environmental problems around the world. Therefore, there is an important requirement to implement ecological regulation using native microorganisms. These beneficial microorganisms are inexpensive, consistent, and more effective than synthetic fertilizers in terms of plant protection against pathogens. These beneficial microorganisms protect plants against pathogens and enhance nutrient availability. Hence, to achieve this goal, better quality strains are needed. Crop improvement relies on the modulation of genes and genomic regions that underlie crucial characteristics, either directly or indirectly. Recombinant biotechnology intends to benefit in reducing the use of synthetic fertilizers; for this function genetically improved microbes could be used. By using recombinant DNA technology, genes of microbes are improved via several genetic modifications depending on the recognition and selection of the desirable characteristics or genes of interest. The current investigation is focused on different strategies used to improve beneficial strain for crop productivity.

Keywords Agriculture · Crop improvement · Gene information · Molecular approaches

18.1 Introduction

Agriculture relies heavily on the use of chemical or synthetic fertilizers and insecticides to achieve higher yields. Issues such as environmental pollution, health threats, disruption of the natural cycle of ecological inputs, and the destruction of biological ecosystems that otherwise support agricultural production are correlated with this reliance. There is a growing use of biological resources to replace chemical

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fertilizers and pesticides. Agricultural development and pest and disease management must therefore be carried out with fewer harmful inputs at shorter periods. In this sense, plant growth-promoting rhizobacteria (PGPR) are potential resources to bring significant benefits to agriculture. Studies have shown that PGPR have great potential to improve crop growth and yield. Cereals are the primary source of food for human nutrition and constitute more than two-fifths of the world population's staple diet. Environmental and genetic factors influence crop productivity (Radhakrishnan et al. 2017). The usage of beneficial microbes alone or as microbial consortia to selected plants with multifunctional properties is a good method to stimulate strength and crop productivity (Ahmad et al. 2018; Maron et al. 2018). Investigation on isolation and characterization of advantageous microbes to plants has been extensively cited, but some of them have been commercialized. It has been demonstrated that many commercial bioinoculants were not effective in the agriculture field but they were effective in laboratory or greenhouse experiments (Vassilev et al. 2015; Arora and Mishra 2016; Sulbhi et al. 2021; Bhandari et al. 2021) due to their low stability and quality of formulation (Bhatt et al. 2021a, b, c). Newly, selective use of beneficial microbiome plants and their combinations to combat biotic and abiotic stress is gaining traction and becoming a stimulating research frontier (Malusá et al. 2016; Bashan et al. 2016; Baez-Rogelio et al. 2017; Stamenkovic et al. 2018). Biofertilizers are formulated from nitrogen-fixing rhizobacteria naturally present in the legume nodules or microbes that are responsible for plant growth promotion. However, these bio-formulations would not be proficient enough for supplying nitrogen to non-leguminous plants. In that circumstances, the practice of genetic engineering is of particular significance, for developing efficient management systems is needed. Consequently, the non-leguminous plants could be cultivated with symbiotic rhizobia root nodules without applying external nitrogen fertilizers (Santi et al. 2013). Foreign genes used to transform microorganisms could be integrated into the genome of the host. For that, the regulatory region of the gene is altered at the promoter or the terminator sites to augment the inserted gene function in the host. The addition of a particular gene that can confer biological control capacity could improve the biological control capacity of microbes that lack these genes (Dash et al. 2016). Various rhizobacteria possess biological control activity that simultaneously produces chitinases. However, few rhizobacteria such as *P. putida* and *R. meliloti* are root colonizer but devoid of chitinase synthesis (Bagwan et al. 2010). Hence, the chitinase gene assimilation into their genome made them competent in the defense of the plants against fungi (Huang et al. 2001). Biofertilizers, when formulated using molecular tools, can enhance cellular pathways for phytohormone production, such as cytokinin, auxin, etc. as well as help in plant growth and development (Fuentes-Ramirez and Caballero-Mellado 2005). Most breeding approaches for biotic and abiotic stress resistance are based on the insertion of a single resistant gene into plants, and therefore crop resistance only lasts for a short duration (Kottapalli et al. 2010; Bhatt et al. 2020a, b, 2021d, e, f). Therefore, the development of multi-stress resistant genotypes is now demonstrated by combining multiple genes from different sources in a single plant (Bhatt et al. 2019a, 2020c, d, e, f). The process of manipulating and

improving microbial strains to enhance their metabolic capabilities is called strain improvement.

Protoplast fusion is an important tool in the selection of strains to provide genetic recombination and develop hybrid strains in filamentous fungi (Steiner et al. 2019). It is used to produce interspecific or even intergeneric hybrids. It has become an important tool for genetic manipulation, as it breaks down obstacles to the genetic exchange performed by conventional mating systems. This technique has great potential for genetic analysis and strain improvement. The stress tolerance capacity in crops has been explained in many studies using the pyramid of various resistance genes (Suresh and Malathi 2013; Muthurajan and Balasubramanian 2010). Abiotic stress also affects the growth and yield of the crop (Pancaldi and Trindade 2020) and can even disturb the survival of plants (Rana et al. 2019; Verma et al. 2008). Salinity is one of the major problems for crop productivity, and maximum crops are subtle to salt during their lifespan and particularly at the seedling period (Bai et al. 2018). Certain varieties of crops that are salt resistant express salt-sensitive genes to tolerate excess salts, and the quantitative trait locus (QTL) linked to these genes can be mapped by microsatellite markers for the selection of salt-tolerant lines (Ruengphayak et al. 2015; Llorens et al. 2020). Numerous drought-tolerant genes have been well discovered and modified in various crops to develop drought resistance (Yu and Yang 2016; Waqas et al. 2020). In many plants, cold resistance genes (OsRAN1 and QTL) have also been acknowledged which is further used in developing cold tolerance in plant varieties using molecular marker enhancement tools (Thitisaksakul et al. 2015; Tiwari et al. 2016; Bimpong et al. 2016). Plant tissue cultures (PTC) also have an important part in modern biotechnology. They are widely used in studies of plant development processes (Sandhu et al. 2019), genetic function (Rai et al. 2018), micropropagation (Zhang et al. 2014), and generation of transgenic plants with specific industrial and agronomic characteristics (Shinada et al. 2014). In this chapter, various techniques involved in beneficial microbes/strain improvement, for the production of biotic and abiotic stress resistivity in different plant varieties, are described. Molecular biological applications for crop improvement like genetic engineering (GE)/recombinant DNA technology (RDT) to adopt better traits of agronomic importance are too elaborated (Almeida et al. 2016; Firn et al. 1994; Bhatt et al. 2015a, b, 2016a, b, 2019b, c; Deng et al. 2010; Kumar 2011; Loyola-Vargas and Ochoa-Alejo 2018).

18.2 Crop Improvement by Genetic Engineering

For many decades, gene transfer among distinct species of plants has played a fundamental role in crop improvement. By transforming genes, many useful traits, such as insect, stress, and disease resistance, have been shifted to many varieties of non-cultivated plant crops (Akhtar et al. 2014; Amin et al. 2014; Dar et al. 2014; Tariq et al. 2014). Recombinant DNA methods and many other methods are used for the transformation of genetic information. Genetic engineering is a technique that

has made possible the transfer of genes between different genera or species using recombinant DNA. This method is an exceptional selection method of expanding the genetic base as compared to conventional breeding. Additionally, because it avoids the skidding problem associated with conventional farming, it is more efficient and takes less time (Khan et al. 2015a). Until now, many genetically modified crops have been developed and commercialized, resulting in higher production efficiency, a greater focus on the market, and better conservation of the environment. These crops include longer postharvest storage tomatoes, insect-resistant cotton and corn, virus-resistant potatoes, herbicide-resistant soybeans, and canola, and many others (Puspito et al. 2015). To improve crops through genetic engineering, an efficient processing system is needed. Different approaches are used to transform different cultures such as recombinant DNA technology, which is used to manipulate genes of microbes via various genetic modifications (Tabashnik et al. 2011). Also, many *Pseudomonas* spp. chelate Fe ions by producing siderophores, thus increasing Fe uptake in plants. *S. meliloti* (RMBPC-2), a genetically modified strain, was made by introducing the genes that drive the plant nitrogenase enzyme to the bacteria (Boccia and Sarnacchiaro 2015). *T. harzianum* is a very effective colonizer that is widely present in soil and also can parasitize pathogenic fungi. In fact, many extracellular enzymes like chitinases, proteases, and glucanases are synthesized by *Trichoderma* which are enhanced by adding chitinase genes. Many extracellular enzymes such as glucanases, chitinases, and proteases synthesized by *Trichoderma* have been enhanced by the addition of chitinase genes (Tabashnik et al. 2011; Boccia and Sarnacchiaro 2015; Awais et al. 2010). Therefore, these genetically altered strains could effectively act as biofertilizers and improve crop yield and quality.

18.2.1 Genetically Modified Microbes

GM microbes provide better access to nutrients for crops and therefore increase plant development. The most important beneficial microbes that are used as biofertilizers are nitrogen-fixing bacteria, such as *Rhizobium* and *Azospirillum*. *Rhizobium* and *Sinorhizobium* are the symbiotic bacteria that form root nodules in legumes and fix nitrogen. It has been reported that these bacteria can stay in soil alive for a long time and in certain cases even without a defined host (Ngwako 2008). These microbes have been widely used as bioinoculants to enhance the growth and yield of crops. However, the improvement in yield is variable and the success of the inoculants appears to depend on the competition with the native strains which are generally the least effective (Qaim 2009). Mycorrhizal fungi signify the group of fungi that form a symbiotic association with plants. An investigation has been carried out to identify if transgenic *Rhizobium* strains enhance nodulation or interfere with a symbiotic association in plants. It was noticed that the strain GM *S. meliloti* does not interfere with the formation of mycorrhizae but improves nodulation. GM sweet clover increased colonization units of arbuscular mycorrhiza and increased the nutrient acquisition capability of mycorrhizal plants (Papagianni 2004; Van Loon 2007).

Azospirillum is recognized for its capability of plant growth promotion by augmenting nitrogen uptake, through phytohormone production (Gonzalez et al. 2015). *Sinorhizobium meliloti* has been genetically modified to promote nodulation in alfalfa roots. This genetic modification includes modification of the expression of nifA gene which is responsible for the management of all other nitrogen fixation (nif) genes (Bakshi 2003). It is assumed that nifA regulates the gene expression other than nif cluster that aids in nodule development (Beyer et al. 2002). In the rhizosphere region of *Pisum sativum*, GM *Rhizobium leguminosarum* strains, labeled with HgCb resistance genes (mer genes) and lacZ genes, were inoculated. In order to observe its impact on crop productivity, *Alcaligenes faecalis*, a non-nodule-forming bacterium, has been genetically engineered and introduced into rice fields in China. By introducing a constitutively expressed nifA regulatory gene, *A. faecalis* was genetically modified and nitrogen fixation got increased as compared to treated fields (Gray and Smith 2004; Huang et al. 2021).

18.3 Intraspecific and Interspecific Gene Transfer

In the nineteenth century, plant breeding began with discoveries about how plant traits are inherited. Plant breeding could be achieved by selecting plants with interesting attributes and manipulation in cross-fertilization. An improved variety with the desired characteristics is formed when a cultivated variety is backcrossed with a wild variety (Goodman et al. 1987; Khan et al. 2015b). In the twentieth century, plant breeders used interspecies hybridization to transfer genes from a non-cultivated plant species to other convertible crop species. For example, *Avena sativa* (oats) and *Beta vulgaris* (sugar beet) were processed and resulted in increased yields of 25–30% and resistance to sugar beet nematodes, respectively (Sharma and Gill 1983). In the 1940s, methods for transferring DNA directly from one organism to another organism were developed as DNA was established as a chemical base of genetic inheritance. Genes can be obtained from plant, animal, bacterial, and viral sources and injected into crops. Tissue specificity, timing, and expression level of genes are under control and they can be modified by gene modification into a fresh host. These methods provide the basis of diversity and permit the regulation of expression of genes (Qamar et al. 2015). In recent times, the expansion of molecular methods has generated different options for the assortment and genetic improvement of livestock (Godrat et al. 2005).

18.4 Genetic Modification Through Somatic Hybridization

18.4.1 Protoplast Fusion

Somatic hybridization is the best technique aimed at the production of interspecific and intergeneric hybrids for plant breeding and crop improvement. In this technique fusion of protoplasts from two different genomes followed by the selection of the desired somatic hybrid cells is carried out for regeneration of hybrid plants (Evans and Bravo 1988). Therefore, it is accepted as an effective approach to generate hybrids by joining two different protoplasts from different plant species or varieties, and hybrids produced via this method are called somatic hybrids. Protoplast fusion is a commonly used method for introducing a group of biosynthetic genes or entire chromosomes into a recipient cell for subsequent genetic manipulation or directed evolutionary approaches. It facilitates the transmission of mitochondrial genomes among taxonomically associated species (Vincelli 2016). This is one of the important or widely studied approaches as a technique to improve fungal strains (Assefa 2018; Nagoshi et al. 2018). In physiology, genetic study and genetic manipulation fungal protoplast are important tools that can be successfully carried by fusing protoplasts into filamentous fungi that lack sexual reproductive ability (Kage et al. 2016; Sharifzadeh et al. 2018). It is admitted as one of the recombinant DNA technologies that provide the tools to increase gene dosage and gene expression from strong promoters, remove unwanted genes from the fungal genome, manipulate the metabolic pathways, and develop fungal strains for the production of heterologous proteins. Several reports have confirmed the isolation and regeneration of protoplasts in different fungi. Protoplast fusion is found to be good for improvement of *Trichoderma* spp. and development of hybrid strains in other filamentous fungi (Atique et al. 2018; Mwobobia et al. 2020). The isolation, fusion, and regeneration of protoplasts were carried out in the genus *Trichoderma* primarily to improve its cellulolytic activity (Federico et al. 2019; Pandeya et al. 2018) and chitinase production (Bowman and Zilberman 2013). However, partial attempts have been done to improve *Trichoderma* species and increase enzyme production (Pandeya et al. 2018; Waddington et al. 2010). Ogawa and his team (Ogawa et al. 1989) revealed an increased cellulase production in *Trichoderma reesei* through interspecific protoplast fusion, while Prabavathy et al. reported an increase in chitinase and biological control activity in *Trichoderma harzianum* through protoplast autofusion; nevertheless, little research has been done on the application of chitinase in the degradation of shellfish waste applying this method (Prabavathy et al. 2006).

18.4.2 Agrobacterium-*Mediated Gene Transfer*

Agrobacterium tumefaciens is a phytopathogenic bacterium capable of transferring part of its genetic material to other plant species through a simple process called

transformation. The genes are encoded in a region of the Ti plasmid called T-DNA. This causes the growth of a tumor termed "crown gall" disease in plants (Gordon and Christie 2015). This bacterium is altered in the laboratory and transfers the gene of interest to plants without causing disease symptoms. The *Agrobacterium* system is quite attractive due to the easy protocol that is associated with minimal cost in terms of equipment and also the resulting transgenic plants have single-copy insertion (Gordon and Christie 2015; Hansen and Wright 1999). With this method, genes for resistance to insects and diseases were transferred. Using recombinant DNA technology, many plant and bacterial genes encoding enzymes have been engineered to make crop plants tolerant of broad-spectrum herbicides and safer for the environment. Because this bacterial gene is designed in such a way that its enzyme is insensitive to the herbicide and then transfers it to the plant, it can also be done by having plants express genes that detoxify the herbicide. The genes obtained from *Bacillus thuringiensis* have been modified and transferred to plants that act as insecticides (Shahid et al. 2016).

18.4.3 Non-Agrobacterium-Based Gene Transfer

Four decades before it was identified, some members of the Rhizobiaceae family also can transfer the gene to the host. *Ensifer adhaerens*, *Ochrobactrum haywardense*, and *Rhizobium etli* are some of the *Agrobacterium*-related species that have been used in gene transfer but have the disadvantage of a limited host range (Mullins et al. 2006).

18.4.4 Viral-Mediated Gene Transfer

Viruses carry complex arrangements and life cycles; many are pathogenic but act as very efficient vehicles in gene delivery (Patel and Misra 2011). RNA and DNA viruses that infect plants can be used as a vector to transfer genes to the target. The gene to be transferred is integrated into the viral genome, and at this instant, the virus acts as a vector to transfer the gene. The virus with the transferred gene infects the target cell and results in a successful transformation. The main disadvantage is the high number of copies per cell, and virus-mediated gene transfer can only produce transient transfer and not stable transformation—that means they cannot be transferred to the offspring. Some of the viral vectors used are a retrovirus, an adenovirus (Chailertvanitkul and Pouton 2010), adeno-associated virus, herpes virus, smallpox virus, human moss virus (HFV), and lentivirus (Patel and Misra 2011; Fiandaca and Federoff 2014).

18.5 Mutagenesis and Crop Improvement

18.5.1 Site-Directed Mutagenesis

In a study, chemical mutagenesis was used to attain fungicide benomyl-resistant strains of *Trichoderma harzianum* (Ahmad and Baker 1987). Remarkably, the mutant strains were better colonizers of the rhizosphere than wild-type strains. The mutation technique will undoubtedly contribute to the upgradation of biological control agents. Genetic engineering proposes stimulating possibilities for the genetic manipulation of fungi both to improve biological control strains and to understand how biological control works. Transformation of filamentous fungi was first reported in the laboratories of Tatum (Mishra and Tatum 1973) and Case (Case et al. 1979). Since then, molecular techniques have become more accessible for use by possible biological control fungi (Fincham 1989; Bhatt et al. 2019d; Sharma and Bhatt 2016; Sharma et al. 2016; Bhatt and Nailwal 2018). There is no doubt that the expansion and use of molecular practices will persist to advance rapidly (Khati et al. 2018a; Gangola et al. 2018a; Bhatt 2018; Bhatt and Barh 2018; Bhatt et al. 2019e; Bhandari and Bhatt 2020; Bhatt and Bhatt 2021).

18.6 Bioinformatics Tools in Crop Improvement

Bioinformatics resources, in addition to various web databases, provide extensive information on genomic data that is widely needed for research purposes. Improving crops using bioinformatics tools is more promising these days (Singh et al. 2021; Zhang et al. 2020a, b; Mishra et al. 2020; Feng et al. 2020; Lin et al. 2020; Zhan et al. 2020; Ye et al. 2019; Huang et al. 2019, 2020). Over time, technology has improved to a surprising level, bioinformatics provides crucial information about crop genomic data, and this technology explores the sequence of many genes. This could help us to sequence the economically important crop and the more beneficial traits. Whole-genome comparisons are accelerating the pace of competent research (Fan et al. 2020; Pang et al. 2020; Gangola et al. 2018b; Gupta et al. 2018; Khati et al. 2017a, 2018b; Kumar et al. 2017). Projects of genome sequencing of economically important crops have been accomplished and are seen as the access to new research. Database of specific data sets in a compiled form with enriched annotations helps to study gene families with greater precision. Genomic comparisons of different crops help pinpoint the conserved regions between crops, providing common adaptation strategies for plants (Nagoshi et al. 2018). After completing the sequencing of the cultures, the data generated was used to create modeled proteomic data that helped to understand the content of certain gene families. Major events, such as gene duplication, as well as other abnormalities, are manipulated using bioinformatics tools (Khati et al. 2017a, b, 2018b; Kumar et al. 2017). Additionally, access to critical data to improve crop traits is positively simplified at a great end by using advances in

technology and data acquisition sites. Therefore, efficient use of genetic data supports sustainable crop improvement. Different techniques, such as high-throughput sequencing, generate a stack of crop data. Omics research works on the prediction of candidate genes and, therefore, on the predicted functions (Mochida and Shinozaki 2010; Lockhart and Winzeler 2000). Data on transcriptomics and metabolomics have also elucidated the regulatory networks that are crucial against plant stressors. As a result, several crops were protected from biotic and abiotic stressors and yield was restored.

18.7 Plant Tissue Culture in Crop Improvement

Advancements in tissue culture methods have very important part in breeding various crops. These in vitro tissue culture techniques offer cloning, screening, micropropagation, micrografting, organogenesis, etc. to assist plant breeders in several ways. In tissue culture practices, the phenomenon of totipotency capacity of the plants (explants) is exploited to introduce variance in genetic organization of plants (Brown and Thorpe 1995). Explants or plants are treated with appropriate treatments such as thermotherapy to eradicate viruses and diseases and allowed to divide to forms a colorless undifferentiated mass of cells (callus) (Jain 2001). The epigenetic alterations induced during tissue culture processes are known as somaclonal variations. Together with molecular and biotechnological interventions, several techniques have been developed to transfer necessary genetic traits that are commercially favored. Clonal multiplication ornamental crop industries operate massively and thus greatly increase cultivars. Plant traits are thus evaluated against different plants in plant breeding (Tazeb 2017). Several genetically modified plants have been established during the last 20 years utilizing technological advancements in genetic engineering (Bawa and Anilakumar 2013). These plants have been developed such that often use either a transforming vector or other techniques that require chemical and enzyme action coupled to favor transformation such as use of liposomes, biolistic particle gun, microinjection, and electroporation techniques (Bhalla 2006). Transformation vector such as *Agrobacterium tumefaciens* induces tumors with its Ti plasmid and subsequently transfers T-DNA (transfer DNA) into host plant parts (typically leaves). The DNA segment of interest was inserted into the T-DNA (transfer DNA), eliminating the nonessential part (the portion of the plasmid that is not required for the act of transfer) (Gheysen et al. 1998; Gelvin 2003). Transformation success modifies the cells, followed by cell harvesting and finally regenerated in vitro into complete plantlets. However, assembly of necessary and advantageous crop traits for any crop enhancement program is certainly most critical and is usually performed by genetic transformation or hybridization program. Single genes are favored for transfer by most molecular and genetic methods. Hybridization is preferred for the successful transfer of more genes in a single reaction time. Tissue culture techniques facilitate the hybridization process when the embryo is aborted and therefore does not favor plant establishment. Tissue culture embryo rescue has

been used successfully to overcome the problem of embryo abortion or the inability of seeds to develop (Tazeb 2017).

There are so many important advantages of plant tissue culture over crops. A wide variety of cultures have been recovered by IVF using pistil pollination and self-pollination and cross-pollination of ovules. A wide series of plants have been recovered by IVF using pistil pollination and self-pollination and cross-pollination of ovules, such as tobacco, corn, clover, poppy, canola, cabbage, cotton, etc. Another type used to give value to cultures is embryo culture and orchids, roses, and bananas are formed by embryo culture. Several other varieties are also successfully formed, such as stress, drought, and heat-tolerant varieties. In vitro propagation by meristem, cell organ and tissue culture, organogenesis, and somatic embryogenesis are presented. These techniques certainly may make breeding programs simpler and overcome some important economic and agronomic factors that might have never occurred with conventional plant breeding and improvement methods (Wang et al. 2016; De Filippis 2013). The method of plant tissue culture plays a dominant role in the second green revolution in which plant biotechnology is considered desirable crops. The yield and quality of the crops are greatly increased through the extensive use of this technology. Increasing nutrition and food safety are the basic points to consider before implementing tissue culture techniques.

18.8 Immobilization of Microbes to Improve Soil Health and Crop Yield

The use of beneficial microbes as bioinoculant increases their number in soil, which in turn increases the availability of nutrients to the plants. Yet, complications in technical handling are often observed with fungal cells when employed as bioinoculants for practical purposes, since satisfactory results are observed during in vitro conditions, but not typically realized in natural agricultural systems (Jain et al. 2010). A number of factors attribute to poor survivability and colonizing ability in rhizosphere, such as competition with native microbiota and abiotic stresses. Encapsulation of the cells in biodegradable capsules can be useful to overcome such hindrances.

18.8.1 Encapsulation of Bacterial Cells

Cell encapsulation facilitates sustainability and stability of biological functions; hence, enhanced cellular activities are realized (Juarez-Jimenez et al. 2012). Besides stability, encapsulation also aids in protecting the cells against all contrary ecological factors and facilitates slow release of cells into the soil in a controlled method, thus

improving the efficiency of microbial fertilizers or biofertilizers (Vassilev and Vassileva 2003).

18.9 Conclusion

The world population is growing rapidly. Thus, in the next few years, it will be the biggest challenge to feed a huge population. Global warming, restricted environmental conditions, and biotic factors limit crop yields. The main challenge for researchers working on different crops is to increase agricultural productivity to counter the demand for foodstuff supply to a rapidly expanding global population. Therefore, crop improvement is the main element of agricultural progress, and there are still a lot of zones left to work on in the field of crop improvement. Applications of RDT or genetic engineering to crop improvement are well suited to deciphering the problem of world hunger and depriving sustainable intensification.

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